

Environmental and Microbial Biotechnology

Mukesh Kumar Meghvansi  
A. K. Goel *Editors*

# Anaerobic Biodigesters for Human Waste Treatment

 Springer

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# **Environmental and Microbial Biotechnology**

## **Series Editor**

Ram Prasad, Department of Botany, Mahatma Gandhi Central University, Motihari,  
Bihar, India

Innovative and novel advances in microbial biotechnology are providing great understandings in to the machineries of nature, presenting fascinating prospects to apply principles of biology to different arenas of science. Sustainable elucidations are emerging to address the concerns on improving crop productivity through microbes, depleting natural resources, environmental pollution, microbial degradation of pollutants, nanomaterials, nanotoxicity & safety issues, safety of food & agricultural products etc. Simultaneously, there is an increasing demand for natural bio-products of therapeutic and industrial significance (in the areas of healthcare, environmental remediation, microbial biotechnology). Growing awareness and an increased attention on environmental issues such as climate change, energy use, and loss of non-renewable resources have carried out a superior quality for research that provides potential solutions to these problems. Emerging microbiome approaches potentially can significantly increase agriculture productivity & human healthcare and henceforth can contribute to meet several sustainable development goals.

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Mukesh Kumar Meghvansi • A. K. Goel  
Editors

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*Editors*

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## Preface

It is estimated that around 1–2 billion kg of human waste is generated per day globally. Unscientific disposal of major chunk of waste in such a huge amount poses serious challenges towards health, hygiene, and environment. Hence, the world over, serious research efforts have been made for the development of various technological solutions for treatment of human waste. Anaerobic digestion (AD) is one of the most commonly used approaches in this regard. During the past few decades, substantial understanding on the process and mechanistic aspects of anaerobic digestion has been developed. Globally, anaerobic digesters of varying capacity and designs have been put under operation. However, there are a lot of challenges associated with the performance and sustainability of anaerobic digesters. These challenges include configuration of appropriate digester design for desired performance, optimization of inoculum for startup and sustained performance, and the suitable interventions related to greater resource recovery. Various researchers have attempted to address these challenges.

Through this volume, sincere efforts have been made to compile the scientific information available on the understanding of paradigms as well as current sustainability approaches with a view to performance enhancement and resource recovery. This volume is divided in two parts. Part I comprises a total eight chapters which deal with various paradigms towards latest understanding of the anaerobic digestion process. Chapter 1 provides a broad and introductory overview of sewage treatment through anaerobic processes where Camila Pesci Pereira and her associates from Brazil discuss various technologies and characteristics. Chapter 2 authored by Anthony Anukam and Pardon Nyamukamba provides the significance of the chemistry of human excreta relevant to biogas production and discusses key criteria and values. Chapter 3 by Niti B. Jadeja and Rohini Ganorkar throws light on various mathematical models that have improved our understanding and operations of AD in recent times. Chapter 4 contributed by Taysnara Simioni and coworkers provides a comprehensive review of the global energy landscape, the production of biogas from sewage sludge, and the use and improvement of the biogas produced. In Chap. 6 authored by Basant Kumar Pillai and colleagues, the microbial community dynamics in anaerobic digesters and recent developments in biotechniques for assessing microbial diversity are discussed. Chapter 7 by the same authors provides an overview of sewage management and advocates the use of sewage sludge as energy

resource. Chapter 8 written by Chukwudi O. Onwosi and colleagues provides a description of basic types of anaerobic digesters, decentralized AD safety issues, and the main factors that drive the practical implementation of decentralized AD in Nigeria. Part II of the book having six chapters deals with sustainability approaches for performance enhancement and resource recovery. In Chap. 9, a critical assessment of various strategies related to performance enhancement of anaerobic digestion has been carried out by Taysnara Simioni and associates. Chapter 10 authored by Deisi Cristina Tápparo and coworkers provides an authoritative account of different technologies for pretreatment, focusing on thermal, ultrasonic, and enzymatic processes, discussing their effects on sludge properties and anaerobic digestion. Chapter 11 contributed by Vidal et al. aims to highlight the circular economy of sewage sludge anaerobic digestion considering the relevance of pretreatments and micropollutants presence for sustainability. Meghvansi and his colleagues critically discuss various aspects of inoculum optimization strategies to improve anaerobic biodigester performance in Chap. 12. Mohamed Mahmoud and Mohamed El-Qelish in Chap. 13 provide a critical assessment of the direct interspecies electron transfer (DIET) mechanism driven by conductive materials that enable value-added resources recovery from different types of organic wastes streams, their current limitations, and their potential scaling-up opportunities. The last but very important chapter contributed by Semiyaga and coworkers presents the potential of AD technology at decentralized FS treatment plants by analyzing the biodegradability characteristics of FS from different sources, BMP, co-digestion, operation and maintenance as well as management options of the produced slurry.

The editors would like to take this opportunity to express their sincere gratitude to all the authors of the book chapters for their valuable contributions. We are also grateful to Professor Ram Prasad, Springer series editor (Environmental Biotechnology), for his valuable insights during conceptualization as well as preparation of this volume. Our gratitude also goes to Rhea Dadra and Aakanksha Tyagi from Springer Nature for their timely support throughout the process of finalizing this volume. We are also thankful to the Director of Defence Research & Development Establishment for his encouragement, moral support, and generous permission to prepare this edited volume. Last but not the least, a special thanks goes to our family members for their support, affection, and care that helped immensely in keeping the editorial work on track.

Gwalior, Madhya Pradesh, India

Mukesh Kumar Meghvansi  
A. K. Goel

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## About the Editors

**Mukesh Kumar Meghvansi** received his PhD from Maharshi Dayanand Saraswati University, Ajmer (India), while working on a multinational research project sponsored by the European Commission. For a stint, he served as lecturer at Bundelkhand University Jhansi (India) and then joined Defence Research & Development Organisation (DRDO, India) in 2008 as a scientist. Since his joining, he is engaged in R&D on organic waste management. He has to his credit 60 publications in various international and national journals/books. He has filed three patents, one design, and one trademark in India. He is life member of Biotech Research Society India, Indian Botanical Society, and annual member of several national/international professional scientific bodies/societies. He is a reviewer of many national and international journals. He has authored/edited five books including two with Springer Nature, Switzerland. He also holds an MBA degree with specialization in project management. He is the recipient of DRDO Science Day Oration Award. In addition, he is empanelled with National Accreditation Board for Testing and Calibration Laboratories (NABL, India) as an Assessor for ISO/IEC 17025:2017. Currently, he is working as Scientist 'E' at Bioprocess Technology Division, Defence Research & Development Establishment, Gwalior (India). His current research area includes anaerobic biodegradation of human waste (night soil). He has been instrumental in design and development of floating biodigester (Mk-II) for Dal lake houseboats and its inland variant for individual household use in Srinagar (UT of J&K, India) for the purpose of human waste treatment. Biodigester Mk-II version has been licensed by DRDO to four Indian industries for commercialization.

**A. K. Goel** received his PhD (Microbiology) from CCS Haryana Agricultural University, Hisar (India), in 1999. As a prolific researcher in the field of microbial biotechnology, he has been recipient of several national awards including AMI-Young Scientist Award, DST (Department of Science & Technology, Govt of India)-Young Scientist Research Fellowship, DRDO-Laboratory Scientist of the Year Award, DRDO Young Scientist Award, DBT (Department of Biotechnology, Govt of India)-Overseas Associate Fellowship, DRDO Technology Group Award,

DRDO Science Day Oration Award, and DRDO Technology Day Oration Award. He has more than 100 research papers, ten patents, radio talks, and several overseas presentations to his credit. He is currently working as Scientist 'F' & Head, Bioprocess Technology Division, Defence Research & Development Establishment, Gwalior (India). Currently, he is leading a research group as a Project Director that is working on development of high performance biodigester (HPBD) for human waste treatment.

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**Part I**

**Towards Understanding of Paradigms**



# Sewage Treatment Through Anaerobic Processes: Performance, Technologies, and Future Developments

1

Camila Pesci Pereira, Adriana Alves Barbosa, and João Paulo Bassin

## Abstract

Biological wastewater treatment relies on the ability of microorganisms to degrade organic matter present in the waste streams, transforming it into inert or less harmful by-products to the environment. Anaerobic biological treatment is a very effective way to minimize aeration energy demand while reducing the organic load concomitant to the generation of valuable resources, such as biogas and compost. The first can be used for energy generation purposes, while the second can be employed as a soil conditioner. Despite these key pictures, anaerobic wastewater treatment processes are more sensitive to disturbance by many environmental factors, mainly because microbial growth is slower than that occurring under anaerobic conditions. However, in recent years, studies have been carried out to improve the biomass retention capacity of the reactors regardless of the hydraulic retention time. This has led to the development of high-rate anaerobic systems, which made it possible to overcome many limitations of conventional ones. This chapter aims to show different anaerobic sewage treatment technologies. First, the main sewage properties and treatment parameters are presented. Then, the main types and characteristics of anaerobic treatment processes will be assessed, focusing on the most used in recent years. The most modern and promising technologies are also described.

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**Keywords**

Sewage treatment · Anaerobic processes · Conventional systems · High-rate systems

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**Abbreviations**

4-NP	4-Nonylphenol
ABR	Anaerobic baffled reactor
AFBR	Anaerobic fluidized bed reactor
AH-ST	Anaerobic hybrid septic tank
AnMBR	Anaerobic membrane bioreactor
APBBR	Anaerobic packed bed biofilm reactor
ASBBR	Anaerobic structured bed biofilm reactor
BOD	Biochemical oxygen demand
CEC	Contaminants of emerging concern
CH <sub>4</sub>	Methane
CIP	Ciprofloxacin
CO <sub>2</sub>	Carbon dioxide
COD	Chemical oxygen demand
CST	Conventional septic tank
CST-M	Modified conventional septic tank
CSTR	Continuously stirred tank reactor
EC	<i>Escherichia coli</i>
EGSB	Expanded granular sludge bed
FC	Fecal chloroforms
GHG	Greenhouse gases
HBAP	Horizontally baffled anaerobic pond
HRAP	High-rate algal ponds
HRT	Hydraulic retention time
JMP	Joint Monitoring Program
N <sub>2</sub>	Molecular nitrogen
NH <sub>3</sub>	Free ammonia
NH <sub>4</sub> <sup>+</sup>	Ammonium ion
NO <sub>2</sub> <sup>-</sup>	Nitrite
NO <sub>3</sub> <sup>-</sup>	Nitrate
OLR	Organic loading rate
SMX	Sulfamethoxazole
SRT	Solids retention time
TC	Total chloroforms
TOC	Total organic carbon
TSS	Total suspended solid
UASB	Upflow anaerobic sludge blanket
UASB-M	Modified upflow anaerobic sludge blanket

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UNICEF	United Nations Children’s Fund
VBAP	Vertically baffled anaerobic pond
VSS	Volatile suspended solid
WHO	World Health Organization

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## 1.1 Introduction

The Population Division of the United Nations (UN) Department of Economic and Social Affairs reported that by mid-2020, the world’s population was estimated to be 7.8 billion and could increase to 9.8 billion by 2050. Furthermore, the report about progress on drinking water, sanitation, and hygiene from 2000 to 2017, prepared by the Joint Monitoring Program (JMP) of the World Health Organization (WHO) and the United Nations Children’s Fund (UNICEF [n.d.](#)), mentioned that approximately 2.2 billion people worldwide do not have access to treated water services, 4.2 billion do not have adequate sanitation services, and three billion do not have basic hand hygiene facilities. These figures only highlight the vast inequalities that exist regarding the accessibility and availability of water and sanitation.

An alternative to minimize these problems is to invest in a sustainable economy of water management, from the catchment, pretreatment, distribution, use, collection, and post-treatment of water to the use of treated wastewater and/or the discharge to the environment. Worldwide, 80% of wastewater is returned to the environment untreated, contributing to 1.8 billion people using contaminated water and acquiring numerous infectious diseases such as cholera, dysentery, typhoid, and polio (UN [2021](#)). Among the different technologies employed for the treatment of wastewaters, biological processes stand out due to their economic advantages. By using natural microorganisms in engineered treatment systems, often referred to as bioreactors, dissolved and particulate organic matter can be converted into gas ( $\text{CO}_2$ ,  $\text{CH}_4$ ) and solid products (bacterial and archaeal biomass).

Biological processes are classified as aerobic (presence of oxygen), anoxic (presence of oxidized nitrogen species and absence of oxygen), and anaerobic (absence of both oxygen and oxidized nitrogen species). This chapter addresses the main characteristics and types of anaerobic processes, both conventional and high-rate anaerobic systems, and their application in domestic sewage treatment.

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## 1.2 Sewage: Definition and General Characteristics

Sewage is wastewater that contains in its composition water, fractions of organic and inorganic compounds, and suspended and dissolved solids, as well as different microorganisms. The characteristics of sewage depend on compounds present in the aqueous phase on the liquid effluent, the climate, the socioeconomic situation of

the region or location where it was generated, the population habits, and the technological development, among countless other situations (Von Sperling 2007).

Sewage can be characterized by several chemical, physical, and biological characteristics. In sewage treatment plants, many parameters are frequently monitored to analyze the efficiency of the treatment and the quality of the final effluent for safe and less harmful disposal into the environment. According to Von Sperling (2007), the most important parameters to be analyzed are solids, organic matter indicators, nitrogen, phosphorus, and fecal contamination indicators. Table 1.1 shows some of these indicators as well as others that often appear in studies involving sewage.

Since there is no need to specifically characterize the organic matter as proteins, carbohydrates, lipids, and so on, it can, for practical reasons, be determined in the laboratory either directly or indirectly. The indirect way is to measure the consumption of oxygen needed to stabilize the organic matter by chemical and biochemical processes, using the chemical oxygen demand (COD) and biochemical oxygen demand (BOD) methods, respectively. On the other hand, organic matter can be directly measured by assessing the total organic carbon (TOC) method. These are the most commonly used analyses in studies on the characterization of wastewaters regarding the organic matter content.

The COD and BOD analyses are the most widely used for the determination of organic matter in wastewaters. In practical terms, the COD is easier to perform because it is faster (approximately 2–3 h). In this method, however, all organic matter fractions are oxidized, not allowing to distinguish between the biodegradable from the inert fraction. BOD analysis is more time-consuming and laborious (BOD<sub>5</sub> takes 5 days to complete); however, it oxidizes only the biodegradable organic matter, which makes it possible to evaluate the organic fraction that will be more easily biodegraded in the biological treatment. For domestic sewage, the COD/BOD ratio generally ranges from 1.7 to 2.4 (Von Sperling 2007). These analyses are important indicators of sewage quality, especially for monitoring biodegradable and recalcitrant organic matter in biological treatment.

The constant monitoring of these parameters and good characterization of the sewage are important because, besides allowing more efficient treatment, they can also predict adjustments in the parameters if changes occur in the effluent quality. Moreover, the disposal of a better-quality final effluent avoids many health risks and environmental pollution problems, such as the eutrophication of water bodies (Dos Santos and van Haandel 2021).

Table 1.2 presents examples of sewage characterization from treatment plants in different countries in the last 5 years.

It is observed that these values may vary not only between sewage samples from different locations but also between those from the same country, region, or even from the same treatment plant, collected and analyzed in different periods. Different samples can present quite discrepant results in studies about the same sewage, for example, for COD results of Sylla et al. (2017), which present a wide range of values (from 246.8 to 940.0). Von Sperling (2007), when surveying physicochemical characterization data of raw domestic sewage in developing countries, observed



**Table 1.1** Indicators commonly used for sewage characterization (Von Sperling 2007)

Characteristics	Indicators	
Physical	<b>Temperature</b>	
	<b>Color</b>	
	Apparent color	
	True color	
	<b>Odor</b>	
	<b>Turbidity</b>	
	Chemical	<b>Solids</b>
		Suspended solids
		<i>Volatile solids (organic matter)</i>
		<i>Fixed solids (inorganic matter)</i>
Solids dissolved		
<i>Volatile solids (organic matter)</i>		
<i>Fixed solids (inorganic matter)</i>		
Sediments		
<b>Carbonaceous organic matter</b>		
Protein compounds		
Carbohydrates		
Fats and oil		
Urea, surfactants, phenols, pesticides, etc.		
<b>Nitrogen</b>		
Molecular nitrogen (N <sub>2</sub> )		
Organic nitrogen		
Free ammonia (NH <sub>3</sub> )		
Ammonium ion (NH <sub>4</sub> <sup>+</sup> )		
Nitrite (NO <sub>2</sub> <sup>-</sup> )		
Nitrate (NO <sub>3</sub> <sup>-</sup> )		
<b>pH</b>		
<b>Phosphorus</b>		
Organic phosphorus		
Inorganic phosphorus		
<b>Oils and grease</b>		
<b>Alkalinity</b>		
<b>Chlorides</b>		
Biological		<b>Pathogenic organisms and indicators of fecal contamination</b>
		Bacteria
	Viruses	
	Protozoa	
	Helminths	
	Total chloroforms (TC)	
	Fecal chloroforms (FC)	
	Escherichia coli (EC)	

**Table 1.2** Examples of sewage characterization studied in different countries in the last 5 years

Wastewater denomination	Location	pH	COD (mg/L)	BOD (mg/L)	NH <sub>4</sub> <sup>+</sup> -N (mg/L)	Suspended solids (SS) (mg/L)	Total nitrogen (mg/L)	Total phosphorus (mg/L)	Reference
Municipal sewage	China	–	522.3	–	42.3	–	55.4	8.5	Gao et al. (2020)
Domestic sewage	China	6.7–8.2	140–160	–	50–70	–	70–80	3–4	Wei et al. (2020)
Urban sewage	China	–	567.1	–	22.81	–	31.6	5.1	Nie et al. (2021)
Raw sewage	Brazil	7.7	422.9	–	34.2	TSS <sup>1</sup> = 251.6 VSS <sup>2</sup> = 207.4	–	–	Vassalle et al. (2020)
Domestic wastewater	UK	8.0	451	196	–	TSS = 277 VSS = 235	–	–	Cruddas et al. (2018)
Domestic sewage	UK	–	300–600	–	–	VSS = from 30 to >450	–	–	Petropoulos et al. (2020)
Sewage	Italy	–	288.5	146.0	–	–	–	2.5	Boni et al. (2020)
Domestic wastewater	Benin (West Africa)	7.2	675–1983	261–440	18–34	211–509	–	6–22	Atinkpahoun et al. (2018)
Sewage	Morocco	7.5–8.6	246.8–940.0	111.5–266.3	22.7–148	TSS = 0.3–0.66	70.3–159.6	–	Sylla et al. (2017)

<sup>1</sup>TSS total suspended solids<sup>2</sup>VSS volatile suspended solids

that organic matter indicators such as COD and BOD present values between 450–800 mg/L and 250–400 mg/L, respectively.

Regarding total nitrogen and total phosphorus, the values in Table 1.2 are closer to those presented by Von Sperling (2007), who reported the values for total nitrogen between 35 and 60 mg/L and total phosphorus within 4–15 mg/L. In raw domestic sewage, nitrogen is generally in organic and ammonia forms, the latter coming mainly from urea, while a large part of the phosphorus in raw domestic sewage may come from detergent substances (Von Sperling 2007). Excess nutrients may cause eutrophication of water bodies.

Thus, taking into account the several polluting compounds present in the sewage, it must undergo appropriate treatment to be disposed of with minimally acceptable quality and avoid damage to the environment.

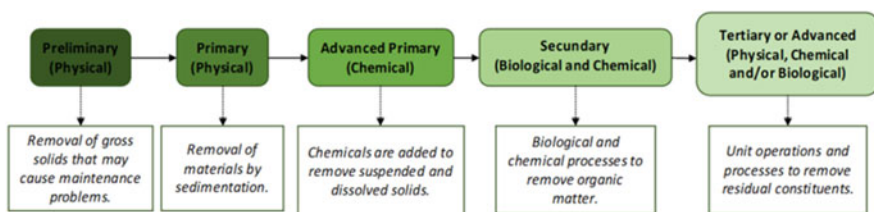
### 1.3 Sewage Treatment

Wastewater treatment methods usually involve several unit operations comprising physicochemical and biological processes. In this sense, wastewater treatment is composed of several steps to improve the quality of the final effluent as each one is performed (Metcalf and Eddy 2003). Figure 1.1 presents a summarized scheme of these steps. The focus of this chapter is on sewage treatment by anaerobic processes, which is part of the secondary treatment stage.

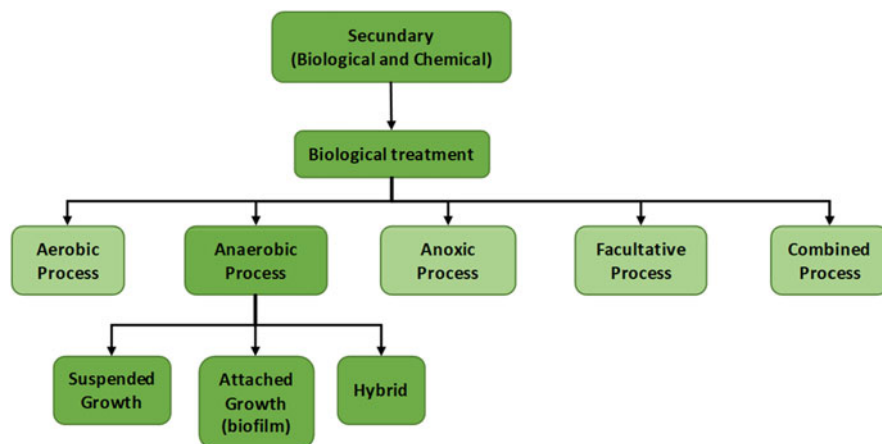
Like the other processes, the anaerobic treatment processes involve different technologies and applications for both sewage treatment and other kinds of wastewaters. The following sections present different modalities of anaerobic processes, from the most traditional to the most modern ones, showing different studies on this topic and the respective performances achieved.

#### 1.3.1 Biological Treatment

The main objectives of biological wastewater treatment are to promote the digestion of biodegradable organic matter into inert material, reduce nutrients (nitrogen and phosphorus), and remove suspended solids and/or other specific compounds.



**Fig. 1.1** Summary scheme of the wastewater treatment steps. (Adapted from Metcalf and Eddy 2003)



**Fig. 1.2** Classification of the biological treatment and anaerobic process. (Adapted from Metcalf and Eddy 2003)

Biological treatment processes can be classified according to their metabolic function. The main processes are aerobic, anaerobic, anoxic, facultative, and the combination of the previous (Metcalf and Eddy 2003).

According to the classification of Metcalf and Eddy (2003), biological processes are classified into three categories: suspended growth, attached growth, and hybrid (combination of the two forms of microbial growth). Figure 1.2 presents a more detailed scheme of the classifications of biological treatment and, specifically, of the anaerobic process.

In suspended growth, the microorganisms responsible for the degradation of organic matter and nutrients are kept in suspension mixed with the liquid. This category is not widely applied in the anaerobic process for sewage treatment but rather to high-organic-strength industrial wastewaters (Metcalf and Eddy 2003). Studies focused on anaerobic treatment over the years have led to the development of high-rate anaerobic systems, allowing the biomass to be retained longer inside the reactors and separating the hydraulic retention time (HTR) from the solids retention time (SRT) in the reactor (Chernicharo 2007). In this way, the reactor performance is improved, and the treatment of high loads at lower HRT becomes feasible.

In attached growth processes, an inert material is used as a support for the adhesion of microorganisms, which form a biofilm capable of degrading organic matter and nutrients. The support media can be stones, sand, plastic materials, and other natural or synthetic materials that can be fully or partially submerged in the liquid (Metcalf and Eddy 2003).

A little different from Metcalf and Eddy (2003), Chernicharo (2007) divides anaerobic treatment systems only into two categories: conventional systems (anaerobic sludge digesters, septic tanks, and anaerobic ponds) and high-rate systems (either attached growth or suspended growth processes). According to the author, there is no defined separation between these two systems.

However, the classification is important, as it determines an important advance in anaerobic reactor technology, the development of high-rate systems, to which much research effort has been directed.

---

## 1.4 Anaerobic Digestion

Anaerobic digestion is a delicate metabolic process that occurs in a sequential manner and in phases, which are interdependent. The main phases are described below (Chernicharo 2007):

**Hydrolysis phase (1):** in this phase, microorganisms break down large-chain molecules into smaller and simpler ones (dissolved material) for better incorporation of these into their cell and facilitate the fermentation. This process can be slow, depending on the material and factors such as reactor temperature, the residence time of the substrate in the reactor, pH, and  $\text{NH}_4^+\text{-N}$  and hydrolysis product concentrations in the liquid.

Among the bacterial genera capable of performing hydrolysis are *Clostridium*, *Micrococcus*, *Staphylococcus*, *Acetivibrio*, *Eubacterium*, *Streptococcus*, *Bacillus*, etc. Hydrolytic and fermentative microorganisms benefit energetically as they are the first to convert the substrates in the anaerobic digestion process.

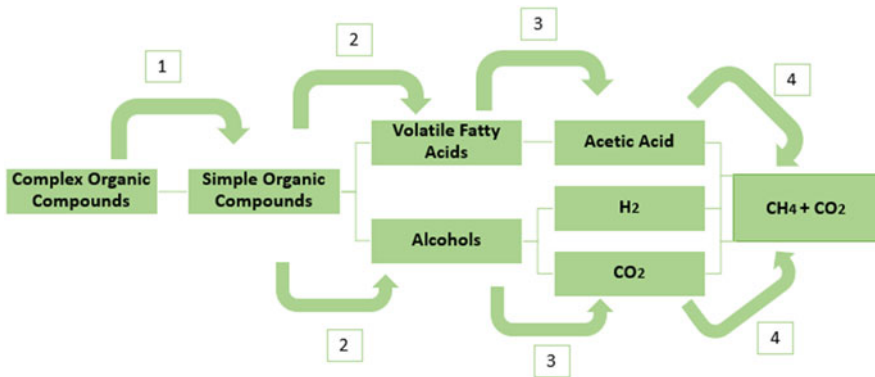
**Acidogenesis phase (2):** this phase is often considered an extension of the previous one. Acidogenic bacteria ferment the substrate metabolized in the hydrolysis phase. The new compounds formed, usually organic acids, are prepared for the subsequent phase, acetogenesis. Some microorganisms responsible for the acidogenic phase are *Bacteroides*, *Ruminococcus*, *Butyribacterium*, *Lactobacillus*, *Streptococcus*, *Pseudomonas*, and others.

**Acetogenesis (3):** in this stage, the microorganisms oxidize intermediate organic compounds to acetate, hydrogen, and  $\text{CO}_2$ . The acetate and hydrogen concentration in this step must be kept low so that the pH does not decrease, which would otherwise compromise the process. For hydrogen to not accumulate in the liquid phase, it must be consumed in subsequent steps. Examples of syntrophic acetogenic bacteria are *Syntrophobacter* and *Syntrophomonas*.

**Methanogenesis (4):** it is the final stage of anaerobic digestion. The organic matter metabolized in the previous steps is converted into methane and  $\text{CO}_2$ . Methanogenic archaea are the main functional groups that carry out this phase. These organisms play an essential role, as they remove excess hydrogen and the fermentation products from the previous stages, resulting in a stable anaerobic process.

A diagram of anaerobic digestion steps is presented in Fig. 1.3.

In the anaerobic treatment process, the characteristics of the sewage, such as nutrients, temperature, pH, alkalinity, toxic substances, etc., are very important for proper treatment. For an efficient biological treatment, the nutrients must be in sufficient concentrations for the growth of the organisms that will perform the



**Fig. 1.3** Anaerobic digestion steps diagram. (1) Hydrolysis, (2) acidogenesis, (3) acetogenesis, (4) methanogenesis. (Adapted from Zeeman et al. 2008)

organic matter digestion. Laboratory analyses are important to evaluate the amount of nutrients necessary for the good progress of the process. Domestic sewage usually has the necessary components for this, which may not be true for some industrial wastewaters (Chernicharo 2007).

Temperature is an important factor in anaerobic treatment since microorganisms do not have the ability to maintain their own cell temperature. In anaerobic digestion, the mesophilic temperature range (30–35 °C) can be considered optimal, but some processes adopt the thermophilic temperature range (50–55 °C). However, this is arguable in terms of advantages due to instabilities in the process, and cost-effectiveness, since the energy consumption to keep the system heated is higher (Chernicharo 2007). More recent studies promoted wastewater treatment by anaerobic processes at lower temperatures (10–20 °C), and good results were reported (Zhang et al. 2013, 2018; Mainardis et al. 2020).

A pH outside the optimal range can also impair the anaerobic process, since it can compromise enzyme activity and lead to toxicity effects, as that posed by free ammonia (NH<sub>3</sub>) at high pH. Although acid-producing bacteria are quite tolerant to a wider pH range (even low pH), the pH control has mainly the purpose of maintaining the activity of methanogenic archaea, whose optimum range is between 6.6 and 7.4, but a stable production is also between 6.0 and 8.0. In general, for the smooth operation of anaerobic reactors, pH in the range of 6.5–8.0 is preferable. Thus, the monitoring of alkalinity is also an important factor, since it will dictate the ability to neutralize the acids formed in the system and, at the same time, promote the buffering effect, thereby avoiding sudden changes in pH (Chernicharo 2007).

The toxicity of some substances in the anaerobic process is more dependent on their concentration than their nature. In fact, any substance at a high concentration can be harmful to anaerobic microorganisms. However, several procedures have been adopted to partially or fully eliminate some of these compounds and minimize the harmful effects they may bring (Chernicharo 2007).

## 1.5 Anaerobic Reactors

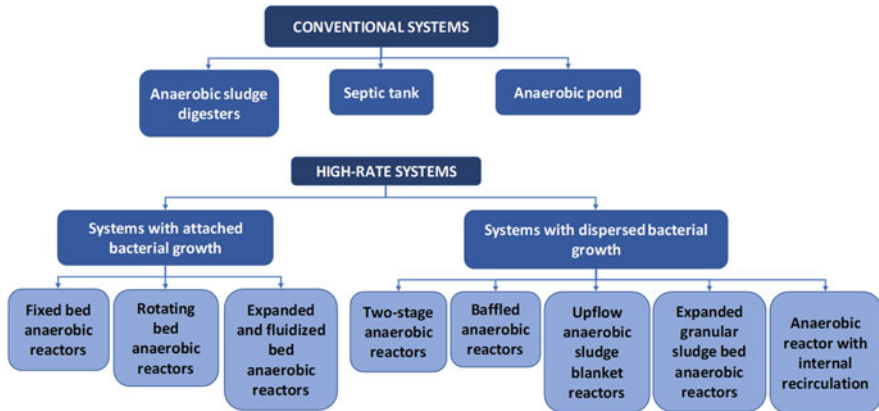
According to Chernicharo (2007), anaerobic systems used for sewage treatment consist of conventional and high-rate systems. In turn, these categories can also be divided into subcategories.

The conventional systems correspond to reactors that do not have the capacity to maintain the biomass for a long time in the reactor, hindering the development of slow-growing microorganisms. These systems usually operate at high HRT and low volumetric load, resulting in lower treatment capacity. Conventional systems are subdivided into (Chernicharo 2007):

- **Anaerobic sludge digesters:** these are often used to stabilize the primary and secondary sludge from sewage treatment. They are not efficient for domestic wastewater treatment, which is usually diluted, being more applied to treat waste streams with a high concentration of suspended material.
- **Septic tank:** low-rate anaerobic digestion process with the function of sedimentation and removal of floating material (mainly oil and grease). The conventional septic tank is used in decentralized systems, being relatively cheap and simple to install. It is considered a low-efficient system, often requiring post-treatment. There are some limitations regarding the treatment performance, the anaerobic conditions of the system, the growth and activity of the microorganisms, and the biodegradation mechanisms (Shaw and Dorea 2020).
- **Anaerobic pond:** it is a low-rate system, as it requires a high HRT to perform the treatment. They are often used to treat wastewaters with high concentrations of organic matter. With a large volume, they are also used for domestic wastewater treatment in tropical regions.

High-rate anaerobic systems arose from the need to reach a higher volumetric treatment capacity by means of better retention of the biomass in the tank. In these processes, the HRT is dissociated from the SRT inside the reactor (Clara et al. 2005; Chernicharo 2007; Carneiro et al. 2020). Under these conditions, even at low HRTs, the proper growth of the microorganisms and the efficiency of the treatment are not hampered. The development of research in this direction has led to the evolution of more compact and efficient systems. Two major groups make up the high-rate anaerobic treatment systems: attached and dispersed (or suspended) growth processes (Chernicharo 2007):

- **Attached growth systems:** these correspond to (1) fixed-bed reactors, which are a kind of anaerobic filters, in which microorganisms adhere to a fixed packing material; (2) rotating biological contactors, also called biodiscs, in which the microorganisms adhere to rotating discs totally or partially submerged, resulting in biofilm formation; and (3) expanded and fluidized bed, in which the biofilm grows forming very thin layers, reducing or eliminating the clogging problem that occurs in the other adhered growth systems.



**Fig. 1.4** Summary flowchart of anaerobic system classifications and their subdivisions. (Adapted from Chernicharo 2007)

- Dispersed growth systems:** the microbial growth occurs in suspension within the reactor and depends on the capacity of the biomass to form flocs that can settle. Examples of systems that fall into this category are (1) two-stage anaerobic reactor, with a complete mixing reactor and a sedimentation and solids recirculation tank, similar to the aerobic activated sludge system; (2) baffled anaerobic reactor, similar to a septic tank, but with multiple layers arranged in series; (3) upflow anaerobic sludge blanket reactor (UASB), which is fed in upflow mode through a dense layer of sludge with high activity; (4) expanded granular sludge bed (EGSB) anaerobic reactor, in which the granular sludge is kept expanded and dispersed due to the high hydraulic rate of the system; and (5) anaerobic reactor with internal circulation (IC), which is a variation of UASB, but allows the treatment of higher organic loads.

Figure 1.4 presents a flowchart that summarizes the anaerobic system classifications and their divisions.

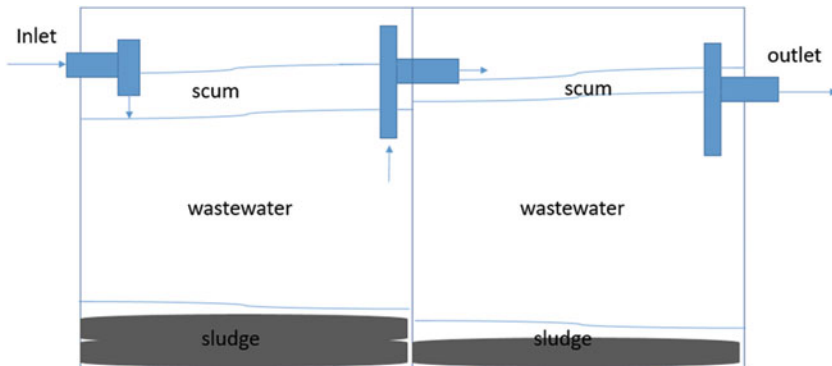
In the subsequent sections, recent studies on anaerobic sewage treatment addressing these different reactor configurations, both conventional and high-rate systems, are presented. Not all reactor categories are presented, but some examples, focusing on the most relevant studies on the topic of anaerobic sewage treatment, making use of the most traditional to the most modern systems, are described.

## 1.5.1 Conventional Systems

### 1.5.1.1 Septic Tank

The conventional septic tank system becomes an important sewage treatment mechanism, since it is a relatively simple and cheap technology. Moreover, its installation can be done in a decentralized manner, in situ. This facilitates bringing basic





**Fig. 1.5** Conventional septic tank diagram. (Adapted from EPA 2021)

sanitation even in more remote regions, where there is no centralized sewage treatment network, thus improving the quality of life of the local population and the ecosystem (Shaw and Dorea 2020). According to a report by the United Nations Children’s Fund (UNICEF) and the World Health Organization (WHO), septic tank coverage in the world is approximately 1.7 billion (data from 2020).

In general, septic tanks receive the wastewater, separate and accumulate solids by sedimentation and/or flotation, promote digestion of organic matter, and then dispose of the final effluent, with or without further treatment, as presented in Fig. 1.5. Although it is a limited system, a good understanding of the process can avoid major pollution problems caused by the digestion of organic matter, such as inadequate disposal of sludge and the final effluent, which may comply with environmental legislations standards due to inefficient treatment and poor management of biogas emitted during digestion (Shaw and Dorea 2020).

Because it is still widely used, but considered inefficient somehow, more recent studies target improving the operation of septic tank systems. These often propose modifications and innovations in the conventional septic tank and promote comparative studies on the treatment performance of both conventional systems and their variation.

This is the case of the study developed by Connelly et al. (2019), in which they compared the performance between a conventional septic tank and a solar septic tank, an innovation of the conventional system, for the treatment of domestic sewage. The authors focused on characterizing the microbiology community in anaerobic digestion to correlate it with the operating conditions and performance of the two systems.

The solar septic tank is an underground chamber heated by solar energy to a temperature range of 50–60 °C. Raw sewage enters the chamber and undergoes a partial pasteurization process. This process promotes the reduction of *E. coli*. In addition, due to this heating throughout the tank, there is increased microbial degradation of organic matter (Connelly et al. 2019).

**Table 1.3** Classifications of anaerobic septic tank systems (adapted from Shaw and Dorea 2020)

Classification	Initials	Description
Conventional septic tank	CST	A system of one or two compartments with no modifications. The purpose is to collect wastewater, separate solids (sludge and scum), and accumulate and discharge effluent
Modified conventional septic tank	CST-M	A system with any modification to conventional design (e.g., baffled, temperature modifications, anaerobic filter, additional treatment, biofilter, mixer)
Anaerobic hybrid septic tank	AH-ST	A system that consists of a sludge bed in the lower part of the tank and an anaerobic filter in the upper part of the tank
Continuously stirred tank reactor	CSTR	An anaerobic reactor system with a mixer, continuously stirred
Upflow anaerobic sludge blanket septic tank	UASB	The reactor is fed from the bottom and is typically divided into four compartments (bottom to top): sludge bed, fluidized zone, gas-liquid separator, and settling compartment
Modified upflow anaerobic sludge blanket septic tank	UASB-M	A system that implemented any modifications to the UASB reactor design (e.g., temperature; post-treatment additions)

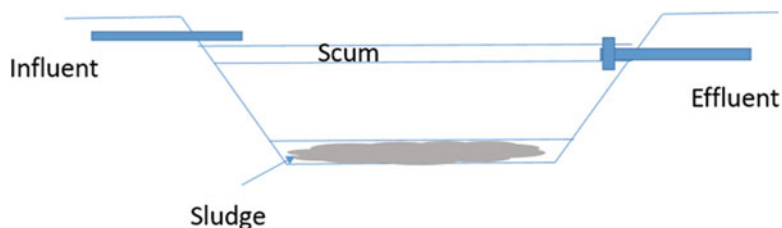
As a result, it was observed that the solar septic tank performed better than the conventional septic tank, showing COD, BOD, and TSS removal of 89%, 75%, and 96%, respectively, while the removal performance of the conventional septic tank were 70%, 70%, and 84%, respectively, for COD, BOD, and TSS. Furthermore, the authors concluded that a good understanding of the system is important for maintaining the microbial community and, consequently, improving treatment performance through anaerobic digestion (Connelly et al. 2019).

In addition to the solar septic tank, there are several other modified septic tank systems. Shaw and Dorea (2020) established a classification of decentralized sanitary systems, shown in Table 1.3.

### 1.5.1.2 Anaerobic Pond

Anaerobic ponds are a type of stabilization pond, in which the wastewater treatment takes place in the absence of oxygen. This system can be combined with others, such as facultative ponds (Chernicharo 2007) and UASB reactors (Dos Santos and van Haandel 2021). Figure 1.6 illustrates an anaerobic pond system.

Anaerobic ponds and stabilization ponds, in general, achieve good removal of organic matter and pathogenic microorganisms from sewage. However, they are not efficient for nutrient removal (nitrogen and phosphorus) and may cause eutrophication by the discharge of these compounds in aquatic ecosystems. In addition, anaerobic digestion can generate a considerable amount of nonbiodegradable solids and biogas (Dos Santos and van Haandel 2021). Table 1.4 presents the main advantages and disadvantages of this system in more detail.



**Fig. 1.6** Anaerobic pond system. (Adapted from DNR 2013)

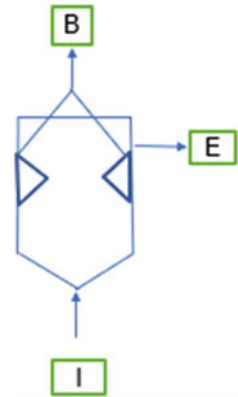
**Table 1.4** Main advantages and disadvantages of the anaerobic lagoon system (Chernicharo 2007; Dos Santos and van Haandel 2021)

Advantages	Disadvantages
It is suitable for wastewaters with a high concentration of organic matter	They are large, high-volume systems and therefore require a large installation area
Suitable for the treatment of wastewaters from tropical regions	They are low-rate systems, so there is a need for higher hydraulic retention times for efficient wastewater treatment
It is a relatively simple system to implement, with lower costs and low energy consumption	They are not efficient in the removal of nutrients (nitrogen and phosphorus). Thus, inadequate treatment or the absence of another type of treatment can lead to eutrophication of the receiving water body
Because they are large-volume systems, they do not require the removal of accumulated solids very often	They are biogas generators that contribute to the greenhouse effect if emitted into the environment without proper treatment In addition, the gases produced can cause odor if emitted without prior treatment
With proper management of the emitted gas, the system can be an interesting biofuel source	They can have a considerable accumulation of nonbiodegradable solids due to sedimentation that needs to be removed from time to time, increasing the operating costs

Although it is a relatively inexpensive system with low cost and energy consumption, it generally applies in combination with other treatment processes, since there is a need for more efficient nutrient removal, for example. In recent years, with the need for sewage treatment at higher rates and with better quality of the final effluent, more modern systems, such as UASB reactors, have replaced the conventional ones or have been combined with the stabilization pond system (Dos Santos and van Haandel 2021). An example of a UASB reactor is displayed in Fig. 1.7. Influent (I) is fed at the bottom of the reactor with ascending flow. At the top of the reactor, there is a three-phase separator with two outlets for treated effluent (E) and biogas (B).

In the study by Dos Santos and van Haandel (2021), for example, a UASB reactor was used in series with a stabilization pond system, with some modifications (polishing ponds), to evaluate the treatment of municipal sewage. The authors evaluated the decay of thermotolerant chloroforms and the removal of nutrients.

**Fig. 1.7** UASB reactor.  
*I* influent, *E* effluent, *B* biogas.  
 (Adapted from Lier et al. 2015, Stazi and Tomei 2018, Akunna 2019)



For conditions more favorable to nutrient removal, it was necessary to increase the HRT so that the pH is raised to a range suitable for efficient nutrient removal.

There is a concern in monitoring and mitigating biogas production in anaerobic pond systems. The reuse of the biogas generated in the digestion process as renewable energy is being implemented, reducing the emission of greenhouse gases (GHG) and thus the adverse effects on the environment. Cruddas et al. (2018) developed a staged anaerobic pond from two others systems, the horizontally baffled (HBAP) and the vertically baffled (VBAP) anaerobic pond, combining one or two stages for the treatment of domestic sewage. Among the aspects evaluated by the authors was the production of biogas. They concluded that the biogas production is higher and more stable using the two-stage system due to a more efficient anaerobic digestion over time.

## 1.5.2 High-Rate Systems

Although there is no clear delineation in the classifications of anaerobic systems, many systems considered to be high-rate processes are variations of conventional systems. The development of the ability to keep the biomass longer inside the reactors has allowed anaerobic treatment processes to be more efficient and work at higher volumetric rates.

Therefore, several studies aimed at improving conventional reactors addressing not only the operational parameters, such as HRT and the organic loading rate (OLR), but also the behavior of microbial growth, such as the activity and microbial community structure within the reactor, which many authors consider to be the main point to obtain an efficient treatment.

### 1.5.2.1 Attached Growth Systems

The use of immobilized biomass systems, such as fixed-bed reactors, has shown good results in anaerobic wastewater treatment processes. Studies in this direction have not only evaluated the reduction of organic matter and nutrients in general but

have been also shown promising in the biodegradation of specific toxic compounds, such as the so-called contaminants of emerging concern (CEC).

These contaminants are composed of a diversity of hygiene products, cosmetics, and pharmaceuticals, among others, eliminated in domestic sewage and various other environmental matrices. These compounds are often persistent to conventional treatment and can cause several health problems if not properly degraded (Carneiro et al. 2020).

Anaerobic treatment systems that form immobilized film have the ability to maintain high SRT with relatively low HRT. A high SRT allows slower microorganisms to grow, maintaining microbial diversity in the biofilm, which is necessary for the capture and biodegradation of these pollutants to be more efficient (Clara et al. 2005; Carneiro et al. 2020).

In this scenario, Carneiro et al. (2020) studied the treatment by a fixed-bed anaerobic reactor system for drug biodegradation. They evaluated not only the relationship of OLR with the operational parameters (SRT and HRT) but also the microbial community of the process and its effect on the removal of two antibiotics, sulfamethoxazole (SMX) and ciprofloxacin (CIP). The result of the study corroborates what has already been observed for this type of treatment, that is, the increase in OLR by decreasing the HRT had a negative effect on the removal of the pharmaceutical compounds, since it decreased the contact time of the biofilm with the antibiotics. Furthermore, the authors compared two different configurations of anaerobic fixed-bed biofilm reactors: the anaerobic structured bed biofilm reactor (ASBBR) and the anaerobic packed bed biofilm reactor (APBBR). The ASBBR presented the best performance in relation to biodegradation and biomethane production because this reactor was richer in archaea, important organisms for the process. For the ASBBR reactor, the removal of SMX was 94% (at OLR of 0.6 kg COD/m<sup>3</sup> day) and 81% (at OLR of 2 kg COD/m<sup>3</sup> day), while CIP removal was 85% (at OLR of 0.6 kg COD/m<sup>3</sup> day) and 64% (at OLR of 2 kg COD/m<sup>3</sup> day). For the APBBR reactor, the removal of SMX was 93% (at OLR of 0.6 kg COD/m<sup>3</sup> day) and 69% (at OLR of 2 kg COD/m<sup>3</sup> day), and that of CIP was 85% (at OLR of 0.6 kg COD/m<sup>3</sup> day) and 66% (at OLR of 2 kg COD/m<sup>3</sup> day).

Like anaerobic fixed-bed reactors, anaerobic fluidized bed reactors are being studied for the removal of micropollutants from domestic sewage. An example is the research by Dornelles et al. (2020), whose goal was to remove 4-nonylphenol (4-NP), an endocrine disruptor, from the degradation of a surfactant widely used in detergents, personal care products, pharmaceuticals, and other products that can be disposed of in both domestic and industrial sewage systems (EPA 2014). The system addressed was the anaerobic fluidized bed reactor (AFBR), using sand as a support. The treatment showed good results not only in the degradation of 4-NP but also in the removal of organic matter which was analyzed by COD removal.

### 1.5.2.2 Dispersed Growth Systems

#### Anaerobic Baffled Reactor (ABR)

According to Pfluger et al. (2020), anaerobic systems such as UASB and anaerobic baffled reactor (ABR) are potentially relevant if combined with other biological processes, since they leave a lot to desire concerning the standards required for the disposal of the final treated effluent, especially regarding nutrient removal. However, these systems generally require low energy consumption and are capable of producing biogas that can be reused as an energy source. The study conducted by these authors evaluated the generation of methane through the treatment of sewage by anaerobic sludge blanket bioreactors compartmentalized at a pilot scale using ABR systems. The observed results suggested that the evaluated system should replace the conventional primary treatment to optimize the production of methane, which can be used as fuel and enhance energy generation. An example of an ABR reactor is shown in Fig. 1.8.

Several studies have been developed aiming to treat numerous wastewaters, among them sewage, using these systems. The UASB system has received much attention in recent years, being one of the most studied and widely used reactors (Mainardis et al. 2020).

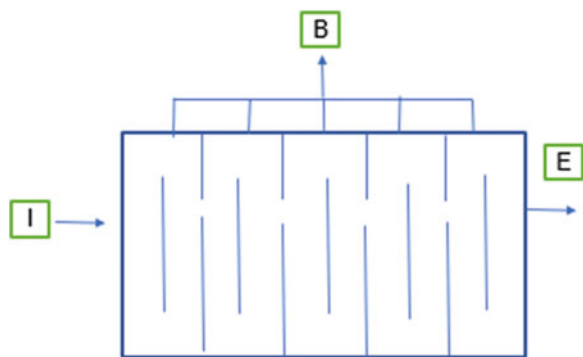
#### Anaerobic Contact Process (ACP)

The anaerobic contact process works like a conventional activated sludge system without aeration. The ACP comprises a biological reactor with mechanical mixing and a sedimentation tank for separating sludge from wastewater. According to Van Haandel and Van Der Lubbe (2019), the ACP is little used in sewage treatment, as it presents difficulties in the separation step taking place in the sedimentation tank due to the presence of residual biogas, resulting in sludge flotation. A schematic representation of the ACP reactor is presented in Fig. 1.9.

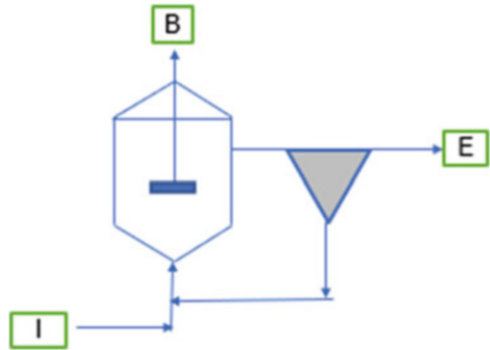
#### Continuously Stirred Tank Reactor (CSTR)

The continuously stirred tank reactor (CSTR) is a fully mixed ideal reactor often used in wastewater treatment due to its simplicity of design and operation, in

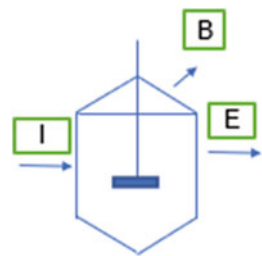
**Fig. 1.8** ABR reactor.  
*I* influent, *E* effluent, *B* biogas.  
 (Adapted from Lier et al. 2015, Stazi and Tomei 2018, Akunna 2019)



**Fig. 1.9** Schematic representation of ACP reactor. *I* influent, *E* effluent, *B* biogas. (Adapted from Van Haandel and Van Der Lubbe 2019)



**Fig. 1.10** Schematic representation of the CSTR reactor. *I* influent, *E* effluent, *B* biogas. (Adapted from Akunna 2019)



addition to providing greater uniformity of system parameters such as temperature, mixing, wastewater composition, intermediates, biotransformation products, and homogenization of microorganisms throughout the reactor. The CSTR works in a steady state with a continuous flow of reactants and products, and the feed assumes a uniform composition throughout the reactor, and the output flow has the same composition as in the tank (Lier et al. 2015). A representative schematic of a CSTR reactor is illustrated in Fig. 1.10.

### Upflow Anaerobic Sludge Blanket (UASB)

UASB is one of the most widely used anaerobic systems in the world. In addition to having the ability to retain solids for a longer time without the need for a long HRT, typical of high-rate systems, which allows a more efficient treatment, they also have other advantages, such as (Mainardis et al. 2020):

- It exhibits good biodegradability performance over a wide range of organic loading rates, achieving satisfactory treatment results even for more dilute wastewaters.
- The granular sludge formed inside the reactor has an excellent sedimentation rate, which allows more efficient solid-liquid separation.
- Its configuration allows excellent mixing between the biomass and the wastewater to be treated, making the anaerobic degradation faster. The movement of the granular sludge that expands vertically in the reactor helps the sludge bind to the

pollutants, creating an interaction between them and forming a highly active biofilm for the treatment.

- The biogas generated in anaerobic digestion facilitates mixing within the reactor, while the three-phase separation system at the top of the reactor allows for efficient separation of the biogas, which can later be treated and used for energy generation.
- Compared to aerobic processes, it has a much lower energy consumption, is efficient even at high organic loading rates, needs limited nutrients, and thus produces less sludge.

However, UASB also has some known disadvantages (Mainardis et al. 2020):

- Low removal capacity of nutrients such as nitrogen and phosphorus, as well as micropollutants.
- At start-up, inoculation must be provided sufficiently to avoid problems such as vulnerability, sensitivity in the process, odor, and a long initiation period.
- Temperature can be a limiting factor in microbial growth. Thus, there is a difficulty in achieving good treatment at low temperatures, typical of colder countries. Since most UASB reactors operate under mesophilic conditions, maintaining them at this temperature range requires energy consumption to heat the system.

Mainardis et al. (2020) have reported that some studies have shown reasonably efficient treatment of domestic sewage (COD removal of 23–60%) at lower temperatures (10–15 °C) and, in some cases, with considerable biogas production.

Zhang et al. (2013) observed an increase in COD removal efficiency from 6% to 23% in municipal sewage treatment in a pilot-scale UASB-digester system operating at 15 °C. The authors used glucose as a co-substrate in the digester for the growth of methanogens and recirculated to the UASB for inoculation; this provided an increase of over 90% in CH<sub>4</sub> production. They concluded that this co-digestion system is feasible for the pretreatment of municipal sewage in colder regions.

Later, Zhang et al. (2018) observed that COD removal decreased with the temperature in the treatment of domestic sewage in a UASB-digester system. At a temperature of 20 °C, 60% COD removal was obtained, while at 10 °C, the removal was 51%. The system operated at an HRT of 6 h. The authors observed that the low efficiency at lower temperatures was due to the slow growth of the methanogens and, even operating with a very oscillating composition of the influent, the final effluent presented stable COD, confirming that the process is viable as a pretreatment for lower temperatures (10–20 °C). In addition, CH<sub>4</sub> production was also possible, reaching 80% of the biological methane potential (BMP) of the influent.

In the previous examples and in recent literature, UASB reactors are well studied, but in most cases, they are combined with other systems. They are often considered a biological pretreatment, and, when associated with other technologies, the treatment performance is enhanced, both in the removal of organic matter and in what concerns biogas production.



In this context, Vassalle et al. (2020) evaluated sewage treatment by co-digestion of the effluent through the UASB system followed by the use of high-rate algal ponds (HRAP). In this configuration, similar to the previous examples, the microalgal biomass produced in the HRAP recirculates to the UASB and was co-treated with raw sewage. This configuration helps the growth of methanogens and, consequently, improves the digestion performance and  $\text{CH}_4$  production. After the treatment, 65% of COD and 61% of  $\text{NH}_4\text{-N}$  were removed, and a 25% higher  $\text{CH}_4$  yield with the co-digestion of sewage and microalgae was obtained.

Finally, a huge potential for the development of the UASB system is observed, not only in sewage treatment plants, combining them with different biological treatment processes, but also in smaller scales, in places where centralized treatment plants cannot be implemented. In this case, the application of UASB-type reactor in rural regions, for example, can be an excellent alternative to sewage treatment, besides allowing a huge gain, not only by improving the basic sanitation of the local population but also due to the reuse of biogas for energy production and, additionally, prevention of GHG emissions (Passos et al. 2020).

### Expanded Granular Sludge Bed (EGSB)

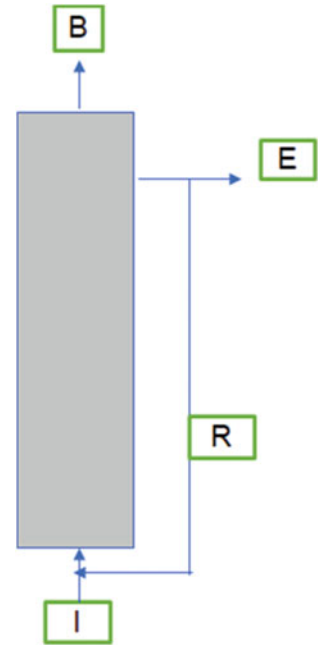
The anaerobic expanded granular sludge bed (EGSB) reactor is similar to the UASB reactor, except for the type of sludge and the degree of expansion of the sludge bed. The granular type is retained in the EGSB reactor, remaining expanded due to the high hydraulic rates applied to the system, intensifying the hydraulic mixture and ensuring better contact between the biomass and the substrate. The high effluent recirculation rate and a height/diameter ratio of around 20 allow for a higher surface velocity of the liquid in the reactor, ranging from 5 to 10 m/h. However, in UASB reactors, the sludge bed remains static due to the low surface velocity of the liquid, ranging from 0.5 to 1.5 m/h. EGSB reactors are mainly used for the treatment of wastewaters with soluble components, since the high surface velocity of the liquid inside the reactor does not allow the efficient removal of particulate materials. Furthermore, the excessive presence of suspended solids in the influent can be detrimental to maintaining the good characteristics of the granular sludge (Chernicharo 2007). A schematic representation of the EGSB reactor is presented in Fig. 1.11.

### Internal Circulation (IC)

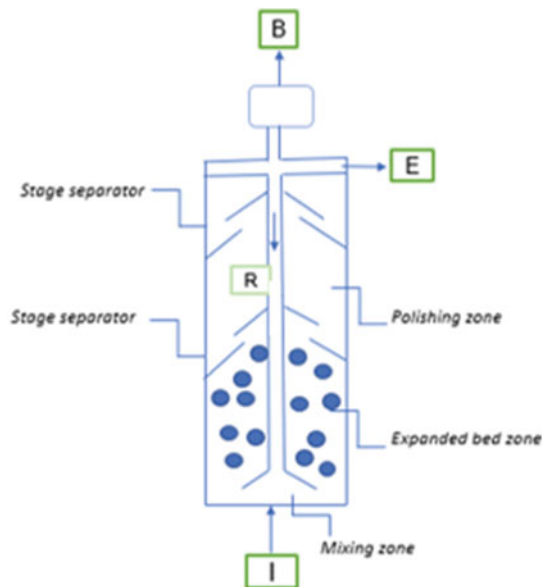
The IC reactor is a special version of the EGSB, consisting of two compartmented UASB reactors, one on top of the other. The first compartment is subject to high organic loads, in the range of 30–40 kg COD/m<sup>3</sup> day.

The separation of gases is carried out in two stages in a reactor with a height between 16 and 20 m, causing the gases collected in the first stage to drag the internal mixture composed of gas, solids, and liquid to the upper part of the reactor. After this step, solids and liquids recirculate to the first compartment, ensuring the mixing and contact of the recirculated biomass with the influent at the bottom of the reactor (Chernicharo 2007).

**Fig. 1.11** Schematic representation of ESBG reactor. *I* influent, *R* recirculation, *E* effluent, *B* biogas. (Adapted from Akunna 2019)



**Fig. 1.12** Schematic representation of ESBG reactor. *I* influent, *R* recirculation, *E* effluent, *B* biogas. (Adapted from Chernicharo 2007)



A schematic representation of the IC reactor is shown in Fig. 1.12. At the bottom of the reactor, called the mixing zone, the influent is mixed with the biomass and the effluent from the recirculation device. Immediately above this step, there is the

expanded bed zone, which contains a high concentration of granular sludge. After that, there is the polishing zone capable of removing additional biomass, allowing for complete removal of biodegradable COD, in addition to the gas collection. Finally, there is the recirculation system, with the capture of biogas between the upstream and downstream flows.

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## 1.6 Future Developments

Currently, the worldwide concern to preserve the environment and maintain the quality of life has led to looking at wastewater and wastes much more as a resource than a product that brings a series of inconveniences (Stazi and Tomei 2018). As presented in the chapter, the anaerobic process is a good option for sewage treatment. Most anaerobic systems are relatively cheap and have the potential to reuse the biogas generated by the process for energy purposes. A treatment using anaerobic systems is able to not only improve sanitary conditions and generate benefits with the production of biogas for energy generation but also make better environmental quality by decreasing the emission of GHGs in the atmosphere and pollutants in the water surface and soil (Passos et al. 2020).

With the development of high-rate anaerobic treatment systems, more efficient and more compact treatment became possible. This category has been widely used in recent decades (Stazi and Tomei 2018). However, they are often insufficient in removing nutrients from the wastewater, making this process need to be combined with other(s) to complement the treatment. Therefore, the anaerobic process is often considered a primary biological treatment.

The combination of some anaerobic systems with subsequent co-digestion ones was also an alternative to optimize not only the treatment itself but also the production of biogas for energy use in some studies. This was especially observed for UASB reactors, in which post-systems after them recirculated certain substrates that were co-digesting with raw sewage (Zhang et al. 2013, 2018; Vassalle et al. 2020). This increased the growth of methanogens in the system and, consequently, the digestion and methane production.

One of the reasons that the UASB reactors are one of the most studied and used anaerobic systems in the world today is because they are high-rate reactors, i.e., they have a high volumetric treatment capacity. In addition, they are relatively compact and have the ability to separate biogas from liquid and biomass by an efficient three-phase separation system.

The characteristics point to a huge potential for implementing compact systems in remote regions, such as rural areas, for example. Passos et al. (2020) evaluated the capacity of using small-scale UASB reactors in rural areas of Brazil, associating them with demographic density data of the regions, where sewage treatment plants do not supply the population demand. In general, the authors observed from the research results that the biogas produced in these systems would supply the energy demand for the stabilization of the biosolids produced in the treatment plants,

sanitizing this sludge for agricultural purposes, and local families could consume the surplus energy.

The anaerobic membrane bioreactors (AnMBRs) represent another technology that has stood out. The membranes can be placed (1) separately from the bioreactor, operating under pressure; (2) inside the bioreactors, submerged, and thus, the membranes work under vacuum; or (3) submerged and external to the bioreactor, in which the suspended biomass is pumped to an external chamber and the retained solids returns to the bioreactor (Stazi and Tomei 2018). This system definitely solves the SRT problem, present in conventional reactors and improved in high-rate reactors, since the membrane system has the ability to completely retain the biomass present in the reactor. However, much still needs to be investigated regarding the operating parameters and some other disadvantages in different areas that decrease the performance of these reactors, such as (Stazi and Tomei 2018):

- Membrane fouling: although a minor problem compared to aerobic membrane bioreactors, AnMBRs have a lower filtration capacity, since they work at lower flows. An alternative is the spraying of biogas generated in the reactor that acts as a cleaning effect and delays scale formation. However, one must consider the shear effects not to impair the process.
- As anaerobic treatment of domestic sewage generally produces little biogas, the reuse of biogas may not generate a positive energy balance, since the operation of the membranes in AnMBR requires more energy consumption than other anaerobic processes. In this case, further studies will be conducted in an effort to enhance CH<sub>4</sub> production.
- The use of AnMBR in domestic sewage treatment is deficient in the removal of nutrients such as nitrogen and phosphorus. However, studies show that struvite can be formed from the soluble phosphorus, magnesium, and ammonium, if present in the wastewater in correct proportions. This approach is interesting for AnMBR systems because, besides removing nutrients from the wastewater, it limits the fouling of the membranes. In addition, there is the possibility of recovering the struvite formed from the wastewater components to use as fertilizer.

Overall, it can be observed that AnMBR systems have a great potential, but there are still many studies in this field to improve and optimize the operation of this type of bioreactor.

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## References

- Akunna JC (2019) Anaerobic waste wastewater treatment and biogas plants. Taylor & Francis Group, LLC, Boca Raton, FL. ISBN: 13 978-0-8153-4639-5
- Atinkpahoun CNH, Soclo HH, Pons M-N, Leclerc J-P (2018) Physico-chemical characterization of domestic wastewaters in the “Vie-Nouvelle” agglomeration, Cotonou, Benin. *Int J Biol Chem Sci* 12(1):542–557. <https://doi.org/10.4314/ijbcs.v12i1.42>

- Boni MR, Copelli S, Raboni M (2020) Study of the performance of disinfection with sodium hypochlorite on a full-scale sewage treatment plant. *Ambiente & Água* 15:1. <https://doi.org/10.4136/ambi-agua.2652>
- Carneiro RB, Mukaeda CM, Sabatini CA, Santos-Neto AJ, Zaiat M (2020) Influence of organic loading rate on ciprofloxacin and sulfamethoxazole biodegradation in anaerobic fixed bed biofilm reactors. *J Environ Manag* 273:111170. <https://doi.org/10.1016/j.jenvman.2020.111170>
- Chernicharo CAL (2007) *Anaerobic reactors*, vol 4. DESA UFMG, IWA Publishing Inc., London. ISBN: 1 84339 164 3
- Clara M, Kreuzinger N, Strenn B, Gans O, Kroiss H (2005) The solids retention time - a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. *Water Res* 39:97–106. <https://doi.org/10.1016/j.watres.2004.08.036>
- Connelly S, Pussayanavin T, Randle-Boggis RJ, Wicheansan A, Jampathong S, Keating C, Ijaz UZ, Sloan WT, Koottatep T (2019) Solar septic tank: next generation sequencing reveals effluent microbial community composition as a useful index of system performance. *Water* 11(2660): 1–14
- Cruddas PH, McAdam EJ, Asproulis N, Antoniadis A, Ansari I, Best D, Jefferson B, Cartmell E, Collins G, Porca E, Peña-Varón MR (2018) Development of a staged anaerobic pond for methane recovery from domestic wastewater. *Ecol Eng* 122:169–176. <https://doi.org/10.1016/j.ecoleng.2018.08.001>
- Dornelles HS, Motteran F, Sakamoto IK, Silva EL, Varesche MBA (2020) 4-Nonylphenol degradation changes microbial community of scale-up Anaerobic Fluidized Bed Reactor. *J Environ Manag* 267:110575. <https://doi.org/10.1016/j.jenvman.2020.110575>
- Dos Santos SL, van Haandel A (2021) Transformation of waste stabilization ponds: reengineering of an obsolete sewage treatment system. *Water* 13:1193. <https://doi.org/10.3390/w13091193>
- EPA (2014) Certain nonylphenols and nonylphenol ethoxylates. Signif New Use Rule 79:59186–59195. <https://www.federalregister.gov/documents/2014/10/01/2014-23253/certain-nonylphenols-and-nonylphenol-ethoxylates-significant-new-use-rule>. Accessed 16 Jul 2021
- Gao Y, Fang Z, Chen C, Zhu X, Liang P, Qiu Y, Zhang X (2020) Evaluating the performance of inorganic draw solution concentrations in an anaerobic forward osmosis membrane bioreactor for real municipal sewage treatment. *Bioresour Technol* 307:123254. <https://doi.org/10.1016/j.biortech.2020.123254>
- Lier JB, Zee FP, Frijters CTM, Ersahin ME (2015) Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. *Rev Environ Sci Biotechnol* 14:681. <https://doi.org/10.1007/s11157-015-9375-5>
- Mainardis M, Buttazzoni M, Goi D (2020) Up-flow anaerobic sludge blanket (UASB) technology for energy recovery: a review on state-of-the-art and recent technological advances. *Bioengineering* 7:43. <https://doi.org/10.3390/bioengineering7020043>
- Metcalf, Eddy (2003) *Wastewater engineering treatment and reuse*, 4th edn. McGraw-Hill, New York, NY
- Nie Z, Huo M, Wang F, Ai S, Sun S, Zhu S, Li Q, Bian D (2021) Pilot study on urban sewage treatment with micro pressure swirl reactor. *Bioresour Technol* 320:124305. <https://doi.org/10.1016/j.biortech.2020.124305>
- Passos F, Bressani-Ribeiro T, Rezende S, Chernicharo CAL (2020) Potential applications of biogas produced in small-scale uasb-based sewage treatment plants in Brazil. *Energies* 13:3356. <https://doi.org/10.3390/en13133356>
- Petropoulos E, Shamurad B, Tabraiz S, Yu Y, Davenport R, Curtis TP, Dolfig J (2020) Sewage treatment at 4 °C in anaerobic upflow reactors with and without a membrane – performance, function and microbial diversity. *Environ Sci Water Res Technol* 7:156. <https://doi.org/10.1039/d0ew00753f>
- Pfluger A, Erickson R, Vanzin G, Hahn M, Callahan J, Munakata-Marr J, Linda Figueroa L (2020) Energy-generating potential of anaerobically enhanced primary treatment of domestic wastewater using multiple-compartment bioreactors. *Environ Sci Water Res Technol* 6:117

- Shaw K, Dorea CC (2020) Biodegradation mechanisms and functional microbiology in conventional septic tanks: a systematic review and meta-analysis. *Environ Sci Water Res Technol* 7: 144. <https://doi.org/10.1039/d0ew00795a>
- Stazi V, Tomei MC (2018) Enhancing anaerobic treatment of domestic wastewater: state of the art, innovative technologies and future perspectives. *Sci Total Environ* 635:78–91. <https://doi.org/10.1016/j.scitotenv.2018.04.071>
- Sylla A, Fatima Ezzahra A, Najwa H, Rihani M, Jamal A, Omar A, Samira E (2017) Disinfection of wastewater treatment plant effluent using anaerobic bacterial bed reactor immersed: operating performance. *J Urban Environ Eng* 11(2):208–218
- United Nation (UN) (2021). <https://www.unwater.org/water-facts/quality-and-wastewater/>. Accessed 31 Jul 2021
- United Nations Children’s Fund (UNICEF) (n.d.). <https://www.unicef.org/brazil/comunicados-de-imprensa/1-em-cada-3-pessoas-no-mundo-nao-tem-acesso-agua-potavel-dizem-unicef-oms>. Accessed 31 Jul 2021
- United States Environmental Protection Agency (EPA) (2021). <https://www.epa.gov/septic/types-septic-systems>. Accessed 23 Jul 2021
- Van Haandel A, Van Der Lubbe J (2019) Anaerobic sewage treatment: optimization of process and physical design of anaerobic and complementary processes. IWA Publishing Alliance House, London
- Vassalle L, Díez-Montero R, Machado ATR, Moreira C, Ferrer I, Mota CR, Passos F (2020) Upflow anaerobic sludge blanket in microalgae-based sewage treatment: codigestion for improving biogas production. *Bioresour Technol* 300:122677. <https://doi.org/10.1016/j.biortech.2019.122677>
- Von Sperling M (2007) Wastewater characteristics, treatment and disposal, vol 1. DESA UFMG, IWA Publishing, Inc., London. ISBN: 1 84339 161 9
- Wei Y, Jin Y, Zhang W (2020) Domestic sewage treatment using a one-stage ANAMMOX Process. *Int J Environ Res Public Health* 17:3284. <https://doi.org/10.3390/ijerph17093284>
- Wisconsin Department of Natural Resources (DNR) (2013) Introduction to Ponds, Lagoons, and Natural Systems study guide. WDNR, Madison, WI. <https://dnr.wi.gov/regulations/opcert/documents/WWSGPondsINTRO.pdf>. Accessed 20 Jul 2021
- World Health Organization and United Nations Children’s Fund (WHO & UNICEF) (2020) Joint Monitoring programme for water supply and sanitation. <https://washdata.org/data/household#!/>. Accessed 13 Jul 2021
- Zeeman, G, Mahmoud, N, Lier JBV (2008). Anaerobic wastewater treatment. In: Biological wastewater treatment: principles, modelling and design. Henze M, Loosdrecht M.C.M.V., Ekama G.A. IWA Publishing, London
- Zhang L, Hendrickx TLG, Kampman C, Temmink H, Zeeman G (2013) Co-digestion to support low temperature anaerobic pretreatment of municipal sewage in a UASB–digester. *Bioresour Technol* 148:560–566
- Zhang L, Vrieze JD, Hendrickx TLG, Wei W, Temmink H, Rijnaarts H (2018) Anaerobic treatment of raw domestic wastewater in a UASB-digester at 10 °C and microbial community dynamics. *Chem Eng J* 334:2088–2097



# The Chemistry of Human Excreta Relevant to Biogas Production: A Review

# 2

Anthony Ike Anukam and Pardon Nyamukamba

## Abstract

As obnoxious as it may sound, studies involving human excreta are of great importance to sanitation, one of the most effective ways by which public health can be improved. The composition of human excreta is highly variable and contains all that enters into the toilet including water, urine, anal cleansing materials, lipids, proteins, polysaccharides, chemical elements, and undigested food residues as well as municipal wastes. A great deal of past and present research has focused on efficient utilization of this waste product of the human digestive system, particularly in biological processes, such as anaerobic digestion, where the waste is used as substrate to produce value-added products like biogas. However, there is very limited data on the chemistry of human excreta and its direct impact on anaerobic digestion process efficiency. This review therefore aims to illustrate the significance of the chemistry of human excreta relevant to biogas production and discuss key criteria and values that will help advance research and development of anaerobic digestion systems using human excreta as a treatment technology.

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**Keywords**

Anaerobic digestion · Biodegradation · Methanogenic bacteria · Pathogens · Urea

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## 2.1 Introduction

Human excreta (HE) are generally perceived as unhealthy, unhygienic waste products that are deleterious to humans. They (HE) include all that enters into the toilet such as urine, flush water, and cleansing materials (Strande et al. 2014). However, as nasty as it may sound, the fact remains that this waste product of the human digestive system (HE) constitutes a valuable resource that can be used to recover energy via anaerobic digestion or as fertilizer substitutes to replenish soil and enhance crop growth. These application potentials of HE are considered as proper management options that can serve to curb the effects of environmental pollution that are often associated with poor sanitation. For instance, in cases where sanitation is poorly managed, HE may build up around homes, within hailing distance to drainage systems, and in refuse dump sites contributing to pollution of the environment (Kulabako et al. 2007). An estimated amount of around 60% of HE generated globally do not undergo any kind of treatment; consequently, more than 50% of all rivers, oceans, and lakes are contaminated with fresh excreta (Baum et al. 2013; Mara 2003).

Studies involving HE and the development of treatment technologies for their conversion into high-value products have been inspired by the issues described above. Anaerobic digestion (AD) is a well-recognized technology for the treatment of organic materials and involves the biological decay of organic matter in an environment starved of oxygen to produce a combustible gas commonly known as “biogas.” The biogas consists of a mixture of methane gas ( $\text{CH}_4$ ) (about 60%) and carbon dioxide ( $\text{CO}_2$ ) (approximately 40%), with trace amounts of hydrogen sulfide ( $\text{H}_2\text{S}$ ), hydrogen (H), and water vapor ( $\text{H}_2\text{O}$ ) respectively. The biogas obtained from AD process can be used for household cooking or for power generation. HE is an organic matter that has the potential to be converted into useful products via AD. In comparison with other treatment technologies, AD shows reduced functional expenses and substantive energy evenness that allows the energy content of its feedstock to be recovered; the nutrients in the feedstock can also be recovered and reused (Gijzen 2002; McCarty et al. 2011). The AD process is robust and efficient, with minimal sludge generation rate; it supports high organic feeding rates and has minimal functional constraints when compared to other waste treatment technologies (Rittmann and McCarty 2001). Previous studies (Kujawa-Roeleveld et al. 2006; Lohri et al. 2010) have shown that biogas production rate from fecal sludge can be up to 28–35 L/day/person, which corresponds to about 120–210 Wh/p/day of recovered energy. Knowledge of the feedstock that enters the AD treatment system is required for optimum efficiency in terms of biogas yield. However, there is very limited report on AD of HE. As a result, the chemistry of HE relative to its influence on the yield of biogas via the AD technology is not completely understood.



The primary purpose of this review therefore is to present a general overview of the chemistry of HE relevant to the yield of methane gas (biogas) from an AD process and discuss essential criteria and measures that will help advance research and development of AD systems using HE as a treatment technology.

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## 2.2 Characteristics of Human Excreta

Human excreta (HE) is composed of urine and feces, which are both considered waste products of body metabolism. The characteristics of HE vary widely and are largely dependent on the health of the individual excreting the waste and the amount and type of food including the type of liquid consumed by the individual (Lentner 1981; Feachem et al. 1983). HE basically contains a combination of undigested materials that pass through the intestines and the materials that are released from the blood stream or exuviated from both the intestines and glands, bile and mucus, which are responsible for the characteristic brown color of HE (Guyton 1992; Featherstone 1999). HE can also contain large proportions of bacteria, pathogens, eggs of helminths, and cysts of protozoa (Feachem et al. 1983; WHO 2006). It exhibits an average pH value of about 6.64 and is composed of approximately 75% water, with bacterial biomass making up about 25–54% of dry solids of the organic portion and the remaining fraction from fiber, protein, undigested carbohydrate, and fat as well as undigested food residues (Rose et al. 2015). The urine fraction of HE also varies in volume and composition, and this variability can be attributed to factors such as discrepancies in physical action and environmental circumstances, including water, salt, and high protein ingestion. The urine has a pH around 6.2 and accommodates large proportions of nitrogen (N), phosphorus (P), and potassium (K) that are often released from the body during excretion (Rose et al. 2015). However, the major elements in HE are oxygen (O), which make up about 74% of the total elements, hydrogen (H) at 10%, and carbon (C) at 5%, respectively (Snyder et al. 1975). Nevertheless, the organic fraction of HE, which makes up a significant portion of dried solids, can have a C content above 40% (Feachem et al. 1984; Strauss 1985). The inorganic portion of HE is primarily composed of calcium and iron phosphates and intestinal secretions, including negligible quantities of dried components of digestive juices like fragmented epithelial cells and mucus (Guyton and Hall 2000; Iyengar et al. 1991).

In their review of the features of HE, Rose et al. (2015) reported that the composition of living and dead bacteria is in the range of 25–54% of the dry weight of HE (Rose et al. 2015). They also alluded that the average water content of HE is around 75%. It has also been reported that disparities in water content and mass of HE are connected with dissimilarities in fiber ingestion because non-disintegrable fiber absorbs more water in the colon, while disintegrable fiber facilitates bacterial growth (Eastwood 1973; Garrow et al. 1993; Reddy et al. 1998). Volatile solids also comprise around 92% of the total solids (TS) proportion of human excreta with a pH that ranges from 5.3 to 7.5 and biochemical oxygen demand (BOD) that falls between 14 and 33.5 g/cap/day, with a chemical oxygen demand (COD) in the

**Table 2.1** The properties of human excreta as reported by previous studies

		Range	Range	Median
		Amount/cap/day	Other units	
Chemical properties	Wet mass	35–796 g	63–86 wt%	128 g/cap/day
	Water content	(Rose et al. 2015; Michael et al. 1972)	2–25 wt% of solids weight (+50% of bacterial biomass)	(Rose et al. 2015)
	Protein	0.5–24.8 g	(Rose et al. 2015)	75 wt% (Rose et al. 2015)
	Fiber	(Rose et al. 2015)	25 wt% of solids weight	6 g/cap/day (Rose et al. 2015)
	Carbohydrates	(Rose et al. 2015)	(Rose et al. 2015)	9 g/cap/day (Rose et al. 2015)
	Fats	4–24 g (Rose et al. 2015)	8.7–16 wt% of solids weight (Rose et al. 2015)	4.1 g/cap/day (Rose et al. 2015)
	Bacteria content	1.9–6.4 g	25–54 wt% of solids weight (Rose et al. 2015)	1.8 g/cap/day (Rose et al. 2015)
	BOD	14–33.5 g (Rose et al. 2015)	100–2200 10 <sup>12</sup> cells/kg (Ciba-Geigy 1977)	6.6f, 7.15 (aver.) (Ciba-Geigy 1977)
	COD	0.9–4 g (Rose et al. 2015)	5–7 wt% of solids weight (Rose et al. 2015)	0.55 MJ/cap/day (Rose et al. 2015)
	TN	0.21–1.45 MJ (Rose et al. 2015)	92 wt% of total solids (Rose et al. 2015)	
	VS		5.3–7.5 (Rose et al. 2015)	
	pH (Ciba-Geigy 1977; Lewis and Heaton 1997)			
	Caloric value			
	Physical properties	Shape		Type 1 (hard lumps)—type 7 (watery diarrhea) (Lewis and Heaton 1997)
Viscosity (Yeo and Welchel 1994)			3500–5500 cPs	1.06–1.09 (aver.) (Ciba-Geigy 1977; Brown et al. 1996)
Density			< 1 g/ml for 10–15% of healthy humans (Michael et al. 1972)	

Note: g/cap/day = daily wet and dry mass of excreta generated by humans

range of 46–96 g/cap/day (Rose et al. 2015). The properties of HE are presented in Table 2.1.

The physical structure of human excreta is equally highly variable, and this structural variability has been characterized by Lewis and Heaton (1997) who first introduced a scale known as the “Bristol Stool Form Scale” for assessing the rate of intestinal transit. They categorized HE into different types, beginning from type 1 (hard lumps) up to type 7 (watery diarrhea), with types 3 (hard, lumpy sausage) and 4 (loose, smooth snake) classified as normal forms of human excreta. The rheological characteristics of fresh human excreta have also been studied by Woolley et al. (2014) who demonstrated that apparent viscosity measurements of the samples of human excreta decrease with increasing shear rate. Higher apparent viscosities have

always been linked to lower moisture contents for any given shear rate (Penn et al. 2018).

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### 2.3 The Chemistry of Human Excreta

As previously stated, human excreta contain a combination of substances that includes urine, water, and a host of chemical elements that are present in varying proportions and which are necessary for the growth of microbial communities that produce biogas in AD processes. The relationship between the concentrations of C and N present in organic materials, often expressed in terms of C/N ratio, is an essential parameter in the performance of AD systems (Herman 2019). On the one hand, if the C/N ratio of the feedstock is exceptionally high, N will be rapidly devoured by methanogenic bacteria to meet the protein requirements of the bacteria with less activity on the C content of the feedstock. This condition will result in low biogas yield. On the contrary, should the C/N ratio be considerably low, N will be given off and accumulate as ammonia (NH<sub>3</sub>). As the digestion process proceeds, the amount of NH<sub>3</sub> increases with the formation of ammonium ions (NH<sub>4</sub><sup>+</sup>) due to digestion of N. This condition will lead to increasing pH values within the digester, with a direct toxic effect on the methanogenic bacteria population (Anukam et al. 2019). The effect of the C/N ratio of HE is further discussed in Sect. 2.4.

Methanogenic bacteria are pH-sensitive and do not survive below a pH value of 6.5; if substantial quantities of organic acids are generated by acid-generating bacteria, the pH of the content of the digester could drop to a value lower than 5 (Anukam et al. 2019). Under this condition, the whole digestion process is inhibited or even halted. Low concentrations of mineral ions like sodium (Na), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S) could also facilitate bacterial growth, while significant amounts of these ions will have toxic effects on AD system performance (Anukam et al. 2019). For instance, the concentration of NH<sub>4</sub><sup>+</sup> that falls between 50 and 200 mg/l will stimulate the growth of bacteria in the digester, whereas concentrations above 1500 mg/l leads to toxicity (Anukam et al. 2019). In a similar manner, heavy metals such as copper (Cu), chromium (Cr), lead (Pb), and zinc (Zn) also facilitate microbial growth when present in relatively small quantities, but their higher amounts will display toxic effects. Admittedly, there is an extensive list of substances with toxic effects on the buildup of microorganisms in AD processes. Table 2.2, in no particular order, shows some of these substances and their toxicity levels under AD conditions.

There are obviously many promoting and hindering factors which play a role in AD processes of organic materials. However, because of the uncertainty about which substances could pose a potential threat in terms of production of toxic effects during AD, it is necessary to undertake a comprehensive analysis that will involve both qualitative and quantitative assessments of HE to determine the exact concentrations of both mineral ions and heavy metals present in the material. Biochemical processes like AD processes need continuous improvements. For this reason, knowledge of the feedstock intended for conversion under this technology is required. This is because

**Table 2.2** Toxic compounds in anaerobic digestion and their level of toxicity (Chengdu Biogas Research Institute 1989)

Compound	Toxicity level
Calcium (Ca <sup>2+</sup> )	2500–4500 mg/l
Sodium (Na <sup>+</sup> )	3500–5500 mg/l
Magnesium (Mg <sup>2+</sup> )	1000–1500 mg/l
Manganese (Mn <sup>2+</sup> )	>1500 mg/l
Potassium (K <sup>+</sup> )	2500–4500 mg/l
Copper (Cu <sup>2+</sup> )	100 mg/l
Chromium (Cr <sup>3+</sup> )	200 mg/l
Sulfate (SO <sub>4</sub> <sup>2-</sup> )	5000 ppm
Sodium chloride (NaCl)	40,000 ppm
Nickel (Ni <sup>2+</sup> )	200–500 mg/l

**Table 2.3** Standard composition of biogas generated from the anaerobic digestion of human excreta (Karki 2009)

Compound	Symbol	% Composition
Methane	CH <sub>4</sub>	50–70
Carbon dioxide	CO <sub>2</sub>	30–40
Hydrogen	H <sub>2</sub>	5–10
Nitrogen	N <sub>2</sub>	1–2
Hydrogen sulfide	H <sub>2</sub> S	Trace amounts
Water vapor	H <sub>2</sub> O	0.3

biogas composition varies with feedstock type and the operating state of the AD process (Surendra et al. 2014). The standard composition of biogas from AD of HE is shown in Table 2.3.

## 2.4 Anaerobically Digesting Human Excreta

The anaerobic digestion (AD) technology is an old alternative energy production technology that has been in existence for many decades and has been used for the treatment of organic materials like HE for the purpose of obtaining value-added products. As previously alluded, however, literature reports on AD of HE have been very limited in recent times; hence, very little is known about the chemistry of this waste product of the human digestive system. In addition, there is also a considerable lack of knowledge about the impact of the chemistry of HE on biogas yield under AD conditions due to the wide variability in the characteristics of HE, which makes it difficult to obtain consistent samples of HE that will allow for experimental reproducibility. Despite these knowledge gaps, it is vital to stress that the most important factors that are likely to have significant effects in the AD process of HE include solids loading, protein, and fat contents of HE as well as energy content, including the amount of urea contained in the urine fraction of HE.

One of the potential obstacles to the prosperous deployment of AD systems using HE as a treatment technology is the high solids loading rate that is associated with fresh HE. According to Rose et al. (2015), the solids content of HE can be up to 25% wt (Rose et al. 2015). AD system operating with solids concentration around 15% w/w is considered more appropriate for optimum efficiency; operation at higher solids loadings will mean using smaller reactor volumes and lesser energy demands

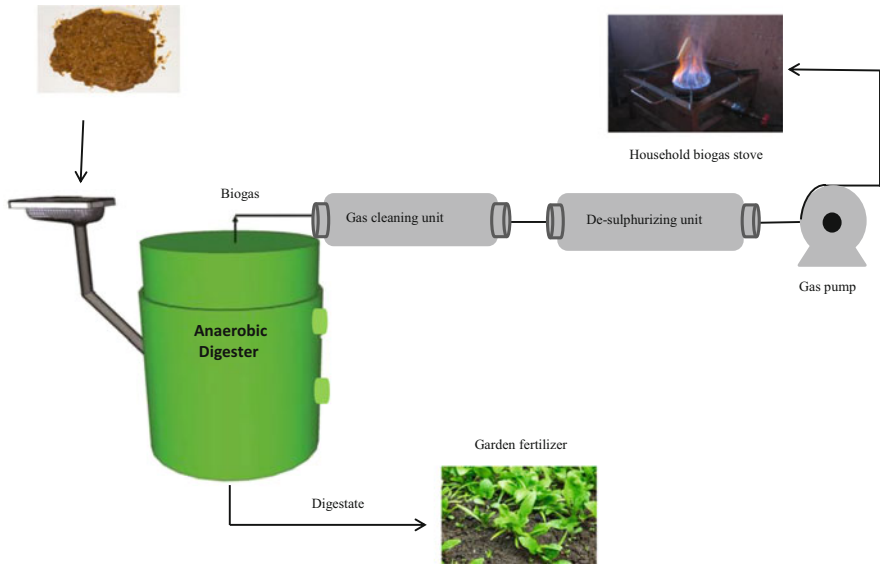
as well as reduced material handling that would most likely lead to lower biogas yields (Rose et al. 2015; Guendouz et al. 2008). The fiber content of HE is considered a key component in AD processes because it plays an integral role in the biodegradation rate of the entire excreta; for instance, lower fiber content will translate to decreased biodegradation rate of the HE, which results in diminished chemical oxygen demand (COD) metamorphosis (Rose et al. 2015).

The potential production of biogas from HE can be significant if the solids content of HE is generated in relatively large quantities per cap/day. This is because large proportions of the solids content of HE relate to considerable quantities of  $\text{CH}_4$  production. However, the efficiency of AD systems for the treatment of HE may be hindered by imbalances in the composition of macronutrients of HE. For example, for AD to proceed optimally, the C/N ratio of the feedstock must be in the range 20:1 and 30:1, respectively (Parkin and Owen 1986). The C/N ratio of HE, which is around 2.3:1 according to reports by Rose et al. (2015), falls way short of the standard C/N ratio required for optimum performance of AD systems. Another potential challenge that could be experienced when treating HE under AD conditions could be related to the high concentrations of sulfide in the excreta, which can pose a potential problem connected to toxicity of methanogenic bacteria, the methane-forming microorganisms (Anukam et al. 2019; Speece 2008). In addition, since HE often contains some amounts of urea in excess of about 50% of total organic solids, it is likely that the HE will also contain large concentrations of nitrogen (N), which has the potential to generate toxic compounds in the form of ammonia ( $\text{NH}_3$ ) under AD conditions. The concentrations of  $\text{NH}_3\text{-N}$  in the AD process of HE depend on the amount of ammonium ions ( $\text{NH}_4^+$ ), temperature, and pH of the process (Speece 1983). Figure 2.1 presents a process diagram showing the anaerobic digestion of HE for biogas production with the possible application of the digestate as garden fertilizer when treated further to remove pathogens.

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## 2.5 Conclusions and Recommendations

This paper has presented a non-exhaustive overview of the chemistry of HE and its influence in biogas yield under AD conditions. Even though the benefits of the AD technology are well documented and well comprehended, the technology HE has had issues being widely applied due to unstable operational performances occurring inside the digesters using HE as feedstock (Rajagopal et al. 2013). These operational issues are mainly caused by the lack of knowledge of the chemistry of HE relative to AD system performance. Furthermore, because of the wide variability in the physicochemical characteristics of HE, treatment technologies such as AD are not lustrous and resilient enough to deal with this wide irregularity in properties. To alleviate this issue, however, it may be necessary to diagnose HE using cutting-edge analytical techniques to understand its chemistry prior to AD as this will help prepare for any operational issues before they occur. Within this context, standardization of the AD system layout using HE as feedstock and the definition of operational parameters aimed at maximizing the quality and quantity of the produced biogas would be realized.



**Fig. 2.1** Setup of a small-scale anaerobic digester using human excreta as a treatment technology (Forbis-Stokes et al. 2016)

Treating HE under anaerobic digestion conditions can provide a meaningful source of energy and a digestate that can be used as a source of fertilizer for improving crop growth. These products have been shown to possess significant economic and environmental benefits at household level (Laramée and Davis 2013; San et al. 2012). However, due to the high content of urea in HE, inhibition of methanogenic bacteria population by increased formation of ammonia is highly likely during AD. Therefore, to overcome this inhibition effect, there may be a need to co-digest HE with a carbon-rich material to allow for an adjustment of the C/N ratio of the co-digesting feedstocks. This will mitigate toxicity effects that are associated with excess ammonia from feedstocks like HE with high contents of N. AD systems can stimulate nutrient recycling/recovery by allowing the products of HE to degrade into an accessible digestate that can serve as a source of fertilizer for soil improvement. However, because the pathogens present in HE may not be completely eliminated during AD, further studies are required to evaluate the potential value of the digestate as a source of fertilizer.

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**Conflict of Interest** The authors have no competing interest to declare.

## References

- Anukam A, Mohammadi A, Naqvi A, Granström K (2019) A review of the chemistry of anaerobic digestion: methods of accelerating and optimizing process efficiency. *Processes* 7(8):504
- Baum R, Luh J, Bartram J (2013) Sanitation: a global estimate of sewerage connections without treatment and the resulting impact on MDG progress. *Environ Sci Technol* 47(4):1994–2000
- Brown DM, Butler D, Orman NR, Davies JW (1996) Gross solids transport in small diameter sewers. *Water Sci Technol* 33(9):25–30
- Chengdu Biogas Research Institute (1989) *The Biogas Technology in China*. Chengdu Biogas Research Institute, Chengdu
- Ciba-Geigy AG (1977) *Wissenschaftliche Tabellen Geigy*. Ciba-Geigy Limited, Basel
- Eastwood M (1973) Vegetable fibre: its physical properties. *Proc Nutr Soc* 32(3):137–143
- Feachem RG, Bradley DJ, Garelick H, Mara DD (1983) Sanitation and disease. Health aspects of excreta and wastewater management. In: *World Bank studies in water supply and sanitation*. Wiley, New York
- Feachem RG, Bradley DJ, Garelick H, Mara DD (1984) Health aspects of excreta and wastewater management. In: Crowdy JP (ed) *Sanitation and disease*, vol 78. Wiley, Chichester, p 760
- Featherstone D (1999) *Why are faeces always brown?* University of Utah, Salt Lake City
- Forbis-Stokes AA, O'Meara PF, Mugo W, Simiyu GM, Deshusses MA (2016) On-site fecal sludge treatment with the anaerobic digestion pasteurization latrine. *Environ Eng Sci* 33(11):898–906
- Garrow JS, James WPT, Ralph A (1993) *Human nutrition and dietetics*, 9th edn. Longman Group, Edinburgh, p 160
- Gijzen HJ (2002) Anaerobic digestion for sustainable development: a natural approach. *Water Sci Technol*. 45(10):321–328
- Guendouz J, Buffiere P, Cacho J, Carrere M, Delgenes J (2008) High solids anaerobic digestion: comparison of three pilot scales. *Water Sci Technol* 58(9):1757–1763
- Guyton AC (1992) *Human physiology and mechanisms of disease*. W. B. Saunders Co, Philadelphia
- Guyton AC, Hall JE (2000) *Textbook of medical physiology*, 10th edn. W. B. Saunders Co, Philadelphia, p 251
- Herman TP (2019) *Optimizing feedstock mixtures for anaerobic digestion of food waste, brewery waste, and crop residues*. Master's Dissertation, Ohio University, USA
- Iyengar V, Albaugh G, Lohani A, Nair P (1991) Human stools as a source of viable colonic epithelial cells. *The FASEB Journal* 5(13):2856–2859
- Karki AB (2009) Biogas as renewable energy from organic waste. In: Doelle HR, Rokem S, Berovic M (eds) *Biotechnology*, vol 10. Eolss, Oxford, p 112
- Kujawa-Roeleveld K, Elmitwalli T, Zeeman G (2006) Enhanced primary treatment of concentrated black water and kitchen residues within DESAR concept using two types of anaerobic digesters. *Water Sci Technol* 53(9):159–168
- Kulabako NR, Nalubega M, Thunvik R (2007) Study of the impact of land use and hydrogeological settings on shallow ground water quality in a peri-urban area of Kampala, Uganda. *Sci Total Environ* 381:180–199
- Laramée J, Davis J (2013) Economic and environmental impacts of domestic bio-digesters: evidence from Arusha, Tanzania. *Energy Sustain Dev* 17(3):296–304
- Lentner C (1981) Units of measurement, body fluids, composition of the body, nutrition. In: *Geigy scientific tables*, 8th edn. Ciba-Geigy Ltd, Basel
- Lewis S, Heaton K (1997) Stool form scale as a useful guide to intestinal transit time. *Scand J Gastroenterol* 32(9):920–924
- Lohri C, Vögeli Y, Oppliger A, Mardini R, Giusti A, Zurbrügg C (2010) Evaluation of biogas sanitation systems in Nepalese prisons. In: *IWA-DEWATS proceedings of decentralized wastewater treatment solutions in developing countries conference and exhibition*, Surabaya, Indonesia

- Mara D (2003) Domestic wastewater treatment in developing countries, 1st edn. Taylor & Francis, London, pp 1–293
- McCarty PL, Bae J, Kim J (2011) Domestic wastewater treatment as a net energy producer-can this be achieved? *Environ Sci Technol* 45(17):7100–7106
- Michael D, Levitt MD, Duane WC (1972) Floating stools– flatus versus fat. *N Engl J Med* 286(18): 973–975
- Parkin GF, Owen WF (1986) Fundamentals of anaerobic digestion of wastewater sludges. *J Environ Eng* 112(5):867–920
- Penn R, Ward BJ, Strande L, Maurer M (2018) Review of synthetic human faeces and faecal sludge for sanitation and wastewater research. *Water Res* 132:222–240
- Rajagopal R, Massé DI, Gursharan S (2013) A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresour Technol* 143:632–641
- Reddy S, Sanders TA, Owen RW, Thompson MH (1998) Faecal pH, bile acid and sterol concentrations in premenopausal Indian and white vegetarians compared with white omnivores. *Br J Nutr* 79(6):495–500
- Rittmann B, McCarty PL (2001) *Environmental biotechnology: principles and applications*. McGraw Hill, New York
- Rose C, Parker A, Jefferson B, Cartmell E (2015) The characterization of feces and urine: a review of the literature to inform advanced treatment technology. *Crit Rev Environ Sci Technol* 45(17): 1827–1879
- San V, Sriv T, Spoann V, Var S, Seak S (2012) Economic and environmental costs of rural household energy consumption structures in Sameakki Meanchey district, Kampong Chhnang Province, Cambodia. *Energy* 48(1):484–491
- Snyder W, Cook M, Tipton I, Nasset E, Karhausen L, Howells G (1975) Reference man: anatomical, physiological and metabolic characteristics (report of Task Group on Reference Man-International Commission on Radiological Protection). Pergamon Press, New York
- Speece RE (1983) Anaerobic biotechnology for industrial wastewaters. *Environ Sci Technol* 17(9): 416–427
- Speece RE (2008) *Anaerobic biotechnology and odor/corrosion control for municipalities and industries*, 1st edn. Archae Press, Nashville
- Strande L, Ronteltap M, Brdjanovic D (2014) *Faecal sludge management: systems approach for implementation and operation*. IWA Publishing, London
- Strauss M (1985) Health aspects of nightsoil and sludge use in agriculture and aquaculture. Part II: pathogen survival (04/85). International Reference Centre of Waste Disposal, Duebendorf
- Surendra KC, Takara D, Hashimoto AG, Khanal SK (2014) Biogas as a sustainable energy source for developing countries: opportunities and challenges. *Renew Sustain Energy Rev* 31:846–859
- WHO (2006) *Guidelines for the safe use of wastewater, excreta and greywater*. Excreta and greywater use in agriculture. WHO, Geneva
- Woolley SM, Buckley CA, Pocock J, Foutch GL (2014) Rheological modelling of fresh human faeces. *J Water Sanit Hyg Dev* 4(3):484–489
- Yeo RS, Welch DN (1994) United State Patent Number 5356626





# Mathematical Modelling for Understanding and Improving the Anaerobic Digestion Process Efficiency

# 3

Niti B. Jadeja and Rohini Ganorkar

## Abstract

Advances in modelling the anaerobic digestion process have helped optimize the complex interplay of biotic and abiotic factors of this multifaceted process. This chapter discusses the ADM1 model and its modifications for effective AD process operations. The biochemical and physicochemical components are explained with reference to the unique microbial community of the multistep AD process. Mathematical models that have led to improved characterization of growth kinetics of anaerobic microbes and their role in the metabolic reactions of the AD process biochemistry are discussed. Advances in instrumentation and computational capacities since the development of the ADM1 model have helped process prediction and identification of the microbial dynamics of AD. The large availability of data has supported scale-up, because of which data-driven models and process digitization make ADs more attractive for energy generation and waste management in recent times. Before entering the era of augmentation of existing AD methods and models, it is critical to understand the basis of process operations, distinct models used and lessons learned which can be incorporated in the face of newer emerging challenges in the future.

## Keywords

Anaerobic digestion · Biogas · Model · ADM · Microbes

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### 3.1 Introduction

The current era aims to achieve zero waste which calls for an effective transition in the green energy sector. More than 11 billion tons of solid waste is collected annually worldwide which contributes to more than 5% of the global greenhouse gas emissions owing to the natural decay of the organic part of the solid waste (UNEP 2020). India is responsible for the highest amount of waste generated globally, and the quantity of waste generation has tripled in the past three decades (Kaushal et al. 2012). Hence, organic waste management remains an integral objective for waste management authorities as well as for averting global warming given the increase in carbon emissions in recent years (Manjusha and Beevi 2016). Anaerobic digestion (AD) process lies at the centre of renewable energy, waste management, agriculture and economy. It serves as an effective method for the degradation of organic material and is less energy intensive compared to other methods (Awe et al. 2017). To meet the growing demands of effective waste management and energy renovation and conservation, AD has undergone impressive improvisations. Since 2015 India has seen the installation of more than 90 industrial- and community-level biogas plants (Balagurusamy and Chandel 2020). Sustained efforts account for decreasing the energy demand of the AD process, managing large quantities of waste and smooth process operations. Efforts to improve and implement large-scale AD processes are integral to a country like India as it has high energy requirements in addition to the need for solid waste management. This multistep process efficiency is governed by variables that are controlled by predictions made through process kinetics, simulations and dynamic modelling. This chapter discusses the models that have improved our understanding and operations of AD in recent times.

### 3.2 Modelling Anaerobic Digestion

The multifaceted AD process runs on effective process design and planning, such that the cost decreases, effective process optimization is achieved and energy generation is increased. The process is a combination of four stages where diverse microbial action occurs to convert organic waste into methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ) that can be used for heat, electricity and biofuel production. The digester residues can be recycled to agriculture as a secondary fertilizer, and the upgraded biogas (biomethane) can be used as a replacement for fossil fuels (Demirel and Yenigün 2002). A typical AD consists of synergic interaction of microorganisms linked by four different steps, namely, hydrolysis or liquefaction, acidogenesis, acetogenesis and methanogenesis. Each reaction recruits unique microorganisms that differ widely regarding physiology, nutritional needs, growth kinetics and sensitivity to the environment of an anaerobic digester. Microbes in acid- and methane-forming phases function when a balance is obtained between the two phases which otherwise leads to reactor instability and low methane yield. Techniques such as membrane separation, kinetic control and pH control have

been used for effective phase separation and process stabilization (Ghosh and Pohland 1974; Massey and Pohland 1978). Hydrolysis and methanogenesis steps can act as a rate-limiting step causing process failure under imposed kinetic stress (Aslanzadeh 2014). Moreover, the AD process is known to operate in thermophilic (120–140 °F), mesophylic (95–105 °F) and psychrophilic (60–75 °F) temperatures. Hence, the process improvisations require consideration of multiple factors which range from physicochemical and technical process parameters to microbial diversity of the process. In fact, the complexity of microbial biochemistry and lack of research on phylogenetic and metabolic pathways adopted by microbes lead to identifying the AD process as a ‘black box’ of archaea and bacteria microbiome (Nelson 2011). Syntrophic and concerted activity of microbes present in AD process is highly responsible for the efficiency and its stability. The third layer of complexity is added by the three phases (aqueous, gas and solid) and the strong and weak acid-bases system that AD deals with. AD is thus one of the most common integrated bioprocesses for the treatment of waste and resource recovery. Some of the studies employing AD for human and animal waste management are listed in Table 3.1. Multiple parallel bioreactions make AD a complex biosystem to which an added layer of complexity is provided by the operational parameters. The development of AD models and assessment of the computational fluid dynamics in AD have been reviewed recently (Manchala et al. 2017; Sadino-Riquelme et al. 2018). We discuss the popular ADM1 model, its modifications and the trending applications of artificial intelligence and machine learning in AD modelling.

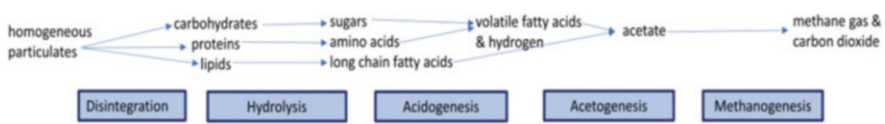
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### 3.3 ADM1

Since 1970, various mechanistic, empirical and mathematical models have been developed for the optimization of the AD process (Donoso-Bravo et al. 2011). One of the most discussed aspects of AD is the mathematical models for predictions and parameter optimization. The very popular model for AD was developed almost two decades ago by the Anaerobic Digestion Modelling Task Group of International Waters Association at the 8th World Congress on Anaerobic Digestion in Sendai, Japan (Batstone et al. 2002). While many different anaerobic models were devised before the ADM1, they contributed to forming the basis of the ADM1. ADM1 was the first generic model of anaerobic digestion that aided in full-scale plant design and operation. It laid the foundation for future optimization of processes for its direct implementation in in situ full-scale plants. The model was based on a reaction system that was classified into biochemical and physicochemical properties of the AD process as depicted in Fig. 3.1. The biochemistry of the process is divided into five sequential steps that include disintegration, hydrolysis, acetogenesis, acetogenesis and methanogenesis.

**Table 3.1** AD studies using human and animal waste and process details

Sr. No.	Study	Place	Waste type	Type of reactor	Reference
1.	Functional bacterial and archaeal diversity study	China	Potato starch processing wastewater	Upflow anaerobic sludge bed reactor	Antwi et al. (2017)
2.	AD for energy recovery in less-developed countries	USA	Human excreta	Lab-scale floating dome anaerobic digester	Colón et al. (2015)
3.	AD for production of biomethane, bioethanol and biodiesel	Oman	Faecal waste	Lab-scale experiments using closed glass bottles	Gomaa and Abed (2017)
4.	AD as a promising low-carbon strategy	China	Human waste	Continuous stirred-tank reactor	Duan et al. (2020)
5.	Archaeal and bacterial community in AD	Mexico	Pig farm waste	Lagoon-type anaerobic digester	Pampillón-González et al. (2017)
6.	Increasing biogas production by thermal sludge pretreatment	Spain	Sludge	Thermophilic lab-scale digester	Ferrer (2008)
7.	Methanogenic microbe diversity of a full-scale AD	China	Sludge and solid waste from municipal wastewater treatment plant	Full-scale anaerobic digester	Zhang et al. (2019)
8.	Alkaline/acid pretreatment	China	Waste activated sludge	Stirred-tank reactor	Wang et al. (2020)
9.	Anaerobic co-digestion and pretreatment	Korea	High-strength organic wastes	Continuous stirred-tank reactor	Choi et al. (2018)
10.	Combination of different substrates to improve anaerobic digestion	Korea	Sewage sludge in a wastewater treatment plant	Lab-scale serum bottles	Park et al. (2016)
11.	AD pasteurization for latrine	Kenya	On-site faecal sludge treatment	On-site floating dome digester and heat pasteurization system	Forbis-Stokes et al. (2016)
12.	Anaerobic co-digestion for methane potential and synergistic effect	Korea	Food waste, human faeces and toilet paper	Lab-scale study	Kim et al. (2019)

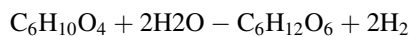


**Fig. 3.1** Overview of sequential biochemical reactions of ADM1 is depicted. The five biochemical reactions, substrates and their products are given

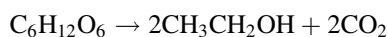
### 3.3.1 Biochemical Reactions

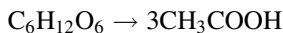
*Disintegration:* Complex organic waste is required to disintegrate into carbohydrate, protein and lipid particulate substrate. This step is integral in AD for human waste and serves as a pretreatment step. Enzymatic and physical methods are the most employed means for the initial treatment of solid waste in AD.

*Hydrolysis:* Extracellular enzymes are produced by microorganisms which cleave complex organic compounds into simpler forms making them available for uptake by microbes to thrive. This conversion process is a result of the hydrolytic microorganism's activity (*Clostridia*, *Micrococci*, *Bacteroides*, *Butyrivibrio*, *Fusobacterium*, *Selenomonas*, *Streptococcus*) and requires the production of exoenzymes like cellulase, cellobiase, xylanase, amylase, protease and lipase which are excreted by the fermentative bacteria (Christy et al. 2014a). This step is the most time-consuming of the AD process owing to the formation of toxic or unwanted compounds. Pretreatment by biological, chemical and mechanical methods or their combination is used to accelerate hydrolysis, as they lyse or disintegrate the substrate and allow the release of intracellular matter, thus allowing greater accessibility of food to anaerobic microorganisms, eventually reducing the retention time in the digester (Ferrer 2008). The following is the basic hydrolysis reaction:

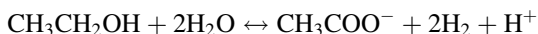
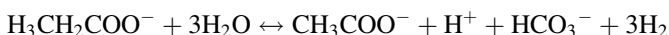


*Acidogenesis:* Soluble organic monomers produced during hydrolysis are degraded further to short-chain organic acids such as acetic acids, propanoic acids, butyric acids, alcohols, hydrogen and carbon dioxide by different facultative and obligatory anaerobic bacteria (*Streptococcus*, *Lactobacillus*, *Bacillus*, *Escherichia coli*, *Salmonella*). The concentration of hydrogen, an intermediate product of this step, influences the final product of the fermentation process (Adekunle and Okolie 2015). A drop in pH (4.5–5.5) is observed in this step which favours the acidogenic and acetogenic microorganisms (Hwang et al. 2001). The overall reactions in acidogenesis are stated below.

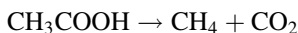
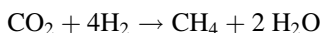




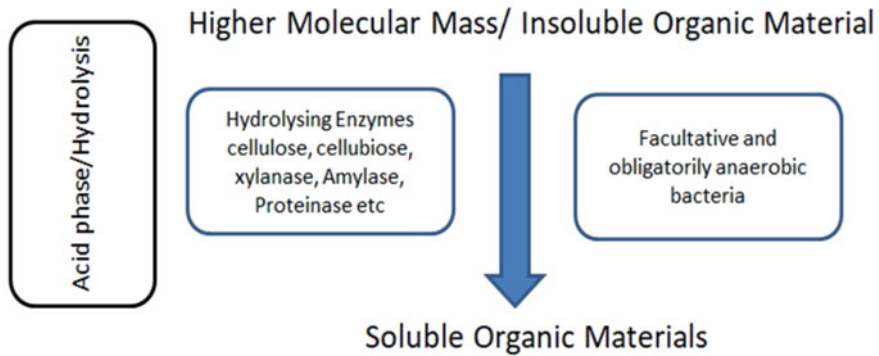
*Acetogenesis*: Acetogenic bacteria convert the compounds generated during the acidogenic phase, producing hydrogen, carbon dioxide and acetate. During acetic and propionic acid formation, a large amount of hydrogen ions is formed, causing a decrease in the pH of the aqueous medium which leads to the accumulation of electron sinks (lactate, ethanol, propionate, butyrate and higher volatile acids) which cannot be consumed directly by the methanogens. The obligate hydrogen-producing acetogenic bacteria (*Syntrophomonas wolfei*, *Syntrophobacter wolinii*) degrade these electron sinks to acetate, carbon dioxide and hydrogen. This transition is important for the successful production of biogas (Christy et al. 2014b). Acetogens make syntrophic associations with hydrogen-consuming methanogens because they rely on low hydrogen partial pressure for their degradation (Salminen et al. 2000). The following are the basic reaction in acetogenesis.



*Methanogenesis*: This is the last step of AD process where methanogenic archaea break down organic compounds derived from acetogenesis. Archaea in this process are divided mainly in two groups, namely, acetoclastic and hydrogenotrophic. The acetoclastic group of microbes degrades acetic acid or methanol to produce methane, whereas the hydrogenotrophic group utilizes hydrogen and carbon dioxide to produce methane. Methanogens prefer slightly alkaline pH around 6.5–8 (Kothari et al. 2014).



Microbial populations capable of carrying out specific metabolic processes have been identified using culture-dependent techniques. However, understanding of the AD microorganisms in syntrophic associations is fragmented creating a bias rather than a wide knowledge gap regarding resource competition and biotic interactions (Zarraonaindia et al. 2013). For bridging this knowledge gap, DNA-based molecular techniques and advanced omics techniques have been implemented and preferred over cultivation-based methods. Microscopic imaging, isotope labelling and biochemical analyses in combination with assessment of anaerobic digestion bioreactor performance provide insights into microbial community dynamics and dominating population functions that ultimately influence and link digester efficiency and stability.



**Fig. 3.2** Enzymes and microbes involved in the hydrolysis reaction of a typical AD are depicted

Methanogens include a subset of microbes capable of hydrogen uptake (hydrogen-utilizing methanogenic group) and acetate uptake by aceticlastic methanogenic microbes. Monitoring the substrate uptake has been well studied using Monod-type kinetics (different from ASM Monod growth kinetics) in order to predict the intracellular biochemical reactions. Parameters like biomass growth and death are represented by first-order kinetics. ASM1 model includes a few inhibition functions such as pH which is implemented as one of the two empirical equations (Fig. 3.2), whereas hydrogen levels and free ammonia are represented by non-competitive functions. Parameter like inorganic nitrogen uptake function is regulated by secondary Monod kinetics (Palanichamy and Palani 2014). The growth kinetics involved at each of the steps of AD have been reviewed and discussed in detail (Pavlostathis and Giraldo-Gomez 1991).

### 3.3.2 Physicochemical Reactions

Reactions such as ion association and dissociation, transformations of state; gaseous to liquid or liquid to solid, are not mediated by microorganisms and are classified as the physicochemical reactions of the AMD1 model. Physicochemical parameters are strong indicators of AD process success. For instance, a change in pH or physical state can help identify inhibiting reactions in AD. Moreover, distinct groups of microbes require effective pH to function, which is why maintaining process pH is crucial in AD accounting for a good proportion of the process costs. Optimizing pH for equilibrium at the multiple steps of the process is described by algebraic equations in the ADM1.

Overall, 26 dynamic state concentration variables and 8 implicit algebraic variables per reactor vessel or element are applied in the form of a differential and algebraic equation set in this model. Implementation of these parameters has been described through equations in previous studies (Manjusha and Beevi 2016). Now

that a basic understanding of the ADM1 is achieved, we dive deep into modifications of this model, its limitations and scope in optimization of AD.

### 3.3.3 Limitations of ADM1

The ADM1 model focused on addressing the over-specificity of previous models and generating a model that was more widely applicable to diverse processes. While these aspects could be addressed in this model, based on the assumptions there were some major limitations. Post-development, the ADM1 model was largely applied to the AD process in multistep processes and theoretic analysis for new parameters of improvised AD processes being developed (Batstone et al. 2002). One of the limitations encountered in the application of ADM was the assumptions of constant volume and uniform mixing in the bioreactors. The process complexity adds to the non-uniform behaviour of biomass in the reactors, especially in large-scale systems. Secondly, consideration of multiple parameters in the model involved multiple equations and balancing considerations. However, the inclusion of metabolic pathways, diverse microbial reactions and population-level interaction of microbes in AD biomass provided a better representation of the AD process when applied at pilot or in situ cases. The inability of accounting for the ion conductivity activity, and its role in governing the process pH, is another major drawback of ADM1. The model does not account for the impact of diverse waste types and varying ratios of biowaste and biomass being used in the AD digesters.

Following the ADM1, several studies have improvised the ADM1 or worked on newer models altogether. Owing to the limitations listed, ADM1 has rarely been applied in industrial-level AD processes. This has inspired previous and recent efforts in simplifying the ADM1 for its wider applicability and ease of use (Weinrich and Nelles 2021). Models specific to reactor design and type have been described in detail (Yu et al. 2013). A few studies on AD models to address the limitations of ADM1 have been tabulated in Table 3.2.

To summarize, ADM1 predicts the bioconversion of organics like methane, carbon dioxide, intermediates and inert products (Batstone et al. 2002). The basic ADM1 considers 26 elements including anions, cations, organics, microbes, acid-base equilibrium and electroneutrality equation and 19 reaction mechanisms including disintegration of solid substrate, hydrolysis of fractions and conversion into soluble compounds, uptake of compounds and disintegration of biomass. pH imbalances, temperature fluctuations and inhibitions due to hydrogen and/or nitrogen are parameters considered in the model. The inclusivity makes the model complicated especially for biologists with no modelling expertise. More than 60 stoichiometric and kinetic parameters in total make this model complicated to apply which led to the writing of simpler models, for example, ADM2, which are gaining popularity (Bernard et al. 2006; Hassam et al. 2015).



**Table 3.2** Different AD models and details of the studies conducted in the recent years

Sr. No.	Model information	Characteristic	Application	Reference
1.	Microbial sim	Dynamic flux balance analysis-based numerical simulator	Addresses high diversity of microbes in AD process in batch or chemostat mode	Popp and Center (2020)
2.	'Enzyme-soup' approach and multiscale microbial community modelling	Genome-scale metabolic networks and reconstructions	Functions of microbial community	Biggs et al. (2015)
3.	Multilevel biogas model for energy balance	Multilevel model for biogas yield prediction	Temperature, hydraulic retention time and dry solids assessment in the incoming sludge	Liu and Smith (2020)
4.	CFD simulation	Sludge properties and mixing	To predict the dynamics of mixing, uniformity index was developed	Terashima et al. (2009)
5.	Modified ADM1	Pilot-scale SBR	Account for variability in parameters	Batstone et al. 2009
6.	Shock loading conditions	Simplified mathematical model	Ion balance for bicarbonate alkalinity	Marsili-Libelli and Beni (1996)
7.	Modelling inhibition	Long-chain fatty acid inhibition	Saturated/unsaturated long-chain fatty acids degraders and the high sensitivity of acetoclastic population	Zonta et al. (2013)
8.	Adaptive neuro-fuzzy modelling	2-stage model	Effluent vs concentration and methane yield prediction	Cakmakci (2007)
9.	MATLAB Neural Network Toolbox	Prediction of trace compounds in biogas from AD	Hydrogen sulphide and ammonia concentrations modelled	Strik et al. (2005)
10.	Extended ADM1	Modelling phosphorus sulphur and iron interactions	Resource recovery	Flores-Alsina et al. (2016)
11.	Kinetics and dynamic modelling of batch AD	Municipal solid waste (MSW) in a stirred reactor	to describe batch digestion of MSW	Nopharatana et al. (2007)

(continued)

**Table 3.2** (continued)

Sr. No.	Model information	Characteristic	Application	Reference
12.	Modelling of two-stage anaerobic digestion using ADM1	2-stage digestion in AD	Simulating dynamic behaviour of a pilot-scale process for two-stage AD	Blumensaat and Keller (2005)
13.	GISCOD model	Integrated model for optimization of co-digestion of combinations of solid wastes	Co-digestion case study of diluted dairy manure and kitchen wastes	Zaher et al. (2009)
14.	Modified ADM1	Non-competitive inhibition function added to ADM1	The rate of acetate uptake for sodium toxicity	Hierholtzer and Akunna (2012)
15.	First-order kinetic transference function model	Anaerobic reactors of 250 mL and thermostatic water bath at a controlled temperature of $35 \pm 2$ °C for olive mill solid waste and brown alga	Kinetic constant and the maximum methane production rates for the co-digestion mixture	de la Lama-Calvente et al. (2021)
16.	Mathematical model	Thermophilic continuous AD at 60 °C using municipal waste, garden waste and industrial organic waste	Three different indicators have been developed to understand matter and energy in closed loop system	Momčilović et al. (2021)
17.	One-dimensional convection-diffusion-reaction equations velocity was used a function of space and time	Plug flow reactor using dry organic waste	Volatile fatty acid concentration profiles for pH prediction and inhibition processes and energy of the process	Panaro et al. (2021)
18.	5 basic models for diverse carbon sources and hydrolysis steps	Continuous stirred-tank reactor using lignocellulose waste	Production of hydrogen and methane from lignocelluloses wastes in two-phase AD	Chorukova et al. (2021)
19.	Gompertz model and first-order model	Rural household bio-digesters using food waste, sewage sludge and poultry litter	Use of temperature enhancement techniques to increase the biogas yield in winter	Lohani et al. (2021)
20.	Multiple-objective mixed-integer linear program model	Augmented e-constraint method for municipal solid waste	Minimize the capital and operational cost, maximize value of final products and minimize greenhouse gas emission	Ooi and Woon (2021)

(continued)

**Table 3.2** (continued)

Sr. No.	Model information	Characteristic	Application	Reference
21.	Gradient-based algorithm	Batch reactor using cattle manure	pH and temperature effects on microbial growth, liquid-gas mass transfer coefficients, dissociation constants and Henry's law for enhanced biogas production	Kegl and Kralj (2021)
22.	Biokinetic model	Mass balances on the substrate degradation, microorganisms' growth and methane production in mesophilic continuous stirred-tank reactor using black water and kitchen waste	Relation of energy production and organic matter in AD	Mohammadi et al. (2021)
23.	Multiple-objective optimization model by mixed-integer linear programming model	Diverse types of waste material	Comprehensive waste management master plan based on energy recovery, carbon footprint, financial profitability estimation for waste to energy	Abdallah et al. (2021)
24.	First-order kinetics and modified Gompertz model	200 ml multi-batch reactor system continuously agitated by magnetic bars at 440 rpm and placed in a thermostatic water bath at mesophilic temperature ( $35 \pm 2$ °C) for co-digestion of waste and alga	Microalga improved digestion, reducing the VFA accumulation	Fernández-Rodríguez et al. (2021)
25.	Modified Gompertz and logistic model	Floating drum digesters of 10 l capacity using cow dung and horse waste	Comparison of modified Gompertz model and logistic model and first-order kinetics model	Moharir et al. (2020)

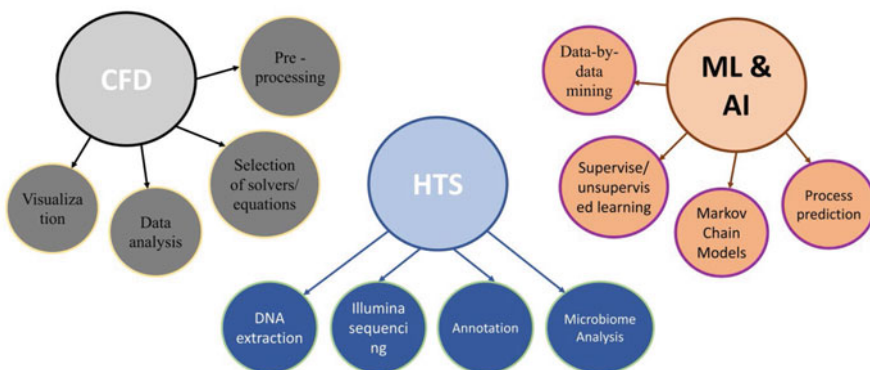
### 3.4 Advances in AD Modelling

#### 3.4.1 CFD

Computational fluid dynamics (CFD) is a modelling approach for processes involving a fluid flow with or without interaction of solids. It is an excellent intersection of mathematical model and a numerical system with a computational facility for faster analysis. With the advancements in informatics, the applications of CFD have grown exponentially, as it eliminates the need for several prototypes and trials. As explained earlier one of the limitations of the AD mathematical models was the assumption of perfect mixing and homogeneity of bioreactor contents. This aspect can be addressed with CFD, a combination of the law of energy conservation, advanced mathematics and computational power. From monitoring basic fluid dynamics and heat transfer to modern-day comprehensive transport analysis in three dimensions, the applications of CFD have greatly enhanced our understanding of AD. CFD allows analysis of aspects like rheology, process medium, dead zones and partial mixing which were overlooked otherwise.

To simplify the coding steps involved in a typical CFD process, various software are available. FLUENT and COMSOL multiphysics are most commonly used CFD software that contain preprogrammed models for AD and other complex processes. OpenFOAM is another popular CFD software that is also open access but requires some knowledge for the selection of codes and models from the vast libraries of C++ and Unix commands (Caillet et al. 2018). The main steps in CFD analysis involve preprocessing, selection of solvers (equations), data analysis and visualization as depicted in Fig. 3.3.

CFD has been widely used to evaluate and analyse the hydraulic and mixing behaviour of anaerobic digesters, and it is expected to play an important role in the use of modelling to study AD (Batstone et al. 2015). A compartment model approach



**Fig. 3.3** Overview of advancements with computational fluid dynamics (CFD), high-throughput technologies in DNA sequencing (HTS) and machine learning-artificial intelligence (ML & AI) in AD process modelling and steps involved in these techniques

of linking kinetics and CFD resulted in differences between compartmental bioreactor and stirred-tank bioreactor for AD which highlights the effect of mixing in a typical AD (Tobo et al. 2020). The simulations in CFD facilitate the simulation of mixing in a typical AD digester by addressing digester velocity, energy transfers and geometry-dependent flow of contents. To reduce errors in CFD application, protocol recommendations for various parameters and assumptions are provided by the International Water Association (IWA) working group on CFD (Wicklein et al. 2016). Temperature gradient and heat energy transfer, issues of grit formation or foaming and biochemical reactions have not been accounted for in the majority of the advanced mathematical models applying CFD. There is a need to analyse integration of these parameters into current CFD models for AD to address these knowledge gaps.

### 3.4.2 High-Throughput Sequencing

The systematic information on the microbial dynamics involved in AD to date is half-understood (Basile et al. 2020). The multistep AD process witnesses mutualism and competitiveness among microbial species (Westerholm et al. 2019). Hence, identification of total microbial diversity and functions (microbiome) is important in improvisations of the AD process. The recent advances in high-throughput technologies that facilitate the whole genome and metagenome sequencing of complex microbiomes have facilitated the prediction of biological functions of the AD process. The metagenomics approach has allowed comprehensive microbiome analysis as it eliminates the need to culture organisms. A typical metagenomics project starts with the extraction of good-quality DNA from the biomass sample. While there exist several protocols for DNA extraction from distinct samples, a few ideal commercial kits are available that make this task an easy-to-do and quick step in any molecular biology experiment (Sahu et al. 2019; Jadeja et al. 2018). The traditional tools for a metagenomics study include Sanger sequencing, polymerase chain reaction, hybridization assays, fluorescent techniques and cloning (Kapley et al. 2016). The Sanger method was the first sequencing technology, from the 1970s, which is still practiced today but largely replaced by next-generation sequencing techniques, especially in human and environmental studies (Jadeja et al. 2018).

In a large-scale genomic reconstruction analysis, more than 800 microbial genomes belonging to 30 diverse phyla were retrieved from metagenomic data available in the public databases specific to the AD process (Basile et al. 2020). Pairwise interactions of species and varying metabolites could be identified in this flux balance-based biological modelling study. *µbialSim* is another dynamic flux balance-based mathematical simulator to incorporate the temporal variable in microbial activity in batch AD and chemostat AD processes (Popp and Centler 2020). The accuracy of this microbial model was validated by using it for monoculture, co-culture and mixed culture scenarios. This model finds special applications in process interruptions like substrate change or bioaugmentation where it can predict

the AD microbiome behaviour. Owing to the key significance of the methanogens, a special microarray, the ANAEROCHIP, for identification of total methanogens in AD was designed (Franke-Whittle et al. 2009). GeoChip 5.0 is a similar array for functional gene identification that has been developed inclusive of a wide variety of more than 160,000 genetic functions of AD (Zhang et al. 2019). With the possibility of understanding multiple microbial functions involved in AD, genome reconstructions have greatly enhanced our understanding of the AD microbiome and its role in process efficiency.

### 3.4.3 Machine Learning

Experience and information on parameters, inputs and outputs generated for the AD process have made it possible to apply the principles of deep learning to improve the prediction-based models. The knowledge generated to date has helped set the priors based on which the runs can be simulated on the principle of Markov chain models and more accurate predictions of process parameters can be made. Random forest, neural networks, support vector machine and partial least squares methods have been extensively used in biological waste management (Cai et al. 2021; Long et al. 2021). Supervised and unsupervised method-based deep learning techniques are found to provide accurate information on limiting factors of the AD process (Lu et al. 2015). Predictive, rather proactive, microbiome management that relies on early-responding microbial indicators can prevent failure (Stenuit and Agathos 2015). Advanced deep learning techniques like recursive neural networks for predicting non-linear parameters can be useful in correlating microbiome dynamics and process variations prior to fluctuations (Seo et al. 2021).

Current AD research is applying these principles of deep learning and machine learning in AD process modelling for optimizing process efficiency. An extension to the previous mathematical model that focused on ammonia inhibition was used to optimize the choice of microorganisms that can be augmented to enhance the AD process efficiency in a recent study (Lovato et al. 2021). The approach can be extended to bioaugment effective microbial population for filling the knowledge gaps of catabolic potential or address inhibiting microbial activity at any stage of the multistep AD process. Strategies combining existing models with machine learning have been found to reduce the errors in process predictions substantially (Hansen et al. 2020). The experience from various AD processes and the data statistics available from previous operations provide a basis for training and testing of data-by-data mining approach. Models based on deep learning involving the use of linear regression, neural networks and sequential regression for optimization are gaining popularity (Ali et al. 2021; Ardabili et al. 2020; Guo et al. 2020). Optimizations by integration of these various techniques in AD modelling to address a regular stream of process input (e.g. daily generated solid waste) have facilitated better decision-making in AD operations.

## References

- Abdallah M, Hamdan S, Shabib A (2021) Development of a dynamic optimization framework for waste management systems. *Methods* 8:101203
- Adekunle KF, Okolie JA (2015) A review of biochemical process of anaerobic digestion. *Adv Biosci Biotechnol* 6:205–212
- Ali RA, Ibrahim NN, Ghani WWAK, Lam HL, Sani NS (2021) Utilization of process network synthesis and machine learning as decision-making tools for municipal solid waste management. *Int J Environ Sci Technol*. <https://doi.org/10.1007/s13762-021-03250-0>
- Antwi P, Li J, Boadi PO, Meng J, Shi E, Xue C et al (2017) Functional bacterial and archaeal diversity revealed by 16S rRNA gene pyrosequencing during potato starch processing wastewater treatment in an UASB. *Bioresour Technol* 235:348–357
- Ardabili S, Mosavi A, Várkonyi-Kóczy AR (2020) Systematic review of deep learning and machine learning models in biofuels research. In: Várkonyi-Kóczy A (ed) *Engineering for sustainable future. INTER-ACADEMIA 2019. Lecture notes in networks and systems*, vol 101. Springer, Cham. [https://doi.org/10.1007/978-3-030-36841-8\\_2](https://doi.org/10.1007/978-3-030-36841-8_2)
- Aslanzadeh S (2014) Pretreatment of cellulosic waste and high rate biogas production. Doctoral dissertation, University of Borås, Borås
- Awe OW, Zhao Y, Nzihou A, Minh DP, Lyczko N (2017) A review of biogas utilisation, purification and upgrading technologies. *Waste Biomass Valoriz* 8:267–283
- Balagurusamy N, Chandel AK (eds) (2020) *Biogas production*. Springer, Cham
- Basile A, Campanaro S, Kovalovszki A et al (2020) Revealing metabolic mechanisms of interaction in the anaerobic digestion microbiome by flux balance analysis. *Metab Eng* 62:138–149
- Batstone DJ, Keller J, Angelidaki et al (2002) The IWA anaerobic digestion model no 1 (ADM1). *Water Sci Technol* 45:65–73
- Batstone DJ, Tait S, Starrenburg D (2009) Estimation of hydrolysis parameters in full-scale anaerobic digesters. *Biotechnol Bioeng* 102:1513–1520
- Batstone DJ, Puyol D, Flores-Alsina X et al (2015) Mathematical modelling of anaerobic digestion processes: applications and future needs. *Rev Environ Sci Biotechnol* 14:595–613
- Bernard O, Chachuat B, Hélias A, Rodriguez J (2006) Can we assess the model complexity for a bioprocess: theory and example of the anaerobic digestion process. *Water Sci Technol* 53:85–92
- Biggs MB, Medlock GL, Kolling GL et al (2015) Metabolic network modeling of microbial communities. *Wiley Interdiscip Rev Syst Biol Med* 7:317–334
- Blumensaatt F, Keller J (2005) Modelling of two-stage anaerobic digestion using the IWA Anaerobic Digestion Model No. 1 (ADM1). *Water Res* 39:171–183
- Cai W, Long F, Wang Y et al (2021) Enhancement of microbiome management by machine learning for biological wastewater treatment. *Microb Biotechnol* 14:59–62
- Caillet H, Bastide A, Chuppa-Tostain G et al (2018) Anaerobic digestion of vinasse and CFD modelling approach. Paper presented at WasteEng 2018-7th International conference on Engineering for Waste and Biomass Valorisation, IMT Mines Albi, Prague, Czech Republic
- Cakmakci M (2007) Adaptive neuro-fuzzy modelling of anaerobic digestion of primary sedimentation sludge. *Bioprocess Biosyst Eng* 30:349–357
- Choi G, Kim J, Lee S et al (2018) Anaerobic co-digestion of high-strength organic wastes pretreated by thermal hydrolysis. *Bioresour Technol* 257:238–248
- Chorukova E, Simeonov I, Kabaivanova L (2021) Volumes ratio optimization in a cascade anaerobic digestion system producing hydrogen and methane. *Ecol Chem Eng* 28:183–200
- Christy PM, Gopinath LR, Divya D (2014a) Microbial dynamics during anaerobic digestion of cow dung. *Int J Plant Anim Environ Sci* 4:86–94
- Christy PM, Gopinath LR, Divya D (2014b) A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. *Renew Sust Energ Rev* 34:167–173

- Colón J, Forbis-Stokes AA, Deshusses MA (2015) Anaerobic digestion of undiluted simulant human excreta for sanitation and energy recovery in less-developed countries. *Energy Sustain Dev* 29:57–64
- de la Lama-Calvente D, Fernández-Rodríguez MJ, Llanos J et al (2021) Enhancing methane production from the invasive macroalga *Rugulopteryxokamurae* through anaerobic co-digestion with olive mill solid waste: process performance and kinetic analysis. *J Appl Phycol* 2021:1–12
- Demirel B, Yenigün O (2002) Two-phase anaerobic digestion processes: a review. *J Chem Technol Biotechnol* 77:743–755
- Donoso-Bravo A, Mailier J, Martín C et al (2011) Model selection, identification and validation in anaerobic digestion: a review. *Water Res* 45:5347–5364
- Duan N, Zhang D, Khoshnevisan B et al (2020) Human waste anaerobic digestion as a promising low-carbon strategy: operating performance, microbial dynamics and environmental footprint. *J Clean Prod* 256:20414
- Fernández-Rodríguez MJ, de la Lama-Calvente D, Jiménez-Rodríguez A et al (2021) Evolution of control parameters in biochemical methane potential tests of olive mill solid waste (OMSW), thermal pretreated OMSW, and its co-digestion with *Dunaliella salina*. *J Appl Phycol* 33:419–429
- Ferrer MI (2008) Study of the effect of process parameters on the thermophilic anaerobic digestion of sewage sludge, evaluation of a thermal sludge pretreatment and overall energetic assessment. Dissertation, Universitat Politècnica de Catalunya, Barcelona Tech, Spain
- Flores-Alsina X, Solon K, Mbamba CK et al (2016) Modelling phosphorus (P), sulfur (S) and iron (Fe) interactions for dynamic simulations of anaerobic digestion processes. *Water Res* 95:370–382
- Forbis-Stokes AA, O’Meara PF, Mugo W et al (2016) On-site fecal sludge treatment with the anaerobic digestion pasteurization latrine. *Environ Eng Sci* 33:898–906
- Franke-Whittle IH, Goberna M, Pfister V et al (2009) Design and development of the ANAEROCHIP microarray for investigation of methanogenic communities. *J Microbiol Methods* 79:279–288
- Ghosh S, Pohland FG (1974) Kinetics of substrate assimilation and product formation in anaerobic digestion. *J Water Pollut Control Fed* 46:748–759
- Gomaa MA, Abed RM (2017) Potential of fecal waste for the production of biomethane, bioethanol and biodiesel. *J Biotechnol* 253:14–22
- Guo HN, Wu SB, Tian YJ et al (2020) Application of machine learning methods for the prediction of organic solid waste treatment and recycling processes: a review. *Bioresour Technol* 319:124114
- Hansen BD, Tamouk J, Tidmarsh CA, Johansen R, Moeslund TB, Jensen DG (2020) Prediction of the methane production in biogas plants using a combined gompertz and machine learning model. In: Gervasi O et al (eds) *Computational science and its applications-ICCSA 2020. Lecture notes in computer science*, vol 12249. Springer, Cham
- Hassam S, Ficara E, Leva A et al (2015) A generic and systematic procedure to derive a simplified model from the anaerobic digestion model No. 1 (ADM1). *Biochem Eng J* 99:193–203
- Hierholtzer A, Akunna JC (2012) Modelling sodium inhibition on the anaerobic digestion process. *Water Sci Technol* 66:1565–1573
- Hwang S, Lee Y, Yang K (2001) Maximization of acetic acid production in partial acidogenesis of swine wastewater. *Biotechnol Bioeng* 75:521–529
- Jadeja NB, Yadav S, Kapley A (2018) Applications of metagenomics approaches in bioremediation of environmental pollutants. In: Bharagava RN (ed) *Recent advances in environmental management*. CRC Press, Boca Raton, pp 483–510
- Kapley A, Jadeja NB, Paliwal V et al (2016) Microbial genomics and bioremediation of industrial wastewater. In: Chandra R (ed) *Environmental waste management*. Taylor and Francis, London, pp 194–225



- Kaushal RK, Varghese GK, Chabukdhara M (2012) Municipal solid waste management in India-current state and future challenges: a review. *Int J Eng Sci Technol* 4:1473–1489
- Kegl T, Kralj AK (2021) Optimization of biogas production from cattle manure by anaerobic digestion using a gradient-based algorithm. *Comput Aided Chem Eng* 50:1909–1915
- Kim J, Kim J, Lee C (2019) Anaerobic co-digestion of food waste, human feces, and toilet paper: methane potential and synergistic effect. *Fuel* 248:189–195
- Kothari R, Pandey AK, Kumar S et al (2014) Different aspects of dry anaerobic digestion for bio-energy: an overview. *Renew Sust Energy Rev* 39:174–195
- Liu J, Smith SR (2020) A multi-level biogas model to optimise the energy balance of full-scale sewage sludge conventional and THP anaerobic digestion. *Renew Energy* 159:756–766
- Lohani SP, Shakya S, Gurung P et al (2021) Anaerobic co-digestion of food waste, poultry litter and sewage sludge: seasonal performance under ambient condition and model evaluation. *Energy Sour A Recov Util Environ Eff* 2021:1–16
- Long F, Wang L, Cai W et al (2021) Predicting the performance of anaerobic digestion using machine learning algorithms and genomic data. *Water Res* 199:117182
- Lovato G, Kovalovszki A, Alvarado-Morales M et al (2021) Modelling bioaugmentation: engineering intervention in anaerobic digestion. *Renew Energy* 175:1080–1087
- Lu J, Behbood V, Hao P et al (2015) Transfer learning using computational intelligence: a survey. *Knowl-Based Syst* 80:14–23
- Manchala KR, Sun Y, Zhang D et al (2017) Anaerobic digestion modelling. In: Li Y, Ge X (eds) *Advances in bioenergy*. Elsevier, Amsterdam, pp 69–141
- Manjusha C, Beevi BS (2016) Mathematical modeling and simulation of anaerobic digestion of solid waste. *Proc Technol* 24:654–660
- Marsili-Libelli S, Beni S (1996) Shock load modelling in the anaerobic digestion process. *Ecol Model* 84:215–232
- Massey ML, Pohland FG (1978) Phase separation of anaerobic stabilization by kinetic controls. *J Water Pollut Control Fed* 50:2204–2222
- Mohammadi S, Álvarez M, de Uribarri P, Eicker U (2021) Decentral energy generation potential of anaerobic digestion of black water and kitchen refuse for eco-district planning. *Energies* 14: 2948
- Moharir S, Bondre A, Vaidya S et al (2020) Comparative analysis of the amount of biogas produced by different cultures using the modified gompertz model and logistic model. *Eur J Sustain Dev Res* 4:0141
- Momčilović A, Stefanović G, Rajković P et al (2021) Possibility of organic waste inclusion and implementation in a closed-loop system. *Facta Univ Work Living Environ Prot*:27–37
- Nelson MC (2011) An integrated investigation of the microbial communities underpinning biogas production in anaerobic digestion systems. Dissertation, The Ohio State University, United States
- Nopharatana A, Pullammanappallil PC, Clarke WP (2007) Kinetics and dynamic modelling of batch anaerobic digestion of municipal solid waste in a stirred reactor. *Waste Manag* 27:595–603
- Ooi JK, Woon KS (2021) Simultaneous greenhouse gas reduction and cost optimisation of municipal solid waste management system in Malaysia. *Chem Eng Trans* 83:87–492
- Palanichamy J, Palani S (2014) Simulation of anaerobic digestion processes using stochastic algorithm. *J Environ Health Sci Eng* 12:1–8
- Pampillón-González L, Ortiz-Cornejo NL, Luna-Guido M et al (2017) Archaeal and bacterial community structure in an anaerobic digestion reactor (Lagoon Type) used for biogas production at a pig farm. *J Mol Microbiol Biotechnol* 27:306–317
- Panaro DB, Mattei MR, Esposito G et al (2021) A modeling and simulation study of anaerobic digestion in plug-flow reactors. preprint arXiv: 2104.04774
- Park KY, Jang HM, Park MR et al (2016) Combination of different substrates to improve anaerobic digestion of sewage sludge in a wastewater treatment plant. *Int Biodeterior Biodegradation* 109: 73–77

- Pavlostathis SG, Giraldo-Gomez E (1991) Kinetics of anaerobic treatment: a critical review. *Crit Rev Environ Sci Technol* 21:411–490
- Popp D, Centler F (2020)  $\mu$ bialSim: constraint-based dynamic simulation of complex microbiomes. *Front Bioeng Biotechnol* 8:574
- Sadino-Riquelme C, Hayes RE, Jeison D et al (2018) Computational fluid dynamic (CFD) modelling in anaerobic digestion: general application and recent advances. *Crit Rev Environ Sci Technol* 48:39–76
- Sahu N, Sharma G, Chandrashekhar B et al (2019) Performance evaluation of methanogenic digester using kitchen waste for validation of optimized hydrolysis conditions for reduction in ammonia accumulation. *Renew Energy* 139:110–119
- Salminen E, Rintala J, Lokshina LY et al (2000) Anaerobic batch degradation of solid poultry slaughterhouse waste. *Water Sci Technol* 41:33–41
- Seo KW, Seo J, Kim K et al (2021) Prediction of biogas production rate from dry anaerobic digestion of food waste: process-based approach vs. recurrent neural network black-box model. *Bioresour Technol* 341:125829
- Stenuit B, Agathos SN (2015) Deciphering microbial community robustness through synthetic ecology and molecular systems synecology. *Curr Opin Biotechnol* 33:305–317
- Strik DP, Domnanovich AM, Zani L et al (2005) Prediction of trace compounds in biogas from anaerobic digestion using the MATLAB Neural Network Toolbox. *Environ Model Softw* 20: 803–810
- Terashima M, Goel R, Komatsu K et al (2009) CFD simulation of mixing in anaerobic digesters. *Bioresour Technol* 100:2228–2233
- Tobo YM, Bartacek J, Nopens I (2020) Linking CFD and kinetic models in anaerobic digestion using a compartmental model approach. *PRO* 8:703
- UNEP (2020). Available at <https://www.unep.org/explore-topics/resource-efficiency/what-we-do/cities/solid-waste-management>. Accessed May 2021
- Wang S, Yu S, Lu Q et al (2020) Development of an alkaline/acid pretreatment and anaerobic digestion (APAD) process for methane generation from waste activated sludge. *Sci Total Environ* 708:134564
- Weinrich S, Nelles M (2021) Systematic simplification of the anaerobic digestion model No. 1 (ADM1)–model development and stoichiometric analysis. *Bioresour Technol* 333: 125124
- Westerholm M, Dolfing J, Schnürer A (2019) Growth characteristics and thermodynamics of syntrophic acetate oxidizers. *Environ Sci Technol* 53:5512–5520
- Wicklein E, Batstone DJ, Ducoste J et al (2016) Good modelling practice in applying computational fluid dynamics for WWTP modelling. *Water Sci Technol* 73:969–982
- Yu L, Wensel PC, Ma J et al (2013) Mathematical modeling in anaerobic digestion (AD). *J Bioremed Biodegr* 4:3
- Zaher U, Li R, Jeppsson U et al (2009) GISCOD: general integrated solid waste co-digestion model. *Water Res* 43:2717–2727
- Zarraonaindia I, Smith DP, Gilbert JA (2013) Beyond the genome: community-level analysis of the microbial world. *Biol Philos* 28:261–282
- Zhang Q, Wang M, Ma X et al (2019) High variations of methanogenic microorganisms drive full-scale anaerobic digestion process. *Environ Int* 126:543–551
- Zonta Ž, Alves MM, Flotats X et al (2013) Modelling inhibitory effects of long chain fatty acids in the anaerobic digestion process. *Water Res* 47:1369–1380



# The Current Energy Panorama and the Production of Biogas from Sewage Sludge

# 4

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## Abstract

It is well accepted that no single alternative energy source will be able to meet humanity's growing energy demand. Instead, the trend is for the energy system of the future to be a mix of various renewable energy sources and, at least for the next few decades, still complemented by fossil energy. The growing demand for alternative sources of sustainable energy, coupled with the challenge that the management of waste produced by population growth and the development of the industry represent, has motivated the research for energy generation technologies based on waste biodegradation. Anaerobic digestion (AD) stands out as a promising technology in this area, as it is capable of converting different types of waste into a highly energetic biogas (50–70% of methane). The AD of sewage sludge (SS) is able to produce the highest biogas capacity worldwide and, besides producing renewable energy in the form of methane, stabilizes sludge and aids in odor and pathogen removal present in this type of waste. This chapter will present a review of the global energy landscape, the production of biogas from SS, and the use and improvement of the biogas produced.

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**Keywords**

World energy panorama · Renewable energy · Biogas · Anaerobic digestion · Sewage sludge · Pretreatment

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## 4.1 The Current Energy Panorama

The growing demand for energy due to the rapid growth of human population and the depletion of nonrenewable energy resources has been the main cause of the search for alternative sustainable energy resources (Khalil et al. 2019; Li et al. 2019). Fossil fuels are nonrenewable energy sources that comprise coal, oil, and natural gas and provide about 80% of the total energy consumed in the production of electricity used for industrial and domestic purposes worldwide (Aziz et al. 2019). Energy consumption has increased worldwide and should keep rising in the upcoming years, with an estimated growth of almost 50% between 2018 and 2050 (Energy Information Administration (EIA) 2019). However, fossil fuels are finite and consumed by humans faster than they could be replenished (Aziz et al. 2019). Furthermore, the use of fossil fuels is considered to be the main reason for various environmental issues, such as air pollution and global warming (Aziz et al. 2019; Khalil et al. 2019). The world's dependence on a single energy source generates energy insecurity, which can lead to economic and political crises (Hagos et al. 2017). At present, the international energy situation is in a stage of new changes and adjustments. The basic trend of the global energy transition is to realize the transition of the fossil energy system into a low-carbon energy system and finally enter the era of sustainable energy mainly based on renewable energy (Li et al. 2020a).

### 4.1.1 Renewable Energy

Renewable energy is an effective way of dealing with the dilemma of meeting the growing energy demand and reducing greenhouse gas emissions. It also plays an important role in ensuring energy security, improving environmental protection, and increasing employment in many countries (Li et al. 2020a). Currently, global renewable resource development and its utilization scale have been continuously expanding, and the application costs have decreased (Lu and Gao 2021). Worldwide renewable energy consumption is expected to increase by 3% per year between 2018 and 2050 and become the leading source of primary energy consumption by 2050 (Energy Information Administration (EIA) 2019).

The most important renewable energy sources are wind, solar, and biomass energy. Hydroelectric, geothermal, and marine are also worth mentioning. Increasingly broad sources of wind and photovoltaic energy already provide reasonably cheap energy (Deshmukh et al. 2021), but these systems are characterized by a highly fluctuating and not always predictable production profile. Similarly, hydroelectric energy depends on the rainfall regime, which can compromise a country's

energy security. Brazil, for example, whose energy matrix is highly dependent on hydro sources, has already experienced major problems of electricity supply as the result of a great period of drought in 2001, which compromised the capacity of the reservoirs, drastically reducing hydro generation capacity (Freitas et al. 2019). Biomass energy conversion techniques, on the other hand, are capable of producing a constant base load and even balance the gaps between supply and demand in the energy sector (Miltner et al. 2017). Biomass is a carbon-neutral resource as well as a source of C/H/O elements to generate organic carbon-based products, such as bioenergy (biofuel and biogas) and chemicals (biorefinery) (Jung et al. 2021). Thus, the valorization of biomass feedstock has received considerable attention in the last few decades, and biomass-based energy sources are expected to have a representative share in the energy system of the future (Miltner et al. 2017; Jung et al. 2021). From an energetic point of view, biomass can be understood as any renewable resource derived from organic matter (OM) (such as animal and vegetable) that can be used for energy production (Aziz et al. 2019).

Renewable energy has grown strongly, and its competitiveness has increased. According to the statistics of the International Renewable Energy Agency (IRENA) (2020a), the global installed capacity of renewable energy has more than doubled in the last decade, with the development and consolidation of new sources of renewable energy. At the end of 2020, the value of 2,789,061 MW of global installed capacity for renewable energy was reached, of which 43.41% corresponds to hydropower, 25.37% to solar, 26.29% to wind, 4.41% to bioenergy, 0.5% to geothermal, and 0.02% to marine. The evolution of the global installed capacity of renewable energy and the distribution profile of the renewable energy sources for the last decade can be seen in Fig. 4.1.

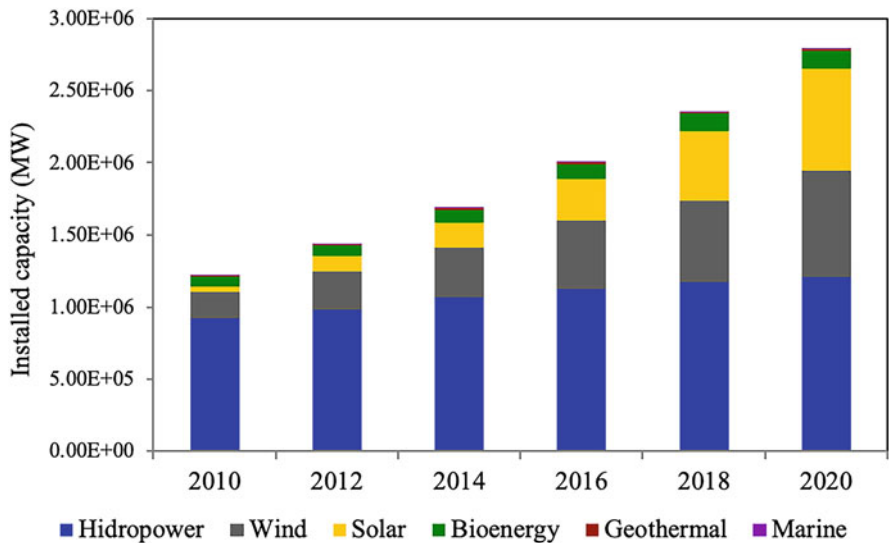


Fig. 4.1 Trends in renewable energy (International Renewable Energy Agency (IRENA) 2020a)

### 4.1.2 Waste for Energy Production

Waste-to-energy processes comprise any waste treatment technology that generates any form of energy, i.e., heat, electricity, or liquid transport fuels (e.g., diesel, petrol, or kerosene), from a waste material feedstock (Rafiee et al. 2021). The so-called waste-to-energy has multiple advantages. Not only it addresses the waste disposal challenge, but it also offers a good opportunity for energy security, as both the processes for production and consumption of energy can be located in the same geographic location, unlike fossil fuels.

The use of waste as biomass for energy production, such as biogas, has emerged as one of the best options to meet the high global demand for energy consumption (Khalil et al. 2019). In literature, studies have reported that several types of organic waste, such as animal waste (Parralejo et al. 2019; Ramos-Suárez et al. 2019), food waste (FW) (Bozym et al. 2015; Kuczman et al. 2018), urban organic solid waste (Tyagi et al. 2018), industrial waste, SS, and agricultural waste (Onthong and Juntarachat 2017; Momayez et al. 2019; Simioni et al. 2021), can potentially be used as sources for biogas production through AD process (Khalil et al. 2019).

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## 4.2 Anaerobic Digestion

AD is a biochemical process of decomposition of OM, carried out by a consortium of microorganisms that live symbiotically in the absence of oxygen. From a technological point of view, AD is a promising alternative for the management of organic materials, as it is capable of converting practically all biomass sources, including different types of wastes, into a highly energetic biogas. This biogas can be used to produce fuel, chemical compounds, electricity, and heat. AD also gives rise to a by-product. Digestate is the residue of degraded material, a product rich in nitrogen and which has potential to be used as agricultural fertilizer (Seadi et al. 2008; Agustini and Gutterres 2017; Xu et al. 2019).

The AD process basically follows the steps of hydrolysis, acidogenesis, acetogenesis, and methanogenesis, sequentially and synergistically. The steps are linked because the different microbial communities involved in each step work in sequence, with the products from one step serving as substrate for the next step. In general, the OM fed into the bioreactor is composed of different percentages of carbohydrates, proteins, and fats. Anaerobic microorganisms' exoenzymes decompose these complex compounds into simpler and more soluble organic compounds such as sugars, amino acids, and fatty acids, which are absorbed and fermented until they are transformed into simple compounds such as acetic acid, H<sub>2</sub>, and CO<sub>2</sub>. In the final step, these simple compounds are directly absorbed by methanogenic microorganisms to produce methane (Seadi et al. 2008; Khalid et al. 2011; Hagos et al. 2017; Parsaee et al. 2019; Xu et al. 2019).

Hydrolysis is the first step of AD, during which complex organic macromolecules (polymers) are converted into simpler and more soluble compounds (monomers and oligomers) (Agustini and Gutterres 2017; Neshat et al. 2017; Li et al. 2019). Lipids,

carbohydrates, and proteins are depolymerized through extracellular enzymes from hydrolytic bacteria (lipase, cellulase, amylase, protease) into long-chain fatty acids, sugars, and amino acids (Seadi et al. 2008). Generally, in AD of waste, hydrolysis is the limiting step of the process, determining the rate and efficiency of degradation (Agustini and Gutterres 2017; Mirmohamadsadeghi et al. 2019).

The monomers and oligomers formed during hydrolysis are then degraded by acidogenic bacteria (fermentative) into short-chain fatty acids (propionate, acetate, butyrate, and lactate), alcohols, and gaseous by-products ( $\text{NH}_3$ ,  $\text{H}_2$ ,  $\text{CO}_2$ , and  $\text{H}_2\text{S}$ ), a step that is known as acidogenesis or fermentation (Appels et al. 2008; Li et al. 2019; Mirmohamadsadeghi et al. 2019).

The third stage of AD is acetogenesis. In this step, acetogenic bacteria convert the organic acids and alcohols of high molecular weight produced in the previous step into acetic acid,  $\text{CO}_2$ , and  $\text{H}_2$ , which are the direct substrates for the next and final step, methanogenesis (Appels et al. 2008; Mirmohamadsadeghi et al. 2019).

In the final stage of AD, methane is produced by two groups of methanogenic bacteria: the acetoclastic, which are responsible for the decomposition of acetate into methane and carbon dioxide, and the hydrogenotrophic methanogenic, which produce methane using hydrogen as electron donor and carbon dioxide as a receptor (Appels et al. 2008; Neshat et al. 2017; Li et al. 2019).

AD is a highly complex process, and many interfering factors are still not fully understood. To achieve the maximum potential of this technology, the control of some parameters is crucial. Composition and chemical structure of the substrate (C/N ratio, biodegradability, bioaccessibility, and bioavailability), temperature, pH, alkalinity, and VFA concentration are among the most important parameters that affect the performance of an AD system. In addition, there are some compounds and conditions that can have an inhibitory effect on the process (Hagos et al. 2017; Neshat et al. 2017).

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### 4.3 Biogas: Characteristics and World Panorama

Biogas is a mixture of gases produced from the anaerobic degradation of organic compounds (Wu et al. 2015; Khan et al. 2017). Landfill waste, SS, animal manure, corn straw, and agricultural waste, among others, are the main sources of biogas generation (Wu et al. 2015). Biogas mainly consists of methane ( $\text{CH}_4$ ) in a range of 50–70% and carbon dioxide ( $\text{CO}_2$ ) in a concentration of 30–50%. The relative content of  $\text{CH}_4$  and  $\text{CO}_2$  in biogas is dependent on the nature of the substrate and the parameters employed in the AD process. In addition to these two main gases, biogas may additionally contain smaller amounts of other compounds: nitrogen ( $\text{N}_2$ ) in concentrations of 0–3%, which may originate in the saturated air of the influent; water vapor ( $\text{H}_2\text{O}$ ) in concentrations of 5–10% or higher in cases that operate at thermophilic temperatures; oxygen ( $\text{O}_2$ ) at concentrations of 0–1%, which is entering the process from the feed substrate or leaks; hydrogen sulfide ( $\text{H}_2\text{S}$ ) at concentrations of 0–10,000 ppmv, which is produced from the reduction of sulfate contained in some wastes; ammonia ( $\text{NH}_3$ ) from the hydrolysis of protein materials;

hydrocarbons in concentrations of 0–200 mg/m<sup>3</sup>; and siloxanes in concentrations of 0–41 mg/m<sup>3</sup>, originating, for example, from cosmetic industries' effluents (Wu et al. 2015; Khan et al. 2017; Angelidaki et al. 2018; Gao et al. 2018).

In addition to CH<sub>4</sub>, all other gases contained in biogas are considered pollutants. The energy content of methane described by the lower heating value (LHV) is 50.4 MJ/kgCH<sub>4</sub> or 36 MJ/m<sup>3</sup>CH<sub>4</sub> (CNP conditions). The higher the CO<sub>2</sub> or N<sub>2</sub> contents in the biogas, the lower its LHV. For example, for biogas with methane content ranging from 60 to 65%, the LHV is approximately 20–25 MJ/m<sup>3</sup> of biogas. There are several treatments to remove undesired compounds from biogas, expanding its range of applications (Angelidaki et al. 2018).

Biogas has characteristics that include low emission of toxic compounds, reduced greenhouse effects, carbon fixation, and other environmental and financial benefits. Its combustion leads to a neutral CO<sub>2</sub>, with a rate of 83.6 kg per GJ, well below to 741 kg CO<sub>2</sub> per GJ from diesel, 733 kg CO<sub>2</sub> per GJ from crude oil, 774 kg CO<sub>2</sub> per GJ from fuel oils, and 1096 kg CO<sub>2</sub> per GJ from wood (Giwa et al. 2020).

In 2019, the maximum biogas generation capacity installed in plants around the world was 19,381 MW, more than twice that observed in 2010, 9519 MW. Germany tops the list of countries with the largest installed capacity (over 7061 MW), followed by the USA (2368 MW), the UK (1775 MW), Italy (1575 MW), China (799 MW), Turkey (534 MW), and Thailand (530 MW) (International Renewable Energy Agency (IRENA) 2020b).

Although it represents only 0.8% of the global renewable energy installed (International Renewable Energy Agency (IRENA) 2020a), compared to other renewable energy sources, biogas production is independent of seasonal fluctuations, can be stably produced, and, therefore, promises a reliable way to produce energy (Koupaie et al. 2019).

### 4.3.1 Biogas Utilization and Improvement

There are four basic ways of using biogas: heat and steam production, electricity generation/cogeneration, use as fuel in vehicles, and, more recently, production of chemical. However, the use of raw biogas is limited by its contaminants, and, in most cases, purifying treatments are necessary to enable its application (Appels et al. 2008). Currently, there are different treatments to remove undesired compounds from biogas, expanding its range of applications.

The first treatment is related to “biogas cleaning” and includes the removal of harmful and/or toxic compounds (such as H<sub>2</sub>S, Si, volatile organic compounds (VOCs), siloxanes, CO, and NH<sub>3</sub>) (Angelidaki et al. 2018). Some authors also recommend drying of biogas, since it can be saturated with water vapor when it leaves the digester (Appels et al. 2008).

The second type of the treatment concerns the upgrading of biogas and aims to increase its LHV, converting it into a standard close to that of natural gas fuel. If the biogas is purified according to specifications similar to natural gas, the final product is called biomethane (Angelidaki et al. 2018; Sahota et al. 2018). Currently, the



specifications of the natural gas composition depend on national regulations, and, in general, methane content higher to 95% is required (Khan et al. 2021). In the biogas upgrading process, the CO<sub>2</sub> present can be removed or converted to CH<sub>4</sub> through reaction with H<sub>2</sub> (Angelidaki et al. 2018; Sahota et al. 2018). There are many commercial biogas upgrading technologies available that are being used to upgrade the raw biogas such as pressure swing adsorption, chemical scrubbing, water scrubbing, organic solvent scrubbing, membrane separation, and cryogenic separation (Khan et al. 2021). In addition, biogas can also be converted through dry reforming of methane (DRM) into more value-added products, such as H<sub>2</sub>, which is considered to be a promising clean energy that is widely used in fuel cells or even used for the synthesis of value-added liquid fuels and chemicals, such as alcohols, plastics, and hydrocarbons (Jung et al. 2021). The selection of the appropriate technology for upgrading the raw biogas depends on the final use of the biogas, the economics involved, and the efficiency of the upgrading process (Khan et al. 2021).

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#### 4.4 Biogas from Sewage Sludge

SS is a by-product of wastewater treatment plant (WWTP) generated from the settling (primary treatment) and activated sludge (secondary treatment) processes (Maragkaki et al. 2018; Zhu et al. 2021). Worldwide, the treatment of municipal wastewater produces large amounts of SS (Grosser et al. 2017), and that amount is expected to increase continuously, due to increasing population connected to sewage networks, building new WWTPs, and upgrading existing plants to meet the more stringent local effluent regulations (Dai et al. 2013). It is estimated that the amount of dry sludge produced per capita on a daily basis is around 60–90 g (Appels et al. 2011).

SS is mainly composed of dehydrated microbial biomass, in addition to pathogens, heavy metals, and other hazardous materials (Di Capua et al. 2020), and must be treated prior to disposal for environmental protection (Maragkaki et al. 2018). Although the generated SS represents approximately 2% of the volume of treated sewage (Khanh Nguyen et al. 2021), its disposal is one of the more expensive steps in a WWTP, representing up to 50% of the total operating costs of the plant (Zhen et al. 2017; Ma et al. 2018; Maragkaki et al. 2018). Therefore, progress in cost-effective SS treatment techniques represents an important research area for waste management companies (Khanh Nguyen et al. 2021). Basic SS disposal practices include agricultural use, landfills, composting, AD, recycling as a construction material, and incineration (Dai et al. 2013; Zhen et al. 2017; Maragkaki et al. 2018; Khanh Nguyen et al. 2021).

Among the treatment methods, AD is considered an effective, economical, and eco-friendly technology for treating these huge amounts of SS since it has the ability to reduce (by circa 40%) the overall load of biosolids to be disposed (Appels et al. 2011; Khanh Nguyen et al. 2021). AD stabilizes sludge, aids in odor and pathogen removal, and, more importantly, produces renewable energy in the form of methane

(Zhen et al. 2017; Khanh Nguyen et al. 2021). The AD of SS has the highest biogas production capacity worldwide. The methane yield obtained through AD is very dependent on the sludge composition; however, theoretically, it should be around  $0.590 \text{ m}^3/\text{kg ODS}$  (Appels et al. 2011).

#### 4.4.1 Characteristics of Sewage Sludge

The physical and chemical characteristics of the SS may have different variations depending on the source and geographical location in which it was generated. SS can be a solid, semi-solid, or muddy liquid waste and is generally a mixture of household and industrial waste (Demirbas et al. 2016). The characteristics of SS are listed in Table 4.1.

SS is characterized by the presence of solid and organic compounds, pathogens, microbial aggregates, filamentous bacteria, extracellular polymeric substances (EPSs), nutrients, and heavy metals (Khanh Nguyen et al. 2021). The various types of toxic substances, microorganisms, and OM produce unpleasant odors, cause environmental pollution, and endanger human health. SS may also possess hazardous organic chemicals such as those existing in pesticides, polychlorinated naphthalene, polycyclic-aromatic hydrocarbons, benzene, toluene, trichloroethylene, and nitrobenzene (Khanh Nguyen et al. 2021), besides nonbiodegradable OM, such as endocrine-disrupting compounds (EDCs) and pharmaceutical and personal care products (PPCPs) (Ma et al. 2018).

The inorganic parts of the SS are mainly composed of iron, phosphorus, calcium, aluminum, and sulfur, including traces of heavy metals (such as zinc, chromium, mercury, lead, nickel, cadmium, and copper) (Demirbas et al. 2016). After treatment, SS can be utilized for agricultural purposes because it contains various beneficial nutrients (Khanh Nguyen et al. 2021). Typically, SS contains the following plant nutrients in dry weight: 1–8% nitrogen (N), 0.5–5% phosphorus in the form of  $\text{P}_2\text{O}_5$ , and <1% potassium (K) as  $\text{K}_2\text{O}$  (LeBlanc et al. 2008).

#### 4.4.2 Pretreatments of Sewage Sludge

Despite the previously mentioned benefits deriving from the anaerobic treatment of SS, AD is generally characterized by long retention times ( $\geq 20$  days) and low VS degradation (30–50%) (Di Capua et al. 2020). The scientific community has identified that hydrolysis is the rate-limiting step in SSAD because of the large amounts of molecular OM and the complex floc structure, constituted by microorganisms held together by EPS, which are generally composed of proteins, polysaccharides, and humic-like substances (Di Capua et al. 2020; Khanh Nguyen et al. 2021). EPS create a three-dimensional matrix bound to the surface of the cells, generating a shield that protects the microorganisms contained in the aggregate, avoiding the rupture and the lysis of the cells and, consequently, decrease the biodegradability of the flocs (Di Capua et al. 2020).

**Table 4.1** Physical and chemical characteristics of SS

Parameter	Value	Reference
Ash	43.4 ± 0.1% (db)	Mu et al. (2020)
Bulk density	1.26–1.38 (kg/L)	Demirbas et al. (2016)
Higher heating value (HHV)	11.3–14.2 (MJ/kg)	Demirbas et al. (2016)
Hydrogen (H)	40–46 (g/kg)	Demirbas et al. (2016)
Organic matter (OM)	418–592 (g/kg)	Demirbas et al. (2016)
Oxygen (O)	185–219 (g/kg)	Demirbas et al. (2016)
Particle density	2.4–2.56 (kg/L)	Demirbas et al. (2016)
pH	7.1–8.2	Demirbas et al. (2016)
Total solids (TS)	2–12% (liquid SS) 12–40% (dewatered SS)	Khanh Nguyen et al. (2021)
Volatile solids (VS)	75–85% (db)	Khanh Nguyen et al. (2021)
Nutrients		
Calcium (Ca)	573.8 (mg/kg TS)	Mu et al. (2020)
Iron (Fe)	5803.2 (mg/kg TS)	Mu et al. (2020)
Magnesium (Mg)	487.5 (mg/kg TS)	Mu et al. (2020)
Nitrogen (N)	5.1 ± 0.0% (db)	Mu et al. (2020)
Organic carbon (OC)	34.8 ± 0.5% (db)	Mu et al. (2020)
Phosphorus (P)	2.5% (db)	Khanh Nguyen et al. (2021)
Potassium (K)	6078.1 (mg/kg TS)	Mu et al. (2020)
Sodium (Na)	731.2 (mg/kg TS)	Mu et al. (2020)
Sulfur (S)	11–17 (g/kg)	Demirbas et al. (2016)
Metals		
Aluminum (Al)	5964.5 (mg/kg TS)	Mu et al. (2020)
Arsenic (As)	9.9 (mg/kg, db)	Khanh Nguyen et al. (2021)
Barium (Ba)	2.8–4.2 (g/kg)	Demirbas et al. (2016)
Cadmium (Cd)	6.94 (mg/kg, db)	Khanh Nguyen et al. (2021)
Chromium (Cr)	119 (mg/kg, db)	Khanh Nguyen et al. (2021)
Cobalt (Co)	18.7 (mg/kg TS)	Mu et al. (2020)
Copper (Cu)	741 (mg/kg, db)	Khanh Nguyen et al. (2021)
Lead (Pb)	134.4 (mg/kg, db)	Khanh Nguyen et al. (2021)
Manganese (Mn)	763.9 (mg/kg TS)	Mu et al. (2020)
Mercury (Hg)	5.2 (mg/kg, db)	Khanh Nguyen et al. (2021)
Molybdenum (Mo)	49.8 (mg/kg TS)	Mu et al. (2020)
Nickel (Ni)	121.3 (mg/kg TS)	Mu et al. (2020)
Selenium (Se)	5 (mg/kg, db)	Khanh Nguyen et al. (2021)
Tim (Sn)	0.1–0.2 (g/kg)	Demirbas et al. (2016)
Titanium (Ti)	0.09–0.13 (g/kg)	Demirbas et al. (2016)
Zinc (Zn)	2.4–3.6 (g/kg)	Demirbas et al. (2016)
Pathogens		
<i>Ascaris lumbricoides</i> – helminth	$2 \times 10^2$ – $1 \times 10^3$ (N°/100 mL)	Khanh Nguyen et al. (2021)
Fecal coliform bacteria	$1 \times 10^9$ (N°/100 mL)	Khanh Nguyen et al. (2021)
Salmonella	$8 \times 10^3$ (N°/100 mL)	Khanh Nguyen et al. (2021)
Virus	$2.5 \times 10^3$ – $7 \times 10^4$ (N°/100 mL)	Khanh Nguyen et al. (2021)

db dry weight basis

In order to accelerate the hydrolysis and enhance subsequent methane productivity, a variety of sludge pretreatment options have been developed to facilitate the release of intracellular substances by rupturing the EPS matrix and cell wall and make them more accessible to subsequent microbial actions (Ma et al. 2018; Khanh Nguyen et al. 2021). By applying different pretreatments (mechanical, thermal, chemical, and/or biological), it is possible to increase the methane yield and to minimize the production of remaining sludge (digestate), but this typically comes with high energy demands and operation cost (Ma et al. 2018).

Physical and mechanical pretreatment (Gil et al. 2018; Nabi et al. 2019) disintegrates the solid particles, reducing their size and thus increasing the particle surface area to enhance the AD process (Khanh Nguyen et al. 2021).

Thermal pretreatment (50–250 °C) (Liao et al. 2016; Neumann et al. 2017; Malhotra and Garg 2019) dissolves the EPS both inside and on the surface of the flocs, thus disintegrating the floc structure and resulting in soluble organic substrates that are easily hydrolyzed during AD (Di Capua et al. 2020; Khanh Nguyen et al. 2021). Thermal pretreatments are beneficial in terms of pathogen sterilization, sludge volume reduction, odor removal, and enhanced sludge dewaterability (Khanh Nguyen et al. 2021). However, they are usually associated with high costs.

Chemical pretreatment (Hallaji et al. 2018; Liu et al. 2018; Wang et al. 2021) is the most promising method for complex organic waste destruction and employs strong reagents to deform the cell wall and membrane, favoring the availability of sludge OM for enzymatic attacks. The major reagents employed in the literature include acids, alkali, and oxidants (ozonation and peroxidation) (Zhen et al. 2017; Khanh Nguyen et al. 2021).

Biological pretreatments (Agabo-garcía et al. 2019) are eco-friendly techniques that utilize aerobic, anaerobic, and enzymatic methods to predigest and enhance the AD hydrolysis stages. These steps can be improved by implementing a complex matrix of microbes that play a synergistic role during the floc structure disintegration of sludge and other organic compounds. Although eco-friendly and cost-effective, this pretreatment technique is time-consuming and requires optimal parameters for microbial proliferation (Khanh Nguyen et al. 2021).

#### 4.4.3 Anaerobic Co-digestion of Sewage Sludge

AD of SS often encountered low methane yields due to the recalcitrant properties of microbial cell wall and extracellular biopolymers. Although the methane production could be improved by mechanical, thermal, chemical, and/or biological pretreatments, the high pretreatment costs limit their applications (Mu et al. 2020). Anaerobic co-digestion (AcoD), which is the AD of two or more different substrates, emerges as a promising option to overcome the disadvantages of mono-digestion and improve the economic viability of AD plants (Hagos et al. 2017). The improved process performance could be attributed to the dilution of potential toxic compounds (heavy metals, pharmaceuticals, and pathogens), balanced macro- and micro-nutrients, synergistic effects of microorganisms, and increased load of biodegradable OM (Ratanatamskul et al. 2015; Grosser et al. 2017; Li et al. 2020b).

There are numerous examples reporting successful co-digestion of SS and organic fraction of municipal solid wastes (OFMSW). In general, the addition of a protein-rich waste (such as SS) to a carbon-rich waste (such as OFMSW) improves the C/N ratio of the mixtures, and the production of biogas through AD increases (Tyagi et al. 2018).

Ghosh et al. (2020) evaluated the potential of co-digestion of OFMSW and SS for enhanced biogas production. The highest cumulative biogas and methane yield of 586.2 mL biogas/gVS and 377 mL CH<sub>4</sub>/gVS, respectively, were observed under an optimum ratio of OFMSW/SS (40:60 w/w). Mono-digested sample of SS showed around 300 mL biogas/gVS of cumulative biogas and CH<sub>4</sub> yield of around 50 mL CH<sub>4</sub>/gVS.

Grosser et al. (2017) investigated the efficiency of the AD of a waste mixture consisting of SS, OFMSW, and grease trap sludge (GTS), on the basis of biogas production and VS reduction. The process was carried out at mesophilic conditions (37 °C), 20 days set as hydraulic retention time (HRT), and the reactors (6 L of working liquid) were constantly mixed (180 rpm). Co-digestion of SS, GTS, and OFMSW provided significant benefits for methane yield and VS removal in comparison with digestion of SS alone. The authors found that anaerobic treatment of SS and GTS at a ratio of 30% resulted in increased methane yield of approximately 52% (from 300 to 456 m<sup>3</sup>/mgVS) compared to digestion of SS alone. Moreover, the addition of OFMSW as a co-substrate significantly improved the efficiency of the SS AD process by enhancing average methane yield up to 82% (300–547 m<sup>3</sup>/mgVS).

FW has also been frequently reported as a co-substrate to improve the AD process of SS. Ratanatamskul et al. (2015) investigated the effect of the AcoDof FW and SS with the mixing ratio (FW/SS) varying to 1:1, 3:1, 5:1, and 7:1. The amounts of biogas production of the mixtures were 761, 998, 1077, and 1504 mL/day, and the methane contents of the obtained biogas were 50.2, 55.5, 55.0, and 60.4%, respectively. The system was operated at total HRT of 33 days, corresponding to organic loading rate (OLR) of 7.0 kg COD/m<sup>3</sup>days.

Mu et al. (2020) conducted a series of co-AD of different urban-derived organic wastes (SS, FW, yard waste – YW) in a semicontinuous mode and with a HRT of 20 days. CH<sub>4</sub> yields (mL/gVS) observed were 448.9 ± 6.6, 484.6 ± 32.6, and 413.4 ± 29.3 for the SS + FW, 49.0 ± 5.0 and 149.0 ± 14.9 for SS + YW, and 164.7 ± 22.7, 232.4 ± 46.7, and 314.9 ± 17.1 for co-AD between the three wastes (SS + FW + YW). The CH<sub>4</sub> variations obtained within the same group of experiments are due to different proportions of mixtures adopted and other particularities.

Maragkaki et al. (2018) performed a series of laboratory experiments in an attempt to optimize biogas production from SS by co-digesting with a dried mixture of FW, cheese whey, and olive mill wastewater (FCO). The experiments were carried out in lab-scale continuous stirred-tank reactors (CSTR), operating under mesophilic conditions (37 ± 2 °C) and with a HRT of 24 days. Four types of influent feedstock were utilized – 100% SS; 97% SS + 3% FCO; 95% SS + 5% FCO; and 93% SS + 7% FCO – prepared on a volume (v/v) basis. It was found that FCO addition can boost biogas yields if the mixture exceeds 3% (v/v) concentration in the feed. The reactor treating the SS produced 287 ml CH<sub>4</sub>/L/days before the addition of

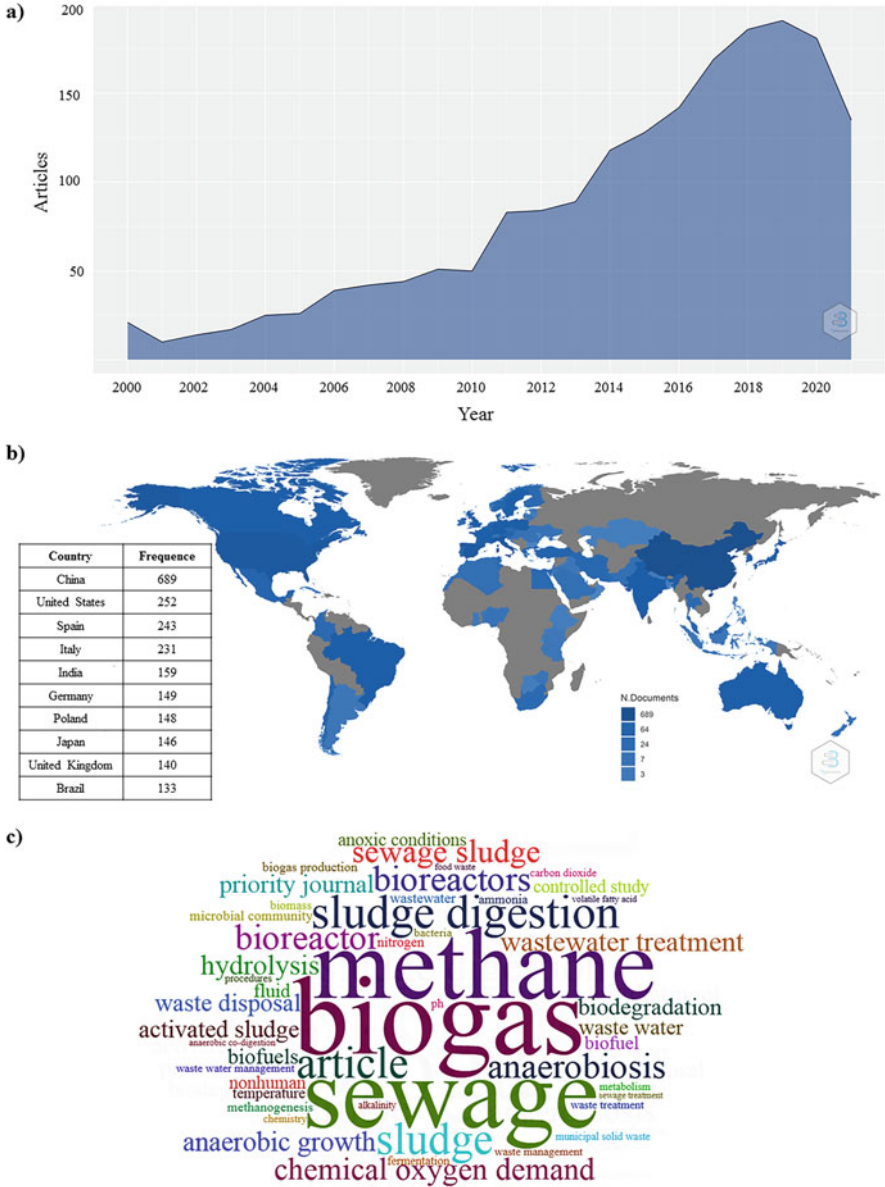
FCO and 815 ml CH<sub>4</sub>/L/days after the addition of 5% FCO (v/v). Any further increase of 5% FCO causes a small increase in biogas production.

Other types of wastes have also been reported as co-substrates for SS in successful AcoD processes. Zhu et al. (2021) assessed the effects on the biogas production of thermophilic AcoD of SS with paper waste (PW) in a continuous experiment with the fixed HRT in 30 days. The mixture ratios of SS/PW content used in this experiment were 4:0, 4:2, 4:4, 4:6, and 4:8 based on the TS. The optimal performance was obtained at the ratio of SS/PW equal to 4:6, where the biogas production increased from 438 ± 53 to 594 ± 72 mL/gVS (+35.6%) compared to the mono-digestion. Vassalle et al. (2020) aimed in their study to evaluate co-digestion between raw sewage and microalgal biomass in terms of biogas production. The results showed that methane yield was increased by 25% after AcoD with microalgae, from 156 to 211 NLCH<sub>4</sub>/kgVS. Considering biogas production, the increase after co-digesting was 10% (from 304.42 to 331.12 NL/kgVS).

Another type of biodegradable wastes, which can be used as co-substrates for SS co-digestion, is fat-rich materials. For example, fat, oil, and grease (FOG) have been reported to increase methane yield in almost threefold when added to the anaerobic digester (Kabouris et al. 2009). However, there can be process inhibition by long-chain fatty acids, sludge flotation, digester foaming, blockades of pipes, and clogging of gas collector (Grosser et al. 2017).

#### 4.4.4 State of the Art

In order to systematize the state of the art and carry out a macroanalysis of the work that has been developed on the production of biogas from sewage, the articles reported in the literature were explored using the “Bibliometrix,” a tool in the RStudio<sup>®</sup> software version 4.1.0. The articles used in this analysis were found by inserting the terms “biogas” and “sewage” and “sludge” and “anaerobic” and “digestion” in the Scopus database, including title, abstract, and keyword. The period delimited for the research was from 2000 to July 2021, resulting in 1845 published articles. According to the bibliometric analysis performed, it was observed that there is a growing interest in the topic in question especially after 2013 and with the peak of publications in 2019 (Fig. 4.2a). China is the country that publishes the most articles on biogas production from SS, followed by the USA and the Spain (Fig. 4.2b). It is worth mentioning the large participation of North America and Europe, with several countries among those that publish the most on the topic, possibly motivated by the stricter environmental policies in these regions. In addition, a WordCloud was generated with the main titles included in the articles that address the topic (Fig. 4.2c). In addition to the words directly related to the theme, some others can be highlighted, such as “chemical oxygen demand,” related to the most used characterization technique, and “municipal solid waste” and “food waste,” as the most studied co-substrate.



**Fig. 4.2** (a) Annual global scientific production of articles that address the theme biogas from sewage. (b) Scientific production by country of articles, addressing the theme biogas from sewage. (c) WordCloud with the 50 most cited words in the titles of the articles studied

## References

- Agabo-garcía C, Pérez M, Rodríguez-Morgado B, Parradob J, Soleraa R (2019) Biomethane production improvement by enzymatic pre-treatments and enhancers of sewage sludge anaerobic digestion. *Fuel* 255:4–9
- Agustini CB, Gutterres M (2017) The range of organic solid wastes from anaerobically digested tanneries. In: Vic A, Artemio N (eds) *Biogas: production, applications and global developments*, 1st edn. Nova Science Publishers, New York
- Angelidaki I, Treu L, Tsapekos P, Luo G, Campanaro S, Wenzel H, Kougias PG (2018) Biogas upgrading and utilization: current status and perspectives. *Biotechnol Adv* 36:452–466. <https://doi.org/10.1016/j.biotechadv.2018.01.011>
- Appels L, Baeyens J, Degrève J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog Energy Combust Sci* 34:755–781. <https://doi.org/10.1016/j.pecc.2008.06.002>
- Appels L, Lauwers J, Degrve J, Helsen L, Lievens B, Willems K, Impe JV, Dewil R (2011) Anaerobic digestion in global bio-energy production: Potential and research challenges. *Renew Sust Energ Rev* 15:4295–4301. <https://doi.org/10.1016/j.rser.2011.07.121>
- Aziz NIHA, Hanafiah MM, Gheewala SH (2019) A review on life cycle assessment of biogas production: challenges and future perspectives in Malaysia. *Biomass Bioenergy* 122:361–374. <https://doi.org/10.1016/j.biombioe.2019.01.047>
- Bozym M, Florczak I, Zdanowska P et al (2015) An analysis of metal concentrations in food wastes for biogas production. *Renew Energy* 77:467–472. <https://doi.org/10.1016/j.renene.2014.11.010>
- Dai X, Duan N, Dong B, Dai L (2013) High-solids anaerobic co-digestion of sewage sludge and food waste in comparison with mono digestions: stability and performance. *Waste Manag* 33: 308–316. <https://doi.org/10.1016/j.wasman.2012.10.018>
- Demirbas A, Taylan O, Kaya D (2016) Biogas production from municipal sewage sludge (MSS). *Energy Sour Part A Recover Util Environ Eff* 38:3027–3033. <https://doi.org/10.1080/15567036.2015.1124944>
- Deshmukh MKG, Sameeroddin M, Abdul D, Abdul Sattar M (2021) Renewable energy in the 21st century: a review. *Mater Today Proc* 2021:5–8. <https://doi.org/10.1016/j.matpr.2021.05.501>
- Di Capua F, Spasiano D, Giordano A et al (2020) High-solid anaerobic digestion of sewage sludge: challenges and opportunities. *Appl Energy* 278:115608. <https://doi.org/10.1016/j.apenergy.2020.115608>
- Energy Information Administration (EIA) (2019) *International Energy Outlook 2019*. <https://www.eia.gov/outlooks/ieo/pdf/ieo2019.pdf>. Accessed 2 June 2020
- Freitas FF, De Souza SSNM, Ferreira LRA et al (2019) The Brazilian market of distributed biogas generation: overview, technological development and case study. *Renew Sust Energ Rev* 101: 146–157. <https://doi.org/10.1016/j.rser.2018.11.007>
- Gao Y, Jiang J, Meng Y et al (2018) A review of recent developments in hydrogen production via biogas dry reforming. *Energy Convers Manag* 171:133–155. <https://doi.org/10.1016/j.enconman.2018.05.083>
- Ghosh P, Kumar M, Kapoor R et al (2020) Enhanced biogas production from municipal solid waste via co-digestion with sewage sludge and metabolic pathway analysis. *Bioresour Technol* 296: 122275. <https://doi.org/10.1016/j.biortech.2019.122275>
- Gil A, Siles JA, Martín MA et al (2018) Effect of microwave pretreatment on semi-continuous anaerobic digestion of sewage sludge. *Renew Energy* 115:917–925. <https://doi.org/10.1016/j.renene.2017.07.112>
- Giwa AS, Ali N, Ahmad I et al (2020) Prospects of China's biogas: fundamentals, challenges and considerations. *Energy Rep* 6:2973–2987. <https://doi.org/10.1016/j.egy.2020.10.027>
- Grosser A, Neczaj E, Singh BR et al (2017) Anaerobic digestion of sewage sludge with grease trap sludge and municipal solid waste as co-substrates. *Environ Res* 155:249–260. <https://doi.org/10.1016/j.envres.2017.02.007>



- Hagos K, Zong J, Li D et al (2017) Anaerobic co-digestion process for biogas production: progress, challenges and perspectives. *Renew Sust Energ Rev* 76:1485–1496. <https://doi.org/10.1016/j.rser.2016.11.184>
- Hallaji SM, Torabian A, Aminzadeh B et al (2018) Improvement of anaerobic digestion of sewage mixed sludge using free nitrous acid and Fenton pre-treatment. *Biotechnol Biofuels* 11:1–12. <https://doi.org/10.1186/s13068-018-1235-4>
- International Renewable Energy Agency (IRENA) (2020a) Trends in renewable energy. Available at <https://www.irena.org/>. Accessed 14 Jul 2021
- International Renewable Energy Agency (IRENA) (2020b) Renewable energy statistics. Available at <https://www.irena.org/>. Accessed 14 Jul 2021
- Jung S, Lee J, Moon DH et al (2021) Upgrading biogas into syngas through dry reforming. *Renew Sust Energ Rev* 143:110949. <https://doi.org/10.1016/j.rser.2021.110949>
- Kabouris JC, Tezel U, Pavlostathis SG et al (2009) Methane recovery from the anaerobic codigestion of municipal sludge and FOG. *Bioresour Technol* 100:3701–3705. <https://doi.org/10.1016/j.biortech.2009.02.024>
- Khalid A, Arshad M, Anjum M et al (2011) The anaerobic digestion of solid organic waste. *Waste Manag* 31:1737–1744. <https://doi.org/10.1016/j.wasman.2011.03.021>
- Khalil M, Berawi MA, Heryanto R, Rizalie A (2019) Waste to energy technology: the potential of sustainable biogas production from animal waste in Indonesia. *Renew Sust Energ Rev* 105:323–331. <https://doi.org/10.1016/j.rser.2019.02.011>
- Khan IU, Othman MHD, Hashim H et al (2017) Biogas as a renewable energy fuel – a review of biogas upgrading, utilisation and storage. *Energy Convers Manag* 150:277–294. <https://doi.org/10.1016/j.enconman.2017.08.035>
- Khan MU, Lee JTE, Bashir MA et al (2021) Current status of biogas upgrading for direct biomethane use: a review. *Renew Sust Energ Rev* 149:111343
- Khanh Nguyen V, Kumar Chaudhary D, Hari Dahal R et al (2021) Review on pretreatment techniques to improve anaerobic digestion of sewage sludge. *Fuel* 285:119105. <https://doi.org/10.1016/j.fuel.2020.119105>
- Koupaie EH, Dahadha S, Lakeh AAB et al (2019) Enzymatic pretreatment of lignocellulosic biomass for enhanced biomethane production: a review. *J Environ Manag* 233:774–784
- Kuczman O, Gueri MVD, De Souza SNM et al (2018) Food waste anaerobic digestion of a popular restaurant in Southern Brazil. *J Clean Prod* 196:382–389. <https://doi.org/10.1016/j.jclepro.2018.05.282>
- LeBlanc RJ, Matthews P, Richard RP (2008) Global atlas of excreta, wastewater sludge, and biosolids management: moving forward the sustainable and welcome uses of a global resource. United Nations Human Settlements Programme (UN-HABITAT), Nairobi
- Li Y, Chen Y, Wu J (2019) Enhancement of methane production in anaerobic digestion process: a review. *Appl Energy* 240:120–137. <https://doi.org/10.1016/j.apenergy.2019.01.243>
- Li L, Lin J, Wu N et al (2020a) Review and outlook on the international renewable energy development. *Energy Built Environ* 17:12–19
- Li Y, Cheng H, Guo G et al (2020b) High solid mono-digestion and co-digestion performance of food waste and sewage sludge by a thermophilic anaerobic membrane bioreactor. *Bioresour Technol* 310:123433. <https://doi.org/10.1016/j.biortech.2020.123433>
- Liao X, Li H, Zhang Y et al (2016) Accelerated high-solids anaerobic digestion of sewage sludge using low-temperature thermal pretreatment. *Int Biodeterior Biodegradation* 106:141–149
- Liu J, Yang M, Zhang J et al (2018) A comprehensive insight into the effects of microwave-H<sub>2</sub>O<sub>2</sub> pretreatment on concentrated sewage sludge anaerobic digestion based on semi-continuous operation. *Bioresour Technol* 256:118–127
- Lu J, Gao X (2021) Biogas: potential, challenges, and perspectives in a changing China. *Biomass Bioenergy* 150:106127. <https://doi.org/10.1016/j.biombioe.2021.106127>
- Ma H, Guo Y, Qin Y, Li Y (2018) Nutrient recovery technologies integrated with energy recovery by waste biomass anaerobic digestion. *Bioresour Technol* 269:520–531

- Malhotra M, Garg A (2019) Performance of non-catalytic thermal hydrolysis and wet oxidation for sewage sludge degradation under moderate operating conditions. *J Environ Manag* 238:72–83
- Maragkaki AE, Vasileiadis I, Fountoulakis M et al (2018) Improving biogas production from anaerobic co-digestion of sewage sludge with a thermal dried mixture of food waste, cheese whey and olive mill wastewater. *Waste Manag* 71:644–651. <https://doi.org/10.1016/j.wasman.2017.08.016>
- Miltner M, Makaruk A, Harasek M (2017) Review on available biogas upgrading technologies and innovations towards advanced solutions. *J Clean Prod* 161:1329–1337. <https://doi.org/10.1016/j.jclepro.2017.06.045>
- Mirmohamadsadeghi S, Karimi K, Tabatabaei M, Aghbashlo M (2019) Biogas production from food wastes: a review on recent developments and future perspectives. *Bioresour Technol Rep* 7:100202. <https://doi.org/10.1016/j.biteb.2019.100202>
- Momayez F, Karimi K, Taherzadeh MJ (2019) Energy recovery from industrial crop wastes by dry anaerobic digestion: a review. *Ind Crop Prod* 129:673–687
- Mu L, Zhang L, Zhu K et al (2020) Anaerobic co-digestion of sewage sludge, food waste and yard waste: synergistic enhancement on process stability and biogas production. *Sci Total Environ* 704:135429. <https://doi.org/10.1016/j.scitotenv.2019.135429>
- Nabi M, Zhang G, Zhang P et al (2019) Contribution of solid and liquid fractions of sewage sludge pretreated by high pressure homogenization to biogas production. *Bioresour Technol* 286:121378
- Neshat SA, Mohammadi M, Najafpour GD, Lahijani P (2017) Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. *Renew Sust Energ Rev* 79:308–322. <https://doi.org/10.1016/j.rser.2017.05.137>
- Neumann P, González Z, Vidal G (2017) Sequential ultrasound and low-temperature thermal pretreatment: process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. *Bioresour Technol* 234:178–187
- Onthong U, Juntarachat N (2017) Evaluation of biogas production potential from raw and processed agricultural wastes. *Energy Procedia* 138:205–210. <https://doi.org/10.1016/j.egypro.2017.05.208>
- Parralajo AI, Royano L, González J, González JF (2019) Small scale biogas production with animal excrement and agricultural residues. *Ind Crop Prod* 131:307–314. <https://doi.org/10.1016/j.indcrop.2019.01.059>
- Parsaei M, Kiani MDK, Karimi K (2019) A review of biogas production from sugarcane vinasse. *Biomass Bioenergy* 122:117–125
- Rafiee A, Khalilpour KR, Prest J, Skryabin I (2021) Biogas as an energy vector. *Biomass Bioenergy* 144:105935. <https://doi.org/10.1016/j.biombioe.2020.105935>
- Ramos-Suárez JL, Ritter A, Mata González J, Camacho Pérez A (2019) Biogas from animal manure: a sustainable energy opportunity in the Canary Islands. *Renew Sust Energ Rev* 104:137–150. <https://doi.org/10.1016/j.rser.2019.01.025>
- Ratanatamskul C, Wattanayommanaporn O, Yamamoto K (2015) An on-site prototype two-stage anaerobic digester for co-digestion of food waste and sewage sludge for biogas production from high-rise building. *Int Biodeterior Biodegrad* 102:143–148. <https://doi.org/10.1016/j.ibiod.2015.03.019>
- Sahota S, Shah G, Ghosh P et al (2018) Review of trends in biogas upgradation technologies and future perspectives. *Bioresour Technol Rep* 1:79–88. <https://doi.org/10.1016/j.biteb.2018.01.002>
- Seadi T, Rutz D, Prassl H et al (2008) *Biogas handbook*. University of Southern Denmark Esbjerg, Esbjerg
- Simioni T, Agustini CB, Dettmer A, Gutterres M (2021) Anaerobic co-digestion of tannery wastes and untreated/pretreated oat straw. *Bioenergy Res*. <https://doi.org/10.1007/s12155-021-10285-1>
- Tyagi VK, Fdez-Güelfo LA, Zhou Y et al (2018) Anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW): progress and challenges. *Renew Sust Energ Rev* 93:380–399. <https://doi.org/10.1016/j.rser.2018.05.051>

- Vassalle L, Díez-Montero R, Machado ATR et al (2020) Upflow anaerobic sludge blanket in microalgae-based sewage treatment: co-digestion for improving biogas production. *Bioresour Technol* 300:122677. <https://doi.org/10.1016/j.biortech.2019.122677>
- Wang S, Han Y, Lu X et al (2021) Microbial mechanism underlying high methane production of coupled alkali-microwave e H<sub>2</sub>O<sub>2</sub> e oxidation pretreated sewage sludge by in-situ bioelectrochemical regulation. *J Clean Prod* 305:127195
- Wu B, Zhang X, Xu Y et al (2015) Assessment of the energy consumption of the biogas upgrading process with pressure swing adsorption using novel adsorbents. *J Clean Prod* 101:251–261. <https://doi.org/10.1016/j.jclepro.2015.03.082>
- Xu F, Li Y, Wicks M et al (2019) Anaerobic digestion of food waste for bioenergy production. *Encycl Food Secur Sustain* 2:530–537. <https://doi.org/10.1016/b978-0-08-100596-5.22433-5>
- Zhen G, Lu X, Kato H et al (2017) Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: current advances, full-scale application and future perspectives. *Renew Sust Energ Rev* 69:559–577. <https://doi.org/10.1016/j.rser.2016.11.187>
- Zhu A, Qin Y, Wu J et al (2021) Characterization of biogas production and microbial community in thermophilic anaerobic co-digestion of sewage sludge and paper waste. *Bioresour Technol* 337:125371. <https://doi.org/10.1016/j.biortech.2021.125371>



# Anaerobic Biodigesters for the Treatment of High-Strength Wastewater

# 5

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and Vivekanand Vivekanand

## Abstract

High-strength wastewater (HSWW) has become a major issue that has been continuously affecting the environment, soil, and other freshwater resources. However, HSWW contains large amounts of organic matter which can be recovered in the form of energy as biogas by using anaerobic digestion. High-rate anaerobic reactors are being employed for treating the wastewater and converting the organics into biogas. In this study, several high-rate anaerobic reactors and the parameters affecting their performance have been discussed. The challenges and future perspectives have been detailed for exploring a better solution to recover bioenergy from the wastewater. Among the different types of reactors, the anaerobic membrane reactor (AMR) with a dynamic module (DM) is the best and most efficient option because it can be employed for treating high-strength wastewater (HSWW) with minimum losses. These reactors also reduce the methane emissions generated from wastewater sludge in an open area.

## Keywords

Biogas · Energy recovery · High-rate anaerobic digestion · Wastewater

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## 5.1 Introduction

Numerous resources such as air, water, sunlight, soil, etc. are available that are employed for the growth of life. Among them, water is one of the most valuable resources which has been existing before the evolution of life; even life cannot exist without it (Shekhawat et al. 2020). As per the World Health Organization (WHO), 97.5% of water present on the earth is salty which requires treatment before it can be used. However, freshwater sources such as groundwater, glaciers, and ice caps are included in the remaining part of total water. It means that only approximately 1% is available which can be used for drinking and other purposes. In the ancient and medieval eras, clean and fresh water was abundantly available because there was a very little number of industries and populations. However, the demand for clean water has been accelerated tremendously owing to population growth, industrialization, construction, and urbanization in the recent decades (Gupta et al. 2009). The use of water by industries, for agricultural and domestic purposes, generates a large amount of wastewater. The major part of industrial wastewater is produced mainly from pulp and paper; meat and poultry; alcohol, beer, and sugar production; chemical production; and food industries (Fito et al. 2018; Njoya et al. 2021). The wastewater produced from pulp and paper and meat and poultry industries contains a higher amount of degradable organic compounds. The quality of this wastewater is variable depending upon from which industry it is produced. It may or may not be biodegradable but contains recalcitrant compounds. According to the “United Nations World Water Development Report 2021,” globally, 80% of total wastewater remains untreated and unused. It goes into the ecosystem and affects around 1.8 billion people who use this contaminated water, resulting in the spread of diseases such as cholera, typhoid, dengue, malaria, and polio. Additionally higher volumes of untreated sewage and agricultural and industrial discharge have affected water quality and contaminated water resources around the world (Bella and Rao 2021). The usage of untreated wastewater for irrigation and other purposes may negatively affect the environment and human beings. Thus, wastewater has become a problematic issue that requires a particular solution so that it can be treated and reused for irrigation and drinking purposes. Other important reasons for the treatment of wastewater are to protect the environment and living things by safeguarding water supplies and preventing them from spreading waterborne diseases (Lohani et al. 2020). However, wastewater contains various types of contaminants such as oxygen-demanding wastes (BOD and COD), radioactive substances, and organic and inorganic compounds. The higher amount of organic matter present in wastewater can be employed for energy recovery which helps to reduce the negative impacts on the environment and living organisms. Anaerobic digestion can be a sustainable and energy-efficient technology to utilize the organic matters present in wastewater compared to the conventional activated sludge (CAS) treatment, which is mainly an aerobic treatment of wastewater (Vinardell et al. 2020).

Aerobic digestion or treatment is employed to convert organic matters into carbon dioxide and treat biomass by using microorganisms in the presence of oxygen. However, aerobic treatment has numerous disadvantages such as not being suitable

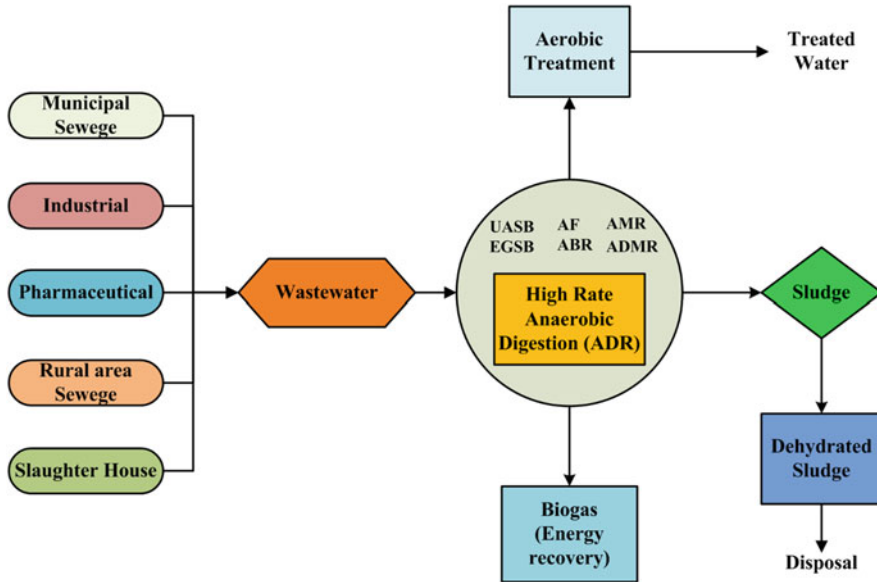
for high-concentration wastewater (HCWW) due to higher energy requirements and scaling up of reactors resulting in the lower efficiency of reactors. Also, higher COD and recalcitrant like polyphenols may prevent the development of aerobic bacteria. Anaerobic digestion is the leading technique to overcome these problems, and proper utilization of organic matter into energy recovery makes the high-strength wastewater free from heavy compounds (Fito et al. 2018). The other main aspect of employing anaerobic digestion for wastewater treatment is to reduce the emission of methane ( $\text{CH}_4$ ).  $\text{CH}_4$  is produced when the contaminants of high-strength untreated wastewater are deposited on the surface; due to these anaerobic zones are formed which enhances the production of  $\text{CH}_4$  owing to anaerobic conditions and it gets exposed to the environment (Johnson et al. 2019). This stabilization of contaminants can help to produce a significant amount of biogas and efficiently treat the water.

Similarly, treatment of HCWW from the pulp and paper industry involves various techniques such as neutralization, screening, sedimentation, and flotation to remove solids. However, the chances of the anaerobic process are very high due to lagoon formation and settlement which results in the generation of unused  $\text{CH}_4$  that is emitted in the environment and degrades it. According to the Climate and Clean Air Coalition,  $\text{CH}_4$  emission generated by human activities and waste is one of the contributors to environmental degradation and climate change.  $\text{CH}_4$  affects the environment temperature 86-fold more than  $\text{CO}_2$  gas. Additionally, the food, sugar, and brewery industries usually produce large volumes of high-organic COD and BOD wastewater, leading to biogas emission. Also, a higher amount of organic matters may cause rapid exhaustion of available oxygen, influencing the aquatic life in the water bodies (Fito et al. 2018). From the abovementioned issues, strict environmental regulations and initiatives are needed for the treatment of wastewater to lessen pollutant concentrations and to recover energy as biogas. Furthermore, the attempts towards environmental protection have led to the development of new wastewater treatment technologies which would be efficient and economical. However, there is still no particular method to treat wastewater that can eliminate all the contamination from the wastewater. AD is the most promising treatment for high-strength organic matters of wastewater. Several anaerobic bioreactors have been developed for anaerobic treatment of HSWW mainly “high-rate anaerobic biodigesters such as upflow anaerobic sludge blanket, expanded granular sludge bed, anaerobic filters, anaerobic baffled reactors, anaerobic membrane bioreactors, and anaerobic dynamic membrane bioreactors.” These bioreactors have their own advantages and disadvantages depending upon the quality of wastewater for which they are applied. A schematic of energy recovery from wastewater by the anaerobic digestion process has been depicted in Fig. 5.1.

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## 5.2 High-Rate Anaerobic Biodigesters

Anaerobic digestion has been recognized as a foremost pre-treatment technology to treat the HSWW and reduce the emission of methane to the environment (Ülgüdür et al. 2019). Since 1880, AD has been into existence formally and used in limited



**Fig. 5.1** Flowchart of wastewater treatment from different sources

application for the utilization of high-strength biodegradable wastes. However, the majority of biowaste was treated by aerobic digestion (McAteer et al. 2020). Anaerobic digestion needs less energy and harvests energy in the form of methane-rich biogas and also produces easily disposable sludges. There are some disadvantages of the application of AD such as slow rate and unstable process. Slow-rate AD means bigger digester volumes are required which leads to higher costs and space requirements, while the unstable process is due to the lack of steady energy supply. To overcome these problematic issues, high-rate anaerobic biodigesters are successfully implemented for treating the industrial and municipal wastewater in the previous few years (Lier et al. 2015). In European countries mainly the Netherlands, an approximately 90% decrease in sludge production knowingly has enhanced the economy of the plant, while higher loading volumes of anaerobic high-rate (AHR) biodigesters allowed for a maximum lessening in space requirement than the traditional methods of activated sludge systems. These merits increase the instant progress of AHR technology for HSWW treatment.

Several configurations of bioreactor have been used for the AD of wastewaters, as studied by McCarty. In 1905, Karl Imhoff designed the first continuous-flow anaerobic reactor which helped to enhance settling and associated digestion of the stabilized solids by using a single flow-through tank. This innovative tank was mainly implemented for municipal wastewaters and is being used in several countries of the world at the present time, mainly in warm climate regions (Lier et al. 2015). Arthur M. Buswell had explored the anaerobic treatment of industrial wastewater intentionally. Buswell had introduced the concept of biochemical

oxidation and reduction reactions that occurred in anaerobic digestion and developed the advanced process. In these bioreactor studies, hydraulic retention time (HRT) was quite alike to solid retention time (SRT). Also, AD is totally dependent on the growing rate of bacteria. Meanwhile, growth rate is quite low; due to this, bioreactors with bigger size and higher volume are required. Thus, completely stirred tank reactors (CSTR) were the prime systems that were employed for AD until the 1960s. These reactors are called low-rate anaerobic reactors. The major drawback of low-rate anaerobic bioreactors is the need for bigger size to provide enough space and attain the exact biomass concentration in the reactor. However, it was reported that the capacity of treatment can be increased by a higher concentration of biocatalysts like methanogenic sludge in an anaerobic reactor. Thus, the new concept of high-rate anaerobic reactors (HRARs) was coined in which the SRT is disengaged from the HRT. HRAR has become an advanced technology that can be used for HSWW and reduce the size of bioreactors that leads to an increase in the rate of the process.

It has gained much attention as a practical interest for cost-effective wastewater treatment. The advancement in HRAR has helped to increase the process rate and reduce the cost and carbon footprint. Njoya et al. have evaluated the treatment of poultry slaughterhouse wastewater in HRAR that removed a higher amount of BOD, COD, and other heavy compounds such as fat, grease, and oil (Njoya et al. 2021). Numerous researches have been investigated for the treatment of heavy organic content present in the wastewater from different industries such as meat, paper and pulp, cheese and dairy, ethanol, and sugar industries by using the HRAR. These studies have opened the way for the utilization of the HRAR for energy recovery and mitigation of unused methane emissions to the environment. There are different types of HRARs such as “upflow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB), anaerobic filters (AF), anaerobic baffled reactors (ABR), anaerobic membrane, and anaerobic dynamic membrane (ADM)” bioreactors which have been used for the treatment of HSWW for further processing of water.

### 5.2.1 Upflow Anaerobic Sludge Blanket (UASB)

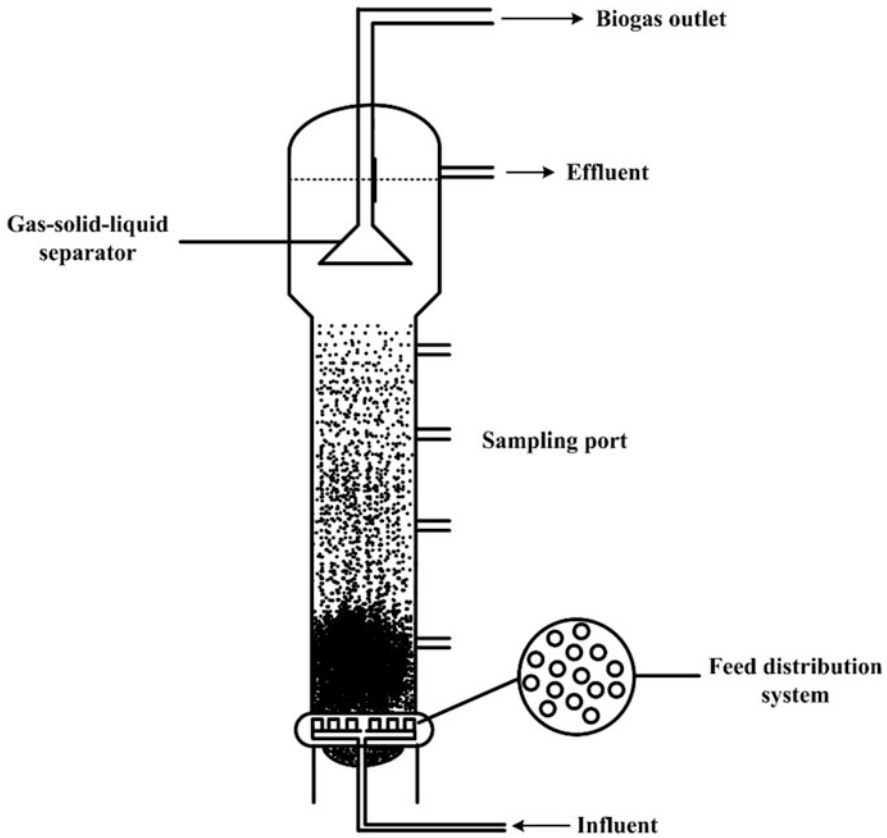
UASB is one of the important bioreactors which was made by Lettinga and his team in Holland in the 1970s–1980s. They had examined that the biomass present in the wastewater exists in free granular aggregates (Dutta et al. 2018). UASB reactor has attained much attention from researchers and has been employed for HSWW treatment owing to a higher biomass concentration and rich microbial diversity (Soares et al. 2019). The higher concentration of biomass in wastewater infers that the transformation of contaminants is very fast; also, the higher concentration and the large amount of organic content present in the wastewater may be treated in compacted reactors (Harihastuti et al. 2021). Thus, UASB is a suitable reactor as compared to other anaerobic techniques and depends on the granulation process with specific wastewater. It means anaerobic granular sludge is the main part of a UASB



reactor. The granules of sludge are dense and have multispecies and microbial communities; this granular ecosystem can degrade the complex organic wastes (Xu et al. 2020). In contrast to improving the effluent quality and less amount of sludge production, UASB has well-known because of its compact design, easy to operate, low capital cost and enhances the calorific of biogas up to certain limits. UASB process involves both physical and biological processes. In the physical process, the solids and gases are separated from the liquid, and in the biological process, the organic matter is degraded under anaerobic conditions. There is no need for a sludge return pump for separating the settler and high-rate effluent recirculation and pumping energy. The other advantage of UASB is no loss of volume of the reactor with any carrier material as compared to an anaerobic filter. As anaerobic sludge has good settling properties and settles without heavy agitation mechanically, therefore, mechanical mixing is usually denied in UASB reactors.

Jiraprasertwong et al. (2021) have used a two-phase UASB system for treating the ethanol production industry wastewater with sludge granulation. They optimized organic loading rate (COD loading of feed) and overall effective liquid holding capacity, was achieved  $32 \text{ kg m}^{-3} \text{ d}^{-1}$  with diameter of granule (1.12 mm) and density ( $1.23 \text{ kg/m}^3$ ). Also, the methane production rate was higher, and up to 90% of COD has been removed. Furthermore, the system resulted in a very high energy yield, and the long time of operation (about 2 years) helped increase the formation of microbial granules (Jiraprasertwong et al. 2021). In Fig. 5.2, the working diagram of UASB reactor is shown for better understanding. The raw material or influent is fed from the bottom side of the reactor which then drifts upwards through a sludge blanket holding a bed and covering with granules. It provides an efficient way of making the mixture of wastewater with the granules that enhance the rate of anaerobic decomposition of the raw material. The biogas is produced and drifts upwards in the reactor, which also helps for proper mixing. However, this system contains a separator that segregates the gas-liquid-solid and granules to improve the quality of biogas. The liquid effluent is separated from the reactor through a vent, and the solid sludge settles down at the bottom of the reactor. Also, biogas is stored in the gas reservoir (Tauseef et al. 2013). The efficiency of the UASB reactor is directly dependent on the quality of granules formed in wastewater.

Wastewater from the different sources may affect the granular sludge, whether readily or slowly, due to the volatility and density of wastes. Hence, it can be a major barrier to establishing the UASB technology (Sahinkaya et al. 2015). However, inoculation with a higher granular sludge from UASB usually assists in increasing the efficiency. In contrast, there is a problem related to retaining the characteristics of sludge granules due to changing the source of wastewater. Preferential flows, dead zones, and hydraulic shortcuts may take place in the UASB. In this context, UASB also has a low stability and requires an initial period required suitable for the growth of anaerobic granules due to varying wastewater sources which limits the application of UASB technology; hence, it requires suitable in-depth research (Sierra et al. 2019).



**Fig. 5.2** Upflow anaerobic sludge blanket (UASB) reactor

### 5.2.2 Expanded Granular Sludge Bed (EGSB)

An EGSB bioreactor is a modified version of the UASB reactor for anaerobic wastewater treatment. There are numerous issues mainly low stability, preferential flows, dead zones, and hydraulic shortcuts which hinder the application of UASB technology. To resolve these issues, researchers have developed a new design of the reactor which is known as the expanded granular sludge bed bioreactor. EGSB has several advantages such as being simple, flexible, capability to treat high influent and velocity of the gas, higher circulation ratios, and higher OLR as compared to UASB technology. EGSB allows the interaction between granular sludge and soluble components of the wastewater (Johnson et al. 2019). EGSB bioreactor has become a popular concept due to its low cost and robust design because it works on a concept of fluidization that helps to increase the organic load and retention times. These positive results enhance the treatment efficiencies (up to 95%) and recover energy in the form of biogas (Cruz-Salomón et al. 2019).

The first EGSB bioreactor with advancement in UASB bioreactor was used in the Netherlands in the 1980s. Additionally, EGSB bioreactor can treat different concentrations of wastewater such as effluents with recalcitrant and the highly toxic ones as compared to conventional UASB. These recalcitrant are primarily biodegradable such as pesticides, methanol, phenol, etc. Frijters et al. have treated the wastewater generated from the textile industry which contains dyes and highly toxic substances (sodium sulfate and chlorinated liquids) and effectively recovered energy without using inhibitors. Furthermore, EGSB bioreactors can be sustained and employed for either inhibitory or toxic conditions, which means wastewater produced from the chemical and pharmaceutical industries. EGSB reactor allows the higher velocities (may be 6–30 m/h) of upflow for liquid owing to the height/diameter ratio that lies in the range 10/1–25/1. The design of this reactor depends upon the hydraulic characteristics that provide long and slender bioreactors and require less space. The height of these reactors recommended for industries lies between 7 and 24 m (Correia et al. 2014). Moreover, the increase in AGS contact with wastewater helps to treat HSWW from “vinasse, palm oil mill effluent (POME), coffee processing wastewater (CPWW), and soft drink industry wastewater” and enhance the rate of AD. Despite the great advantages of the EGSB bioreactor, EGSB is not suitable for completely removing the suspended solids, pathogens, nutrients, and organics because of the higher flow velocity of liquid as compared to the AGSB reactor. Due to this, post-treatment is required as per environmental regulations. Also, the formation of hydrogen sulfide ( $H_2S$ ) in the reactor produces a strong stench and abrasion, particularly the higher amount of sulfate ( $SO_4^{2-}$ ) present in the influent of wastewaters. However, the start-up time of the bioreactor may be prolonged due to the biological and chemical composition of the wastewater. Several factors such as the wastewater characteristics, particle size distribution (PSD), acclimatization of AGS, bioreactor design, HRT, SRT, OLR, and environmental parameters (pH and temperature) can influence the performance of the EGSB bioreactor. The variation in wastewaters can be due to the composition of different sources such as domestic, industrial, and agricultural wastewater. Thus, it is essential to know which type of wastewater can be treated by AD. For this, an important index is introduced which is called the biodegradability index (BOD/COD ratio). The recommended index is greater than  $>0.3$ , and was also investigated for the very low value of the index that wastewater is not used to treat in EGSB reactor due to low efficiency. Moreover, there are many toxic compounds like ammonia, metals, volatile fatty acid (VFA), and chlorinated and aromatic hydrocarbons that have resulted in operational problems occurring in EGSB.

### 5.2.3 Anaerobic Filters (AF)

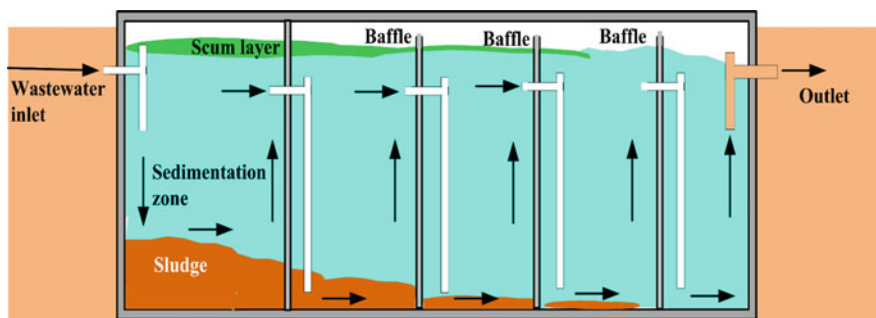
Anaerobic filters are an integral part of a reactor which are employed to entrap the sludge aggregates or bio-solid materials between packing materials. These types of filters have been used for wastewater treatment for a long period, and the search for appropriate filters (operating conditions and anaerobic filter treatment performances)

for different technologies (biological methods) is still in process. Generally, the anaerobic filters are classified based on the feeding type and packing media. As per the feeding type, the filters are individual-feeding based (upflow, downflow, and horizontal direction) and multiple-feeding (Goli et al. 2019) based which are preferred for numerous industrial applications. For packing media-type anaerobic filters, a lot of focus has been on the different materials of filters (which is an important criterion for the selection). Although the size, shape, porosity, and specific surface area of the filter are also important performance parameter criteria, they are still not explored much (Zhao et al. 2020). Some of the important packing media used for different applications (treatment of slaughterhouse wastewater, dairy wastewater, domestic wastewater etc.) are polyethylene, Floccor, polypropylene, packing ring, ceramic, ultraviolet-stabilized media matrix, pumice stones, tezontle (volcanic rock), plastic-corrugated cylinders, sand filter, etc. (Goli et al. 2019; Lohani et al. 2020; Shende and Pophali 2021). The operating conditions and performance characteristics on which these anaerobic filters are designed such as “operating temperature, pH value, hydraulic retention time (HRT), organic loading retention (OLR), COD, CH<sub>4</sub> yield,” etc. (Zhao et al. 2020). Tonon et al. (2015) have examined the wastewater treatment process that included anaerobic and sand filters, as well as the use of coconut shells to eliminate particles, organic debris, phosphate, and pathogens. The anaerobic filters with coconut shells as fillers have a COD and BOD removal efficiency of 65–80%. At all times, the combination of anaerobic filters and sand filters resulted in a 95% efficiency. Oliveira Cruz et al. (2019) have checked the feasibility of green coconut husks (*Cocos nucifera*) in an anaerobic filter which is generally used as waste. The results showed that coconut husks in combination with a sand filter will be a promising technology at a small scale. Kaetzel et al. (2018) have used biochar and woodchips in an anaerobic filter for the wastewater filtration and also checked their suitability as compared to gravel as a reference material.

The results showed that the performance of biochar filters is best compared to the wood chips and gravel filters in terms of “COD, TOC, turbidity, and FIB removal,” indicating the better characteristics of biochar for wastewater treatment. Lohani et al. (2020) have used a sand filter and a combination of ST-UASB-sand filters for domestic wastewater treatment which helped eliminate the organics and fecal coliform. The sand filter contributes significantly to the overall removal and also stabilizes the effluent quality at an acceptable level (TSS < 110 mg/L and COD < 210 mg/L). Márquez et al. (2021) created a novel method to frame a novel UASB reactor that is divided into two-stage separation (UASB-2SS) and three-stage separation (UASB-3SS). Further, the authors have proposed three models for the evaluation of efficiency of UASB-2SS and UASB-3SS reactors (modified model of the Monod’s equation, model based on combining Monod’s equation and Velz’s law, coupled model) that achieved best results through the coupled model.

### 5.2.4 Anaerobic Baffled Reactors (ABR)

An ABR is considered a sustainable technology for treating domestic wastewater. It requires a lesser amount of energy and supports to protect the environment efficiently. It has a higher potential and flexibility than other wastewater technologies owing to its flexibility to hydraulic shock load. This is caused by variation in the flow because of the presence of inhibitor in the wastewater. Especially, the designs of ABR contribute to the formation of microorganisms for AD and the segregation of liquid-solid phases and provide system stability (Reynaud and Buckley 2016). ABR passage prevents the effect of pathogenic bacteria by destroying them and retains the exact level of phosphorus and nitrogen in the sludge. Thus, the treated water could be employed for irrigation purposes. Also, it is replaced the septic tank usage for domestic wastewater treatment. ABR has overcome the problems related to the incapability of hydraulic shock absorption and inferior treatment of sludge in septic tanks (Richards et al. 2016; Withers et al. 2014). ABRs consist of an arrangement of baffles for treating the wastewater that flows up and down the baffles. In compartments, the speed of flow is kept less than 0.6 m/h, and the number of compartments generally lies in range 3–6. These compartments are connected either with vertical pipes or baffles as depicted in Fig. 5.3 (Shende and Pophali 2021). ABRs could be used for handling low-strength wastewater (300 mg/L of COD) with 95% COD removal efficiency at 10 h HRT. ABR is also known as a sludge retention and digestion device with alternating standing and hanging baffles that force the wastewater to drift continually through the settled sludge. This phenomenon enhances the association of organics and biomass which increases the AD and retention of particulate and organic matter. The design of this reactor shows decoupling of HRT from SRT and has different compartments, so that COD retention and digestion take place separately (Reynaud and Buckley 2016). As per literature, researchers have investigated that the reactor requires a high SRT and reactor volume that is evaluated hydraulically as compared to organic loading for anaerobic treatment. In the design of ABR, the solid retention is dependent upon the upflow velocity of the wastewater that is retained in the compartments holding the



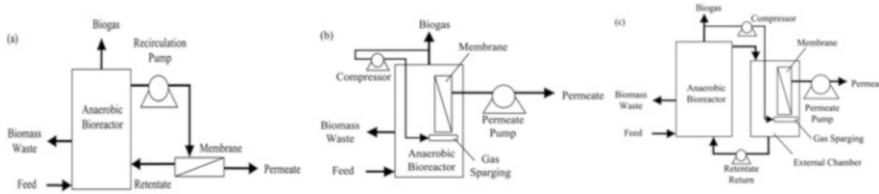
**Fig. 5.3** Schematic diagram of anaerobic baffled reactor

sludge also. The volume of solid matters does not float for the release of biogas in low-strength wastewater applications.

Putra et al. (2020) have investigated the utilization of the ABR with a volume of 60 L for the treatment of fishmeal wastewater with higher organic matter (140 g COD·L<sup>-1</sup>). The wastewater contains 60% (w/w) of oil and grease, 27% (w/w) protein, and 13% (w/w) mixture of suspended solids and soluble organics. The reactor worked on HRT for 20 days and resulted in the removal of the higher amount of total and soluble COD approximately 98% and 94%, respectively. Zha et al. (2019) have employed the ABR for the treatment of blackwater produced from the domestic rural area under the optimized HRT of 48 h. The reported overall results of average removal efficiencies of COD, nitrogen (N<sub>2</sub>), ammonium nitrogen (NH<sub>4</sub><sup>+</sup>), and phosphorus (P) are 94.05%, 28.78%, 14.21%, and 32.54%, respectively, during the continuous operation of 112 days. Fujihira et al. (2018) have worked on ABR for the treatment of wastewater at a laboratory scale. The authors have obtained better results with >90% of COD removal and >70% of COD removal converted into biomethane at steady-state conditions. Li et al. (2021) have studied the performance of ABR for wastewater containing oxytetracycline (OTC) under acidic condition. It was stated that overall, 95% and 60% of COD and OTC were removed, respectively. As mentioned in literature, the overall efficiency of ABR is approximately >90%. It is examined that COD removal is decreased for lower HRT up to 0.9 day, and the COD removal efficiency obtained is less than 60%. As it contains a compartmentalized configuration which works as anaerobic treatment in two phases with segregation of acidogenic and methanogenic biomass, ABR can be employed for all soluble wastewater which may be low or high strength. The demerits of ABR reactors are higher solid loss, elongated start-up phase, disruption of microbial communities, and requirement of an additional treatment technique to reduce the pathogens remaining in the wastewater (Pal 2017). Owing to its simple design and fluent operation, it can be efficiently employed for treating municipal and domestic wastewater in tropical regions of developing countries. However, it has not been used and developed too much on full scale.

### 5.2.5 Anaerobic Membrane Bioreactors (AMR)

An AMR is a biological treatment process that works without oxygen and employs a membrane to isolate the solids and liquids completely. Membrane bioreactors (MBRs) are more efficient in that it can deal with biomass accumulation and work at higher inlet concentrations of wastewater. AMRs, a combination of MBR and anaerobic bioreactors, are commonly used to treat “synthetic wastewaters, food processing wastewaters, industrial wastewaters, high-solid-content waste streams, and other waste streams” (Liao et al. 2006; Skouteris et al. 2012; Jensen et al. 2015; Robles et al. 2020). Microbial activity, operational temperature, SRT, HRT, reactor design, and membrane location are all factors that influence AMR treatment performance. Further, the AMR’s performance is heavily influenced by the type of membrane and its location. Flux, membrane pore size and materials, operational



**Fig. 5.4** Schematics of different types of AMBR: (a) pressure-driven membrane; (b) vacuum-driven (internally and externally) membrane (c) pressure and vacuum driven membrane

pressure and temperature, hydrodynamics, mixed liquor suspended solids, etc. are the membrane performance parameters of AMRs (Ozgun et al. 2013; Cheng et al. 2018; Lei et al. 2018).

As per membrane design and operation, AMRs can be further divided into three categories: side-stream AMR, submerged AMR (SAMBR), and external submerged AMR (Liao et al. 2006; Ji et al. 2020). The different types of AMR being used are shown in Fig. 5.4. The first is a pressure-driven membrane, whereas the second and third are a vacuum-driven immersed membrane. The membrane is separated from the bioreactor in the first approach, and a pump is needed to force bioreactor effluent into the membrane unit and through the membrane (Fig. 5.4a). There are two ways to employ the vacuum-driven immersed membrane method. Figure 5.4b shows how the membrane can be immersed directly in the bioreactor or a separate chamber (Fig. 5.4c) (Liao et al. 2006). Schneider et al. (2021) have investigated the performance of anaerobic membrane bioreactors with forward osmosis membranes (FO-AMR) in terms of biomethane generation, anaerobic microbiome cell integrity (shear stress impact), and FO filtering efficiency of brewery wastewater during the start-up period. FO-AMR could be an efficient and quick procedure for biologically treating brewery wastewater and producing bioenergy as per the findings of the study. However, for a long-term FO-AMR operation, more process optimization is required, particularly in the areas of HRT/SRT, OLR, C/N ratios, and cleaning techniques, washout, excess VFA, and salts.

The efficacy of AMR for livestock wastewater treatment in terms of biogas production and pollutant removal was reviewed by Zhang et al. (2019). The authors have found that suspending numerous fundamental mechanisms in the air, such as contaminant removal pathways and membrane fouling behavior, had a limited effect on AMR efficacy for livestock wastewater treatment. Membrane fouling in AMR is a challenging problem that reduces membrane performance and shortens its lifespan. Sohn et al. (2021) have conducted a study of the literature on the various types of fouling reduction enhancers in AMR and their effects on membrane fouling reduction. The use of fouling reduction enhancers such as activated carbon, charcoal, zeolite, and polyaluminum chloride might effectively lessen membrane fouling in AMRs, as per the findings. Furthermore, while enhancers have the potential to be a foulant, overdosing or using large particle sizes could have the opposite effect. Ji et al. (2020) have examined the current advances and obstacles for removing contaminants (ECs) from the environment using AMR technology, as well as the

mechanisms and factors that influence the removal of ECs by AMR. ECs are synthetic organic chemicals that have been released into the environment, posing a major hazard to the ecosystem as well as human health. AMR can significantly boost EC removal and biogas output. Despite the influence of several factors, the AMR's ability to remove ECs and its overall system performance can be improved by the optimization of AMR design and technical operation.

### 5.2.6 Anaerobic Dynamic Membrane Bioreactors (ADMB)

The issue related to retention of anaerobic biomass in the reactor is still a major obstacle in the previously discussed reactors for wastewater treatment to energy recovery. To optimize this issue to a great extent, the combination of anaerobic treatment reactors and membrane filter technology has been employed at the lab and in full scale to maintain the microorganisms, which is known as anaerobic membrane reactor (AMR) (Visvanathan and Abeynayaka 2012; Ozgun et al. 2013). As discussed in the previous section of AMR, HRT and SRT can be easily controlled to enhance the process quality for high-strength wastewater treatment, and HRT has been reduced. However, SRT can be stabilized for a long time (50–700 days) to remove the effluent free from solids and higher COD due to the retention of slowly decomposed organic matter in the reactor (Smith et al. 2014; Tang et al. 2017). The characterization of anaerobic sludge in AMRs depends upon the high viscosity and the concentration of liquor suspended solids. In addition, the higher content of biopolymers and inorganics have emanated the issue of fouling in the membrane which is mostly higher than aerobic MRs (Meng et al. 2017). This phenomenon normally takes place on the surface of the membrane of AMR which can reduce the efficiency of the reactor. Membrane fouling is categorized into two types, namely, cake layer formation on the surface of the membrane and pore-clogging, the former being the major contributor to membrane fouling. As the cake layer contributes to 80% of overall filtration in many applications; due to this, cake layer filtration can prevent pollutants and growing pathogens during the process (Hu et al. 2018a, b). The cake layer is referred to as a dynamic layer for filtering purposes and retaining the organics for a long time period. To enhance the performance of dynamic module (DM) layer or cake layer, a support material is used which may be woven or nonwoven, and it provides homogeneity to the layer (Ersahin et al. 2016). The DM filtration technology has been employed and integrated with other anaerobic reactors such as UASB and EGSB for making the ADMR and reducing the membrane fouling (Ding et al. 2015; Quek et al. 2017). DM technology has been considered as a substitute for conventional microfiltration/ultrafiltration membranes that were used in AMR, and it has a number of advantages like low cost, less fouling, and higher filtration flux (Yu et al. 2015). For better results, different types of dynamic modules such as hollow fiber, flat-sheet, and tubular (Loderer et al. 2012) have been used. The selection of DM has been done based on the size, cleaning method, and operation with which they are used. Furthermore, on the basis of relative location and membrane configurations, ADMRs have been categorized



**Table 5.1** Comparison of the different types of high-rate anaerobic reactors

Type of reactor	Wastewater source	Biogas recovery	COD removal (%)	Limitations	Reference
UASB	Low strength	Low	72–80	Incomplete removal of nutrients, unable to remove pathogens, odor, toxicity, and corrosion problem	Sierra et al. (2019)
EGAB	Medium strength	Moderate	80–90	Low rate of removal of nutrients and pathogens	Cruz-Salomón et al. (2019)
AMR	High strength	High	<95	Membrane fouling, high cleaning cost, poor efficiency at lower temp	Dvořák et al. (2016)
ADMR	High strength	High	<99	Formation of DM layer is a complex process	Lei et al. (2018)

into two types, namely, submerged (it may be internally and externally submerged) and side-stream types (Shoener et al. 2016).

In the case of submerged configuration, the DM is directly attached to the reactor (internally or externally), and it is operated under vacuum (Ersahin et al. 2016; Quek et al. 2017). On the other side, a side-stream configured DM is installed in the bioreactor externally, and a membrane tank is also installed; it works under atmospheric pressure and above (Alibardi et al. 2016). Numerous researches have been conducted with flat-sheet DM (may be inner and outer) with submerged ADMR effectively than side-stream ADMR. DM formation takes place in four steps, namely, substrate formation, separation layer formation, fouling layer formation, and filtration cake formation. The investigation of DM characteristics can be done by using physicochemical methods mainly particle size distribution (PSD), scanning electron microscopy and energy-diffusive X-ray (SEM-EDX), atomic force microscope (AFM), excitation-emission matrix (EEM), Fourier transform infrared spectroscopy (FTIR), gel filtration chromatography (GFC), confocal laser scanning microscopy (CLSM), and specific methanogenic activity (SMA). The cleaning of DM is carried out by the following methods: biogas sparging, mixed liquor recycling, liquid cross-flow, membrane relaxation, backwashing, vibration, and brushing; some chemicals (NaClO) can be used (Hu et al. 2018a, b). Additionally, the comparison of ADMR with other reactors is tabulated in Table 5.1. There are several parameters like reactor design and configuration, sludge characteristics, and properties of membrane and wastewater which are reported to influence the overall efficiency of ADMR. The relative location of configuration are submerged and side-stream ADMRs which have different efficiencies depending upon the removal of COD and methane production (Alibardi et al. 2016). It is reported from the literature that approx. 99% COD removal rate and a higher methane production were obtained by submerged ADMRs which were higher than side-stream ADMRs (Ersahin et al. 2016). Membrane properties are defined by mesh material, pore size, surface

properties, and membrane module type (flat-sheet or tubular). In terms of mesh pore size, a higher range (10–200  $\mu\text{m}$ ) has resulted in higher flux and lower retention of particles; therefore, 30–100  $\mu\text{m}$  pore size was preferred (Ersahin et al. 2017). To date, there is a limited number of researches conducted on different particle sizes and mesh materials. Researchers have used different types of mesh supporting material such as hollow fiber (Isik et al. 2020), nylon mesh (having pore sizes of 20, 53, and 100  $\mu\text{m}$ ) (Yurtsever et al. 2020), and carbon cloth (Jia et al. 2020). Moreover, the sources of wastewater mainly solid wastes and municipal and industrial wastewater affect the performance of ADMRs. This is due to properties like composition, biodegradability, and nature (pH) of wastewater. ADMRs have better results for municipal wastewater owing to a high biodegradability, better removal of pollutants, higher filtration performance, low membrane fouling, and less toxicity to biomass than industrial wastewater. However, a low methane production was obtained due to the low content of organic matter (Kim et al. 2011).

Sludge and operational characteristics also influence the performance of ADMRs; however, limited research has been conducted on considering the few factors (mainly stirring intensity, OLR, SRT, and HRT, without temperature) of operational characteristics. Furthermore, ADMRs have several advantages over AMRs in relation to various aspects of reactor performance. Firstly, membrane modules used in ADMR are less expensive than the membrane of AMR and need less cleaning. Secondly, biomass retention is also higher for ADMRs than AMR owing to prolonged SRT and increased DM layer formation (Lei et al. 2018). ADMR technology has become the leading technology for energy recovery from organic matter present in wastewater.

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### 5.3 Additives for Enhancing the Anaerobic Performance of Wastewater

Numerous technologies have been employed to treat the HSWW for energy recovery in the form of biogas and for irrigation purposes. However, there are several flaws related to COD removal rate, inhibitors, metal ions, growth of pathogens, and degradation and lower efficiency of the reactor, factors that hinder the implementation of the technology. In order to solve these problems, nanotechnology is introduced and employed to make the process sustainable and economically viable and to reduce the negative downstream impacts (Park et al. 2018). The various types of nanomaterials (additives) such as magnetite, activated carbon, graphite, key trace elements (cobalt, nickel, copper, iron, molybdenum, and zinc), carbon nanotubes (CNT), and biochar have been investigated for enhancing methane production (Abdelsalam et al. 2017; Martins et al. 2018; Zhang et al. 2019; Tetteh and Rathilal 2021). The metal and nonmetallic oxides like titanium, iron, aluminum, zinc, and silver oxides have been used as nanoparticles for the treatment of wastewater as antimicrobial agents for balancing the microbial activities (Mu et al. 2011; Suanon et al. 2016). Tetteh and Rathilal (2021) have investigated the impact of four biomagnetic nanoparticles ( $\text{CuO}$ ,  $\text{Fe}_2\text{O}_3$ ,  $\text{TiO}_2$ , and  $\text{Cu/Fe-TiO}_2$ ) on the treatment

of sugar industrial wastewater by high-rate anaerobic digestion. Better results were achieved by Cu/Fe-TiO<sub>2</sub> nanoparticles as compared to others. Achi et al. (2022) have employed biochar and zeolite for treating the cassava wastewater when it was co-digested with poultry litter and dairy manure. The outcomes from study were in form of a higher COD removal and enhancement of methane potential. The enhancement of acetotrophic and hydrogenotrophic microbes can be achieved by the addition of nanomaterials which leads to a higher rate of AD. However, the use of nanomaterials for the treatment of wastewater with high-rate AD is still under research and needs in-depth study to make the process eco-friendly and cost-effective.

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## 5.4 Summary and Future Perspective

High-rate anaerobic technology for energy recovery from HSWW has gained much attention and has a new pathway from wastewater to bioenergy and irrigation purposes. The concerns related to the degradation of environment and depletion of fossil fuels have driven the search for alternate solutions. In this context, high-rate anaerobic technology can become the solution for these problems. The large amounts of sludge generated from wastewater treatment plants are considered the key source, as organic matter is converted into methane. Traditional anaerobic reactors have been utilized for treating wastewater, but they consume high energy and provide bad-quality effluent. For this, AMR technology with a dynamic module (DM) provides better results such as a higher COD removal rate, longer retention time, and enhanced biogas production. This technology has been also employed with nanomaterials for enhancing the performance of the reactor and mitigating the growth of pathogens (Achi et al. 2022). Because the traditional filters show significant result for remaining the pathogens in the treated water are accumulated on the top layer of the sand filter which can create health hazards. Further research is required to find out some safety procedures to reuse the treated water and its nutrients to grow valuable crops (Lohani et al. 2020). Some of the authors have emphasized implementing the single intermittent sequencing batch reactor or combined sequential assemblies to reduce the organic and inorganic content efficiently (Aziz et al. 2019). Stazi and Tomei (2018) reviewed the work performed on the anaerobic treatment of domestic wastewater (DWW) and recommended to focus the future research activities on evaluating the technological effectiveness of anaerobic domestic wastewater under ambient conditions. Further, the potential of anaerobic (Kaetzl et al. 2018) DWW treatment to be energy-producing and cost-effective and to meet environmental discharge requirements has been demonstrated and should be considered as a priority in the future guidelines for domestic wastewater treatment technologies. Furthermore, ADMR is an advanced technology in which DM is coupled with AMR, and this process is cost-effective and highly efficient than other reactors. However, the ongoing research for this field is not yet fully developed, meaning it is still in the infancy phase. There is a lot of scope for optimizing the process and operational parameters, modelling and controlling the different DM

materials used, and employing the various types of nanomaterials for further advanced technology.

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## References

- Abdelsalam E, Samer M, Attia Y, Abdel-Hadi M, Hassan H, Badr Y (2017) Effects of Co and Ni nanoparticles on biogas and methane production from anaerobic digestion of slurry. *Energy Convers Manag* 141:108–119
- Achi CG, Hassanein A, Lansing S (2022) Enhanced biogas production of cassava wastewater using zeolite and biochar additives and manure co-digestion. *Energies* 13(2):491
- Alibardi L, Bernava N, Cossu R, Spagni A (2016) Anaerobic dynamic membrane bioreactor for wastewater treatment at ambient temperature. *Chem Eng J* 284:130–138
- Aziz A, Basheer F, Sengar A, Khan SU, Farooqi IH (2019) Biological wastewater treatment (anaerobic-aerobic) technologies for safe discharge of treated slaughterhouse and meat processing wastewater. *Sci Total Environ* 686:681–708
- Bella K, Rao PV (2021) Anaerobic digestion of dairy wastewater: effect of different parameters and co-digestion options—a review. *Biomass Convers Biorefin* 2021:1–26
- Cheng D, Ngo HH, Guo W, Liu Y, Chang SW, Nguyen DD et al (2018) Anaerobic membrane bioreactors for antibiotic wastewater treatment: performance and membrane fouling issues. *Bioresour Technol* 267:714–724
- Correia GT, Perez-Perez T, Pereda-Reyes I, Merencio DO, Zaiat M, Kwong WH (2014) Mathematical modeling of the hydrodynamics of an EGSB reactor. *J Chem Chem Eng* 8:602–610
- Cruz-Salomón A, Ríos-Valdovinos E, Pola-Albores F, Lagunas-Rivera S, Meza-Gordillo R, Ruíz-Valdiviezo V et al (2019) Expanded granular sludge bed bioreactor in wastewater treatment. *Glob J Environ Sci Manage* 5(1):119–138
- Ding A, Pronk W, Qu F, Ma J, Li G, Li K et al (2015) Effect of calcium addition on sludge properties and membrane fouling potential of the membrane-coupled expanded granular sludge bed process. *J Membr Sci* 489:55–63
- Dutta A, Davies C, Ikumi DS (2018) Performance of upflow anaerobic sludge blanket (UASB) reactor and other anaerobic reactor configurations for wastewater treatment: a comparative review and critical updates. *J Water Supply Res Technol AQUA* 67(8):858–884
- Dvořák L, Gómez M, Dolina J, Čermín A (2016) Anaerobic membrane bioreactors—a mini review with emphasis on industrial wastewater treatment: applications, limitations and perspectives. *Desalinat Water Treat* 57(41):19062–19076
- Ersahin ME, Tao Y, Ozgun H, Spanjers H, van Lier JB (2016) Characteristics and role of dynamic membrane layer in anaerobic membrane bioreactors. *Biotechnol Bioeng* 113(4):761–771
- Ersahin ME, Tao Y, Ozgun H, Gimenez JB, Spanjers H, van Lier JB (2017) Impact of anaerobic dynamic membrane bioreactor configuration on treatment and filterability performance. *J Membr Sci* 526:387–394
- Fito J, Tefera N, Kloos H, Van Hulle SW (2018) Anaerobic treatment of blended sugar industry and ethanol distillery wastewater through biphasic high rate reactor. *J Environ Sci Health Part A* 53(7):676–685
- Fujihira T, Seo S, Yamaguchi T, Hatamoto M, Tanikawa D (2018) High-rate anaerobic treatment system for solid/lipid-rich wastewater using anaerobic baffled reactor with scum recovery. *Bioresour Technol* 263:145–152
- Goli A, Shamiri A, Khosroyar S, Talaiekhosani A, Sanaye R, Azizi K (2019) A review on different aerobic and anaerobic treatment methods in dairy industry wastewater. *J Environ Treat Technol* 6(1):113

- Gupta VK, Carrott P, Ribeiro Carrott M, Suhas (2009) Low-cost adsorbents: growing approach to wastewater treatment—a review. *Crit Rev Environ Sci Technol* 39(10):783–842
- Harihastuti N, Yuliasni R, Djayanti S, Handayani NI, Rame R, Prasetyo A (2021) Full-scale application of up-flow high-rate anaerobic reactor with substrate modification and effluent recirculation for sugarcane vinasse degradation and biogas generation. *J Ecol Eng* 22(4): 314–324
- Hu Y, Wang XC, Ngo HH, Sun Q, Yang Y (2018a) Anaerobic dynamic membrane bioreactor (AnDMBR) for wastewater treatment: a review. *Bioresour Technol* 247:1107–1118
- Hu Y, Yang Y, Yu S, Wang XC, Tang J (2018b) Psychrophilic anaerobic dynamic membrane bioreactor for domestic wastewater treatment: effects of organic loading and sludge recycling. *Bioresour Technol* 270:62–69
- Isik O, Hudayarizka R, Abdelrahman AM, Ozgun H, Ersahin ME, Demir I (2020) Impact of support material type on performance of dynamic membrane bioreactors treating municipal wastewater. *J Chem Technol Biotechnol* 95(9):2437–2446
- Jensen P, Yap S, Boyle-Gotla A, Janoschka J, Carney C, Pidou M (2015) Anaerobic membrane bioreactors enable high rate treatment of slaughterhouse wastewater. *Biochem Eng J* 97:132–141
- Ji J, Kakade A, Yu Z, Khan A, Liu P, Li X (2020) Anaerobic membrane bioreactors for treatment of emerging contaminants: a review. *J Environ Manag* 270:110913
- Jia R, Sun D, Dang Y, Meier D, Holmes DE, Smith JA (2020) Carbon cloth enhances treatment of high-strength brewery wastewater in anaerobic dynamic membrane bioreactors. *Bioresour Technol* 298:122547
- Jiraprasertwong A, Karnchanapaisal P, Seneesrisakul K, Rangsunvigit P, Chavadej S (2021) High process activity of a two-phase UASB (upflow anaerobic sludge blanket) receiving ethanol wastewater: operational conditions in relation to granulation development. *Biomass Bioenergy* 148:106012
- Johnson I, Ali MAS, Kumar M (2019) Cyanobacteria/microalgae for distillery wastewater treatment—past, present and the future. In: Shah MP (ed) *Microbial wastewater treatment*. Elsevier, Amsterdam, pp 195–236. <https://doi.org/10.1016/B978-0-12-816809-7.00010-5>
- Kaetzl K, Lübken M, Gehring T, Wichern M (2018) Efficient low-cost anaerobic treatment of wastewater using biochar and woodchip filters. *Watermark* 10(7):818
- Kim J, Kim K, Ye H, Lee E, Shin C, McCarty PL et al (2011) Anaerobic fluidized bed membrane bioreactor for wastewater treatment. *Environ Sci Technol* 45(2):576–581
- Lei Z, Yang S, Li Y-y, Wen W, Wang XC, Chen R (2018) Application of anaerobic membrane bioreactors to municipal wastewater treatment at ambient temperature: a review of achievements, challenges, and perspectives. *Bioresour Technol* 267:756–768
- Li C, Wang R, Yang X, Zhou M, Pan X, Cai G et al (2021) Deeper investigation on methane generation from synthetic wastewater containing oxytetracycline in a scale up acidic anaerobic baffled reactor. *Bioresour Technol* 333:125156
- Liao B-Q, Kraemer JT, Bagley DM (2006) Anaerobic membrane bioreactors: applications and research directions. *Crit Rev Environ Sci Technol* 36(6):489–530
- Lier JV, Van der Zee F, Frijters C, Ersahin M (2015) Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. *Rev Environ Sci Technol* 14(4):681–702
- Loderer C, Wörle A, Fuchs W (2012) Influence of different mesh filter module configurations on effluent quality and long-term filtration performance. *Environ Sci Technol* 46(7):3844–3850
- Lohani SP, Khanal SN, Bakke R (2020) A simple anaerobic and filtration combined system for domestic wastewater treatment. *Water Energy Nexus* 3:41–45
- Márquez RA, Maldonado MJ, Guevara PE, Rey LD, Pérez PS (2021) An approach to models for the design of upflow anaerobic filters. *J Appl Water Eng Res* 9(2):107–132
- Martins G, Salvador AF, Pereira L, Alves MM (2018) Methane production and conductive materials: a critical review. *Environ Sci Technol* 52(18):10241–10253

- McAteer PG, Trego AC, Thorn C, Mahony T, Abram F, O'Flaherty V (2020) Reactor configuration influences microbial community structure during high-rate, low-temperature anaerobic treatment of dairy wastewater. *Bioresour Technol* 307:123221
- Meng F, Zhang S, Oh Y, Zhou Z, Shin H-S, Chae S-R (2017) Fouling in membrane bioreactors: an updated review. *Water Res* 114:151–180
- Mu H, Chen Y, Xiao N (2011) Effects of metal oxide nanoparticles (TiO<sub>2</sub>, Al<sub>2</sub>O<sub>3</sub>, SiO<sub>2</sub> and ZnO) on waste activated sludge anaerobic digestion. *Bioresour Technol* 102(22):10305–10311
- Njoya M, Basitere M, Ntwampe SKO, Lim JW (2021) Performance evaluation and kinetic modeling of down-flow high-rate anaerobic bioreactors for poultry slaughterhouse wastewater treatment. *Environ Sci Pollut Res* 28(8):9529–9541
- Oliveira Cruz LM, Gomes BGLA, Tonetti AL, Figueiredo ICS (2019) Using coconut husks in a full-scale decentralized wastewater treatment system: the influence of an anaerobic filter on maintenance and operational conditions of a sand filter. *Ecol Eng* 127:454–459
- Ozgun H, Dereli RK, Ersahin ME, Kinaci C, Spanjers H, van Lier JB (2013) A review of anaerobic membrane bioreactors for municipal wastewater treatment: integration options, limitations and expectations. *Sep Purif Technol* 118:89–104
- Pal P (2017) Industry-specific water treatment: case studies. In: *Industrial water treatment process technology*. Elsevier, Amsterdam, pp 243–511
- Park J-H, Kang H-J, Park K-H, Park H-D (2018) Direct interspecies electron transfer via conductive materials: a perspective for anaerobic digestion applications. *Bioresour Technol* 254:300–311
- Putra AA, Watari T, Maki S, Hatamoto M, Yamaguchi T (2020) Anaerobic baffled reactor to treat fishmeal wastewater with high organic content. *Environ Technol Innov* 17:100586
- Quek PJ, Yeap TS, Ng HY (2017) Applicability of upflow anaerobic sludge blanket and dynamic membrane-coupled process for the treatment of municipal wastewater. *Appl Microbiol Biotechnol* 101(16):6531–6540
- Reynaud N, Buckley C (2016) The anaerobic baffled reactor (ABR) treating communal wastewater under mesophilic conditions: a review. *Water Sci Technol* 73(3):463–478
- Richards S, Paterson E, Withers PJ, Stutter M (2016) Septic tank discharges as multi-pollutant hotspots in catchments. *Sci Total Environ* 542:854–863
- Robles Á, Durán F, Giménez JB, Jiménez E, Ribes J, Serralta J et al (2020) Anaerobic membrane bioreactors (AnMBR) treating urban wastewater in mild climates. *Bioresour Technol* 314:123763
- Sahinkaya E, Yurtsever A, Toker Y, Elcik H, Cakmaci M, Kaksonen AH (2015) Biotreatment of As-containing simulated acid mine drainage using laboratory scale sulfate reducing upflow anaerobic sludge blanket reactor. *Min Eng* 75:133–139
- Schneider C, Oñoro AE, Hélix-Nielsen C, Fotidis IA (2021) Forward-osmosis anaerobic-membrane bioreactors for brewery wastewater remediation. *Sep Purif Technol* 257:117786
- Shekhawat SS, Kulshreshtha NM, Gupta AB (2020) Investigation of chlorine tolerance profile of dominant gram negative bacteria recovered from secondary treated wastewater in Jaipur, India. *J Environ Manag* 255:109827
- Shende AD, Pophali GR (2021) Anaerobic treatment of slaughterhouse wastewater: a review. *Environ Sci Pollut Res* 28(1):35–55
- Shoener BD, Zhong C, Greiner AD, Khunjar WO, Hong P-Y, Guest JS (2016) Design of anaerobic membrane bioreactors for the valorization of dilute organic carbon waste streams. *Energy Environ Sci* 9(3):1102–1112
- Sierra JDM, Oosterkamp MJ, Wang W, Spanjers H, van Lier JB (2019) Comparative performance of upflow anaerobic sludge blanket reactor and anaerobic membrane bioreactor treating phenolic wastewater: overcoming high salinity. *Chem Eng J* 366:480–490
- Skouteris G, Hermosilla D, López P, Negro C, Blanco Á (2012) Anaerobic membrane bioreactors for wastewater treatment: a review. *Chem Eng J* 198:138–148
- Smith AL, Stadler LB, Cao L, Love NG, Raskin L, Skerlos SJ (2014) Navigating wastewater energy recovery strategies: a life cycle comparison of anaerobic membrane bioreactor and conventional treatment systems with anaerobic digestion. *Environ Sci Technol* 48(10):5972–5981

- Soares JL, Cammarota MC, Gutarra MLE, Volschan I (2019) Reduction of scum accumulation through the addition of low-cost enzymatic extract in the feeding of high-rate anaerobic reactor. *Water Sci Technol* 80(1):67–74
- Sohn W, Guo W, Ngo HH, Deng L, Cheng D, Zhang X (2021) A review on membrane fouling control in anaerobic membrane bioreactors by adding performance enhancers. *Journal Water Process Eng* 40:101867
- Stazi V, Tomei MC (2018) Enhancing anaerobic treatment of domestic wastewater: state of the art, innovative technologies and future perspectives. *Sci Total Environ* 635:78–91
- Suanon F, Sun Q, Mama D, Li J, Dimon B, Yu C-P (2016) Effect of nanoscale zero-valent iron and magnetite ( $\text{Fe}_3\text{O}_4$ ) on the fate of metals during anaerobic digestion of sludge. *Water Res* 88: 897–903
- Tang J, Wang XC, Hu Y, Ngo HH, Li Y (2017) Dynamic membrane-assisted fermentation of food wastes for enhancing lactic acid production. *Bioresour Technol* 234:40–47
- Tauseef S, Abbasi T, Abbasi S (2013) Energy recovery from wastewaters with high-rate anaerobic digesters. *Renew Sust Energy Rev* 19:704–741
- Tetteh EK, Rathilal S (2021) Application of biomagnetic nanoparticles for biostimulation of biogas production from wastewater treatment. *Mater Today Proc* 45:5214–5220
- Tonon D, Tonetti AL, Coraucci Filho B, Bueno DAC (2015) Wastewater treatment by anaerobic filter and sand filter: hydraulic loading rates for removing organic matter, phosphorus, pathogens and nitrogen in tropical countries. *Ecol Eng* 82:583–589
- Ülgüdür N, Ergüder TH, Uludağ-Demirer S, Demirer GN (2019) High-rate anaerobic treatment of digestate using fixed film reactors. *Environ Pollut* 252:1622–1632
- Vinardell S, Astals S, Peces M, Cardete M, Fernández I, Mata-Alvarez J et al (2020) Advances in anaerobic membrane bioreactor technology for municipal wastewater treatment: a 2020 updated review. *Renew Sust Energy Rev* 130:109936
- Visvanathan C, Abeynayaka A (2012) Developments and future potentials of anaerobic membrane bioreactors (AnMBRs). *Membr Water Treat* 3(1):1–23
- Withers PJ, Jordan P, May L, Jarvie HP, Deal NE (2014) Do septic tank systems pose a hidden threat to water quality? *Front Ecol Environ* 12(2):123–130
- Xu H, Wang K, Zhang X, Gong H, Xia Y, Holmes DE (2020) Application of in-situ  $\text{H}_2$ -assisted biogas upgrading in high-rate anaerobic wastewater treatment. *Bioresour Technol* 299:122598
- Yu H, Wang Z, Wu Z, Zhu C (2015) Dynamic membrane formation in anaerobic dynamic membrane bioreactors: role of extracellular polymeric substances. *PLoS One* 10(10):e0139703
- Yurtsever A, Basaran E, Ucar D (2020) Process optimization and filtration performance of an anaerobic dynamic membrane bioreactor treating textile wastewaters. *J Environ Manag* 273: 111114
- Zha X, Ma J, Tsapekos P, Lu X (2019) Evaluation of an anaerobic baffled reactor for pre-treating black water: potential application in rural China. *J Environ Manag* 251:109599
- Zhang M, Ma Y, Ji D, Li X, Zhang J, Zang L (2019) Synergetic promotion of direct interspecies electron transfer for syntrophic metabolism of propionate and butyrate with graphite felt in anaerobic digestion. *Bioresour Technol* 287:121373
- Zhao K, Wu Y, Young S, Chen X (2020) Biological treatment of dairy wastewater: a mini review. *J Environ Inform Lett* 4:22–31



# Microbial Community Dynamics in Anaerobic Digester Treating Human Waste: A Review

# 6

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## Abstract

Microbial communities (bacteria and archaea) play the most important part in the production of biogas in anaerobic digesters. A comprehensive understanding of microbial diversity, composition, abundance, interactions and their behaviour is required to yield biogas optimally. Their active genes, metabolic products and proteins help to speed up the anaerobic digestion. High-throughput sequencing and appropriate bioinformatics analysis can easily assess the diversity and quantity of microbial communities, which is vital for the overall process. High-throughput sequencing provides detailed information on microbial diversity and resilience of anaerobic digester system. Sequencing tools like next-generation sequencing and 16S rRNA amplicon sequencing help in understanding the underlying causes of anaerobic digestion through exploring the microbial population in biogas reactors and interaction among microbiomes and process parameters. Anaerobic digestion of human waste has gained popularity due to its ability to transform organic waste into biogas. In the current chapter, the microbial community in anaerobic digesters and recent developments in biotechniques for assessing microbial diversity have been reviewed.

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**Keywords**

Anaerobic digestion · Microbial diversity · Biotechniques · Human waste · Biogas

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## 6.1 Introduction

Understanding the microbial community dynamics is crucial to promote microbial interactions and to enable the metabolic co-dependencies. Microbial communities' physiology and ecology are influenced by temporal dynamics and spatial structure of its members. Microbial interactions can be facilitated by spatial structure, allowing metabolic co-dependencies to increase community resilience and homeostasis (Mark Welch et al. 2016; Ronda and Wang 2022). Microbial communities are sensitive to temporal dynamics, with changes in metabolism, community composition and function leading to phenotypically complex community trajectories. Understanding about the governing spatio-temporal principles within a microbiome is critical for its physiology.

Intrinsic and extrinsic variables influence the temporal dynamics of microbial communities (Ryo et al. 2019). Individual species metabolism and colonisation capacity and intra- and inter-species interactions are intrinsic determinants, while periodic alterations in ambient environments such as pH and nutritional availability are extrinsic impacts. Microbial communities have piqued researchers' interest because they decompose organic matter using carbon and nitrogen as energy sources, as well as oxygen, and produce CO<sub>2</sub> and soil-enriching compost. The resulting compost contains a high percentage of biologically stable humic compounds, making it an excellent soil addition (Białobrzewski et al. 2015).

Traditional systems such as aerobic and anaerobic digestion (AD) provide organic waste management and sustainable energy production (Choi et al. 2021). AD is commonly used in the treatment of organic waste, and it is gaining attention as biogas produced by AD is widely employed as a sustainable energy source. The AD process is used to treat a variety of organic wastes (liquid and solid), and it is increasingly being utilised to treat human waste.

In anaerobic digestion environments, microbes from a variety of taxonomic groups play an essential role in interactions that occur during biomass breakdown and methanogenesis (Li et al. 2017). A wide range of environmental conditions may have an impact on microbes (Table 6.1), viz. pH, alkalinity, organic matter, ammonia concentration and volatile fatty acids (VFA); also the variances in physiology, nutrition-dependent growth kinetics and sensitivities to environmental conditions affect the microbial population (Kovacs et al. 2015; Li et al. 2017).

Understanding the ecology of anaerobic digesters and how it relates to system's function necessitates the identification of active and numerous microorganisms, connecting their identities to their functional responsibilities. Several 16S rRNA gene amplicon analyses have showed that in comparably operating anaerobic digesters, there appears to be a group of abundant microorganisms that are stable throughout time (Venkiteshwaran et al. 2015; Werner et al. 2011). Other biological

**Table 6.1** Environmental factors' influence on anaerobic digestion process

S. no.	Factors	Effects	Optimal range	Influence on AD process
1	pH, alkalinity	Biochemical metabolism	Acid-forming bacteria: pH 5 Methanogenic bacteria: pH 7	pH ranges distinguish the hydrolytic, acidogenic and methanogenic stages
2	Temperature	Microbial density and diversity	Mesophilic: 30–35 Thermophilic: 50–60	Influence acetoclastic and hydrogenotrophic methanogenesis processes
3	Ammonia	Methanogen community structure	AD—function up to 1000 mg TAN/L	Higher concentration inhibits the methanogenic activity
4	Hydraulic retention time	Process efficiency	Short/long	Short retention time favours hydrolytic-acidogenic phase. Long retention time aids methanogenic degradation
5	Organic loading rate	Microbial community structure	High/low	High OLR increases VFA production/accumulation. Organic shock loading condition favours hydrogenotrophs
6	Nutrients	Enzymatic activity	Macronutrients: Ca, K, Mg, Na, P and S Micronutrients: B, Co, Cu, Fe, Mn, Mo and Zn	Enrich the archaea community, faster VFA degradation, improved process stability

systems, such as wastewater treatment plants and the human digestive system, have also been linked to this activity (Saunders et al. 2015). However, in anaerobic digesters, a significant portion of the visible microbial population may come from dead or inactive cells that arrive with the influent biomass and retain DNA. As a result, reported microbial population dynamics are unlikely to accurately reflect changes in process performance or stability. This can lead to erroneous inferences and relationships (Fodor et al. 2012).

Molecular approaches have been developed to address this issue, but the complex matrix of anaerobic digester sludge samples will likely result in undesirable chemical reactions. As a result, monitoring the microbial composition of the influent to detect the abundant organisms sustained by immigration could be a viable alternative (Lee et al. 2015; Seib et al. 2016). Various microbial communities involved in the anaerobic digestion process for treating different organic waste with a special focus on human waste are reviewed here in this chapter.

## 6.2 Microbial Communities in Anaerobic Digestion Process

The microbiological processes of AD can be conceptualised as hydrolysis, acidogenesis, acetogenesis and methanogenesis. These four processes are carried out via guild of microbes, and it is necessary to uphold a balanced reaction rate for stable digestion. Table 6.2 depicts the microbial communities observed during the four phases of the anaerobic digestion process.

The impact of microbe's structure on digester operation and stability has received little attention. Researchers recently began to apply data on the community structure of microbes to better understand or forecast how it affects digester performance (Venkiteshwaran et al. 2015). Microbial diversity has been proven to play a crucial influence in natural and engineered ecosystem performance, as measured by species richness and relative abundance of species. It's a type of functional insurance that allows an ecosystem's richness and evenness to be maintained through compensating growth (Fernandez et al. 2000). System's perturbation may change in the population of one species within a functional group, i.e. one species decreased or

**Table 6.2** Microbial community in anaerobic digestion process

S. no.	Anaerobic digestion process	Major microbial community	References
1	Hydrolysis	<i>Firmicutes</i> and <i>Bacteroidetes</i> <i>Acetivibrio</i> <i>Clostridium</i> <i>Bacteroides</i> <i>Thermotoga</i> (phylum <i>Thermotogae</i> )	Amekan (2020) De Vrieze et al. (2015) Hassa et al. (2018) Venkiteshwaran et al. (2015)
2	Acidogenesis	<i>Bacteroidetes</i> <i>Chloroflexi</i> <i>Firmicutes</i> <i>Proteobacteria</i>	Stiles and Holzapfel (1997) Balk et al. (2002) Dong et al. (2000)
3	Acetogenesis	<i>Smithllea</i> <i>Syntrophobacter</i> <i>Pelotomaculum</i> <i>Syntrophus</i> <i>Syntrophomonas</i>	Liu et al. (1999) de Bok et al. (2001) Imachi et al. (2007) Sousa et al. (2007)
4	Methanogenesis	<i>Methanobacterium</i> <i>Methanobrevibacter</i> <i>Methanoculleus</i> <i>Methanospirillum</i> <i>Methanothermobacter</i> <i>Methanosaeta</i> <i>Methanosarcina</i>	Amekan (2020) Hori et al. (2006) Leclerc et al. (2004) Savant et al. (2002) Cuzin et al. (2001)

eliminated; a different species belonging to the same functional group and more resistant to the perturbation may quickly take its place if it was there in sufficient numbers at the outset (Fernandez et al. 2000; Briones and Raskin 2003; Wittebolle et al. 2009; Werner et al. 2011).

In the operational phase, the AD process is used to treat municipal and industrial wastes based on their solid content. Han et al. (2017) examined AD's (full-scale) operating under wet condition (total solids  $\leq 10\%$ ) and semi-dry condition (total solids  $\leq 20\%$ ). In wet systems, *Methanobacteriaceae*, *Porphyromonadaceae*, *Sphingobacteriaceae* and *Syntrophomonadaceae* were the dominant bacterial and archaeal groups. In semi-dry digester, *Clostridiaceae*, *Lachnospiraceae*, *Methanomicrobiaceae*, *Patulibacteraceae*, *Pseudonocardiaceae* and *Rikenellaceae* species were predominant.

In single and two-stage thermophilic digesters, the effects of vegetable and fruit waste and swine manure co-digestion on microbial structure were compared by Merlino et al. (2013). The single-stage process produced highly diverse microbial population (archaea (Methanosarcinales); bacteria (*Bacilli*, *Clostridia* and *Firmicutes*)) than the two-stage method, which was linked to the increased substrate degradation and, as a result, better process performance.

Anaerobic co-digestion significantly balances the C/N ratio, maintains buffering of medium through pH/alkalinity equilibrium, supplements micro- and macronutrients, attenuates inhibitors or any toxic composites and enhances biodegradability of organic matter (Hartmann et al. 2002). Digestion of a wide range of feedstocks improves not only biogas production and process stability but also the diversity and dynamic range of microbial populations (Cuetos et al. 2008). C/N balance in anaerobic co-digestion has been shown to alter bacterial and archaeal association in previous investigations. Under varying operating circumstances, *Firmicutes* and *Chloroflexi* were found to be capable of degrading a wide range of organics (Tyagi et al. 2021). Both groups were found in abundance in a wide spectrum of anaerobic co-digesters and exhibited resistance to heavy organic loading (Rong et al. 2018).

Kirkegaard et al. (2017) used 16S rRNA gene sequencing to investigate the microbiota in anaerobic digesters (full-scale) processing suspended particles. In mesophilic, mesophilic plus thermal hydrolysis and thermophilic digesters, diverse microbial communities were discovered (Kirkegaard et al. 2017). *Candidatus Methanofastidiosa* (WCHA1-57) belonging to archaea is the dominant in mesophilic digesters. Acetoclastic methanogens, viz. *Methanothermobacter*, *Methanosarcina* and *Methanobrevibacter*, were dominant in thermophilic digestion. Abundance population of *Methanosaeta* and *Methanoculleus* were seen in mesophilic digestion combined with thermal hydrolysis process. *Methanoculleus* might be present in AD due to increased levels of ammonia in the system.

Microbial populations in AD, viz. mono-digesters, mesophilic co-digesters and thermophilic co-digesters, were examined by Sundberg et al. (2013). Two major elements that determine the organisation of microbial populations in digesters are the operational temperature and feedstock content. *Actinobacteria*, *Chloroflexi*, *Euryarchaeota*, *Proteobacteria* and *Spirochetes* remained dominant in mono-

digesters, whereas *Firmicutes* dominated in co-digesters. The development of *Thermotogae* species existed in the thermophilic digesters. The makeup of the microbial population in a digester is substantially influenced by operational parameters and substrate type. A healthy and diverse microbial population that can endure process perturbations is aided by a good nutritional balance. The link between the functional microbial populations and process parameters can be exploited to generate tools for designing and operating and in controlling the process of AD (Tyagi et al. 2021; Supaphol et al. 2011).

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### 6.3 Microbial Diversity: Biotechniques

The spectrum of microorganisms and their proportional abundance in a given community is referred to as microbial diversity. Microbial diversity is significant because it affects the resilience of processes (Torsvik et al. 1998; Mirmohamadsadeghi et al. 2021). It can provide detailed information about biological diversity in (1) genetic variation within the species, (2) the number and distribution of different species and (3) community diversity. The classification of unknown bacteria, on the other hand, can be the most difficult aspect of determining microbial diversity (Fakruddin and Mannan 2013).

The variance in the molecular features, i.e. nucleic acid homology, can be used to determine biodiversity. The community's stability is linked to the system's stability, and stress in the AD system can result in unstable system and fluctuation in species diversity (Yannarell and Triplett 2005). As a result, diversity analysis is appealing since it allows for a deeper understanding of (1) organisms' genetics and distribution in a community, (2) diversity and functional role, (3) species type and (4) specified amount of individual species in the system (Fakruddin and Mannan 2013).

Recently emerging molecular and chemical ecology approaches have opened up new possibilities for studying microbial diversity (Giovannoni et al. 1990; Akyol et al. 2019). These techniques can be used to look into the diversity and structure of microbial communities. Polymerase chain reaction amplification, a common molecular biology technique, allows specific DNA sequences to be amplified and used to assess the makeup of microbial communities. Many microbial systems use the rRNA genes (i.e. 16S rRNA) to investigate biodiversity and microbial composition (Vanwonderghem et al. 2014).

Assessment of microbial community in ADs has been successful using conventional molecular fingerprinting approaches or first-generation sequencing techniques. These procedures, however, are time-consuming and result in a low community resolution (Leclerc et al. 2004). High-throughput techniques, often known as next-generation sequencing (NGS), are newly developed sequencing technologies that can sequence numerous DNA molecules simultaneously at low cost, in a short amount of time and with high resolution (Churko et al. 2013). These characteristics result in the creation of enormous data sets, which can help with correlation analysis statistically (Vanwonderghem et al. 2014).

Anaerobic digester's microbial communities can be studied using metagenomic and bioinformatics techniques. Next-generation sequencing-based metagenomics is a fast emerging study that aids in the knowledge of the diversity and functional complexity of biological systems such as the human body, animals, soil, ocean and anaerobic habitats. In anaerobic digester, a metagenomic technique can reveal the progress of a digester, i.e. ability to progress from the first phase to an acidic state in which volatile fatty acids build and come back to normal operation (Jünemann et al. 2017; Pore et al. 2016; Lei et al. 2019). The primary goal of metagenomic approaches, particularly in less complicated environments, is to reconstruct substantial portions of genomes from species found in the microbial community (Mirmohamadsadeghi et al. 2021).

Gene-centric metagenomics has demonstrated to be more effective in complex environments like anaerobic digester by delivering a snapshot of gene frequency (Fontana et al. 2018). Metagenomic approaches have shown a large quantity of gene reads, the majority of which have yet to be identified, limiting the functional information derived from these reads. Regardless, metagenomics has shed light on the evolutionary connections among diverse species as well as the microbial community's metabolic functionality in AD (Vanwonterghem et al. 2014). The microbial diversity and their function can be considerably affected by different feedstocks, pretreatment of substrate and operational conditions (Duan et al. 2021).

The functional redundancy can be estimated using an approach that combines metagenomics with AD performance data. Furthermore, by maintaining the amount of metabolic diversity, it is feasible to achieve a steady operational situation (Mirmohamadsadeghi et al. 2021). Future amplicon sequencing methods with a higher resolution and longer read length, as well as enhanced algorithms and genome binning procedures, may usher in future improvements in metagenomics (Muller et al. 2013; Albertsen et al. 2013). In the future, metagenomics paired with meta-omic approaches such as meta-proteomes and meta-transcriptomes will aid in the creation of genomic database for anaerobic digester and also offer information on various functional groups and interactions among them (Vanwonterghem et al. 2014). Metagenomic and bioinformatics methodologies include the following series of steps (Rudakiya and Narra 2021; Zhang et al. 2019; Sun et al. 2016).

1. Sample collection from different AD processes that are feed stock dependent.
2. Bioinformatics study of metagenomic data related to microbial populations requires DNA extraction.
3. Following that, polymerase chain reaction (PCR) is performed via 16S rRNA or particular primers.
4. Products from PCR can be cloned to appropriate vectors, and vector library is created through vector cloning techniques.
5. Roche GS FLX454 pyrosequencing platform forms the basis for DNA sequencing.
6. Following the capture of metagenomic data, raw next-generation sequencing reads are obtained.

7. Raw sequence pretreatment is a crucial step in obtaining high-quality readings for downstream processing (Tools: Trimmomatic software, ACE Pyrotag Pipeline, HMMER, MG-RAST, ChimeraSlayer (Campanaro et al. 2016; Ho et al. 2014; Azizi et al. 2016; Wirth et al. 2012; Martinez et al. 2014))
8. Eliminating adapters and linkers, without chimaeras and replication, de-multiplexing barcoded samples and quality control are all part of the sequence pretreatment.  
Sequences are allied through MOTHUR, INFERNAL aligner and ClustalW (Martinez et al. 2014; Cardinali-Rezende et al. 2016; Zhang et al. 2019).
9. Consequently, aligned sequences are grouped into operational taxonomic units (OTUs) using average neighbouring clustering algorithm (Tools: Usearch software, sequence classifiers – RDP Bayesian Classifier, UCLUST-RDP classifier and MEGA/MEGA5 (Cardinali-Rezende et al. 2016; Pope et al. 2013; Rudakiya et al. 2019)).
10. Investigation of biological diversity of microbial communities (Tools: MOTHUR package, R software package having VEGAN library and RDP Pipeline (Zhang et al. 2019; Oksanen et al. 2007; Cardinali-Rezende et al. 2016)).
11. Taxonomic composition analysis is a bioinformatics investigation used for the anaerobic microbial populations and performed via (1) filtering and comparing databases and (2) taxonomic groups of sequences.

Metagenomic techniques, though not optimal for online use due to long processing times and expensive costs, offer a wealth of information about the microbial phylogeny in anaerobic digester systems. However, a biomarker database must be built before these strategies can be fully realised in AD (Hashemi et al. 2021).

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## 6.4 Human Waste Anaerobic Digestion: Microbial Dynamics

Anaerobic digestion is a wastewater treatment method that converts organic matter into biomethane (Lettinga et al. 2001). AD is commonly used to treat a wide range of faecal wastes since it can be a cost-effective solution to lessen the environmental impact of faeces storage while simultaneously releasing methane. Human waste has been found to be a worthy substrate for generating biogas in many investigations, with equal performance in laboratory conditions (Duan et al. 2020; Lalander et al. 2018; Colon et al. 2015; Zhang and Angelidaki 2015). Human waste is high in organic matter and nutrients, making it a sustainable feedstock for a variety of applications (Singh et al. 2017) to yield biofuels, viz. methane, bioethanol and biodiesel, by pyrolysis, AD, hydrothermal liquefaction etc. (Gomaa and Abed 2017).

The ability of a large number of microbes capable of degrading complex organic polymers to work together is critical to the success of the AD process (Bedoya et al. 2020). In anaerobic digester, the microbial community gets influenced by temperature, organic load, amount of toxins, sludge retention duration, influent's

composition, topographical location and annual seasons. However, little is known about the diversity and functional features of microbes in anaerobic digesters (Hao et al. 2016; Bedoya et al. 2020). A greater understanding of the dynamics and ecology of microbes in these systems can help predict their performance better and also throw the limelight on the desirable microbial structure for improved organic matter decomposition, biogas generation and pathogen control (Hao et al. 2016; De Francisci et al. 2015).

In anaerobic reactor, almost 90% of microbial population is represented by bacteria and archaea (Bedoya et al. 2020; Guo et al. 2015). Bacteria play a role in the early stages of AD, such as pathogen race, whereas archaea are in charge of the final step, which creates methane, a useful renewable energy source (Ariesyady et al. 2007). Samples from soil, ocean, human gut and sewage sludge have all been effectively used to describe phylogenetic compositions and functional potentiality of complex microbial populations using high-throughput sequencing technologies (Li et al. 2018; Nascimento et al. 2018). Bacterial communities were studied commonly in wastewater treatment plants, by sequencing of 16S rRNA gene amplicon libraries (metataxonomic method) (Iwai et al. 2016). Despite the fact that a large number of research have already focused on human waste AD, little is known about the process's stability and inhibitor variables during human waste AD treatment. Sequencing from 16S rRNA has been developed for functional inference (Duan et al. 2020; Iwai et al. 2016).

Sequencing tools like next-generation and 16S rRNA amplicon sequencing might help researchers in understanding the fundamental causes of AD by exploring the microbial populations in biogas reactors and the interaction among microbiomes and process parameters. In a recent batch experiment, researchers compared the microbial population composition of former and latter anaerobic digestion process of human faeces and showed that *Methanomicrobia* and *Cloacimonetes* were the most abundant archaea and bacteria, respectively (Gomaa and Abed 2017).

Aeration, nitrification and denitrification technologies have been designed to reduce COD and eliminate nitrogen from wastewaters in existing wastewater treatment plants (Khoshnevisan et al. 2018; Shirzad et al. 2019). Life cycle assessment (LCA) is a well-established instrument for assessing a variety of environmental effects over the course of a product or process's lifespan (Khoshnevisan et al. 2020). This method can be utilised to evaluate the entire environmental effects of anaerobic digestion of human waste, as it eliminates issues that arise, that is, the formation of intermediate elements. A life cycle energy and environmental assessment method was employed by Chen et al. (2012) to investigate the performance of the biogas-digestive system in China. Arafat et al. (2015) investigated the treatment technologies of municipal solid waste having energy recovery potential and its environmental impacts. Gao et al. (2017) compared present human excreta sanitation machinery to comprehensive Chinese rural toilet designs, which included rainwater harvesting flushing systems, standard flushing, urine segregation and composting schemes, using LCA. However, the environmental benefits of a well-designed human waste anaerobic digestion system have yet to be explored, and their evaluation is urgently required.



Duan et al. (2020) studied the AD of human waste at higher influent feedstock concentrations, ideal conditions, inhibitory variables and changes in microbial population in biogas reactors fed continuously. *Methanosaeta* and WSA2 were the dominant archaeal species among microbial populations during stable period. Microbial groups (WWE1 and WSA2) that were uncharacterised were observed, and the possible syntrophic interaction among the two groups would be critical in producing a high-performing process (Duan et al. 2020).

Up-flow anaerobic sludge blanket reactors to treat orthodox toilet and vacuum toilet black water with loading increments were effectively operated by Gao et al. (2019). The archaeal and bacterial populations clearly diverged between the conventional and vacuum toilet reactors, indicating that archaeal community evolved at a slower rate compared to bacterial community. Archaea members were hydrogenotrophic methanogens: *Methanolinea* in the conventional toilet reactor accounted for 56.6% and *Methanogenium* in the vacuum toilet reactor for 62.3%. Bacterial members were *Porphyromonadaceae* in both conventional (15.9%) and vacuum (13.4%) toilet reactors, sulphate-reducing bacteria in conventional and *Fibrobacteraceae* in vacuum toilet reactor (Gao et al. 2019).

Mesophilic AD (full scale) to treat sewage sludge and food wastewater was examined to investigate microbial communities and the effects of total ammonia nitrogen concentration and sodium ion concentration on changes in these communities (Lee et al. 2018). The addition of food waste and sewage sludge formed very distinct microbial community structures; and the variation among these two digesters was mainly influenced by total ammonia nitrogen and sodium ions. The bacterial populations of sewage sludge digesters are greatly influenced by microorganisms from influent sludge. *Methanoculleus* may be tolerant to high ammonia levels in AD.

High-solids AD, a promising approach having a smaller reactor and reduced heating energy consumption, has shown poorer digesting efficiency and increased tolerance to certain inhibitors in some cases. Archaeal and bacterial populations in anaerobic digesters handling sewage sludge having 10–19% of total solids were studied to learn more about the phenomenon (Liu et al. 2016). Genus *Methanosarcina* drove the acetoclastic methanogenesis in producing methane, and their total ratio decreased with increased total solids, which are contrary to the relative abundance of hydrogenotrophic methanogens. Microbial communities of different waste treatment in anaerobic digestion process are shown in Table 6.3. Understanding the prevalent microbial population is critical for improving biogas production and, as a result, the overall process efficacy. However, research on bacterial populations and abundance is relatively restricted. Precise databases for bacterial identification and sequencing methodologies should be developed. Validation of sequencing data is essential, and the isolation and screening of genes and proteins with potential industrial applications should be investigated.

**Table 6.3** Microbial communities of different wastes in anaerobic digester

S. no.	Type of waste	Microbial dynamics	Reference
1	Toilet flushed black water	<i>Methanospirillaceae Methanoculleus Methanospirillum Methanogenium Porphyromonadaceae Fibrobacteraceae Ruminococcaceae Bacteroidaceae Clostridiales</i>	Gao et al. (2019)
2	Food waste and animal waste	<i>Methanobacterium beijingense Methanobacterium petrolearium Methanoculleus bourgensis Methanoculleus receptaculi</i>	Koo et al. (2017)
3	Food wastewater or sewage sludge	<i>Methanoculleus Methanobacterium Methanomassiliicoccus Methanomethylophilaceae Candidatus methanoplasma Methanosarcina Methanimicrococcus</i>	Lee et al. (2018)
4	Food waste-recycling wastewater	<i>Fastidiosipila Petrimonas vadin BC27 Syntrophomonas Proteiniphilum</i>	Kim et al. (2018)
5	Rice straw	<i>Enterobacteriaceae Clostridiaceae Prevotellaceae Peptostreptococcaceae</i>	Wachemo et al. (2019)
6	Human waste	<i>Methanosaeta</i> and WSA2	Duan et al. (2020)
7	Raw food wastewater	<i>Methanomicrobiales Methanosarcinales Methanobacteriales</i>	Kim et al. (2014)
8	Dairy manure	<i>Methanobacterium Methanoculleus</i>	Lv et al. (2013)

## 6.5 Conclusion

Human waste poses a threat to the environment and public health, making its long-term management a severe concern. Anaerobic digestion (AD) has long been promoted as a waste management process that is both environmentally beneficial and sustainable, producing biomethane as a by-product. AD covers a wide range of communities with a high level of functional interdependence among individual or group of organisms. Combination of meta-omics, virtualisation techniques and chemical analysis could be a potent device for extracting very important information from anaerobic digester. It is critical to recognise distinct species, understand their roles during the process, separate their functions and establish a stable AD process. Microbial populations of AD can be analysed via metagenomic and bioinformatics methodologies. Next-generation sequencing-based metagenomics is a fast emerging study that aids in the knowledge of the diversity and functional complexity of biological systems

Human waste is rich in organic matter and nutrients, making it a sustainable feedstock to yield biofuels like methane, bioethanol and biodiesel. The performance of AD process greatly depends on the synergic interactions of numerous microorganisms capable of degrading complex organic polymers. Understanding

microbial dynamics and their ecology allied with the systems may forecast their performance better and also throw the limelight on the desirable microbial population structure for better organic matter degradation, biogas production and pathogen reduction.

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## References

- Akyol C, Ince O, Ince B (2019) Crop-based composting of lignocellulosic digestates: focus on bacterial and fungal diversity. *Bioresour Technol* 288:121–549
- Albertsen M, Hugenholtz P, Skarshewski A, Nielsen KL, Tyson GW, Nielsen PH (2013) Genome sequences of rare, uncultured bacteria obtained by differential coverage binning of multiple metagenomes. *Nat Biotechnol* 31:533–538
- Amekan Y (2020) The influence of microbial community dynamics on anaerobic digestion efficiency and stability: a review. *Int J Renew Energy Dev* 9(1):85–95
- Arafat HA, Jijakli K, Ahsan A (2015) Environmental performance and energy recovery potential of five processes for municipal solid waste treatment. *J Clean Prod* 105:233–240
- Ariesyady HD, Ito T, Okabe S (2007) Functional bacterial and archaeal community structures of major trophic groups in a full-scale anaerobic sludge digester. *Water Res* 41:1554–1568
- Azizi A, Kim W, Lee JH (2016) Comparison of microbial communities during the anaerobic digestion of *Gracilaria* under mesophilic and thermophilic conditions. *World J Microbiol Biotechnol* 32(10):158
- Balk M, Weijma J, Stams AJM (2002) *Thermotoga lettingae* sp. nov., a novel thermophilic, methanol-degrading bacterium isolated from a thermophilic anaerobic reactor. *Int J Syst Evol Microbiol* 52:1361–1368
- Bedoya K, Hoyos BO, Zurek E, Cabarcas F, Alzate J (2020) Annual microbial community dynamics in a full-scale anaerobic sludge digester from a wastewater treatment plant in Colombia. *Sci Total Environ* 726:138–479
- Białobrzewski I, Miks-Krajnik M, Dach J, Markowski M, Czekala W, Głuchowska K (2015) Model of the sewage sludge-straw composting process integrating different heat generation capacities of mesophilic and thermophilic microorganisms. *Waste Manag* 43:72–83
- Briones A, Raskin L (2003) Diversity and dynamics of microbial communities in engineered environments and their implications for process stability. *Curr Opin Biotechnol* 14(3):270–276
- Campanaro S, Treu L, Kougias PG, De Francisci D, Valle G, Angelidaki I (2016) Metagenomic analysis and functional characterization of the biogas microbiome using high throughput shotgun sequencing and a novel binning strategy. *Biotechnol Biofuels* 9(1):26
- Cardinali-Rezende J, Rojas-Ojeda P, Nascimento AM, Sanz JL (2016) Proteolytic bacterial dominance in a full-scale municipal solid waste anaerobic reactor assessed by 454 pyrosequencing technology. *Chemosphere* 146:519–525
- Chen S, Chen B, Song D (2012) Life-cycle energy production and emissions mitigation by comprehensive biogas–digestate utilization. *Bioresour Technol* 114:357–364
- Choi G, Kim H, Lee C (2021) Long-term monitoring of a thermal hydrolysis-anaerobic co-digestion plant treating high-strength organic wastes: process performance and microbial community dynamics. *Bioresour Technol* 319:124–138
- Churko JM, Mantalas GL, Snyder MP, Wu JC (2013) Overview of high throughput sequencing technologies to elucidate molecular pathways in cardiovascular diseases. *Circ Res* 112:1613–1623
- Colon J, Forbis-Stokes AA, Deshusses MA (2015) Anaerobic digestion of undiluted simulant human excreta for sanitation and energy recovery in less-developed countries. *Energy Sustain Dev* 29:57–64

- Cuetos MJ, Gomez X, Otero M, Morn A (2008) Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). *Biochem Eng J* 40(1):99–106
- Cuzin N, Ouattara AS, Labat M, Garcia JL (2001) *Methanobacterium congolense* sp. nov., from a methanogenic fermentation of cassava peel. *Int J Syst Evol Microbiol* 51:489–493
- de Bok FA, Stams AJ, Dijkema C, Boone DR (2001) Pathway of propionate oxidation by a syntrophic culture of *Smithella propionica* and *Methanospirillum hungatei*. *Appl Environ Microbiol* 67(4):1800–1804
- De Francisci D, Kougias PG, Treu L, Campanaro S, Angelidaki I (2015) Microbial diversity and dynamics of biogas reactors due to radical changes of feedstock composition. *Bioresour Technol* 176:56–64
- De Vrieze J, Saunders AM, Hey Y, Fang J, Nielsen PH, Verstraete W, Boon N (2015) Ammonia and temperature determine potential clustering in the anaerobic digestion microbiome. *Water Res* 75:312–323
- Dong X, Xin Y, Jian W, Liu X, Ling D (2000) *Bifidobacterium thermacidophilum* sp. nov., isolated from an anaerobic digester. *Int J Syst Evol Microbiol* 50:119–125
- Duan N, Zhang D, Khoshnevisan B, Kougias PG, Treu L, Liu Z, Lin C, Liu H, Zhang Y, Angelidaki I (2020) Human waste anaerobic digestion as a promising low-carbon strategy: perating performance, microbial dynamics and environmental footprint. *J Clean Prod*. <https://doi.org/10.1016/j.jclepro.2020.120414>
- Duan N, Kougias PG, Campanaro S, Treu L, Angelidaki I (2021) Evolution of the microbial community structure in biogas reactors inoculated with seeds from different origin. *Sci Total Environ* 773:144981. <https://doi.org/10.1016/j.scitotenv.2021.144981>
- Fakrudin M, Mannan KS (2013) Methods for analyzing diversity of microbial communities in natural environments. *Ceylon J Sci* 42:19–33
- Fernandez AS, Hashsham SA, Dollhopf SL (2000) Flexible community structure correlates with stable community function in methanogenic bioreactor communities perturbed by glucose. *Appl Environ Microbiol* 66(9):4058–4067
- Fodor A, DeSantis T, Wylie K, Badger J, Ye Y, Wohlever T, Hu P, Sodergren E, Liolios K, Huot CH, Birren B, Earl A (2012) The “most wanted” taxa from the human microbiome for whole genome sequencing. *PLoS One* 7:41294. <https://doi.org/10.1371/journal.pone.0041294>
- Fontana A, Campanaro S, Treu L, Kougias PG, Cappa F, Morelli L, Angelidaki I (2018) Performance and genome-centric metagenomics of thermophilic single and two-stage anaerobic digesters treating cheese wastes. *Water Res* 134:181–191
- Gao H, Zhou C, Li F, Han B, Li X (2017) Economic and environmental analysis of five Chinese rural toilet technologies based on the economic input–output life cycle assessment. *J Clean Prod* 163:379–391
- Gao M, Guo B, Zhang L, Zhang Y, Liu Y (2019) Microbial community dynamics in anaerobic digesters treating conventional and vacuum toilet flushed blackwater. *Water Res* 160:249–258
- Giovannoni SJ, Britschgi TB, Moyer CL, Field KG (1990) Genetic diversity in Sargasso Sea bacterioplankton. *Nature* 345:60–63
- Gomaa MA, Abed RMM (2017) Potential of fecal waste for the production of biomethane, bioethanol and biodiesel. *J Biotechnol* 253:14–22
- Guo J, Peng Y, Ni BJ, Han X, Fan L, Yuan Z (2015) Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing. *Microb Cell Factories* 14:33
- Han G, Gu S, Lee J, Shin J, Hwang S (2017) A comparative study on the process efficiencies and microbial community structures of six full-scale wet and semi-dry anaerobic digester treating food waste. *Bioresour Technol* 245:869–875
- Hao L, Bize A, Conteau D, Chapleur O, Courtois S, Kroff P, Desmond-Le QE, Bouchez T, Mazéas L (2016) New insights into the key microbial phylotypes of anaerobic sludge digesters under different operational conditions. *Water Res* 102:158–169

- Hartmann H, Angelidaki I, Ahring BK (2002) Co-digestion of the organic fraction of municipal waste with other waste types. In: Mata-Alvarez J (ed) *Biomethanization of the organic fraction of municipal solid waste*. IWA Publishing, London, pp 181–199
- Hashemi S, Hashemi SE, Lien KM, Lamb JJ (2021) Molecular microbial community analysis as an analysis tool for optimal biogas production. *Microorganisms* 9:1162
- Hassa J, Maus I, Off S, Puhler A, Scherer P, Klocke M, Schluter A (2018) Metagenome, metatranscriptome, and metaproteome approaches unravelled compositions and functional relationships of microbial communities residing in biogas plants. *Appl Microbiol Biotechnol* 102(12):5045–5063
- Ho D, Jensen P, Batstone D (2014) Effects of temperature and hydraulic retention time on acetotrophic pathways and performance in high-rate sludge digestion. *Environ Sci Technol* 48(11):6468–6476
- Hori T, Haruta S, Ueno Y, Ishii M, Igarashi Y (2006) Dynamic transition of a methanogenic population in response to the concentration of volatile fatty acids in a thermophilic anaerobic digester. *Appl Environ Microbiol* 72(2):1623–1630
- Imachi H, Sakai S, Ohashi A (2007) *Pelotomaculum propionicicum* sp. nov., an anaerobic, mesophilic, obligately syntrophic, propionate-oxidizing bacterium. *Int J Syst Evol Microbiol* 57:1487–1492
- Iwai S, Weinmaier T, Schmidt BL, Albertson DG, Poloso J, Dabbagh K, Desantis TZ (2016) Piphillin: improved prediction of metagenomic content by direct inference from human microbiomes. *PLoS One* 11:e0166104. <https://doi.org/10.1371/journal.pone.0166104>
- Jünemann S, Kleinbölting N, Jaenicke S, Henke C, Hassa J, Nelkner J, Stolze Y, Albaum SP, Schluter A, Goesmann A (2017) Bioinformatics for NGS-based metagenomics and the application to biogas research. *J Biotechnol* 261:10–23
- Khoshnevisan B, Tsapekos P, Alvarado-Morales M, Rafiee S, Tabatabaei M, Angelidaki I (2018) Life cycle assessment of different strategies for energy and nutrient recovery from source sorted organic fraction of household waste. *J Clean Prod* 180:360–374
- Khoshnevisan B, Tabatabaei M, Tsapekos P, Rafiee S, Aghbashlo M, Lindeneg S, Angelidaki I (2020) Environmental life cycle assessment of different biorefinery platforms valorizing municipal solid waste to bioenergy, microbial protein, lactic and succinic acid. *Renew Sust Energy Rev*. <https://doi.org/10.1016/j.rser.2019.109493>
- Kim W, Kim S, Heo S, Kim D, Kim J, Jo H, Kwon J, Yang J (2014) Quantitative analysis of microbial community structure in two-phase anaerobic digesters treating food wastewater. *Korean J Chem Eng* 31:381–385
- Kim E, Lee J, Han G, Hwang S (2018) Comprehensive analysis of microbial communities in full-scale mesophilic and thermophilic anaerobic digesters treating food waste-recycling wastewater. *Bioresour Technol* 259:442–450
- Kirkegaard RH, McIlroy SJ, Kristensen JM, Nierychlo M, Karst SM, Dueholm MS, Albertsen M, Nielsen PH (2017) The impact of immigration on microbial community composition in full-scale anaerobic digesters. *Sci Rep* 7:1–11
- Koo T, Shin SG, Lee J, Han G, Kim W, Cho K, Hwang S (2017) Identifying methanogen community structures and their correlations with performance parameters in four full-scale anaerobic sludge digesters. *Bioresour Technol* 228:368–373
- Kovacs E, Wirth R, Maróti G, Bagi Z, Nagy K, Minárovits J, Rákhely G, Kovács KL (2015) Augmented biogas production from protein-rich substrates and associated metagenomic changes. *Bioresour Technol* 178:254–261
- Lalander C, Nordberg A, Vinneras B (2018) A comparison in product-value potential in four treatment strategies for food waste and faeces- assessing composting, fly larvae composting and anaerobic digestion. *Glob Change Biol Bioenergy* 10:84–91
- Leclerc M, Delgènes JP, Godon JJ (2004) Diversity of the archaeal community in 44 anaerobic digesters as determined by single strand conformation polymorphism analysis and 16S rDNA sequencing. *Environ Microbiol* 6(8):809–819

- Lee S, Kang HJ, Park HD (2015) Influence of influent wastewater communities on temporal variation of activated sludge communities. *Water Res* 73:132–144
- Lee J, Kim E, Han G, Tongco JV, Shin SG, Hwang S (2018) Microbial communities underpinning mesophilic anaerobic digesters treating food wastewater or sewage sludge: a full-scale study. *Bioresour Technol* 259:388–397
- Lei Y, Sun D, Dang Y, Feng X, Huo D, Liu C, Zheng K, Holmes DE (2019) Metagenomic analysis reveals that activated carbon aids anaerobic digestion of raw incineration leachate by promoting direct interspecies electron transfer. *Water Res* 161:570–580
- Lettinga G, Rebac S, Zeeman G (2001) Challenge of psychrophilic anaerobic wastewater treatment. *Trends Biotechnol* 19(9):363–370
- Li N, Xue Y, Chen S, Takahashi J, Dai L, Dai X (2017) Methanogenic population dynamics regulated by bacterial community responses to protein-rich organic wastes in a high solid anaerobic digester. *Chem Eng J* 317:444–453
- Li H, Li T, Li X, Wang G, Lin Q, Qu J (2018) Gut microbiota in Tibetan herdsmen reflects the degree of urbanization. *Front Microbiol* 9:1745. <https://doi.org/10.3389/fmicb.2018.01745>
- Liu Y, Balkwill DL, Aldrich HC, Drake GR, Boone DR (1999) Characterization of the anaerobic propionate-degrading syntrophs *Smithella propionica* gen. nov., sp. nov. and *Syntrophobacter wolinii*. *Int J Syst Bacteriol* 49:545–556
- Liu C, Li H, Zhang Y, Si D, Chen Q (2016) Evolution of microbial community along with increasing solid concentration during high-solids anaerobic digestion of sewage sludge. *Bioresour Technol* 216:87–94
- Lv B, Xing M, Yang J, Qi W, Lu Y (2013) Chemical and spectroscopic characterization of water extractable organic matter during vermicomposting of cattle dung. *Bioresour Technol* 132:320–326
- Mark Welch JL, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG (2016) Biogeography of a human oral microbiome at the micron scale. *Proc Natl Acad Sci* 113:791–800
- Martinez MA, Romero H, Perotti NI (2014) Two amplicon sequencing strategies revealed different facets of the prokaryotic community associated with the anaerobic treatment of vinasses from ethanol distilleries. *Bioresour Technol* 153:388–392
- Merlino G, Rizzi AR, Schievano A, Tenca A, Scaglia B, Oberti R, Adani F, Daffonchio D (2013) Microbial community structure and dynamics in two-stage vs single-stage thermophilic anaerobic digestion of mixed swine slurry and market biowaste. *Water Res* 47:1983–1995
- Mirmohamadsadeghi S, Karimi K, Azarbaijani R, Yeganeh LP, Angelidaki I, Nizami AS, Bhat R, Dashora K, Vijay VK, Aghbashlo M (2021) Pretreatment of lignocelluloses for enhanced biogas production: a review on influencing mechanisms and the importance of microbial diversity. *Renew Sust Energ Rev* 135:110173
- Muller EE, Glaab E, May P, Vlassis N, Wilmes P (2013) Condensing the omics fog of microbial communities. *Trends Microbiol* 21:325–333
- Nascimento AL, Souza AJ, Avelino P, Andrade M, Andreote FD, Coscione AR, Oliveira FC (2018) Sewage sludge microbial structures and relations to their sources, treatments, and chemical attributes. *Front Microbiol*. <https://doi.org/10.3389/fmicb.2018.01462>
- Oksanen J, Kindt R, Legendre P, O'Hara B, Stevens MHH, Oksanen MJ, Suggests M (2007) The vegan package. *Commun Ecol Package* 10:631–637
- Pope PB, Vivekanand V, Eijsink VG, Horn SJ (2013) Microbial community structure in a biogas digester utilizing the marine energy crop *Saccharina latissima*. *3 Biotech* 3(5):407–414
- Pore SD, Shetty D, Arora P, Maheshwari S, Dhakephalkar PK (2016) Metagenome changes in the biogas producing community during anaerobic digestion of rice straw. *Bioresour Technol* 213:50–53
- Ronda C, Wang H (2022) Engineering temporal dynamics in microbial communities. *Curr Opin Microbiol* 65:47–55
- Rong X, Kai Z, Pu L, Khan A, Xiong J, Tian F, Li X (2018) A critical review on the interaction of substrate nutrient balance and microbial community structure and function in anaerobic co-digestion. *Bioresour Technol* 247:1119–1127

- Rudakiya D, Narra M (2021) Microbial community dynamics in anaerobic digesters for biogas production. In: Microbial rejuvenation of polluted environment. Springer, New York. [https://doi.org/10.1007/978-981-15-7459-7\\_7](https://doi.org/10.1007/978-981-15-7459-7_7)
- Rudakiya DM, Patel SH, Narra M (2019) Structural insight into the fungal  $\beta$ -glucosidases and their interactions with organics. *Int J Biol Macromol* 138:1019–1028
- Ryo M, Aguilar-Trigueros CA, Pinek L, Muller LAH, Rillig MC (2019) Basic principles of temporal dynamics. *Trends Ecol Evol* 34:723–733
- Saunders A, Albertsen M, Vollertsen J, Nielsen P (2015) The activated sludge ecosystem contains a core community of abundant organisms. *ISME J* 10:117. <https://doi.org/10.1038/ismej.2015.117>
- Savant DV, Shouche YS, Prakash S, Ranade DR (2002) *Methanobrevibacter acididurans* sp. nov., a novel methanogen from a sour anaerobic digester. *Int J Syst Evol Microbiol* 52:1081–1087
- Seib MD, Berg KJ, Zitomer DHH (2016) Influent wastewater microbiota and temperature influence anaerobic membrane bioreactor microbial community. *Bioresour Technol* 216:446–452
- Shirzad M, Panahi HKS, Dashti BB, Rajaeifar MA, Aghbashlo M, Tabatabaei M (2019) A comprehensive review on electricity generation and GHG emission reduction potentials through anaerobic digestion of agricultural and livestock/slaughterhouse wastes in Iran. *Renew Sust Energy Rev* 111:571–594
- Singh R, Chang H, Yan D, Lee K, Ucmak D, Wong K, Abrouk M, Farahnik B, Nakamura M, Zhu T, Bhutani T, Liao W (2017) Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 15:73
- Sousa DZ, Smidt H, Alves MM, Stams AJ (2007) *Syntrophomonas zehnderi* sp. nov., an anaerobe that degrades long-chain fatty acids in co-culture with *Methanobacterium formicicum*. *Int J Syst Evol Microbiol* 57:609–615
- Stiles ME, Holzapfel WH (1997) Lactic acid bacteria of foods and their current taxonomy. *Int J Food Microbiol* 36:1–29
- Sun L, Liu T, Müller B, Schürer A (2016) The microbial community structure in industrial biogas plants influences the degradation rate of straw and cellulose in batch tests. *Biotechnol Biofuels* 9(1):128
- Sundberg C, Al-Soud WA, Larsson M, Alm E, Yekta SS, Svensson BH, Sørensen SJ, Karlsson A (2013) Pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. *FEMS Microbiol Ecol* 85(3):612–626
- Supaphol S, Jenkins S, Intomo P, Waite I, O'Donnell A (2011) Microbial community dynamics in mesophilic anaerobic co-digestion of mixed waste. *Bioresour Technol* 102:4021–4027
- Torsvik V, Daae FL, Sandaa RA, Ovreas L (1998) Novel techniques for analysing microbial diversity in natural and perturbed environments. *J Biotechnol* 64:53–62
- Tyagi V, Bhatia A, Kubota K, Rajpal A, Ahmed B, Khan A, Kazmi AA, Kumar M (2021) Microbial community dynamics in anaerobic digesters treating organic fraction of municipal solid waste. *Environ Technol Innov* 21:101–303
- Vanwonterghem I, Jensen PD, Ho DP, Batstone DJ, Tyson GW (2014) Linking microbial community structure, interactions and function in anaerobic digesters using new molecular techniques. *Curr Opin Biotechnol* 27:55–64
- Venkiteswaran K, Benjamin B, James M, Daniel Z (2015) Relating anaerobic digestion microbial community and process function. *Microbiol Insights* 8:37–44
- Wachemo AC, Tong H, Yuan H, Zuo X, Korai RM, Li X (2019) Continuous dynamics in anaerobic reactor during bioconversion of rice straw: rate of substance utilization, biomethane production and changes in microbial community structure. *Sci Total Environ* 687:1274–1284
- Werner JJ, Knights D, Garcia ML (2011) Bacterial community structures are unique and resilient in full-scale bioenergy systems. *Proc Natl Acad Sci* 108(10):4158–4163
- Wirth R, Kovács E, Maróti G, Bagi Z, Rákhely G, Kovács KL (2012) Characterization of a biogas producing microbial community by short-read next generation DNA sequencing. *Biotechnol Biofuels* 5(1):41

- Wittebolle L, Marzorati M, Clement L (2009) Initial community evenness favours functionality under selective stress. *Nature* 458:623–626
- Yannarell AC, Triplett EW (2005) Geographic and environmental sources of variation in lake bacterial community composition. *Appl Environ Microbiol* 71:227–239
- Zhang Y, Angelidaki I (2015) Counteracting ammonia inhibition during anaerobic digestion by recovery using submersible microbial desalination cell. *Biotechnol Bioeng* 112:1478–1482
- Zhang L, Loh KC, Lim JW, Zhang J (2019) Bioinformatics analysis of metagenomics data of biogas-producing microbial communities in anaerobic digesters: a review. *Renew Sust Energy Rev* 100:110–126





# Recent Trends in Performance Assessment of Anaerobic Biodigestion for Sewage Waste Management: A Critical Review

# 7

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## Abstract

Sewage originated as an issue when humanity began to live in settlements, and the task of disposing of large quantities of wastewater, mainly excreta, has gained momentum. Equally, water contamination from urbanization and industrial developments necessitated wastewater treatment and building sewage treatment plants. Anaerobic digestion (AD) of sewage has emerged as a feasible well-proven procedure among the technologies created, as it provides essential waste-to-energy technology for developing countries like India. Co-digestion of AD with two or more substrates has been the most widely published research work. The majority of sludge produced in India is dumped in landfills and has a shortage of proper sewage sludge management and treatment facilities. AD promotes the sewage sludge to renewable energy with a calorific value of 8–21 MJ/kg and has a high energy recovery potential around 315–608 kWh/ton by anaerobic biodigestion. The review of various studies on anaerobic digestion advises that sludge must be classified as an energetic substance with a potential for energy recovery across the country, which aids in energy recovery and addresses sludge management in India.

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**Keywords**

Sewage · Ancient systems · Anaerobic biodigestion · Sewage sludge · Energy potential

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## 7.1 Introduction

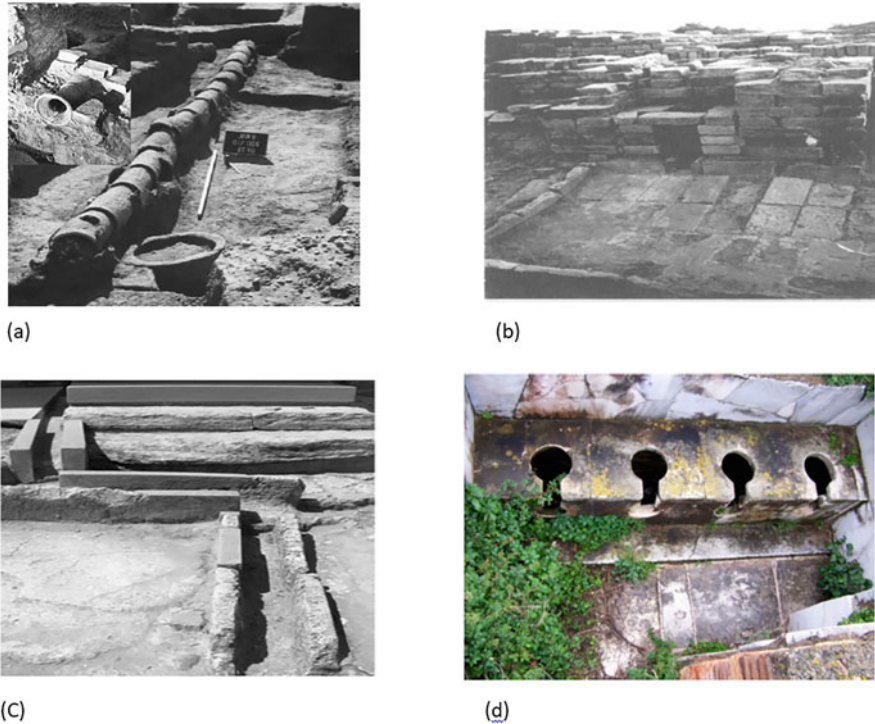
Wastewater management has received little attention for centuries and was usually dumped in most civilizations' streets and near population centers, posing significant health and environmental risks. The handling of this situation has now become a top priority globally. Way back, humans were apathetic with sewage during the hunter-gatherer era, and they treated waste in the same way that wild animals did (Vuorinen et al. 2007). The subject of sewage originated when humanity began to live in settlements, and the task of disposing of large quantities of wastewater, mainly excreta, has gained momentum. Although historians and economists have written extensively about the evolution and management of sewage over time, engineering knowledge was highly limited (Serneri 2007).

Humans have been on the planet for almost 2 lakh years, majorly as hunter-gatherers by ever-growing numbers (Vuorinen et al. 2007). The earliest human societies were dispersed across large areas, and the garbage they created was returned to the soil and degraded through natural processes. Disposal difficulties were limited since they existed as small nomadic hunter-gatherer societies. A new era began 10,000 years ago when people established permanent settlements and adopted an agrarian lifestyle (De Feo et al. 2014).

Humans practiced the disposal of excreta through holes made into the ground, covered after use, until the emergence of the first advanced civilization. The history of sanitation in ancient civilizations enclosed Africa, Southern Europe, the Middle East, and Asia to India (Lofrano and Brown 2010). According to historical documents, the Mesopotamian Empire was the first civilization that formally addressed sanitary issues originating from communal living. There are ruins of Babylonia linked to the drainage system to take waste away and latrines leading to cesspits (Jones 1967).

The Indus Valley has achieved tremendous progress in terms of wastewater management. There are signs of a sophisticated and scientifically progressive urban culture in the area (Pathak 2001) (Fig. 7.1). The community's quality of life reflected a thorough understanding and application of urban planning and effective governance by municipal authorities and a strong emphasis on hygiene. The world's foremost urban sanitation systems were uncovered in Harappa and Mohenjo-Daro and discovered in Rakhigarhi (Webster 1962). Houses and drainage channels were connected, and wastewater entered the street sewers only after preliminary treatment.

The Greeks were pioneers in the development of modern sanitation systems. Archaeological investigations have proven indisputably that current water management practices have their origins in ancient Greece (Angelakis et al. 2007). In the Palace of Minos at Knossos and the west side of the "Queen's room" at Phaistos,



**Fig. 7.1** Sewage systems in (a) Mesopotamian civilization, (b) Indus civilization, (c) Egyptian civilization, and (d) Roman civilization. (Source: Antoniou et al. 2016; Lofrano and Brown 2010)

toilets resembling Egyptian ones were found. These were connected to a closed sewer system still under operational after 4000 years (Angelakis et al. 2005). The Ancient Greeks had public latrines that discharged wastewater and stormwater into pipes that led to a collection basin outside the city (300 BC to 500 AD).

The Romans were excellent administrators and engineers with technologically advanced systems. The water systems of Rome are one of the wonders of the ancient world, and their systems rivaled modern technology (Hopkins 2007; Fraisse et al. 2007). The Romans continued the Assyrians' engineering work, transforming their ideas into substantial infrastructure to serve all populations. The sanitary dark ages began when the Roman Empire fell apart, lasting about a thousand years (476–1800) (Lofrano and Brown 2010; Aiello et al. 2008). The Roman civilization and many other societies before it had a culture of water as a source of health and wellness, which was later abandoned. The magnificent water conveyance infrastructure that would have been a source of pride for the Romans for generations was overlooked. It's hard to think that just half of the Italian communes had drinking water pipelines at the end of the nineteenth century, and more than 77% lacked sewers (Sorcinelli 1998).

The necessity of waste and wastewater disposal became apparent as the pace of urbanization and industrialization increased all through the eighteenth century, preceding and escorting the industrial revolution (Tarlow 2007; Lofrano and Brown 2010). The United Kingdom was the earliest country to experiment with coordinated action to enhance the conditions of an urban environment. “The remedy to pollution is dilution” was the guiding philosophy, and governments began to impose waste treatment requirements. Before the 1st World War, when construction facilities to treat wastewater were halted, they were built in Europe’s major cities (Seeger 1999; Cooper 2007).

The 2nd World War also slowed the development of wastewater treatment until 1948, resulting in increased water pollution. By 1950, the focus of pollution disputes had shifted to water quality standards and stream categorization, which were crucial prerequisites for establishing a waste management policy (Shifrin 2005). This shift has emphasized the need to comprehend diverse wastewater treatment methods and critical advancements in wastewater treatment technology. This paper aims to analyze the evolution of sanitation systems, improvements in wastewater treatment, and the anaerobic digestion process in particular.

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## 7.2 Evolution of Sewage Treatment

It is well recognized that the human-water-sanitation interaction has undergone significant modifications over time due to cultural, societal, and religious influences. For example, wastewater was treated in Harappa and Mohenjo-Daro using tapered terra-cotta pipes that led to a tiny pit (Jansen 1989; Good et al. 2009). The liquids flooded into drainage pipes in the roadway, while solids settled and pooled in the sump. During maintenance and cleaning work, drainage channels were removed and concealed with bricks and chipped stones (Wolfe 1999). It was perhaps the first attempt at treating wastewater on record, and the timeline of the historical evolution of the sewerage system is given in Table 7.1.

Egyptians used portable toilets, bowls made of ceramic and clay, and the excrement was emptied into pits outside the house, on streets, or to the river (Breasted 1906). The wealthier had the provisions wherein wastewater was drained from the bathroom by placing a basin underneath the spout of the floor slab or by way of drainage pipes flowing through the exterior wall into a vessel or directly into the desert sand (Lofrano and Brown 2010). In the ancient cities of Pompeii and Herculaneum, different designs were used, and in Pompeii cesspools were a common way to manage wastewater. Also, pipes made of copper were developed by ancient Egyptians and the techniques of making copper alloys (Ahmed et al. 2020).

The Romans invented superior sanitary technologies, such as baths with running water and underground sewers and drains. They reused wastewater from the baths by flushing latrines before dumping it into sewers and ultimately into the river (Vuorinen 2010). Unfortunately, the Romans’ developments in sanitation were lost in the course of the Middle Ages. The United States and Europe had forgotten about the Romans’ achievements by the 1800s, and chamber pots were trendy again. Street

**Table 7.1** Historical evolution of sewage treatment

S. no.	Civilization	Time	Wastewater history	Reference
1	Babylonians	4000 BC	Introduce clay sewer pipes	Schladweiler (2002), De Feo et al. (2014)
2	Mesopotamian Empire	3500–2500 BC	Latrines with cesspits	Lofrano and Brown (2010)
3	Indus civilization	26–1700 BCE	Houses connected to drainage channels Wastewater discharged with treatment	Wolfe (1999)
4	Greek civilization	300 BC to 500 AD	Public latrines with piped drainage	Tolle-Kastenbein (2005)
5	Roman civilization	800 BCE and 300 CE	Advanced water and sewage infrastructural systems Ancient sewer—cloaca maxima	Hopkins (2007)
6	Egyptian civilization	2000–500 BC	Bathrooms and toilets made of limestone with a drainage system	Lofrano and Brown (2010)
7	Etruscan civilization	800–100 BC	Drainage channels on sides of streets	Angelakis et al. (2013)
8	Hellenistic periods	480–67 BC	Systematized baths, toilets, sewerage and drainage systems	Yannopoulos et al. (2017)
9	Western Han dynasty	206 BC–24 AD	Toilet made of stone and connected with running water	Antoniou et al. (2016)
10	Classic Mayans	250–900 AD	Flush toilets and underground aqueducts	Markonis et al. (2013)
11	Medieval Europe	1200	Toilets in castles, with a stone seat on top	Gray (1940) Burian and Edwards (2002)
12	Paris	1370	First closed sewer	Gandy (1999)
13	Scotland	1775	Flushing lavatory	Theoharidis (1991)
14	France	1860	The invention of the septic tank	De Feo et al. (2014)
15	England	1912	Discovery of the sewage treatment process	Stanbridge (1976)

gutters became the favored disposal destination for chamber pots in large cities like New York, Paris, and London (Wolfe 1999; Cooper 2007; Lofrano and Brown 2010).

Unsanitary conditions and illnesses resulted from the contamination in streets and rivers. Cholera and typhoid fever were among the diseases spread by poor waste disposal. It was established in the mid-1800s that mixing drinking water sources with sewage caused illness/plague (cholera) (Taylor 1996). The connection between sewage disposal and disease emerged when Louis Pasteur established that germs transmit disease (Gal 2008). Cholera became a global epidemic in 1817 when a

deadly outbreak began in Jessore, India, about halfway between Kolkata and Dhaka, and subsequently spread across much of India, Myanmar, and Sri Lanka (Gill et al. 2001).

Local conditions quickly deteriorated since cesspools were rarely drained and regularly overflowed in densely populated areas, and threats to public health became apparent. Following the cholera outbreak in Paris in 1832, authorities began to recognize the link between public health and the city's unhygienic environments (Cooper 2007). Cholera epidemics killed many people in Europe and North America up until the nineteenth century. For a long time, everyone assumed it was due to poor air quality (Tulchinsky 2018). John Snow revealed that the 1854 London cholera pandemic was caused by pollution in the drinking water. London suffered from two more cholera in 1866 and 1872 (De Feo et al. 2014; Tulchinsky 2018).

Yellow fever struck Memphis, Tenn, in 1873, and 5 years later, people died in the same city of the same cause. This paved the way for separate wastewater sewage systems and got introduced in 1880 (Sawchuk and Burke 1998). This principle was highly advocated by Edwin Chadwick, named the "Father of Sanitation" in England. In larger cities, water closets were connected directly to storm sewers, and sewage was moved from the ground to neighboring water bodies, resulting in a new issue of surface water pollution (Winter et al. 1998). The general relationship between chemical water pollution and toxicity was understood and a timeline depicting the development of analytical procedures made at the beginning of the twentieth century (Shelford 1912; Lofrano and Brown 2010).

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### 7.3 Developments in Wastewater Legislations

In ancient times, human activities were administered by dharma (law and order), which is codified in the Hindu sacred scriptures, the Shrutis and Smritis in Vedas (Cullet and Gupta 2009). During this historical period, the Laws of Manu provided evidence of the water law during the time. Diversion of waters was discouraged, and those who polluted, stole, or diverted the water were subjected to a system of social reprimands and punishments (Gupta and van der Zaag 2008). This suggests that Islamic regulations were most likely implemented during this time. According to Islamic law, water is God's gift, neither individual nor government can own it, and everyone should have access to it (Faruqui 2001; Naff and Dellapenna 2002).

The theory of controlling surface water by the government was first established by the British. The East India Company was primarily concerned with expanding trade and transportation, and law evolved due to practice and judicial process (Siddiqui 1992). The Northern India Canal and Drainage Act (1873), which governed drainage, navigation, and irrigation, was one of the most critical enactments. In terms of water, colonial legislation established a split of responsibilities between centers and states (Cullet and Gupta 2009). The Government of India Act (1935) gave provinces authority over water supply, drainage, canals, irrigation, embankments, and water storage and hydropower (Getzler 2004).

The early 1970s witnessed the signs of emerging changes fundamentally in water access, possibly as a result of declining per capita water availability, increased contamination of prevailing water supplies, rapidly increasing irrigation use, and increased competition among water users for a larger share of finite supplies (Amerasinghe et al. 2013). As a result, a regulatory framework for pollution was established in the early 1970s. Initially, environmental regulations concentrated mainly on defining the problem, setting standards, and funding essential services, all targeted at reducing surface water contamination (Shifrin 2005). While water remained predominantly a state topic by the 1980s, it became clear that the nonexistence of a national water strategy was a foremost hindrance to establishing coherent water programs (Muralidhar 2006).

The twentieth century saw significant changes in managing wastewater, environmental awareness, and public attitudes toward pollution (Shifrin 2005). The 8th Report of the Royal Commission on Sewage Disposal (1912) pioneered the concept of biochemical oxygen demand (BOD) and recognized tests related to sewage and its disposal requirements, and many countries adopted these testing methods. Streeter and Phelps in 1925 and Imhoff and Mahr in 1932 developed aeration/deaeration models that helped in predicting the acceptable BOD loads in surface waters (Juwarkar et al. 1995).

Management of stream pollution focused solely on dissolved oxygen, biochemical oxygen demand, significant nutrients, and pathogenic pollutants until Clean Water Act (1972), which laid the groundwork for hazardous chemical pollution prevention (Schellenberg et al. 2020). The Environment (Protection) Act of 1986 was then formulated to address issues related to environmental protection. The development of the National Water Policy (1987) enhanced the process and was reformulated in 2002. Water (Prevention and Control of Pollution) Act (1974) was formulated to prevent and control water pollution and to maintain water resources in India. The Water (Prevention and Control of Pollution) Cess Act (1977) tariffs a cess on water consumption by industries and local authorities (Singh et al. 2019). Ambient water quality standards are typically in the form of scientific judgments or recommendations produced nationwide by committees of the Federal Water Pollution Control Agency under the 1965 Water Quality Act (Shifrin 2005).

Controlling wastewater discharge is mainly dependent on the carrying capacity of receiving waters for over a century. These rules were in place until the Safe Drinking Water Act (1974) prompted the Environmental Protection Agency (EPA) to develop hazardous chemical drinking water limits (Cullet and Gupta 2009). The EPA's efforts in this area coincided with the development of the Water Quality Criteria, which formed the list of maximum contamination levels (MCL) for around 90 chemicals, microorganisms, physical properties, and radionuclides (Pandey et al. 2014). The MCLs are created by taking into account both health-related factors and economic or practical considerations. The analytical methods were developed to detect the contaminants in specific (Kaur et al. 2012).

## 7.4 Technological Evolvement of Wastewater Treatment

In response to the environmental challenges posed by water contamination, several researchers have dedicated their efforts to inventing unique wastewater treatment methods (Nassar et al. 2017). For example, breakthroughs in microbiology promoted wastewater treatment during the end of the nineteenth century, and Arden and Lockett ascertained active sludge in 1914, one of the wastewater treatment technologies that we presently utilize (Shifrin 2005; Pandey et al. 2014; Schellenberg et al. 2020).

Several contaminants and their derivatives are also discharged into the water environment due to increased urbanization and industrialization. Organics, nutrients, and pollutants in low concentrations create the majority of pollution, which is very harmful to humans and aquatic life (Saravanan et al. 2019; Rashid et al. 2021). On the other hand, water scarcity resulting from economic and population growth is one of humanity's greatest fears and a limitation to sustainable development. Therefore, wastewater treatment for reuse and recycle has become the need of the hour (Ahmed et al. 2020).

Wastewater is the water discharged from domestic, industries, institutions, and commercial activities directed to treatment plants via a designed and engineered network of pipelines (Crini and Lichtfouse 2019). Technologies have been developed to reduce wastewater discharges and pollutant hazards that include adsorption, coagulation/flocculation, oxidation (electrochemical/photo-electrochemical/Fenton's), membrane filtration (nano/UV) and biological treatment, etc. (Xu et al. 2018). However, distinct types of wastewater necessitate different treatment processes and procedures, and sewage wastewater takes precedence because it originates from household waste (Rashid et al. 2021).

Sewage water is wastewater released from households and consists of gray water (bathing, washing dishes, laundry) and black water (used water from toilets). It contains detritus, for instance, paper casings, hygiene produces, soap deposits, and dirt, and has a foul odor due to the chemical makeup of the numerous waste components (Schellenberg et al. 2020). Overpopulation in metropolitan areas has led to sewage pollution, and its hazard to human health and the environment has become a subject of concern. It also impacts biodiversity, aquatic life, and agriculture, as well as contributes to eutrophication and a rise in biological oxygen demand (BOD) (Shafiq et al. 2019). In addition, the presence of pathogenic or disease-causing bacteria and hazardous substances in sewage water and its disposal contaminate the land or water body, necessitating sewage wastewater treatment and safe removal (Chahal et al. 2016).

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## 7.5 Sewage Water Treatment Methods

The purpose of sewage treatment is to reduce the concentration of dangerous substances to the standards set by the Ministry of Environment and Forests (MoEF) of the Indian government, which cannot be modified by the State Pollution



Control Boards (PCB), and the fecal coliform limits set by the National River Conservation Directorate (NRCD) (MoEF 2005).

The sewage treatment plants (STP) were designed to remove the suspended matter during the end of the nineteenth century using simple gravity settling. However, the presence of organic matter (colloidal/dissolved) in sewage rendered primary treatment unsuccessful, and in the early twentieth century, secondary treatment (biological methods) was developed to eliminate the organic matter (Elsayed and Manar 2019). However, the sewage disposal from the secondary treatment resulted in a higher concentration of ammonia, nitrate, and phosphate, which led to eutrophication. This nurtured the development of tertiary treatment systems to remove nutrients (Lofrano and Brown 2010).

Biological treatment of wastewater appears to be a viable method, with the benefits of decreased treatment costs and no secondary pollution. Organic wastewaters are treated using aerobic biological processes to obtain maximum treatment efficiency. Methane gas from anaerobic digestion helps secure revenue from the Clean Development Mechanism's mainly carbon credits and may be used as renewable energy (Anukam et al. 2019). Significant progress has been made in anaerobic treatment's biotechnology for waste treatment based on resource recovery and utilization, along with complementing the goal of pollution management (Cruz et al. 2021). The present paper aims to address anaerobic digestion and its significance in sewage treatment in particular.

### 7.5.1 History of Anaerobic Digestion

Anaerobic biological treatment has advanced in recent years, and anaerobic digesters are frequently employed to treat complex organic solid wastes, including primary and secondary wastewater sludge (Neves et al. 2018). However, it hasn't been widely employed in treating organic wastewaters of low strength either from industrial or residential uses in the past, while, aerobic treatment processes are preferred due to its operational easiness and can abide fluctuations during the process (Breitenmoser et al. 2019).

Technological advancements have greatly improved anaerobic therapy, and in the seventeenth century, Jan Baptista van Helmont found that combustible fumes might emerge from decaying organic substances (Rufai 2010). In 1776, Count Alessandro Volta established a link between the amount of organic matter degrading and flammable gas created. Sir Humphry Davy discovered that methane was produced during the anaerobic digestion (AD) of cattle manure in 1808 (Ghosh et al. 2019). The first digestion plant was created in 1859 in a leper colony in Bombay, India. With numerous benefits over aerobic treatment, anaerobic treatment has been nurtured as a viable and cost-effective alternative today (Abbasi et al. 2012). The historical evolution of AD is given in Table 7.2.

Anaerobic systems have much potential for treating low-concentration wastewaters since they are likely to operate with low hydraulic retention times and high solids retention periods (Singh et al. 2020). In anaerobic systems, 70–90% of

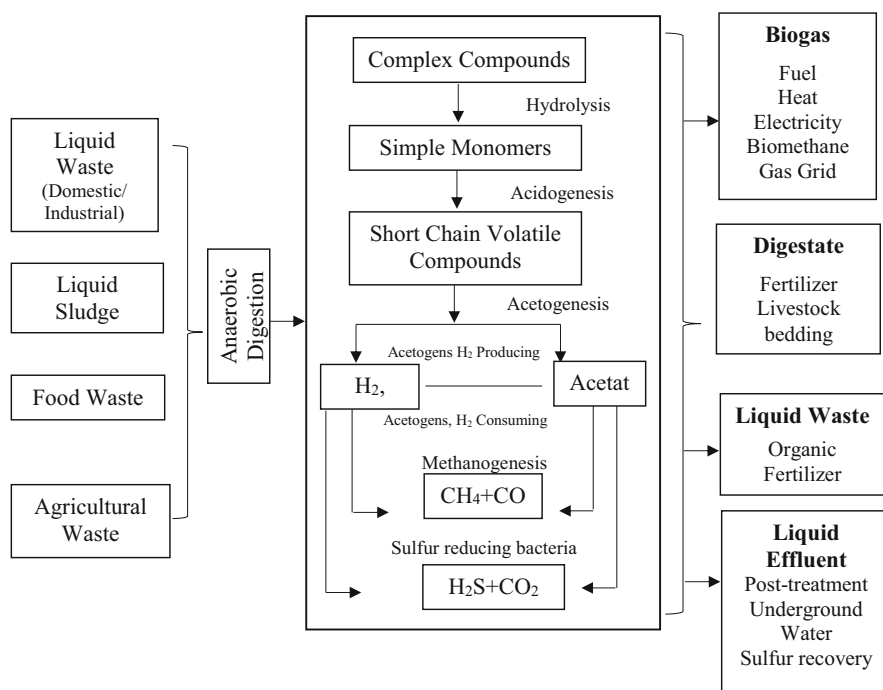
**Table 7.2** Historical evolution of anaerobic digestion

Time	Employed/ developed	Occurrence	Reference
Tenth century	Assyria	Biogas was utilized to heat water	Lusk (1998)
Sixteenth century	Persia		
Seventeenth century	Jan Baptista van Helmont	First to discover that combustible gases could form when organic matter decomposes	Al Mamun and Torii (2015)
1776	Count Alessandro Volta	The amount of organic matter decomposed and amount of flammable gas produced is proportionate	Lusk (1998)
1808	Sir Humphry Davy	It has been proven that anaerobic manure decomposition produces methane	Gashaw and Abile (2014)
1859	Bombay (Matunga Leper Asylum)	First anaerobic digestion plant in India	Ghosh et al. (2019)
1864	Pasteur	Fermentation process	Rufai (2010)
1876	Herter	Developed biomass-methane model and first internal combustion engine	Zehnder et al. (1982) Heywood (2018)
1895	Donald Cameron	Exeter's streets were lit with biogas from a septic tank in England	Cheremisnoff et al. (1980)
1904	Hampton	Installed first dual-purpose tank sedimentation and sludge treatment	Humenik et al. (2004)
1911	England	Anaerobic digestion of sludge in a lagoon	Humenik et al. (2004)
Early twentieth century	Germany	First patent issued for Imhoff tank	Imhoff (1938)
1930s	Buswell	Anaerobic conditions of bacteria required to promote methane production	Buswell and Heave (1930)
1937	S.V. Desai	First successful attempt for anaerobic digestion in India	Ghosh et al. (2019)
1939–1945	Germany and France	Digestion of manure for methane was increased	Markonis et al. (2013)
1970s	United States	Farm-scale AD was first used in the United States during the 1970s' oil crisis and developed a plug-flow digester for dairy manure	Humenik et al. (2004)
1980s	United States	Anaerobic digester failure rates were approaching 50% in manure-fed AD systems	Faulhaber et al. (2012)
1990s	EPA AgSTAR Program	Interest in energy and waste stabilization has resurfaced. Around 75 dairy and swine	Humenik et al. (2004)

(continued)

**Table 7.2** (continued)

Time	Employed/developed	Occurrence	Reference
		digesters were produced as a result of the EPA AgSTAR Program	
2010s	Biogas fuel	Utilization of biogas as fuel for internal and external combustion engines; development of biogas fueled co-generation and tri-generation systems	Rufai (2010) Kim et al. (2016)
Twenty-first century	Recent advances	Anaerobic digestion—bio-electrochemical system/microbial fuel cells/anaerobic co-digestion, digestion, thermodynamic analysis, microbial community analysis, anaerobic digestion biorefinery	Khanal et al. (2020)

**Fig. 7.2** Process and energy potential of anaerobic biodigestion

biodegradable organic matter gets converted into biogas, and about 5–15% of the organic material gets converted into microbial biomass as sludge (Fig. 7.2). Sludge is typically more concentrated and has higher dewatering properties (Ghosh et al. 2019).

Anaerobic decomposition is a biologically mediated, natural process that can be simulated to treat pollutants generated by municipal, agricultural, and industrial

activities. Anaerobic digesters have been used to stabilize sewage sludge (SS) for decades, but their successful and cost-effective usage to treat liquid wastes is a relatively new phenomenon, owing to the development of innovative reactor designs (Weiland 2010). Anaerobic bioreactors have the ability to hold a more significant biomass content than typical digesters; sludge retention is independent of influent retention time. This can be achieved by (1) attachment of biomass to the medium and (2) non-attached biomass as a suspended growth process (Ghosh et al. 2019).

Anaerobic digestion relies on a community of microbes known as the microbiota to stabilize organic materials in oxygen-free conditions. Organic substrates undergo a variety of metabolic transformations during anaerobic digestion, with methane ( $\text{CH}_4$ ) and carbon dioxide ( $\text{CO}_2$ ) as the significant gas products (Weiland 2010). Extracellular enzymes hydrolyze complicated organic materials into soluble products in the first stage. Next, sugars, amino acids, and long-chain fatty acids are fermented in the cell to produce volatile fatty acids, alcohols,  $\text{CO}_2$ , molecular hydrogen ( $\text{H}_2$ ), nitrogen, and sulfur compounds. Finally, the methanogenesis process converts  $\text{H}_2$ ,  $\text{CO}_2$ , and acetate ( $\text{CH}_3\text{COO}^-$ ) into  $\text{CH}_4$  (Jain et al. 2015; Ghosh et al. 2019).

The anaerobic process depends on the stability of various biological processes such as substrate utilization, specific growth rate, decay rate, and gas output, which might influence anaerobic digestion. For example, a higher concentration of volatile fatty acids during the first reaction stage indicates process instability (Rao and Saroj 2011). Methane-producing bacteria thrive in a pH range of 6.6–7.6, whereas non-methanogenic bacteria thrive in 5–8.5. The anaerobic process can occur at temperatures between 4 and 60 °C. The sludge digester operates in a mesophilic temperature range (30–40 °C), with 35 °C or above as the ideal temperature for anaerobic microbial growth with an optimal nitrogen-to-phosphorus ratio of 7 (Jena et al. 2017). The advantages and limitations of anaerobic digestion are given in Table 7.3.

The anaerobic process has been used to generate energy and treat waste for many years. It's employed in closed systems where microorganisms can be kept in controlled conditions. The purpose of this technique is to reduce the amount of waste that ends up in alternative treatment systems like landfills and incineration plants and to recycle the nutrients from the waste into agriculture (Breitenmoser et al. 2019). Furthermore, the process can be utilized for the efficient degradation of different waste materials, and today the technique is used mainly in four significant sectors of waste treatment: (1) municipal sewage; (2) industrial wastewater from biomass, food-processing, or fermentation industries; (3) livestock waste; and (4) organic portion of municipal solid waste (Angelidaki et al. 2003). The present paper aims to discuss the advancements of anaerobic digestion for sewage waste.

## 7.5.2 Anaerobic Digestion for Sewage Waste

Anaerobic digestion (AD) is an established technology for sewage sludge (SS) treatment. The high water content of sewage sludge can be processed in AD

**Table 7.3** Advantages and limitations of anaerobic digestion

Advantages	Disadvantages
Methane is produced through anaerobic digestion, a high-calorie fuel that may be used to generate heat and electricity while simultaneously creating fewer sediments	Anaerobic bacteria are harmful at obtaining energy from organic foods in comparison with their aerobic cousins
Digestion residues are highly valuable organic fertilizers that can be used instead of artificial fertilizers	pH, temperature, and alkalinity factors must be precisely managed, necessitating stricter process controls for optimum performance
Liquor is high in a variety of nutrients	Due to the presence of sulfur in waste feeds, hydrogen sulfide is produced during digestion
Anaerobic digestion lowers the BOD and COD of effluents, lowering the risk of contamination	Heavy metals are not removed by digestion; the only way to keep the system under control is to feed the cleanest feedstock possible
Pathogen populations are reduced when pasteurization is combined	Usually, some type of posttreatment is required
Anaerobic digestion can help to eliminate odor problems	
Anaerobic digestion can be carried out in small on-site agriculture projects to sizeable urban waste disposal facilities	
The use of anaerobic digestion to treat residential organic waste minimizes the amount of garbage in landfills	
Low construction and operational cost with low energy consumption	

without any pre-treatment (Ward et al. 2008; Hanum et al. 2019). Matunga Leper Asylum was the first AD invented in 1897 in Mumbai, India (Abbasi et al. 2012). They employed human feces to fuel the AD process to produce gas and minimize their electricity consumption. The first successful attempt for AD was undertaken in 1937 by S.V. Desai, a microbiologist at the Indian Agricultural Research Institute (IARI) (Ghosh et al. 2019).

AD is a major waste-to-energy technology for developing countries such as India, and a variety of reactor technologies and digester models are available presently (Ghosh et al. 2019). It can be done in batch or continuous mode. Batch operations carry out all phases of biochemical reaction in a single tank, and continuous procedures can be carried out by loading either continuously or semi-continuously, with single-stage or two-stage processes (Zupancic and Grlic 2012). In the traditional continuous AD method, biochemical stages are completed in a single step. At the same time, in a two-stage procedure, decomposition of organic wastes occurs first in hydrolysis-acidogenic reactor and methanogenic reaction in an upflow anaerobic sludge blanket (UASB) reactor, which increases biodegradation efficiency (Rajeshwari et al. 2001).

Digester designs might vary based on waste composting, its volume, and regional characteristics. Biogas plant models have been recognized by India's Ministry of New Renewable Energy (Ghosh et al. 2019). AD model of a floating dome with a

cylindrical digester fabricated using steel was developed by Khadi and Village Industries Commission (KVIC). The Deenbandhu model was a fixed-dome type with a hemisphere digester, which is considerably less expensive than the KVIC model (Thomas et al. 2017). The Pragati model is a hybrid with a hemisphere digester and floating drum and can yield higher gas. Tubular digesters are prepared with a polyethylene (tubelike) bag and a PVC pipeline (gas collection), but they are not suitable for systems with a high gas pressure (Ambulkar and Shekdar 2004; Thomas et al. 2017).

With a focus on India, Baral et al. (2020) evaluated the novelty of anaerobic digestion for sewage treatment followed by CO<sub>2</sub> capture by microalgae and aiming for zero waste discharge. The attention was given to sewage generation and treatment of pipelines in India's Class-I and Class-II metropolitan cities. The sewage COD would be transformed to CH<sub>4</sub> and CO<sub>2</sub>, with the latter being turned to microalgae in the photo-bioreactor deriving energy source from sunshine. The process is expected to provide roughly  $1.69 \times 10^8$  kWh day<sup>-1</sup> of energy, replacing 3% of India's current total petroleum product usage.

Anaerobic digestion (AD) is a viable and well-established method for SS disposal that provides stability, energy recovery (predominantly methane), and environmental protection (Zhen et al. 2016). However, the mono-digestion of sludge is sluggish and unstable due to a lack of nutrients, a low organic loading rate, limited biodegradability, and a high level of pollutant toxicity (Mata-Alvarez et al. 2014; Zhang et al. 2018; Pan et al. 2019). Co-digestion involving two or more substrates could effectively overcome these limitations by balancing the composition of an unbalanced substance, which can increase buffering capacity, speed up hydrolysis, and improve system stability and biogas output (Mehariya et al. 2018).

Researchers are paying more attention to co-digestion since it has a higher energy recovery efficiency (Pan et al. 2019). The second most common substrate for anaerobic co-digestion is sewage sludge (AcoD). Traditionally, the most extensively published co-digestion study has been AcoD between SS and the organic portion of municipal solid waste, and it has steadily increased the co-digestion of SS with fats, oils, and carbohydrates (Mata-Alvarez et al. 2014). Many positive experiences were reported when SS and fats and oils from various sources were co-digested. Wastewater treatment plants (WWTP) and industrial operations are the two primary sources of fats and oils.

Fats and oils account for 25–40% of total chemical oxygen demand (COD) in WWTPs, and they are typically eliminated (50–90%) before biological treatment, and its employment as a co-substrate saves the cost of residual treatment (Davidsson et al. 2008; Noutsopoulos et al. 2013). The methane yield was boosted by 60% when SS mix was co-digested with fat, oil, and grease from a meat processing factory (46% volatile solids (VS) added to the feed) (Luostarinen et al. 2009). The SS was co-digested with oil and grease from restaurants (48% of total VS load), and methane output was 2.6 times higher (Kabouris et al. 2009). Food waste (FW) was mixed in the range of 30–40% VS with SS in mesophilic conditions, and the maximum rate of methane was produced (Kim et al. 2003; Koch et al. 2016).

Incorporating SS into a mixed digester with around 1 m<sup>3</sup> of organic content from municipal solid waste per day increased biogas production by 25% (Edelmann et al. 2000). In addition, the size reduction of organic waste before the digestion process enhanced 20% of the digester's organic loading rate without affecting the plant's working conditions or functioning (Edelmann et al. 2000). A study on co-digestion of SS and microalgae showed improved digestate dewaterability and stability of the process (Sole-Bundo et al. 2017). Fountoulakis et al. (2010) observed that the methane generation rate increased to  $2353 \pm 94$  mL/day when SS was mixed with 1% glycerol, which was more than double that of SS mono-digestion (Fountoulakis et al. 2010). Co-digestion of sewage sludge with waste from coffee, poultry, and agricultural residues also improved performance (Neves et al. 2006; Borowski et al. 2014; Aylin Alagoz et al. 2018).

Pan et al. (2019) investigated SS and food waste's combined effect during AcoD and its biodegradation kinetics. The findings of the experiments confirmed the supremacy of anaerobic co-digestion of SS and food waste, and adding food waste might enhance the system stability leading to an increase in methane emission. The ratios of 0.5:0.5 of SS/FW achieved the maximum methane recovery with the shortest lag phase (0.182 days) and fastest hydrolysis rate (0.334/day) (Pan et al. 2019).

The banana plant was researched to improve the efficiency of the AD process, and several elements of the banana plant, including semi-dried banana leaves, were employed to produce biogas (Jena et al. 2017). In contrast, sewage water is a reliable source of microorganisms, allowing for efficient biomass biodegradation, while urea ( $\text{CO}(\text{NH}_2)_2$ ) helps to maintain the C/N ratio of digester mass and regulates the bacterial activities as well (Yao et al. 2018). Furthermore, with the synthesis of lactic acid and acetic acid, urea improves CH<sub>4</sub> output. According to reports, adding urea to lignocellulosic biomass exposes cellulose to the degradation process, which creates CH<sub>4</sub>. However, higher levels of urea addition (>3%) restrict methanogenic operations, resulting in lower CH<sub>4</sub> generation (Liu et al. 2015; Zhu et al. 2010; Jiang et al. 2012).

Several nanoparticles with precise physicochemical qualities such as high reactivity and large surface area were recently added to AD to speed up the biogas production rate (Baniamerian et al. 2019). Small amounts of heavy metallics (ferrous nanoparticles) increase CH<sub>4</sub> generation during anaerobic digestion processes, prevent hydrogen sulfide (H<sub>2</sub>S) production, and determine the associated reactions connected to organic matter biodegradation (Luna-delRisco et al. 2011; Su et al. 2013; Wang et al. 2016). Jena et al. (2020) investigated the suitability of AD for semi-dried banana leaves in liquid sewage. This study looked at the quantity and quality of biogas and the rate of biogas (v/v) evolution, which was shown to be lowest without additives and raised with urea, FeCl<sub>3</sub>, and a combination of urea FeCl<sub>3</sub> additions, respectively.

Zahan et al. (2016) investigated the impacts of food waste co-digestion with SS. With the addition of 1–5% food waste to SS, specific biogas production increased by 25–50%, which is significantly higher than the  $284 \pm 9.7$  mLN/g produced with the addition of VS. Different ratios of SS/FW were investigated in

batch biochemical methane potential studies, and the optimum balance of 47–48% food waste can be co-digested with SS for better efficiency. Municipal sludge from primary settling tanks of STP is potentially valuable biomass for biogas production. Sludge is produced in large quantities every day at wastewater treatment plants with anaerobic digesters. The combination of wastewater treatment with a microalgal culture is now regarded as a promising path for producing renewable bioenergy through biodiesel or biogas (Subhadra and Edwards 2010; Ajeer et al. 2015).

Upflow anaerobic sludge blanket (UASB) offers a long-term option for cleaning domestic wastewater in developing countries. The failure of the UASB procedure to satisfy the intended disposal requirements, on the other hand, has been directed at posttreatment of effluent. UASB-based STPs can be upgraded using a variety of technological ways to obtain acceptable effluent quality for disposal or reuse, viz., primary posttreatment (eliminates organic and inorganic compounds and suspended matter) and secondary posttreatment (removes degradable, soluble, and colloidal matter and nutrients) (Lettinga 2008).

Lettinga et al. (1993), Seghezzi et al. (2002), and Lettinga (2008) investigated the application of the UASB method for sewage treatment and found that in countries with warm temperatures, roughly 70% reduction of chemical oxygen demand (COD) may be achieved (Siddiqi 1990; Khan et al. 2011). India has installed approximately 30 UASB-based STPs since the late 1980s, with another 20 under progress (MoEF 2005, 2006). The single-step UASB approach has proven to be a viable option in warm temperature zones, but the treatment's efficiency reduces with a drop in temperature achieving only 50% removal of COD at 15 °C (Singh and Viraraghavan 2002; Lew et al. 2003).

Elmitwalli et al. (2003) investigated a two-step anaerobic system operating at low temperatures and an anaerobic system containing an anaerobic filter, an anaerobic hybrid reactor, and a trickling filter. Working conditions were identical to those of other two-step systems. However, adding a trickling filter improved the performance of a two-step anaerobic system from 63% to 85%, and effluent was reused for controlled irrigation, and nutrients could be reclaimed.

The natural biological mineralization (NBM) treatment strategy for low- and high-strength wastewater treatment relies on anaerobic processes. In combination with the NBM system, high-rate anaerobic treatment systems are unquestionably a viable path to long-term environmental preservation and, ultimately, a more sustainable society. Anaerobic pre-treatment, followed by NBM treatment methods, can yield the maximum reuse or recovery of resources (Khan et al. 2011).

There has been documentation of a wide range of posttreatment arrangements based on various UASB combinations. The complete eradication of pathogens from the effluent of the UASB reactor treating sewage would be a distinct component of the posttreatment to protect human health (Khan et al. 2011). Prakash et al. (2007) used coagulation and flocculation as a posttreatment method to analyze the effluent of a 38 MLD UASB reactor handling sewage in India.

Sewage sludge is a by-product of wastewater treatment and a type of biomass. Therefore, SS could be used for energy recovery or biofuel production via anaerobic digestion. In Italy, Bianchini et al. (2015) chose SS as a waste-to-energy substrate



with the addition of a drying process, with the effluent sludge having a water content of 71.8–79.0% and a heating value of 12.7–15.5 MJ/kg. Magdziarz and Wilk (2013) related the calorific values of coal, biomass, and sewage sludge which were estimated to be ~23.5, 17.6, and 12.8 MJ/kg, respectively.

In India, emphasis on sewage sludge management studies is considered limited (Singh et al. 2020). Around 62,000 MLD of sewage is produced in India, of which 20,120 MLD gets treated; Facilities to treat 3157 MLD are being constructed; and 38,722 MLD has no treatment facility (CPCB 2015). Sewage sludge treatment produces 3955 thousand metric tons of dry sewage sludge per year, resulting in proper sludge management. Batcha and Kirubakaran (2018) and Kale et al. (2017) described the basic energy recovery technique from SS via the thermal pathway.

In India, there is a shortage of proper sewage sludge management and treatment facilities. As a result, the majority of the sludge produced in India is dumped in landfills. Although some WWTPs feature anaerobic sludge digesters, the majority of them flare the methane delivered. Singh et al. (2020) looked into the energy recovery potential of SS in India via incineration and anaerobic digestion. Sludge dry matter has been shown to have a calorific value of 8–21 MJ/kg. The energy recovery perspective of SS was 555–1068 kWh/ton under incineration and 315–608 kWh/ton for anaerobic digestion on a dry matter basis (Singh et al. 2020). The review of various studies on anaerobic digestion advises that sludge must be classified as an energetic substance with a potential for energy recovery across the country, which aids in energy recovery and addresses sludge management in India.

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## 7.6 Conclusion

Anaerobic digestion (AD) is a potential method for producing significant hydrogen and methane from sewage wastes. This clean energy could be used as a fuel source in combustion engines and lower greenhouse gas emissions. Furthermore, the optimum temperature, pH, retention time, organic loading rate, moisture, and carbon-to-nitrogen ratio are significant, which might influence the activity of microorganisms in producing biogas. Thus, AD has enormous potential to help cities and industries manage organic waste, add to the country's energy grid diversification, promote innovative bioenergy research and knowledge generation, and produce new products.

Sewage treatment using AD can help India implement a wide range of policies across industries and contribute to the achievement of crucial sustainable development goals (SDGs 2, 3, 6, 7, 11–15). AD converts waste into a sustainable energy source, as encouraged by India's renewable energy projects for the past 30 years, and creates digestate, a valuable bio-fertilizer or soil enhancer. It also reduces the harmful health and environmental effects. However, land availability, installation costs, financial backing, and institutional capacity are vital for the long-term viability of AD treatment. Despite being a well-established biotechnological process, anaerobic digestion continues to attract a great scientific interest, owing to its diversity in

terms of substrate and product spectrum, as well as its suitability for anaerobic microbial ecology.

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## References

- Abbasi T, Tauseef SM, Abbasi SA (2012) A brief history of anaerobic digestion and “biogas”. In: *Biogas energy*. Springer, New York, pp 11–23
- Ahmed A, El-Gohary F, Tzanakakis V, Angelakis A (2020) Egyptian and Greek water cultures and hydro-technologies in ancient times. *Sustain For* 12:9760
- Aiello AE, Larson MS, Sedlak R (2008) Hidden heroes of the health revolution. Sanitation and personal hygiene. *Am J Infect Control* 36:128–151
- Ajeeb A, Joseph T, Narayan CM, Senthilkumar R (2015) An overview of bio augmentation of methane by anaerobic co-digestion of sewage sludge along with microalgae and waste paper. *Renew Sust Energ Rev* 50:270–276
- Al Mamun MR, Torii SH (2015) Enhancement of production and upgradation of biogas using different techniques—a review. *Int J Earth Sci Eng* 8(2):877–892
- Ambulkar AR, Shekdar AV (2004) Prospects of biomethanation technology in the Indian context: a pragmatic approach. *Resour Conserv Recycl* 40(2):111–128
- Amerasinghe P, Bhardwaj RM, Scott C, Jella K, Marshall F (2013) Urban wastewater and agricultural reuse challenges in India. *International Water Management Institute Research Report* p 147
- Angelakis AN, Koutsoyiannis D, Tchobanoglous G (2005) Urban wastewater and storm water technologies in ancient Greece. *Water Res* 39:210–220
- Angelakis AN, Savvakis YM, Charalampakis G (2007) Aqueducts during the Minoan Era. *Water Sci Technol* 7:95–101
- Angelakis AN, De Feo G, Laureano P, Zourou A (2013) Minoan and Etruscan hydro-technologies. *Watermark* 5:972–987
- Angelidaki I, Ellegaard L, Ahring BK (2003) Applications of the anaerobic digestion process. *Adv Biochem Eng Biotechnol* 82:1–33
- Antoniou GP, De Feo G, Fardin F, Tamburrino A, Khan S, Tie F, Reklaityte I, Kanetaki E, Zheng XY, Mays LW, Angelakis AN (2016) Evolution of toilets worldwide through the millennia. *Sustain For* 8:1–55
- Anukam A, Ali M, Muhammad N, Karin G (2019) A review of the chemistry of anaerobic digestion: methods of accelerating and optimizing process efficiency. *Processes* 7:1–19
- Aylin Alagoz B, Yenigün O, Erdiñçler A (2018) Ultrasound assisted biogas production from co-digestion of wastewater sludges and agricultural wastes: comparison with microwave pre-treatment. *Ultrason Sonochem* 40:193–200
- Baniamerian H, Isfahani PG, Tsapekos P, Alvarado-Morales M, Shahrokhi M, Vossoughi M, Angelidaki I (2019) Application of nano-structured materials in anaerobic digestion: current status and perspectives. *Chemosphere* 229:188–199
- Baral S, Davide D, Dileep M, Akash G, Ankit K, Gourav G, Parag J (2020) Biofuel production potential from wastewater in India by integrating anaerobic membrane reactor with algal photobioreactor. *Biomass Bioenergy* 133:105445
- Batcha K, Kirubakaran V (2018) Feasibility study of sewage sludge for thermochemical conversion. *Int J Appl Eng Res* 13:12611–12615
- Bianchini A, Bonfiglioli L, Pellegrini M, Saccani C (2015) Sewage sludge drying process integration with a waste-to-energy power plant. *Waste Manag* 42:159–165
- Borowski S, Domański J, Weatherley L (2014) Anaerobic co-digestion of swine and poultry manure with municipal sewage sludge. *Waste Manag* 34(2):513–521
- Breasted JH (1906) *Ancient records of Egypt, vol. V: historical documents from the earliest times to the Persian conquest*. University of Chicago Press, Chicago

- Breitenmoser L, Gross T, Huesch R, Rau J, Dhar H, Kumar S, Hugi C, Wintgens T (2019) Anaerobic digestion of biowastes in India: opportunities, challenges and research needs. *J Environ Manag* 236:396–412
- Burian SJ, Edwards FG (2002) Historical perspectives of urban drainage. *Glob Solut Urban Drain* 284:1–16
- Buswell AM, Heave SL (1930) State water survey, bulletin No. 30. State of Illinois, Urbana
- Chahal C, van den Akker B, Young B, Franco C, Blackbeard J, Monis P (2016) Pathogen and particle associations in wastewater: significance and implications for treatment and disinfection processes. *Adv Appl Microbiol* 97:63–119
- Cheremisinoff NP, Cheremisinoff PN, Ellerbusch F (1980) Biomass: applications, technology, and production. Marcell Dekker Inc, New York
- Cooper PF (2007) Historical aspects of wastewater treatment. In: Lens P, Zeeman G, Lettinga G (eds) Decentralised sanitation and reuse: concepts, systems and implementation. IWA Publishing, London
- CPCB (2015) Inventorization of sewage treatment plant. Available at [https://nrcd.nic.in/writereaddata/FileUpload/NewItem\\_210\\_Inventorization\\_of\\_Sewage-Treatment\\_Plant.pdf](https://nrcd.nic.in/writereaddata/FileUpload/NewItem_210_Inventorization_of_Sewage-Treatment_Plant.pdf). Accessed 17 July 2021
- Crini G, Lichtfouse E (2019) Advantages and disadvantages of techniques used for wastewater treatment. *Environ Chem Lett* 17:145–155
- Cruz I, Larissa A, Ram B, Ashok N, Muhammad B, Renan T, Luiz Fernando F (2021) An overview of process monitoring for anaerobic digestion. *Biosyst Eng*. <https://doi.org/10.1016/j.biosystemseng.2021.04.008>
- Cullet P, Gupta J (2009) Evolution of water law and policy. In: The evolution of the law and politics of water. Springer, New York, pp 157–173
- Davidsson A, Lovstedt C, la Cour JJ, Gruvberger C, Aspegren H (2008) Co-digestion of grease trap sludge and sewage sludge. *Waste Manag* 28:986–992
- De Feo G, Antoniou G, Fardin F, El-Gohary F, Zheng X, Reklaityte I, Butler D, Yannopoulos S, Angelakis A (2014) The historical development of sewers worldwide. *Sustain For* 6:3936–3974
- Edelmann W, Engeli H, Gradenecker M (2000) Co-digestion of organic solid waste and sludge from sewage treatment. *Water Sci Technol* 41:213–221
- Elmitwalli TA, Sayed S, Groendijk L, van Lier J, Zeeman G, Lettinga G (2003) Decentralised treatment of concentrated sewage at low temperature in a two-step anaerobic system: two upflow-hybrid septic tanks. *Water Sci Technol* 48(6):219–226
- Elsayed M, Manar EA (2019) Wastewater treatment methodologies. *Int J Environ Agri Sci* 3(1): 1–18
- Faruqui NI (2001) Islam and water management: overview and principles. In: Faruqui NI, Biswas AK, Bino MJ (eds) Water management in Islam. United Nations University Press, Tokyo
- Faulhaber C, Raman D, Robert B (2012) An engineering-economic model for analyzing dairy plug-flow anaerobic digesters: cost structures and policy implications. *Trans ASABE* 55:201–209
- Fountoulakis MS, Petousi I, Manios T (2010) Co-digestion of sewage sludge with glycerol to boost biogas production. *Waste Manag* 30(10):1849–1853
- Fraisse PH, Moretti JC, Theatre L (2007) Exploration archéologique de Délos XLII E.F. Athens, Hellas
- Gal J (2008) The discovery of biological enantioselectivity: Louis Pasteur and the fermentation of tartaric acid, 1857 - A review and analysis 150 yr later. *Chirality* 20(1):5–19
- Gandy M (1999) The Paris Sewers and the Rationalization of Urban Space. *Trans Inst Br Geogr* 24(1):23–44
- Gashaw A, Abile M (2014) Co-digestion of Ethiopian food waste with cow dung for biogas production, co-digestion of Ethiopian food waste with cow dung for biogas production. *Int J Res* 1(7):475–500
- Getzler J (2004) A history of water rights at common law. Oxford University Press, Oxford
- Ghosh A, Sarkar JP, Das B (2019) A critical analysis on anaerobic digestion of OFMSW in India. In: Ghosh S (ed) Waste valorisation and recycling. Springer, Singapore

- Gill G, Burrell S, Brown J (2001) Fear and frustration - the Liverpool cholera riots of 1832. *Lancet* 358:233–237
- Good IL, Kenoyer JM, Meadow RH (2009) New evidence for early silk in the Indus civilization. *Archaeometry* 51:457–466
- Gray HF (1940) Sewerage in ancient and medieval times. *Sew Work J* 12(5):939–946
- Gupta J, van der Zaag P (2008) Interbasin water transfers and integrated water resources management: where engineering, science and politics interlock. *Phys Chem Earth* 33:28–40
- Hanum F, Yuan LC, Kamahara H, Aziz HA, Atsuta Y, Yamada T, Daimon H (2019) Treatment of sewage sludge using anaerobic digestion in Malaysia: current state and challenges. *Front Energy Res* 7:19
- Heywood JB (2018) *Internal combustion engine fundamentals*. McGraw-Hill Education, New York
- Hopkins JNN (2007) The cloaca maxima and the monumental manipulation of water in archaic Rome. *Waters* 4:1–15
- Humenik F, Martin J, Rice M, Roos K (2004) *Anaerobic digestion of animal manure*. EPA, Washington
- Imhoff K (1938) (L. Pearse, ea.) *modern se-wage disposal*. Lancaster Press, Lancaster, p 47
- Jain S, Jain S, Wolf IT, Lee J, Tong YW (2015) A comprehensive review on operating parameters and different pre-treatment methodologies for anaerobic digestion of municipal solid waste. *Renew Sust Energy Rev* 52:142–154
- Jansen M (1989) Water supply and sewage disposal at Mohenjo-Daro. *World Archaeol* 21(2): 177–192
- Jena SP, Mishra S, Acharya SK, Mishra SK (2017) An experimental approach to produce biogas from semi dried banana leaves. *Sustain Energy Technol Assess* 19:173–178
- Jena SP, Mohanty UK, Mahapatra S (2020) Anaerobic digestion of semi-dried banana leaves in sewage water under the influence of certain additives. *Mater Today Proc.* <https://doi.org/10.1016/j.matpr.2020.02.830>
- Jiang Y, Heaven S, Banks CJ (2012) Strategies for stable anaerobic digestion of vegetable waste. *Renew Energy* 44:206–214
- Jones DE (1967) Urban hydrology a redirection. *Civ Eng* 37:58–62
- Juwarkar AS, Oke B, Juwarkar A, Patnaik SM (1995) Domestic wastewater treatment through constructed wetland in India. *Water Sci Technol* 32:291–294
- Kabouris JC, Tezel U, Pavlostathis SG (2009) Methane recovery from the anaerobic codigestion of municipal sludge and FOG. *Bioresour Technol* 100(15):3701–3705
- Kale VB, Aashirvad V, Rathod S, Nikumbh C, Bhere R, Rasgotra SS (2017) Effective utilization of sewage sludge for production of energy. *Int Res J Eng Technol* 65:3353–3357
- Kaur R, Wani S, Singh A, Lal K (2012) *Wastewater production, treatment and use in India*. Available at [www.ais.unwater.org/ais/pluginfile.php/356/mod\\_page/content/111/CountryReport\\_India.pdf](http://www.ais.unwater.org/ais/pluginfile.php/356/mod_page/content/111/CountryReport_India.pdf)
- Khan A, Rubia G, Vinay T, Anwar K, Beni L, Indu M, Kazmi AA (2011) Sustainable options of post treatment of UASB effluent treating sewage: a review. *Resour Conserv Recycl* 55:1232–1251
- Khanal S, Wong J, Sanchez A, Insam H (2020) Recent advances in anaerobic digestion. *Bioresour Technol* 316:123955
- Kim HW, Han SK, Shin HS (2003) The optimisation of food waste addition as a co-substrate in anaerobic digestion of sewage sludge. *Waste Manag Res* 21(6):515–526
- Kim Y, Kawahara N, Tsuboi K, Tomita E (2016) Combustion characteristics and NOX emissions of biogas fuels with various CO<sub>2</sub> contents in a micro co-generation spark-ignition engine. *Appl Energy* 15:539–547
- Koch K, Plabst M, Schmidt A, Helmreich B, Drewes JE (2016) Co-digestion of food waste in a municipal wastewater treatment plant: comparison of batch tests and full-scale experiences. *Waste Manag* 47:28–33
- Lettinga G (2008) Towards feasible and sustainable environmental protection for all. *Aquat Ecosyst Health Manage* 11(1):116–124

- Lettinga G, deMan A, van der Last ARM, Wiegant W, van Knippenberg K, Frijns J (1993) Anaerobic treatment of domestic sewage and wastewater. *Water Sci Technol* 27(9):67–73
- Lew B, Belavski M, Admon S, Tarre S, Green M (2003) Temperature effect on UASB reactor operation for domestic wastewater treatment in temperate climate regions. *Water Sci Technol* 48(3):25–30
- Liu S, Ge X, Liew LN, Liu Z, Li Y (2015) Effect of urea addition on giant reed ensilage and subsequent methane production by anaerobic digestion. *Bioresour Technol* 192:682–688
- Lofrano G, Brown J (2010) Wastewater management through the Ages: a history of mankind. *Sci Total Environ* 408:5254–5264
- Luna-delRisco M, Orupold K, Douraguier HC (2011) Particle-size effect of CuO and ZnO on biogas and methane production during anaerobic digestion. *J Hazard Mater* 189:603–608
- Luostarinen S, Luste S, Sillanpaa M (2009) Increased biogas production at wastewater treatment plants through co-digestion of sewage sludge with grease trap sludge from a meat processing plant. *Bioresour Technol* 100(1):79–85
- Lusk P (1998) Methane recovery from animal manures: a current opportunities casebook, 3rd edn. National Renewable Energy Laboratory, Golden
- Magdziarz A, Wilk M (2013) Thermal characteristics of the combustion process of biomass and sewage sludge. *J Therm Anal Calorim* 114(2):519–529
- Markonis Y, Angelakis AN, Christy J, Koutsoyiannis D (2013) Climatic variability and the evolution of water technologies in Crete. *Water Res Manage* 8(2):1–35
- Mata-Alvarez J, Dosta J, Romero-Güiza MS, Fonoll X, Peces M, Astals S (2014) A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renew Sust Energ Rev* 36: 412–427
- Mehariya S, Patel AK, Obulisamy PK, Punniyakotti E, Wong JWC (2018) Co-digestion of food waste and sewage sludge for methane production: current status and perspective. *Bioresour Technol* 265:519–531
- MoEF (2005) Management information system, technical report, National River Conservation Directorate. Ministry of Environment and Forests, New Delhi
- MoEF (2006) Management information system, technical report, National River Conservation Directorate. Ministry of Environment and Forests, New Delhi
- Muralidhar S (2006) The right to water: an overview of the Indian legal regime. In: Riedel E, Rothen P (eds) *The human right to water*. Berliner Wissenschafts, Berlin, pp 65–81
- Naff T, Dellapenna JW (2002) Can there be a confluence? A comparative consideration of western and Islamic fresh water law. *Water Policy* 4:465–489
- Nassar MY, Ali EI, Zakaria ES (2017) Tunable auto-combustion preparation of TiO<sub>2</sub> nanostructures as efficient adsorbents for the removal of an anionic textile dye. *RSC Adv* 7: 8034–8050
- Neves L, Oliveira R, Alves MM (2006) Anaerobic co-digestion of coffee waste and sewage sludge. *Waste Manag* 26(2):176–181
- Neves GN, Berni M, Dragone G, Mussatto S, Forster-Carneiro T (2018) Anaerobic digestion process: technological aspects and recent developments. *Int J Environ Sci Technol* 15:2033–2046
- Noutsopoulos C, Mamais D, Antoniou K, Avramides C, Oikonomopoulos P, Fountoulakis I (2013) Anaerobic co-digestion of grease sludge and sewage sludge: the effect of organic loading and grease sludge content. *Bioresour Technol* 131:452–459
- Pan Y, Zhongxiang Z, Guangyin Z, Xueqin L, Peter B, Yu-You L, Youcai Z, Rajesh B (2019) Synergistic effect and biodegradation kinetics of sewage sludge and food waste mesophilic anaerobic co-digestion and the underlying stimulation mechanisms. *Fuel* 253:40–49
- Pandey P, Philip K, Michelle S, Sagor B, Vijay S (2014) Contamination of water resources by pathogenic bacteria. *AMB Express* 4:51
- Pathak B (2001) Our toilets—Indian experience. First world toilet summit 2001 conference. Rest Room Associations, Singapore

- Prakash KJ, Tyagi VK, Kazmi AA, Kumar A (2007) Post-treatment of UASB reactor effluent by coagulation and flocculation process. *AIChE Environ Prog* 26(2):164–168
- Rajeshwari KV, Lata K, Pant DC, Kishore VVN (2001) A novel process using enhanced acidification and a UASB reactor for biomethanation of vegetable market waste. *Waste Manag Res* 19: 292–300
- Rao P, Saroj B (2011) Experimental design of mixture for the anaerobic co-digestion of sewage sludge. *Chem Eng J* 172:977–986
- Rashid R, Iqrash S, Parveen A, Iqbal J, Hussain M (2021) A state-of-the-art review on wastewater treatment techniques: the effectiveness of adsorption method. *Environ Sci Pollut Res* 28:1–17
- Rufai I (2010) A review of the evolution and development of anaerobic digestion technology. *J Eng Technol* 5(1):100–111
- Saravanan A, Kumar PS, Yaashikaa PR, Kanmani S, Varthine RH, Muthu CMM, Yuvaraj D (2019) Modelling on the removal of dye from industrial wastewater using surface improved *Enteromorpha intestinalis*. *Int J Environ Res* 13:349–366
- Sawchuk L, Burke S (1998) Gibraltar's 1804 yellow fever scourge: the search for scapegoats. *J Hist Med Allied Sci* 53:3–42
- Schellenberg T, Subramanian V, Ganeshan G, Tompkins D, Pradeep R (2020) Wastewater discharge standards in the evolving context of urban sustainability—The Case of India. *Front Environ Sci* 8:1–23
- Schladweiler JC (2002) Tracking down the roots of our sanitary sewers. In: Kurz GE (ed) *Proceedings of the ASCE pipeline division of ASCE*, Cleveland, OH, USA. American Society of Civil Engineers, Reston, pp 1–27
- Seeger H (1999) The history of German wastewater treatment. *Eur Water Manag* 2:51–56
- Seghezze L, Guerra R, González S, Trupiano AP, Figueroa M, Cuevas C, Zeeman G, Lettinga G (2002) Removal efficiency and methanogenic activity profiles in a pilot-scale UASB reactor treating settled sewage at moderate temperatures. *Water Sci Technol* 45:243–248
- Sermeri SN (2007) The construction of the modern city and the management of water resources in Italy, 1880–1920. *J Urban Hist* 33:813–827
- Shafiq I, Hussain M, Shehzad N, Maafa IM, Akhter P, Shafique S, Razaq A, Yang W, Tahir M, Russo N (2019) The effect of crystal facets and induced porosity on the performance of monoclinic BiVO<sub>4</sub> for the enhanced visible-light driven photocatalytic abatement of methylene blue. *J Environ Chem Eng* 7:1–10
- Shelford VE (1912) An experimental study of the effects of gas waste upon fishes with especial reference to stream pollution. *Bull Illinois State Lab Nat Hist* 11:379–425
- Shifrin N (2005) Pollution management in the twentieth century. *J Environ Eng* 131(5):676
- Siddiqi RH (1990) Treatment of municipal wastewater by UASB reactor, performance of 5 MLD Plant at Kanpur, Aligarh, India. Dept. of Civil Engineering, Aligarh Muslim University
- Siddiqui IA (1992) History of water laws in India. In: Singh C (ed) *Water law in India*. New Delhi, Indian Law Institute, pp 289–319
- Singh KS, Viraraghavan T (2002) Impact of temperature on performance, microbiological and hydrodynamic aspects of UASB reactors treating municipal wastewater. In: *Proceedings of Seventh Latin American Workshop and Symposium on Anaerobic Digestion*, p 613–620
- Singh A, Sawant M, Kamble SJ, Herlekar M, Starkl M, Aymerich E (2019) Performance evaluation of decentralized wastewater treatment system in India. *Environ Sci Pollut Res* 26:21172–21188
- Singh V, Harish P, Munish C (2020) Estimation of energy recovery potential of sewage sludge in India: waste to watt approach. *J Clean Prod* 276:122538
- Sole-Bundo M, Cucina M, Folch M, Tapias J, Gigliotti G, Garfí M (2017) Assessing the agricultural reuse of the digestate from microalgae anaerobic digestion and co-digestion with sewage sludge. *Sci Total Environ* 586:1–9
- Sorcinelli P (1998) *Storia sociale dell'acqua*. Ritie culture. Mondadori, Milano
- Stanbridge HH (1976) *History of sewage treatment in Britain* (in 12 volumes). Institute of Water Pollution Control, Maidstone

- Su L, Shi X, Guo G (2013) Stabilization of sewage sludge in the presence of nanoscale zero-valent iron (nZVI): abatement of odor and improvement of biogas production. *J Mater Cycles Waste Manage* 15:461–468
- Subhadra B, Edwards M (2010) An integrated renewable energy park approach for algal biofuel production in United States. *Energy Policy* 38:4897–4902
- Tarlow S (2007) The archaeology of improvement. In: Britain. Cambridge University Press, Cambridge, pp 1750–1850
- Taylor RH (1996) The rise and fall of cholera in the nineteenth century. *Gastroenterol Int* 9:159
- Theoharidis P (1991) Examples of residence in Mt Athos Forts, castles and monasteries cells of the early Ottoman period. The monastery of Simonos Petra. In: Lavas G, Philipides D (eds) Greek traditional architecture: Macedonia B. Melissa, Athens, pp 271–276
- Thomas P, Soren N, Rumjit NP, James JG, Saravanakumar MP (2017) Biomass resources and potential of anaerobic digestion in Indian scenario. *Renew Sust Energ Rev* 77:718–730
- Tolle-Kastenbein R (2005) *Archeologia dell' Acqua*, Longanesi. Casa Editrice Longanesi and C. S. r.l. Società a Socio Unico, 20145
- Tulchinsky TH (2018) John Snow, Cholera, the Broad Street Pump; Waterborne diseases then and now. *Case Studies in Public Health*, p 77–99
- Vuorinen HS (2010) Water, toilets and public health in the roman era. *Water Sci Technol* 10:411–415
- Vuorinen HS, Juuti PS, Katko TS (2007) History of water and Health from ancient civilizations to modern times. *Water Sci Technol* 7:49–57
- Wang T, Zhang D, Dai L, Chen Y, Dai X (2016) Effects of metal nanoparticles on methane production from waste-activated sludge and microorganism community shift in anaerobic granular sludge. *Nat Sci Rep* 6:25857
- Ward AJ, Hobbs PJ, Holliman PJ, Jones DL (2008) Optimization of the anaerobic digestion of agricultural resources. *Bioresour Technol* 99:7928–7940
- Webster C (1962) The sewers of Mohenjo-Daro. *J Water Pollut* 34:116–123
- Weiland P (2010) Biogas production: current state and perspectives. *Appl Microbiol Biotechnol* 85(4):849–860
- Winter T, Harvey J, Franke O, Alley W (1998) Ground water and surface water a single resource: U.S. Geol Surv Circ, p 1139
- Wolfe P (1999) History of wastewater. *World of water 2000—the past, present and future*. Water World/Water and Wastewater International Supplement to Penn Well Magazines, Tulsa
- Xu Z, Wei C, Jin J, Xu W, Wu Q, Gu J, Ou M, Xu X (2018) Development of a novel mixed titanium, silver oxide polyacrylonitrile nanofiber as a superior adsorbent and its application for MB removal in wastewater treatment. *J Braz Chem Soc* 29:560–571
- Yannopoulos S, Yapijakis C, Kaiafa-Saropoulou M, Antoniou G, Angelakis A (2017) History of sanitation and hygiene technologies in the Hellenic World. *J Water Sanit Hyg Dev* 7:163–180
- Yao Y, Bergeron AD, Davaritochae M (2018) Methane recovery from anaerobic digestion of urea-pretreated wheat straw. *Renew Energy* 115:139–148
- Zahan Z, Othman M, Rajendram W (2016) Anaerobic codigestion of municipal wastewater treatment plant sludge with food waste: a case study. *Biomed Res Int* 2016:1–13
- Zehnder AJ, Ingvorsen K, Marti T (1982) Anaerobic digestion. In: Hughes DE et al (eds) *Anaerobic digestion*. Elsevier Biomedical Press, Amsterdam

- Zhang X, Chen J, Idossou V, Tyagi RD, Li J, Wang H (2018) Lipid accumulation from *Trichosporon oleaginosus* with co-fermentation of washed wastewater sludge and crude glycerol. *Fuel* 226:93–102
- Zhen G, Lu X, Kobayashi T, Kumar G, Xu K (2016) Anaerobic co-digestion on improving methane production from mixed microalgae (*Scenedesmus* sp., *Chlorella* sp.) and food waste: kinetic modeling and synergistic impact evaluation. *Chem Eng J* 299:332–341
- Zhu J, Wan C, Li Y (2010) Enhanced solid-state anaerobic digestion of corn stover by alkaline pretreatment. *Bioresour Technol* 101:7523–7528
- Zupancic GD, Grilc V (2012) Anaerobic treatment and biogas production from organic waste. InTech, Shanghai





# Decentralized Anaerobic Digestion Technology for Improved Management of Human Excreta in Nigeria

# 8

Chukwudi O. Onwosi, Victor C. Igbokwe, and Flora N. Ezugworie

## Abstract

The management of human excreta is unarguably a serious problem in Nigeria. This problem is even more pronounced in rural and semi-urban settings with inefficient or no waste management plans and structures. Anaerobic digestion is an innovative technology that has been widely utilized in treating fecal wastes because of its minimal environmental impacts and simultaneous generation of biogas and a nutrient-rich end product with great agronomic value. Incorporating small-scale AD technology into sewage management is an innovative, low-cost, waste-free strategy. Although AD can be implemented in various settings, establishing a functioning decentralized AD will be the most attractive option to valorize human excreta in Nigeria. The purpose of this chapter is to present decentralized anaerobic digestion (DAD) as a viable alternative to Nigeria's current human excreta management schemes. Decentralized AD of human excreta is a cost-effective option to minimize waste transportation costs while maximizing community benefits in terms of clean environment and maintenance of public health and safety. The risks and hazards of decentralized AD are

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explored in this study, as well as measures for controlling and preventing it. Practical suggestions to achieve mass adoption of decentralized AD in Nigeria are also discussed here.

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**Keywords**

Human excreta · Decentralized anaerobic digestion · Nigeria · Biogas · On-site sanitation system

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## 8.1 Introduction

Human excreta pose a significant environmental and public health concern, thereby making its management a severe problem (Duan et al. 2020). In developing nations like Nigeria, excreta disposal is mostly by non-sewerage water-dependent methods in urban areas and non-sewerage water-independent methods in rural and semi-urban regions, both of which do not require any prior treatment(s) of the waste before disposal (Igwe et al. 2020). Centralized wastewater or sewage treatment plants are usually absent in Nigeria largely due to the high cost of installation and maintenance (Ehizemhen et al. 2018), insufficient space, and lack of information on the reuse benefits of fecal waste (Olafeju et al. 2019).

In the stead of wastewater or sewage treatment plants, septic tanks, soak-away pits, and their variants are conventional on-site sanitation systems (OSS) constructed and connected to toilets in households to contain the excreta generated. Disappointingly, a WHO/UNICEF report published in 2019 estimated that less than 1% of the urban population in low-income countries evacuated and processed the fecal sludge generated from their OSS (WHO/UNICEF 2019; Rotowa and Ayadi 2020). Interestingly, this is very much the obvious scenario in the majority of Nigerian cities. This is a serious threat to the sustainability of OSS and its variants as long-term retention of the excreta will put more pressure on the installations and eventually cause a collapse of the structures. Thus, it is no longer sufficient to build on-site sanitation facilities to manage human excreta but to find innovative ways to transform the threats of long-term excreta retention to benefits especially at the household or communal level. Anaerobic digestion is one of such innovative technology that has been widely utilized for treating various fecal wastes, since it can minimize environmental impacts of excrement storage (Andriani et al. 2015; Duan et al. 2020) simultaneously leading to the generation of biogas and a nutrient-rich end product with great agronomic value (Gohil et al. 2018).

The high organic matter and nutrients composition of human excreta has made them suitable feedstock for a variety of applications, including the production of biofuels like biomethane, bioethanol, and biodiesel via pyrolysis, anaerobic digestion (AD), hydrothermal liquefaction, and other processes (Duan et al. 2020; Somorin 2020). Anaerobic digestion and biogas generation can be a cost-effective way to achieve a range of energy, environmental, and waste management policy objectives (Adnan et al. 2019). Biogas is widely considered one of the most

significant bioenergy forms for dealing with the world's current environmental and energy challenges (Kapoor et al. 2019). AD of human excreta has gained popularity over the years, but the spotlight has been on establishing large facilities to anaerobically valorize this human waste. This approach will require transportation of huge amounts of fecal waste from residences to large processing facilities. This is not a sound approach in terms of environmental friendliness and economic viability. A better approach will be to establish decentralized AD systems in households and/or communities (González et al. 2020).

Installing small-scale anaerobic digesters in homes and communities to manage human excreta from these local sources is termed as decentralized AD. Decentralized AD is beneficial especially where significant volumes of organic waste are handled inadequately due to the high costs of transporting them to large centralized AD facilities (Vaneekhaute et al. 2018). This will also help to drastically minimize carbon emissions during the transport of human excreta to central treatment facilities (Falconer et al. 2020). The aim of this chapter is to explore decentralized AD technology as a feasible alternative to Nigeria's current human excreta management schemes. The focus will be on practical approaches to establish a fully operational decentralized AD technology in Nigeria, using human excreta as feedstock, to reduce waste transportation costs while maximizing community benefits in terms of clean environment and energy while safeguarding public health and safety. This chapter also covers the description of the basic types of anaerobic digesters, decentralized AD safety issues, and the main factors that drive the practical implementation of decentralized AD in Nigeria.

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## 8.2 Current Human Excreta (HE) Management Schemes in Nigeria

A global projection by the WHO/UNICEF reported that 2.6 billion people or 39% of the world population do not have access to adequate human excreta disposal systems (WHO/UNICEF 2010). In a more recent report, approximately 121.9 million Nigerians do not have access to proper excreta disposal facilities, and 38.8 million defecate in the open space. Consequently, only 29% of Nigerian homes have access to upgraded sanitation facilities (UNICEF/WHO 2015). These estimates are very worrisome given the grave consequences improper excreta disposal has on the health and socioeconomic status of the population (Fadairo et al. 2020). For instance, Desmond et al. (2021) investigated the impact of indiscriminate excreta disposal on the infestation of intestinal worms in Olomoro community in Oleh LGA in Delta State. Their study showed that indiscriminate excreta disposal was indeed a major cause of intestinal worm infestation in the studied area. This observation was attributed to the proximity between the excreta disposal system and the drinking water supply in the studied area. Similarly, there are reports of increasing prevalence of neglected tropical diseases (NTDs) like schistosomiasis, trachoma, and intestinal worms in regions of the country with very poor sewage management methods. More worrisome is the fact that in 771 out of 774 local government areas in Nigeria

(UNICEF 2016), open defecation is still being practiced translating to nearly 25% of the national population still defecating openly (Vanguard 2018).

In almost every case, human excreta is mixed with black water, water-carried wastes, and liquid household waste from residences or commercial and industrial establishments. This composite waste is channeled to septic tanks and brick and cement constructed soak-away pits. Thus, the term “sewage” is most commonly used to refer to all liquid and semi-solid household waste sewage that is characterized by extreme foul smell, mainly because of the significant portion of human excreta it contains (Igbinomwanhia et al. 2017).

According to Igwe et al. (2020), excreta disposal systems can be broadly grouped as:

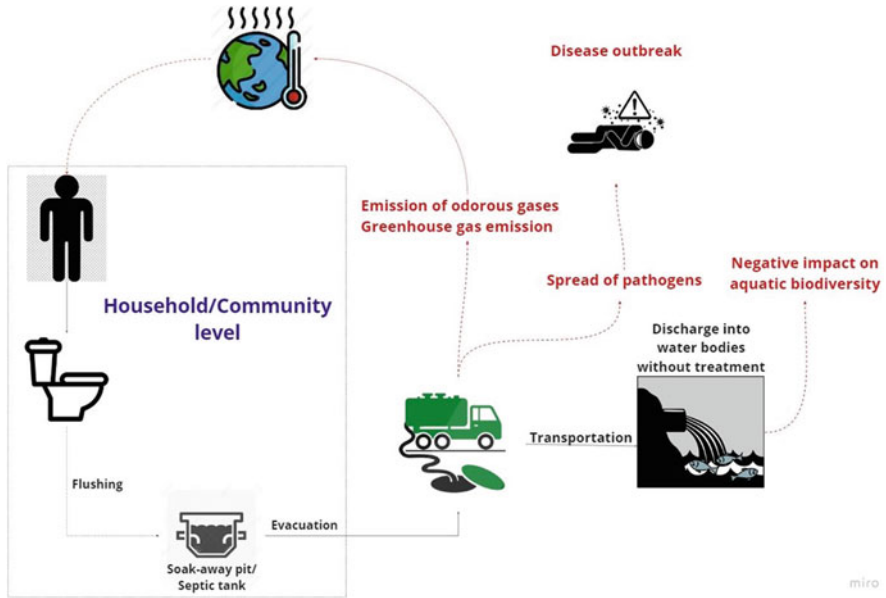
- Non-sewerage (non-pipe network) water-independent methods, e.g., bush disposal, compost latrine, and pit latrine. These toilet facilities do not ensure hygiene separation of human excreta from human contact (Onyeabor and Umeh 2019).
- Non-sewerage (non-network) water-dependent methods are used where water is available, e.g., pour-flush latrine, aqua privy, and septic tank.
- Sewerage (network) water-dependent methods involve a mixture of human excreta and other waterborne waste products from houses and commercial layouts. These discharges are transported through pipes that are linked to sewage treatment facilities, where the sewage is treated before being released into the rivers or seas, as mandated by various municipal legislations.

Conventionally, there are three dominant practices of managing human excreta in Nigeria as outlined by Ehizemhen et al. (2018), namely:

- Disposal into exposed drains and aquatic bodies (rivers and canals)
- Land disposal
- Burial in shallow channels

As indicated by the health records from Akwa Ibom State Hospital’s Management Board (2009), the discharge of untreated human waste into water or tidal mudflats in shoreline settlements is detrimental to public health, and this is the biggest concern confronting Nigerian coastal districts. This situation makes coastal communities more vulnerable to feco-oral diseases spread by contaminated food and water (Okon et al. 2017). As a result, some populations may have access to water even if their water is of poor quality. In the case of land disposal, leachates from sewage eventually end up in boreholes, lakes, wells, and other water bodies (Igbinomwanhia et al. 2017). In rural and semi-urban dwellings in Nigeria, sewage plus wastewater treatment plants are usually absent largely due to the high cost of installation and maintenance (Ehizemhen et al. 2018), insufficient space, and lack of information on the reuse benefits of fecal waste (Olapeju et al. 2019). Thus, it is very common to see built-up coverages in rural areas than in metropolises.

Like other countries across the world, there has been a steady migration of people from rural and semi-urban areas to Nigeria’s metropolitan areas for obvious reasons



**Fig. 8.1** Current scheme of managing human excreta in Nigeria

of searching for better living and economic opportunities. According to a 2018 WHO/UNICEF report, 50% of Nigerians live in urban areas, and the numbers continue to grow exponentially. The urban population would eventually double in the next two decades at a rate of 3.2% (Aliyu and Amadu 2017; Rotowa and Ayadi 2020). Septic tanks, latrines, and their variants are on-site sanitation systems (OSS) used by 64% of these urban populations which means that more OSS will be constructed since an increased amount of fecal sludge is expected to be generated. According to Rose et al. (2015), the average daily amount of excreta generated by an adult human is around 130 g of feces and 1.4 L of urine per capita. Considering this reality, Rotowa and Ayadi (2020) highlighted the dire need to depart from the “business as usual” depicted in Fig. 8.1 to a more proactive OSS for the competent management of human excreta both in urban and regional settings, especially at the household level. This is a key towards ensuring effective public health protection and promotion as well as other significant socioeconomic advantages (Olapeju et al. 2019; Orji et al. 2020).

Nigeria’s National Environmental Sanitation Policy (NESP) (2005) aims to create an environment that promotes good health and prevents sickness. This policy also promotes sanitary conditions to alleviate poverty and increase socioeconomic status in the home and community (Yaradua and Muhammad 2016). In this regard, integrating small-scale AD technology in sewage management is an ambitious low-cost, non-waste approach in managing human excreta. AD technology offers the benefits of converting organic waste to value-added products (Anukam et al. 2019), in this case converting human excreta to biogas and using digestate

eventually as biofertilizer for crop application. Besides, AD technologies have an outstanding potential to minimize the emissions of greenhouse gas (GHG), and this would play an important role in achieving the Paris Agreement of 2016, targeted at reducing the average world temperature increase to below 2 °C (Zamri et al. 2021).

### 8.3 Anaerobic Digestion Technology

Anaerobic digestion (AD) is a technology widely used to degrade organic waste into biogas and nutrient-dense digestate residue. In comparison with other approaches such as pyrolysis, torrefaction, incineration, gasification, and composting method (Zamri et al. 2021), AD has gained popularity as a choice solution to manage organic wastes (Meegoda et al. 2018) because of its low cost, low environmental impact, and high potential for energy recovery. The nutrient-rich digestate (end product of AD) is very rich in phosphorus and remineralized nitrogen (Mothe and Polisetty 2020), and it can be applied to agricultural fields (Logan et al. 2019) in their crude unprocessed form (Vaneekhaute et al. 2018) to increase crop productivity and effectively replace mineral fertilizers (Achinas et al. 2017).

Several studies have demonstrated and even described AD as a preferential approach of intensively biodegrading organic components of municipal solid waste (Adekunle and Okolie 2015). The AD process is based on the effective conversion of organic waste into a useful product known as biogas, which contains the flammable gas methane (CH<sub>4</sub>) (Anukam et al. 2019). Biogas from AD is a renewable energy resource that has the capacity to meet a quarter of the world's gas demand and 6% of primary energy demand. In fact, however, AD-generated electricity accounted for 0.2–0.4% of world power generation (Lü et al. 2021). The major components of biogas are methane (40–75%) and CO<sub>2</sub> (15–60%) in volume (Achinas et al. 2017). The numerous environmental and socioeconomic benefits of AD have garnered international attention and policy support (Yang et al. 2021).

Distinct groups of microorganisms participate in AD in a multiphase/multistage operation to degrade organic substrates and yield, in most cases, CH<sub>4</sub> and CO<sub>2</sub>, under stringent anaerobic conditions (oxygen reduction potential below 200 mV) (Achinas et al. 2017). The multistep activities of the AD process involve both facultative and stringent anaerobic organisms functioning together in a synergistic manner (Onwosi et al. 2019). Bacteria and the archaea carry out AD under strict anaerobic environments (Adekunle and Okolie 2015). The ratio of CO<sub>2</sub> and CH<sub>4</sub> in the final products is associated with the degree of carbon oxidation in the organic substrate (Mothe and Polisetty 2020).

The AD process is an integrated physiological and biochemical process that links microbial and energy metabolism processes under particular conditions (Mao et al. 2015). AD occurs in three major phases which are hydrolysis, acidogenesis (acetogenesis), and methanogenesis (Tian et al. 2018; Qiu et al. 2019). The organic waste is initially hydrolyzed to simple components like sugars (Lee and Lee 2019). Following suit, fermentative bacteria convert simple monomers, sugar, amino acids, and fatty acids into intermediate propionic, butyric acid, and other acids in the

following stage, acidogenesis. Acetogenesis is the process of homoacetogens converting the result of acidogenesis into acetate. Methanogenesis occurs at the end of the process, where two types of methanogens, acetoclastic (acetate consumers) and hydrogen-using methanogens, synthesize methane (biogas) from acetate and carbon dioxide (carbon dioxide-reducing methanogens) (Nguyen and Khanal 2018; Kainthola et al. 2019; Lee and Lee 2019; Onwosi et al. 2019). The rate-determining phase for AD is either hydrolysis or methanogenesis, depending on the substrate type. If the substrate has a more complex structure, hydrolysis becomes the rate-limiting stage, whereas if the substrate is readily broken down, methanogenesis becomes the rate-determining phase (Atelge et al. 2020). The predominant groups of microorganisms actively involved in the AD process include hydrolytic-fermentative bacteria, proton-reducing acetogenic bacteria, hydrogenotrophic methanogens, and acetoclastic methanogens (Onwosi et al. 2019; Wu et al. 2020; Zhang et al. 2018). These microorganisms differ greatly in their physiology, nutritional requirements, growth kinetics, and sensitivity to the surrounding media (Adekunle and Okolie 2015). In AD, the metabolic products excreted by one group of microorganisms are vital substrates for another group of microorganisms (Nguyen and Khanal 2018).

A variety of organic wastes, including human excreta, have been investigated for use in AD to produce biogas. Municipal solid wastes, industrial effluents, animal dung, and agricultural processing wastes are among the most often utilized wastes. Because excreta is rich in organic matter and nutrients, it has been used as a renewable feedstock for a wide range of applications such as pyrolysis, AD, hydrothermal liquefaction, and other techniques to make biofuels like methane, bioethanol, and biodiesel (Duan et al. 2020). AD of human excreta has also been of immense interest to researchers in this field. The use of raw materials such as human excreta is advantageous in terms of the process because it eliminates the need for supplemental starter (microorganism's seed) and ensures a constant supply of microorganisms throughout the feeding of raw materials. These microbes that abound in human excreta originate from the gastrointestinal tract where they perform a similar AD process (Andriani et al. 2015). The abundance of the starter organisms in human excreta substantially contributes to its long-term viability in biogas generation (Andriani et al. 2015) and has over time made human excreta a biomass of interest to explore for simultaneous biogas generation and nutrient/resource recovery (Duan et al. 2020).

Employing AD as a strategy to manage human excreta in Nigeria, and indeed Africa, is a realistic solution that provides dual advantages of reducing the burdens of environmental pollution caused by human excreta while also generating clean energy in the form of biogas. Despite the fact that carrying out AD of human excreta on-site provides the best potential to greatly improve the advantages of AD, only a tiny fraction of human excreta collected is currently efficiently managed, particularly in cities in low-income countries (Decrey and Kohn 2017). In terms of agriculture, the digestate generated by the AD of human excreta will not only provide high-quality organic fertilizer, but it will also be a more low-cost and readily accessible fertilizer for growing crops than synthetic fertilizers. This will be extremely

beneficial to developing nations such as Nigeria, who rely heavily on expensive, imported synthetic fertilizers for agriculture. Together, all these make AD a realistic alternative in Nigeria's human excreta management, which will do a lot to improve the country's socioeconomic and environmental quality, a demand that is very vital in Nigeria and even other developing countries at present (Halder et al. 2016).

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#### **8.4 Concept of Decentralized Anaerobic Digestion (DAD) Technology**

Most policymakers continue to focus on centralized and network sewerage systems, which are constructed without much input or engagement from beneficiaries and fail to fulfill the demands of the people who require basic sanitation (Eawag 2005). A panel of experts in the field of environmental sanitation from a wide range of international organizations met in Bellagio, Italy, in February 2000 and proposed certain guiding principles as the basis of a planning process and implementation of environmental sanitation services. These principles are now referred to as the "Bellagio Principles." The Bellagio Principles inspired the Water Supply and Sanitation Collaborative Council's (WSSCC) Environmental Sanitation Working Group to conceptualize household-centered environmental sanitation (HCES) as a practical approach to realize the ultimate vision of "water and sanitation for all in an environment that balances the needs of the population to healthy life on Earth."

Instead of exporting issues downstream, HCES attempts to fix them where they originate (Sherpa et al. 2012). According to Schertenleib (2000), HCES reverses the traditional order of centralized top-down planning by making households the main point of environmental sanitation planning. The HCES is founded on the idea that service users should have a say in how the service is designed and that environmental sanitation issues should be addressed as near to the source as possible. In fact, only issues that cannot be resolved at the household level should be "exported" to the neighborhood, town, city, and so on, until they reach a bigger authority.

Community-led urban environmental sanitation (CLUES) was later predicated on the validation of the HCES methodology. Hence, CLUES is an updated version of the HCES guidelines. CLUES is an improved set of planning recommendations based on the lessons learnt from the HCES approach's demonstration. CLUES was established to support small communities with environmental sanitation facility development and execution (Mtika and Tilley 2020). The change in nomenclature from HCES to CLUES emphasizes the importance of a wide community participation in the planning and decision-making processes (beyond the household level) (Eawag 2009). Despite the name change, the primary characteristics remain the same: a multi-sector and multi-actor strategy that considers water supply, sanitation, solid waste management, and storm drainage, as well as prioritizing the involvement of relevant stakeholders from the start of the planning process. The CLUES model was adopted exclusively for the development and implementation of environmental sanitation infrastructure and services in low-income small towns, which makes it unique (Lüthi et al. 2011).

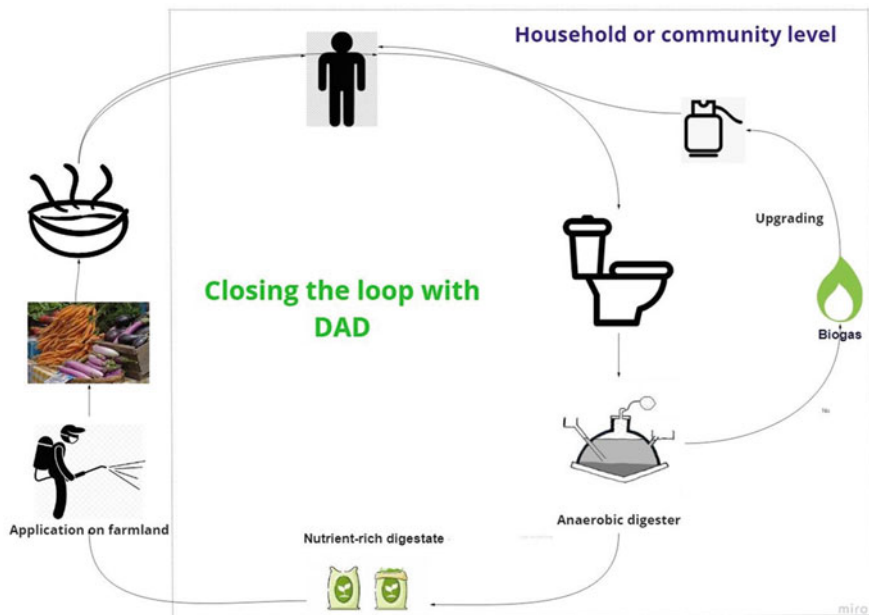


The Bellagio Principles laid the groundwork for radical rethinking, which led to the creation of HCES and then CLUES. Decentralized AD systems, on the other hand, are well suited for the management of HE. Decentralized AD, in tandem with the goals and objectives of HCES and CLUES, is a suitable technique and ambitious way to manage HE in Nigeria and many other African nations. Source separation and control-based approaches are typically referred to as new ecological and resources-related sanitation, wastewater management, or decentralized sanitation (Harder et al. 2019).

Based on the scale of operation, there are two main types of AD systems: centralized and decentralized. A centralized AD plant is a large-scale waste and biomass treatment facility. Because of its large scale, it provides benefits of pretreating waste, modifying bioprocesses, and training operators. Because of the low energy density of biomass, a centralized AD plant is required for large-scale energy generation and waste management. This necessitates the transportation and storage of a massive amount of raw feedstock (Wang 2014), thereby leading to the release of bio-aerosols, odorous gases, significant vehicle traffic, and other attendant negative environmental impact(s) (O'Shea et al. 2017). The alternative to a centralized AD is a decentralized AD. Decentralized AD systems are typically managed by privates independently from municipal or commercial waste utilities (Bortolotti et al. 2018). The decentralized approach's relevance and feasibility are dependent on a seamless integration of the whole waste management and processing chain, starting from generation to product valorization, as well as improved connectedness between the various phases of the waste processing route. As a result, the transition to a decentralized valorization network necessitates a change in biowaste management at the local and territorial levels, which in turn entails strategizing to optimize its spatial structure (Thiriet et al. 2020). Decentralized anaerobic digestion is a viable option for low-density regions or for the treatment of a modest volume of waste generated seasonally (González et al. 2020).

Although AD of HE can be implemented at various scales (from small scale to industrial scale), the most interesting for Nigeria will be to establish a functional decentralized AD system. In some Latin American countries and even Asia, the use of family-sized anaerobic digesters has been in vogue and is gaining traction and showing significant potentials (Halder et al. 2016). Decentralized AD appears to be a more sustainable form of AD, especially in countries like Nigeria with no viable alternative sewage treatment plan. One of the main objectives of decentralizing AD systems is to get them adapted and well suited for territorial waste management (Thiriet et al. 2020) which will be more tailored than a wider spectrum waste management approach. It is critical to develop small-scale AD units for waste treatment in rural areas (González et al. 2020) since decentralized AD is typically characterized by ease of maintenance and operation likewise low cost of installation and unlike large-scale off-site AD.

Several countries' renewable energy objectives are even incentivizing the implementation of community-scale waste-to-energy facilities through subsidies and incentives (Logan et al. 2019). Decentralized AD systems will provide some operational benefits such as simplified waste management, the capacity to generate energy



**Fig. 8.2** Closing the loop with decentralized anaerobic digestion (DAD) of human excreta

independently, and economic benefits associated with thermal energy generation and the production of organic fertilizers and amendments (Anyaku and Baroutian 2018). All of these are summarized in Fig. 8.2.

In addition, installation of household digesters requires several competent professionals for design, planning, and construction, as well as other unskilled labor for daily maintenance and operation; hence, new job opportunities will be created for the local populace (Yasar et al. 2017). Household digesters are a clean, ecologically friendly technology which may well support rural communities in meeting their energy requirements for lighting, cooking, and power, resulting in better living circumstances (Garfí et al. 2016).

## 8.5 Operational Units in Decentralized AD of Human excreta

Decentralized AD is carried out with the construction and design of anaerobic digesters, in this case called domestic digesters, household digesters, or small-scale anaerobic digesters. In practice, toilets must be connected directly to the digester tank in order to maintain a continuous supply of the substrate, which in this case is excreta. Septic tanks have traditionally been used to collect human waste from human habitations. Because the entire process that occurs in the septic tank is anaerobic, advancement has identified the possibility of using septic tanks as a bio-digester (Claribelle et al. 2020). After decomposition, the sludge and scum that

enter the septic tanks produce a variety of gases. This gas released, the majority of which is methane, is usually not collected for economic use. The alternative way of getting the excreta for AD is to transfer the excreta from a septic tank or pit latrine to the tank of the anaerobic digester. However, this is always not a good option because both aerobic and anaerobic processes contribute to the breakdown of rapidly degradable organic waste; hence, only biologically inert particles will remain in excreta residue after a period of time, thereby making it unsuitable for AD and biogas generation (Surendra et al. 2013). Again, huge amounts of odorous gases will be released in the course of the transfer. Likewise, the possibility of polluting the surrounding environment with sewage sludge cannot be overlooked especially in rural dwellings where specialized equipment that would effectively carry out the transfer may be lacking.

Once the excreta has been fed to the digester tank via delivery pipes, all of the anaerobic digestion process commences, leading to the fermentation of the excreta and the consequent production of biogas under suitable anoxic conditions. The biogas generated can be channeled to a gas storage tank, which should be separate from the main anaerobic digester or, in some circumstances, located at the top of the digester. It is worth noting that the biogas generated in this scenario contains impurities like  $\text{CO}_2$  and  $\text{H}_2\text{S}$ , as well as levels of water vapor, which might limit its immediate usage to supply energy in the form of cooking gas or electricity (Mudasar and Kim 2017; Kapoor et al. 2019). Water scrubbing, membrane systems, pressure swing adsorption, chemical absorption, and amine gas treatment are some of the classical methods for removing or treating these contaminants to obtain biomethane (Miltner et al. 2017; Claribelle et al. 2020; Lombardi and Francini 2020). While these methods may lead to the purification of biogas from the AD of excreta, it is also necessary to examine the costs and simplicity of implementation at the home or community level. Because this is not an industrial setting, caution must be taken in ensuring that integrating these biogas purification methods is not time-consuming in the practical sense. This is owing to the fact that the majority of anaerobic digester users are homes and/or communities with daily obligations.

### **8.5.1 Basic Types of Household Digesters/Design and Construction of Digesters**

Anaerobic digesters are essentially enclosed chambers where organic waste is digested anaerobically (DeRouchey 2014). Anaerobic digesters are classified into two main categories based on their intended function and scale: small-scale and medium-/large-scale digesters. The small-scale digester is mostly used to generate energy for domestic heating and cooking. Medium-/large-scale biogas digesters, on the other hand, are built for the treatment of industrial and municipal organic wastes, with chemical oxygen demand (COD)/biochemical oxygen demand (BOD) reduction as the primary goal and biogas production as a compensation (Bhol et al. 2011).

Household anaerobic digesters are often built relatively near to small residences, mostly in rural regions, to supply biogas for home consumption while the digestate

from the reactors being applied to crops as organic manure (Jegade et al. 2019). Usually a small-scale biogas digester consists of an inlet for the substrate (in this case, excreta), an airtight chamber, a reservoir for biogas collection (which could be at the upper part of the digester, a floating drum or plastic balloons), and an expansion chamber (Lehtinen 2017).

For the design of the digester, several materials and geometrical configurations have been adopted. Horizontal, spherical, cylindrical, and dome shapes are examples of these geometric configurations. Brick, cement, fiber glass for the dome structure, and metals (stainless steel and mild steel) are some of the materials typically used in its construction (Ali et al. 2019). For a household digester, the maximum capacity of the tank is around 10 m<sup>3</sup> (Jegade et al. 2019). Household digesters are relatively inexpensive and simple to operate (Rajendran et al. 2012). The most popular types of anaerobic digesters constructed for household, community, or small-scale applications include

- Fixed dome
- Floating drum
- Plug flow

One feature of these digesters that makes them suitable for household use is that they do not have mechanical mixers and, in many cases, do not require supplementary heating; hence, they're economical and can easily be operated and maintained (Jegade et al. 2019). Each of these types of household digesters has its own set of benefits, but they are not particularly efficient especially in hilly regions. To make up for the inadequacies of these typical household digester models, prefabricated biogas digesters (PBDs) are still being designed, tested, and widely used in developing nations. PBDs prototypes are largely based on the fixed dome, floating drum, and plug-flow digesters models (Cheng et al. 2014). The size of a household or community-scale anaerobic digester is largely determined by the location, number of residents, and daily substrate supply. For a household of nine, the size of these digesters is normally approximately 6m<sup>3</sup> for Nigeria (Rajendran et al. 2012). But instead of having a digester for each individual home, community-type anaerobic digesters employ a huge volume of sewage stream for 10–20 households. The biggest benefit of small-scale digesters is that they can serve both rural and urban families by allowing them to produce biogas for cooking, heating, and even electric applications (Ajay et al. 2021) while properly managing their sewage. These sorts of digesters are more practicable in nations where households are concentrated, such as in Nigeria (Rajendran et al. 2012).

### 8.5.1.1 Fixed-Dome Digesters

Fixed-dome digesters (FDD), often known as “Chinese” or “hydraulic” digesters, are the most prominent household digester model developed and utilized mostly in China. FDD comes in a variety of designs, including Indian Deebandhu, Chinese fixed dome, and so on. Regardless, all of the variants have the same semicircular dome casing (Yasar et al. 2017). The majority of fixed dome digesters are

constructed underground. This design of the FDD is based on mono-feedstock wet anaerobic digestion (WAD) concept (Mungwe et al. 2016). The overall size of the FDD ranges from 4 to 20 m<sup>3</sup>, and it is carefully designed to allow substrates such as human excreta and animal manure to be fed into the digester with minimal introduction of air, and it varies greatly depending on geographic location and climatic conditions (Surendra et al. 2013).

In design, the dome positioned above the digester essentially functions as a gas holder. The feedstock is fed into the digester by an intake pipe, and the pressure necessary for biogas emission is provided by a displaced level of feedstock inside the digester. The biogas produced is eventually stored in a gas holding unit (Ajay et al. 2021). FDD can be constructed using bricks and mortar (Mutungwazi et al. 2018). It can also be constructed using prefabricated plastics (Jegede et al. 2019).

The materials required to construct this type of digester are readily available especially in rural regions. Again, the cost required for installation and recurring maintenance is very minimal since there are no moving or rust-prone metallic parts of the digester (Mutungwazi et al. 2018). Maintenance is also relatively easy since it just generally requires the inspection and, if required, repair of pipes and fittings on a regular basis (Budiman 2020). These advantages make the fixed dome digester a good choice for implementing anaerobic digestion of HE, although it is important to mention that it is very difficult to build a dome with brick and mortar as it should only be done by a highly experienced bricklayer and it is a time-consuming task too (Bhol et al. 2011). Generally, the FDD has an average lifespan of approximately 20 years (Surendra et al. 2013).

### 8.5.1.2 Floating Drum Digesters

The chamber of the floating drum digester is constructed using reinforced concrete, and it is usually installed underground just like the fixed dome digester. The floating drum digester does not have any component for mixing or heating (Garfi et al. 2016); it consists of a subsurface digester and an aboveground moveable gas container or drum, which can be cylindrical or dome-shaped (Yasar et al. 2017). The drum moves up when biogas is generated and down when biogas is spent. Even on a substrate with a high solid content, if the drum floats in a water jacket, it cannot get stuck (Budiman 2020). A supporting structure is also installed to keep the gas holder from tipping (Yasar et al. 2017). The floating drum digester is made of a moving steel gas holder at the top. This moveable gas holding unit is usually made of mild steel which accounts for roughly 40% of the overall cost of the digester (Ajay et al. 2021). Steel drums are both costly and time-consuming to maintain (Bhol et al. 2011). The addition and removal of weight from the gas holder to increase or reduce the pressure of gas flow from the digester in order to meet the appropriate pressure of 7–20 mbar at the burner is one of the essential operations of the floating drum digester (Itodo et al. 2013).

Floating drum digesters are simple to use and manage. Typical operations and maintenance tasks include digestate management, purging debris accumulated at the bottom of the reactor, controlling biogas leakage, and painting the drum on a regular basis to prevent rust (Garfi et al. 2016). Floating drum digesters release biogas at a

constant pressure, and the stored gas volume is easily detected by the drum's location. Provided that derusting and painting are done regularly, the tightness of the gas holder is preserved (Bhol et al. 2011). If not painted, the drum could corrode, and this effectively reduces the lifespan of the digester (Garfí et al. 2016). Installing the floating drum digester requires skilled personnel; as such, it is more expensive than the fixed dome digester (Ajay et al. 2021).

### 8.5.1.3 Plug-Flow Anaerobic Digester

Plug-flow anaerobic digester (PFAD) is a long, narrow horizontal tank typically constructed with reinforced concrete, steel, or fiberglass (Ghosh and Bhattacharjee 2013). In the design of the PFAD, as the substrate (in this case could be sewage) is continuously introduced, it flows through the tank and forces the ejection of the digested substrate (Ramatsa et al. 2014). This arrangement allows for the evaluation of separation of the different phases of the AD along the reactor (Escalante-Hernández et al. 2017); acidogenic and methanogenic phases are well separated inside the digester. This separation improves the stability and efficiency of the AD process in the reactor. The length of the PFAD is five times its breadth with some insulated parts (Ramatsa et al. 2014) but no means of internal agitation (Ghosh and Bhattacharjee 2013). The advantages of plug-flow anaerobic digesters include a relatively simple design, low capital cost and operational energy consumption (Ramatsa et al. 2014), prevention of short circuiting and regulated retention time, sufficient pathogen elimination, and the ability to handle high solid content (Adl et al. 2012). However, some disadvantages exist, such as decreased mass transfer due to poor mixing, and this causes sand, soil, or dirt to easily settle, and it is very difficult to clean up (Ramatsa et al. 2014). Other disadvantages include lower efficiency at low solid content, temperature stratification, and solid sedimentation issues (Adl et al. 2012).

## 8.5.2 Criteria for Designing and Situating Household Digesters

It is very difficult to decide on a single type of digester for installation at the household level or small scale. The digester design varies depending on the geographical location, substrate availability, and climatic conditions. To reduce gas loss, a digester utilized in hilly areas, for example, is constructed with a smaller gas capacity. Due to geothermal energy, it is preferable to build digesters underground in tropical nations (Rajendran et al. 2012). The majority of small-scale biogas digesters are actually built underground, and the biogas generated is primarily utilized for cooking (Bruun et al. 2014).

Anaerobic reactors are designed to degrade organic matter and reduce biochemical oxygen demand and nutritional content from influent sewage streams via the coordinated metabolic activity of microorganisms under anaerobic conditions. The first and most significant requirement in anaerobic reactor design should be how to achieve effective mass and energy transfer from the bulk sewage stream to microorganisms native to the biomass and reactor (Rowse 2011). Two other key

design considerations include the prevailing weather and climatic conditions and the socioeconomic situation in the geographical location (Pérez et al. 2014). Some other design parameters to be considered in designing a household anaerobic digester include simplification of design, ease of handling and maintenance, ease of building, local accessibility of construction and maintenance materials, low cost, required amount of animal waste, volume of required water, volume of biogas generated, effluent volume produced, durability, digester location (temperature), cultural acceptance, and cooking time (Rowse 2011; Usack et al. 2014). Services of highly specialized professional employees, contractors, designers, and operators are required for the successful installation of anaerobic digesters. Training can be conducted at a modest cost for the respective parties to overcome the skills disparities. Meanwhile, planners must be well aware of the current price standards and product quality criteria before commissioning the construction of the household- or community-scale digesters (Matheri et al. 2018).

In the construction of small-scale anaerobic digesters, appropriate models are needed to adapt to a variety of geological, topographical, and climatic conditions in a locality, such as those seen in areas with a high groundwater table, rocky soils, and cold winter temperatures (Cheng et al. 2014). However, a defective design or construction of these may necessarily cause a leak after a short time of operation, and this presents serious worries since these digesters cannot be readily fixed for regular operation once they have experienced a fault in design or construction. Furthermore, due to the reliance on prevailing weather conditions, repairing or rebuilding the faulty digester might take a lengthy period, sometimes up to several months (Cheng et al. 2014).

### **8.5.3 Management of Digestate Resulting from Anaerobic Digestion of Human Excreta**

The effluent from the AD process is called digestate. The quantity and quality of anaerobic digestate made from human excreta are largely determined by the anaerobic digester's operating parameters, the frequency with which excreta are fed to the digester, and the mixing regime used (Logan and Visvanathan 2019). In many cases, digestate contains substantial amounts of methane, which contributes to global warming. Furthermore, digestate may emit ammonia, carbon dioxide, and nitrous oxide, which cause pollution both locally and worldwide (Visvanathan 2014). It is important to develop an efficient digestate management plan to improve the overall eco-image of human excreta management via decentralized anaerobic digestion. It is also critical that the techniques chosen for digestate management be attractive, safe, practical, and simple to implement.

Digestate is composed of two fractions: the solid residue, which is the fiber, and the liquid effluent, which can be cured and utilized as an organic fertilizer in agriculture (Iorhemen et al. 2016). Digestate is often high in macronutrients such as N, P, and K, as well as micronutrients such as Zn, Fe, Mo, and Mn (Muhmood et al. 2018), all of which are necessary for plant growth and development, without

causing harm to the soil. This also helps to increase food production to meet the needs of the growing populace, therefore achieving SDGs 1, 2, and 13, which are poverty eradication, zero hunger, and climate action, respectively (Dahunsi et al. 2021). Because the fiber is thick and contains few plant nutrients, it can be used as an organic low-grade fertilizer, though further processing, via composting, can result in high-quality compost. Composting is a natural aerobic biochemical process in which thermophilic bacteria break down organic materials at 40–70 °C into a stable soil-like product. Composting can help lessen the volume of digestate by 40–50%, metabolically eradicate pathogens in the thermophilic phase, and generate biofertilizer as end product (Ezechi et al. 2017). On the other hand, the liquid effluent has a high amount of nutrients and may be utilized as organic fertilizer. The high water content of the liquid makes it suitable to be applied as fertilizer using traditional irrigation methods. As a result, using fiber and liquid from anaerobic digestion has resulted in better fertilizer use and, as a result, less chemical use in cropping systems (Alfa et al. 2014).

Digestates are widely utilized as organic fertilizers because of their capacity to enhance and change soil structures while also improving soil nutrient status and augmenting a load of beneficial microbes for specific activities, especially in marginal or nutrient-depleted soils (Dahunsi et al. 2021). Digestate from the AD of human excreta can also be used to promote subsistence farming in growing food crops and flowers for aesthetics at the home level (Amasuomo and Ojukonsin 2015). This may lead to a plethora of local economic opportunities, with people taking the further step of collecting digestates from neighboring homes, processing them, and selling them to consumers, mostly farmers, at wholesale and retail settings.

Ajieh et al. (2021) conducted a study to determine the sociocultural acceptability of biogas generation from human excreta in certain Benin city neighborhoods (a state in the southern part of Nigeria). Their research revealed that human excreta is a viable feedstock for biogas generation and that utilizing the digestate produced in agriculture would considerably increase food production and availability to the growing populace. Owing to the high cost and limited availability of synthetic fertilizers to farmers in Nigeria (Sekumade 2017; Hassan and Hussain 2018), employing digestate as an organic fertilizer is indeed a viable digestate management practice. Despite the benefits of using digestate as organic fertilizer, there are still some concerns regarding the presence of heavy metals and pathogens (particularly antibiotic-resistant bacteria) such as *Salmonella*, *Escherichia coli*, *Klebsiella*, *Clostridium*, and *Campylobacter* which are potentially harmful to soil organisms, plants, and people (Ndubuisi-Nnaji et al. 2020; Seruga et al. 2020). All of these present some sociocultural barriers to its full-scale utilization in agriculture.



## 8.6 Safety Considerations in Decentralized Anaerobic Digestion of Human Excreta

When considering decentralized anaerobic digestion as a strategy for managing human excreta in Nigeria, it is clear that this strategy will bring a number of benefits, including appropriate sanitation and sewage containment. In the same vein, the biogas produced may be leveraged to augment existing power supply and save the country from the obvious energy crisis (Kapoor et al. 2019) and excessive generator noise as well as the financial and environmental costs of burning fossil fuels. Despite the enormous advantages of AD technology in managing human excreta, it is only appropriate to evaluate the technology's inherent risks. Safety is critical to the long-term implementation of decentralized AD technologies, particularly in a nation like Nigeria where there is a lack of adequate urban and regional planning as well as the construction of unauthorized residential constructions (Obi-Ani and Isiani 2020). Nigeria has undergone and is still undergoing rapid urbanization, which has resulted in a slew of issues including violations of building development standards, pollution, overpopulation, and flood (Adeleye et al. 2019). All of this highlights the necessity to evaluate the inherent hazards and risks involved with encouraging the use of AD systems to manage human excreta in a decentralized context. This would aid in the prevention of accidents that might result in serious injuries, human deaths, environmental degradation, and property destruction.

The risks associated with AD systems have long been overlooked (Raboni et al. 2015), which may be due to the fact that the majority of AD installations are typically at the household or community level, falling well below baselines for the implementation of policy benchmarks intended to control or prevent major hazards and risks (Moreno and Cozzani 2015). The most prevalent risks connected with AD systems are fire and explosion, which are primarily caused by the methane composition of biogas (Scarponi et al. 2015). Meanwhile, CO<sub>2</sub> and NH<sub>3</sub>, which are also constituents of biogas, are toxic to both humans and the environment, depending on the concentration and length of exposure (Stolecka and Rusin 2021). CO<sub>2</sub> has a myriad of effects on humans, including abnormal respiratory rates, headaches, loss of consciousness, and even immediate death. In most cases, NH<sub>3</sub> poisoning causes severe respiratory failure, chemical burns, and deep ulcers (Stolecka and Rusin 2021). On the other hand, hazardous emissions are usually caused by the presence of hydrogen sulfide (ranging between 50 and 20,000 ppm in biogas) (Moreno et al. 2018). The feedstock, in this case human excreta, and even the digestate left over at the end of AD, if not correctly managed, can pollute aquifers and introduce pathogens and other harmful elements into the surrounding environment (Trávníček and Kotek 2015).

A number of innovative and attractive solutions can be adopted to prevent and control accidents in AD systems. One of these solutions will be to install automated safety systems alongside anaerobic digesters (Sovacool et al. 2016). Automated safety systems require a constant energy source to avoid outages and device failure. However, because of the country's inconsistent power supply in many regions, particularly in rural and semi-urban areas, the potential for optimum reliability of

these systems in a country like Nigeria is rather restricted. Again, the use of automated safety systems will need the skills of experienced technicians and engineers, resulting in higher costs of installation and maintenance which will perhaps discourage extensive decentralized use of AD systems to manage human excreta. In the Nigerian context, a more realistic alternative would be to address inherent safety throughout the design phase of anaerobic digester, their accessories, and installation steps (Moreno et al. 2018). This can be regulated by government organizations such as the National Environmental Standards and Regulation Agency and the Standard Organization of Nigeria (SON). The SON is the official regulatory agency in Nigeria mandated with the task of standardizing and maintaining the quality of all merchandise and manufacturing practices. Although different strategies to prevent and manage accidents and hazards in AD systems have been devised and assessed, it is critical that these strategies be tailored to the dynamics of the locale and its climatic conditions for decentralized anaerobic digestion of human excreta. Affordability and efficiency are other important considerations.

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## **8.7 Roadmap to Implementing Decentralized Anaerobic Digestion Technology in Nigeria**

In tandem with the objectives of HCES and CLUES, decentralized AD will be a feasible management solution for managing human excreta in Nigeria. In Nigeria, human excreta are handled in a decentralized way, as opposed to the centralized organization seen in many developed countries. As a result, installing AD systems in households and/or communities will necessitate rebuilding or redesigning conventional soak-away pits and septic tanks to become efficient anaerobic digesters. Decentralizing the management of human excreta will increase the host community and households' sense of responsibility in waste management (Anyaoku and Baroutian 2018), incentivizing homes to generate biogas for domestic cooking thereby reducing overdependence on fossil fuels and eventually yield digestate as organic fertilizer. This will, again, serve to reduce the environmental and financial implications of transporting huge quantities of sewage using trucks from houses to central facilities. However, there are sociocultural, technological, financial, and regulatory concerns that must be addressed for widespread realization of decentralized AD in Nigeria.

### **8.7.1 Technology and Education**

In comparison with developed counterparts, establishing a sustainable technology remains a key issue for AD deployment in developing countries like Nigeria (Patinvoh and Taherzadeh 2019). This is linked to the lack of qualified and experienced craftsmen to construct and maintain anaerobic digesters, and this is a barrier to the widespread adoption of decentralized AD systems in Nigeria. The benefits of decentralized AD technology must first and foremost be communicated to

legislators, who are the ultimate decision-makers (Bundhoo and Surroop 2019). All other stakeholders must also be properly enlightened, reoriented, and motivated to implement, integrate, and develop decentralized AD technology in Nigeria (Audu et al. 2020). To communicate the potential of the AD technology to government authorities, the commercial sector, and potential investors, training and capacity-building workshops should be organized. This training and capacity-building may be done by local university personnel who are specialists on the subject, and if that is not possible, foreign expertise could be sought. The training should cover all parts of AD technology, from the fundamentals through digester construction, commissioning, operation, and maintenance (Bundhoo and Surroop 2019). Pilot projects should also be done to show the technology's viability, as well as adequate marketing; these may be required to overcome whatever initial apprehensions about adopting and adapting this technology in Nigeria (Audu et al. 2020).

### 8.7.2 Funding

In developing countries, a lack of finance is a key impediment to AD adoption (Kemausuor et al. 2018). For instance, a locally fabricated household digester with a daily input capacity of 50 kg costs over \$1500, which is a huge investment for low-income households when additional expenses of storage tanks, operation, purification, biogas compressor, and maintenance are included (Patinvoh and Taherzadeh 2019). Also, for successful biogas generation, mechanical pretreatment is sometimes necessary before the digestion process; the expense of this is also rather high. Due to the obvious high costs, any attempt to stimulate the deployment of household anaerobic digesters in Nigeria may not gain traction (Ahonle and Adeoye 2019). Many potential users who are low-income earners and do not have access to appropriate credit schemes or other financial assistance from the government or aid groups would face a significant financial burden as a result of this (Nevzorova and Kutcherov 2019). Monetary incentives should be offered to individuals interested in installing anaerobic digesters to replace or complement soak-away pits for containing human excreta in order to overcome this financial constraint (Hasan et al. 2020; Paul 2021). Incentives for venture capitalists, commercial banks, and companies to invest should also be in place. Nigeria is one of the recipients of USD 60 million from the African Development Bank's Sustainable Energy Fund for Africa (Akuru et al. 2017).

### 8.7.3 Policy

For any technology to be effectively implemented in a country, well-developed policies are necessary. Nigeria presently lacks a comprehensive policy on renewable energy and its associated technologies (Oyedepo et al. 2019), particularly when it comes to decentralized AD technology. As a result, government intervention is necessary, notably from both the federal and state government of Nigeria, in the

form of plans and policies as well as an adequate legal and institutional framework for the development and implementation of this technology in the management of human excreta in communities and households. In Nigeria, sustainable decentralized AD management of human excreta is practicable if the Federal Government can mobilize the political will to put in place the necessary legislative framework (Imoisi and Okongwu 2020). In fact, a key government policy would positively drive investors' confidence and encourage Nigerians to install anaerobic digesters in their homes, workplaces, and communities (Akuru et al. 2017). Governments and nongovernmental groups can work together to promote the adoption and dissemination of decentralized AD technology in Nigeria. In different nations, public policies have played a significant role in promoting and expanding the use of AD technology especially to generate biogas. Governments in Africa, particularly Nigeria, should follow the lead of European, Asian, and South American countries, as well as other nongovernmental organizations, who formulate schemes and provide financial subsidies, loans, and incentives for the installation of anaerobic digesters in homes and communities located in urban, semi-urban, and rural areas.

#### **8.7.4 Public Awareness**

People's awareness, perceptions, and attitudes about new technologies are low in the public and political realms. The majority of Nigerians are not acquainted with the benefits of AD technology and bioenergy in any sort (Oyedepo et al. 2019). There is a general lack of understanding of the alternatives available and the advantages that may be derived from each of these solutions, particularly in the treatment of human excreta. This is a fundamental issue, because decentralized AD cannot be optimally operational in the country if potential end users are unaware of its possibilities. This scenario is the consequence of a variety of reasons, including a lack of access to effective mass communication to enlighten the populace on the benefits of AD management of human excreta, particularly in rural areas of the country, as well as hesitancy to adopt new and developing technologies over traditional choices (Oyedepo et al. 2019). To address this issue, trained government officials, in conjunction with other trained stakeholders, should organize sensitization and awareness-raising programs to communicate the benefits of AD technology to the general public in an easy-to-understand way (Bundhoo and Surroop 2019).

#### **8.7.5 Research and Development**

The inadequacy of research and development (R&D) funding is considered in developing nations, such as Nigeria, as a key barrier to driving technology innovations. Obviously, due to a lack of financing, there are issues with inadequate R&D and a shortage of adequately trained researchers. In order to improve AD technologies, lower the cost of installation, and make it accessible for poorer families, institutional networking for R&D and coordinated efforts in solving

R&D challenges should be established (Igwe et al. 2020; Nevzorova and Kutcherov 2019). Pilot projects can be carried out in universities to assess the technology's practicality and to provide the best conditions for its effective implementation at household and community levels (Patinvoh and Taherzadeh 2019). More training, consultation, and instructional programs for technicians and engineers manufacturing and installing AD systems will be established if R&D efforts are intensified. Also, it is important that universities in Nigeria develop or implement adequate technology education and training programs to support R&D efforts in AD technology (Ahonle and Adeoye 2019).

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## 8.8 Conclusion

The use of AD technology in organic waste management is becoming more prevalent due to its low environmental impact, relative simplicity of operation, and lower operating and maintenance costs. Recognizing the enormous potential of human excreta as a valuable resource in the production of value-added bioproducts via AD, there is an urgent need to implement decentralized AD as an ambitious strategy to create wealth for Nigeria's teeming population. This will also be a great step towards reducing overreliance on fossil fuels and synthetic chemicals. Hence, both governmental and nongovernmental agencies must work together to develop decentralized AD technology in Nigeria.

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## References

- Achinas S, Achinas V, Euverink GJW (2017) A technological overview of biogas production from biowaste. *Engineering* 3(3):299–307
- Adekunle KF, Okolie JA (2015) A review of biochemical process of anaerobic digestion. *Adv Biosci Biotechnol* 6(3):205
- Adeyeye B, Popoola A, Sanni L, Zitta N, Ayangbile O (2019) Poor development control as flood vulnerability factor in Suleja, Nigeria. *Town Reg Plan* 74:23–35
- Adl M, Sheng KC, Xia YH, Gharibi A, Chen X (2012) Examining a hybrid plug-flow pilot reactor for anaerobic digestion of farm-based biodegradable solids. *Int J Environ Res* 6(1):335–344
- Adnan AI, Ong MY, Nomanbhay S, Chew KW, Show PL (2019) Technologies for biogas upgrading to biomethane: a review. *Bioengineering* 6(4):92
- Ahonle JE, Adeoye PA (2019). Potential, barriers and prospects of biogas production in North-Central Nigeria. 3rd International Engineering Conference (IEC 2019) Federal University of Technology, Minna
- Ajay CM, Mohan S, Dinesha P (2021) Decentralized energy from portable biogas digesters using domestic kitchen waste: a review. *Waste Manag* 125:10–26
- Ajeh MU, Isagba ES, Ihoeghian N, Edosa VI, Amenaghawon A, Oshoma CE, Erhunmwunse N, Obuekwe IS, Tongo I, Emokaro C, Ezemonye LI (2021) Assessment of sociocultural acceptability of biogas from faecal waste as an alternative energy source in selected areas of Benin City, Edo State, Nigeria. *Environ Dev Sustain* 2021:1–18
- Akuru UB, Onukwube IE, Okoro OI, Obe ES (2017) Towards 100% renewable energy in Nigeria. *Renew Sust Energ Rev* 71:943–953

- Alfa MI, Adie DB, Igboro SB, Oranusi US, Dahunsi SO, Akali DM (2014) Assessment of biofertilizer quality and health implications of anaerobic digestion effluent of cow dung and chicken droppings. *Renew Energy* 63:681–686
- Ali AM, Chindo YM, Yaro SA (2019) Improvement on the design of a small scale anaerobic floating drum biogas digester. *Int J Eng Technol Res* 17(5):228–237
- Aliyu AA, Amadu L (2017) Urbanisation, cities, and health: the challenge to Nigeria - a review. *Ann Afr Med* 16(4):149–158
- Amasuomo E, Ojukonsin T (2015) Exploring the potential of organic waste as a source of methane gas for electricity generation in Nigeria. *J Manag Sustain* 5:99
- Andriani D, Wresta A, Saepudin A, Prawara B (2015) A review of recycling of human excreta to energy through biogas generation: Indonesia case. *Energy Procedia* 68:219–225
- Anukam A, Mohammadi A, Naqvi M, Granström K (2019) A review of the chemistry of anaerobic digestion: methods of accelerating and optimizing process efficiency. *Processes* 7(8):504
- Anyako CC, Baroutian S (2018) Decentralized anaerobic digestion systems for increased utilization of biogas from municipal solid waste. *Renew Sust Energy Rev* 90:982–991
- Atelge MR, Atabani AE, Banu JR, Krisa D, Kaya M, Eskicioglu C, Kumar G, Lee C, Yildiz YS, Unalan SE, Mohanasundaram R, Duman F (2020) A critical review of pretreatment technologies to enhance anaerobic digestion and energy recovery. *Fuel* 270:117494
- Audu IG, Barde A, Yila OM, Onwualu PA, Lawal BM (2020) Exploring biogas and biofertilizer production from abattoir wastes in Nigeria using a multi-criteria assessment approach. *Recycling* 5(3):18
- Bhol J, Sahoo BB, Mishra CK (2011) Biogas digesters in India: a review. In: National Conference on Renewable and New Energy Systems, Odisha
- Bortolotti A, Kampelmann S, De Muynck S (2018) Decentralised organic resource treatments—classification and comparison through extended material flow analysis. *J Clean Prod* 183:515–526
- Bruun S, Jensen LS, Sommer S (2014) Small-scale household biogas digesters: an option for global warming mitigation or a potential climate bomb? *Renew Sust Energy Rev* 33:736–741
- Budiman I (2020) The role of fixed-dome and floating drum biogas digester for energy security in Indonesia. *Indones J Energy* 3(2):83–93
- Bundhoo ZM, Surroop D (2019) Evaluation of the potential of bio-methane production from field-based crop residues in Africa. *Renew Sust Energy Rev* 115:109357
- Cheng S, Li Z, Mang HP, Huba EM, Gao R, Wang X (2014) Development and application of prefabricated biogas digesters in developing countries. *Renew Sust Energy Rev* 34:387–400
- Claribelle NN, Chinedu IN, Nnaemeka OP, Toochukwu ES (2020) Production of electricity from human waste as a strategy for curbing electricity generation problem in Nigeria. *Energy* 9(4): 85–96
- Dahunsi SO, Oranusi S, Efevbokhan VE, Adesulu-Dahunsi AT, Ogunwole JO (2021) Crop performance and soil fertility improvement using organic fertilizer produced from valorization of *Carica papaya* fruit peel. *Sci Rep* 11(1):1–16
- Decrey L, Kohn T (2017) Virus inactivation in stored human urine, sludge and animal manure under typical conditions of storage or mesophilic anaerobic digestion. *Environ Sci Water Res Technol* 3(3):492–501
- DeRouchey JM (2014) Manure/waste management. In: Encyclopedia of meat sciences, 2nd edn. Springer, New York, pp 152–156
- Desmond O, John M, Bariyaa WT, Barikuura DF, Blessed DOP (2021) Indiscriminate excreta disposal, a major cause of intestinal parasite infestation in Olomoro in Delta state Nigeria. *Inte Res J Modern Eng Technol Sci* 3(3):1–7
- Duan N, Zhang D, Khoshnevisan B, Kougias PG, Treu L, Liu Z, Lin C, Liu H, Zhang Y, Angelidaki I (2020) Human waste anaerobic digestion as a promising low-carbon strategy. Operating performance, microbial dynamics and environmental footprint. *J Clean Prod* 256:120414

- Eawag (2005) Household-centered environmental sanitation, implementing the bellagio principles in urban environmental sanitation, provisional guideline for decision-makers. Swiss Federal Institute of Aquatic Science and Technology, Dübendorf
- Eawag (2009) Community-led urban environmental sanitation planning: CLUES Complete guidelines for decision-makers with 30 tools. Swiss Federal Institute of Aquatic Science and Technology, Nairobi
- Ehizemhen CI, Agashua LO, Abubakar S (2018) Case study of household sludge management and viable development in Bwari, Nigeria. *Euro J Appl Eng Sci Res* 6(3):24–28
- Escalante-Hernández H, Castro-Molano L, Besson V, Jaimes-Estévez J (2017) Feasibility of the anaerobic digestion of cheese whey in a plug flow reactor (PFR) under local conditions. *Ing Invest Tecnol* 18(3):265–277
- Ezechi EH, Nwabuko CG, Enyinnaya OC, Babington CJ (2017) Municipal solid waste management in Abuja, Nigeria: challenges and prospects. *Environ Eng Res* 22(3):231–236
- Fadairo O, Alabi-Adelakun O, Adetunji A (2020) Promoting community-led water, sanitation and hygiene interventions in rural settlements in Oyo state, Nigeria: Lessons from Agele and Mogba communities of Ibarapa-East Local Government Area. *Rural-Urban Link Sustain Dev* 163:176–201
- Falconer RE, Haltas I, Varga L, Forbes PJ, Abdel-Aal M, Panayotov N (2020) Anaerobic digestion of food waste: Eliciting sustainable water-energy-food nexus practices with agent based modelling and visual analytics. *J Clean Prod* 255:120060
- Garfi M, Martí-Herrero J, Garwood A, Ferrer I (2016) Household anaerobic digesters for biogas production in Latin America: a review. *Renew Sust Energ Rev* 60:599–614
- Ghosh R, Bhattacharjee S (2013) A review study on anaerobic digesters with an Insight to biogas production. *Int J Eng Sci Invent* 2(3):8–17
- Gohil SN, Shilpkar PG, Shah MC, Shah AJ, Acharya PB (2018) Methane from human excreta: comparative assessment of batch and continuous biomethanation process. *J Pure Appl Microbiol* 12(4):2143–2148
- González R, Hernández JE, Gómez X, Smith R, Arias JG, Martínez EJ, Blanco D (2020) Performance evaluation of a small-scale digester for achieving decentralised management of waste. *Waste Manag* 118:99–109
- Halder PK, Paul N, Joardder MUH, Khan MZH, Sarker M (2016) Feasibility analysis of implementing anaerobic digestion as a potential energy source in Bangladesh. *Renew Sust Energ Rev* 65:124–134
- Harder R, Wielemaker R, Larsen TA, Zeeman G, Öberg G (2019) Recycling nutrients contained in human excreta to agriculture: pathways, processes, and products. *Crit Rev Environ Sci Technol* 49(8):695–743
- Hasan AM, Kabir MA, Hoq MT, Johansson MT, Thollander P (2020) Drivers and barriers to the implementation of biogas technologies in Bangladesh. *Biofuels* 2020:1–13
- Hassan Y, Hussain N (2018) Agricultural pollution: an assessment of synthetic fertilizer application in the Sudano-Sahelian zone of Nigeria. *Ann Soc Sci Manag Stud* 2(1):55579
- Igbinomwanhia DI, Opoke IS, Omorose SO, Yakubu YU, Oniovusa SC (2017) Preliminary investigation of the status of domestic and commercial sewage management in Benin Metropolis, Nigeria. *J Sustain Dev Stud* 10(2):34–44
- Igwe CN, Amadi AN, Onwuzulike NM, Ozims SJ, Amadi COA, Achigbu KI, Azuamah YC, Okereke EE (2020) Anal cleansing and excreta disposal practices among young adults in southeastern Nigeria. *Int J Sci Healthcare Res* 5(4):1–6
- Imoisi S, Okongwu J (2020) Need for a policy framework for sustainable renewable energy technologies in Nigeria: biogas utility. *Chukwuemeka Odumegwu Ojukwu Univ J Com Prop Law* 2(1):11
- Iorhemen OT, Alfa MI, Onoja SB (2016) The review of municipal solid waste management in Nigeria: the current trends. *Adv Environ Res* 5(4):237–249
- Itodo IN, Yusuf PO, Obeta SE (2013) Determination of appropriate operational parameters of a floating-drum biogas plant for rural Nigeria. *Int J Renew Energy Technol* 4(3):313–321

- Jegede AO, Zeeman G, Bruning H (2019) A review of mixing, design and loading conditions in household anaerobic digesters. *Crit Rev Environ Sci Technol* 49(22):2117–2153
- Kainthola J, Kalamdhad AS, Goud VV (2019) A review on enhanced biogas production from anaerobic digestion of lignocellulosic biomass by different enhancement techniques. *Process Biochem* 84:81–90
- Kapoor R, Ghosh P, Kumar M, Vijay VK (2019) Evaluation of biogas upgrading technologies and future perspectives: a review. *Environ Sci Pollut Res* 26(12):11631–11661
- Kemausuor F, Adaramola M, Morken J (2018) A review of commercial biogas systems and lessons for Africa. *Energies* 11(11):2984. <https://doi.org/10.3390/en11112984>
- Lee YJ, Lee DJ (2019) Impact of adding metal nanoparticles on anaerobic digestion performance—a review. *Bioresour Technol* 292:121926
- Lehtinen S (2017) Building a small scale anaerobic digester in Quelimane
- Logan M, Visvanathan C (2019) Management strategies for anaerobic digestate of organic fraction of municipal solid waste: Current status and future prospects. *Waste Manag Res* 37(1):27–39
- Logan M, Safi M, Lens P, Visvanathan C (2019) Investigating the performance of internet of things based anaerobic digestion of food waste. *Process Saf Environ* 127:277–287
- Lombardi L, Francini G (2020) Techno-economic and environmental assessment of the main biogas upgrading technologies. *Renew Energy* 156:440–458
- Lü F, Wang Z, Zhang H, Shao L, He P (2021) Anaerobic digestion of organic waste: recovery of value-added and inhibitory compounds from liquid fraction of digestate. *Bioresour Technol* 333:125196
- Lüthi C, Morel A, Tilley E, Ulrich L (2011) Community-led urban environmental sanitation planning: clues complete guidelines for decision-makers with 30 tools. Swiss Federal Institute of Aquatic Science and Technology (Eawag), Dübendorf
- Mao C, Feng Y, Wang X, Ren G (2015) Review on research achievements of biogas from anaerobic digestion. *Renew Sust Energ Rev* 45:540–555
- Matheri AN, Mbohwa C, Ntuli F, Belaid M, Seodigeng T, Ngila JC, Njenga CK (2018) Waste to energy bio-digester selection and design model for the organic fraction of municipal solid waste. *Renew Sust Energ Rev* 82:1113–1121
- Meegoda JN, Li B, Patel K, Wang LB (2018) A review of the processes, parameters, and optimization of anaerobic digestion. *Int J Environ Res Public Health* 15(10):2224
- Miltner M, Makaruk A, Harasek M (2017) Review on available biogas upgrading technologies and innovations towards advanced solutions. *J Clean Prod* 161:1329–1337
- Moreno VC, Cozzani V (2015) Major accident hazard in bioenergy production. *J Loss Prev Process Ind* 35:135–144
- Moreno VC, Guglielmi D, Cozzani V (2018) Identification of critical safety barriers in biogas facilities. *Reliab Eng Syst Saf* 169:81–94
- Mothe S, Polisetty VR (2020) Review on anaerobic digestion of rice straw for biogas production. *Environ Sci Pollut Res* 28:24455–24469
- Mtika WM, Tilley E (2020) Environmental sanitation planning: feasibility of the CLUES framework in a Malawian small town. *Front Environ Sci* 7:204
- Mudasar R, Kim MH (2017) Experimental study of power generation utilizing human excreta. *Energy Convers Manag* 147:86–99
- Muhmood A, Majeed A, Niaz A, Shah SSH, Shahid MO (2018) Evaluation of anaerobic digestate potential as organic fertilizer in improving wheat production and soil properties. *Int J Plant Soil Sci* 24(1):1–10
- Mungwe JN, Colombo E, Adani F, Schievano A (2016) The fixed dome digester: an appropriate design for the context of Sub-Saharan Africa? *Biomass Bioenergy* 95:35–44
- Mutungwazi A, Mukumba P, Makaka G (2018) Biogas digester types installed in South Africa: a review. *Renew Sust Energ Rev* 81:172–180
- Ndubuisi-Nnaji UU, Ofon UA, Ekponne NI, Offiong NAO (2020) Improved biofertilizer properties of digestate from codigestion of brewer's spent grain and palm oil mill effluent by manure supplementation. *Sustain Environ Res* 30(1):1–11



- Nevzorova T, Kutcherov V (2019) Barriers to the wider implementation of biogas as a source of energy: a state-of-the-art review. *Energy Strat Rev* 26:100414
- Nguyen D, Khanal SK (2018) A little breath of fresh air into an anaerobic system: how microaeration facilitates anaerobic digestion process. *Biotechnol Adv* 36(7):1971–1983
- Obi-Ani NA, Isiani MC (2020) Urbanization in Nigeria: the Onitsha experience. *Cities* 104:102744
- Okon AJ, Eja ME, Kalu RE (2017) A study of access to sanitation profiles of rural upland and coastal communities of Akwa Ibom State, Nigeria. *Global J Pure Appl Sci* 23(2):207–212
- Olapeju OO, Majid MR, Nyakuma BB (2019) Cultural and perception dimensions of faecal waste applications for sustainable reuse in Ogun State, Nigeria. *Chem Eng Trans* 72:193–198
- Onwosi CO, Eke IE, Igbokwe VC, Odimba JN, Ndukwe JK, Chukwu KO, Aliyu GO, Nwagu TN (2019) Towards effective management of digester dysfunction during anaerobic treatment processes. *Renew Sust Energy Rev* 116:109424
- Onyebor EN, Umeh GN (2019) Access to improved toilet facilities among predominantly farming communities in Izzi Local Government Area of Ebonyi State, Nigeria. *J Environ Earth Sci* 9(11):1–9
- Orji SM, Ede AO, Onwuagba CG, Nwazunku AA, Okorie PC, Abonyi IC (2020) Assessment of water and excreta sanitation in Owerri Municipal and Owerri North Local Government Area, Imo State, Southeast, Nigeria
- O'Shea R, Wall DM, Murphy JD (2017) An energy and greenhouse gas comparison of centralised biogas production with road haulage of pig slurry, and decentralised biogas production with biogas transportation in a low-pressure pipe network. *Appl Energy* 208:108–122
- Oyedepo SO, Dunmade IS, Adekeye T, Attabo AA, Olawole OC, Babalola PO, Oyebanji JA, Udo MO, Kilanko O, Leramo RO (2019) Bioenergy technology development in Nigeria—pathway to sustainable energy development. *Int J Environ Sustain Dev* 18(2):175–205
- Patinvoh RJ, Taherzadeh MJ (2019) Challenges of biogas implementation in developing countries. *Curr Opin Environ Sci Health* 12:30–37
- Paul UO (2021) Biogas production from waste: a case study of Ekenwan Benin city Nigeria. Doctoral dissertation, Vytautas Magnus University)
- Pérez I, Garfí M, Cadena E, Ferrer I (2014) Technical, economic and environmental assessment of household biogas digesters for rural communities. *Renew Energy* 62:313–318
- Qiu L, Deng YF, Wang F, Davaritouchae M, Yao YQ (2019) A review on biochar-mediated anaerobic digestion with enhanced methane recovery. *Renew Sust Energy Rev* 115:109373
- Raboni M, Torretta V, Urbini G, Viotti P (2015) Automotive shredder residue: a survey of the hazardous organic micro-pollutants spectrum in landfill biogas. *Waste Manag Res* 33(1):48–54
- Rajendran K, Aslanzadeh S, Taherzadeh MJ (2012) Household biogas digesters—a review. *Energies* 5(8):2911–2942
- Ramatsa IM, Akinlabi ET, Madyira DM, Huberts R (2014) Design of the bio-digester for biogas production: a review. In: *Proceedings of the world congress on engineering and computer science*, vol. 2, p 22
- Rose C, Parker A, Jefferson B, Cartmell E (2015) The characterization of Feces and Urine: a review of the literature to inform advanced treatment technology. *Crit Rev Environ Sci Technol* 45 (17):1827–1879. <https://doi.org/10.1080/10643389.2014.1000761>
- Rotowa OO, Ayadi PA (2020) Faecal sludge management in the residential cores of Akure, Nigeria. *J Environ Prot Sustain Dev* 6(2):32–47
- Rowse LE (2011) Design of small scale anaerobic digesters for application in rural developing countries. Graduate Thesis and Dissertations, University of Florida
- Scarponi GE, Guglielmi D, Moreno VC, Cozzan V (2015) Risk assessment of a biogas production and upgrading plant. *Chem Eng Trans* 43:1921–1926
- Schertenleib R (2000) The Bellagio principles and a household centered approach in environmental sanitation. *Ecosan—closing the loop in Wastewater Management and Sanitation*, p 52
- Sekumade AB (2017) Economic effect of organic and inorganic fertilizers on the yield of maize in Oyo State, Nigeria. *Int J Agric Econ* 2(3):63–68

- Seruga P, Krzywonos M, Paluszak Z, Urbanowska A, Pawlak-Kruczek H, Niedźwiecki Ł, Pińkowska H (2020) Pathogen reduction potential in anaerobic digestion of organic fraction of municipal solid waste and food waste. *Molecules* 25(2):275
- Sherpa MG, Lüthi C, Koottatep T (2012) Applying the household-centered environmental sanitation planning approach: a case study from Nepal. *J Water Sanit Hyg Dev* 2(2):124–132
- Somorin TO (2020) Valorisation of human excreta for recovery of energy and high-value products: a mini-review. In: Daramola M, Ayeni A (eds) *Valorization of biomass to value-added commodities: current trends, challenges, and future prospects*. Springer, Cham, pp 341–370
- Sovacool BK, Andersen R, Sorensen S, Sorensen K, Tienda V, Vainorius A, Schirach OM, Bjørn-Thygesen F (2016) Balancing safety with sustainability: assessing the risk of accidents for modern low-carbon energy systems. *J Clean Prod* 112:3952–3965
- Stolecka K, Rusin A (2021) Potential hazards posed by biogas plants. *Renew Sust Energ Rev* 135: 110225
- Surendra KC, Takara D, Jasinski J, Kumar Khanal S (2013) Household anaerobic digester for bioenergy production in developing countries: opportunities and challenges. *Environ Technol* 34(13-14):1671–1689
- Thiriet P, Bioteau T, Tremier A (2020) Optimization method to construct micro-anaerobic digesters networks for decentralized biowaste treatment in urban and peri-urban areas. *J Clean Prod* 243: 118478
- Tian H, Karachalios P, Angelidaki I, Fotidis IA (2018) A proposed mechanism for the ammonia-LCFA synergetic co-inhibition effect on anaerobic digestion process. *Chem Eng J* 349:574–580
- Trávníček P, Kotek L (2015) Risks associated with the production of biogas in Europe. *Process Saf Prog* 34(2):172–178
- UNICEF (2016) Water sanitation and hygiene: the situation. <https://www.unicef.org/nigeria/wes.html>
- UNICEF/WHO (2015) *Progress on sanitation and drinking water—2015 update and MDG assessment*. World Health Organization, Geneva
- Usack JG, Wiratni W, Angenent LT (2014) Improved design of anaerobic digesters for household biogas production in Indonesia: one cow, one digester, and one hour of cooking per day. *Sci World J* 2014:318054
- Vaneekhaute C, Styles D, Prade T, Adams P, Thelin G, Rodhe L, Gunnarsson I, D’Hertefeldt T (2018) Closing nutrient loops through decentralized anaerobic digestion of organic residues in agricultural regions: a multi-dimensional sustainability assessment. *Resour Conserv Recycl* 136:110–117
- Vanguard (2018) Nigeria ranks No 2 in open defecation – Buhari. <https://www.vanguardngr.com/2018/11/nigeria-ranks-no-2-in-open-defecation-buhari/>. Accessed March 2, 2022
- Visvanathan C (2014) Evaluation of anaerobic digester for greenhouse gas emissions at various stages of its management. *Int Biodeterior Biodegrad* 95:167–175
- Wang J (2014) Decentralized biogas technology of anaerobic digestion and farm ecosystem: opportunities and challenges. *Front Energy Res* 2:10
- WHO/UNICEF (2010) *Progress on sanitation and drinking water*. UNICEF, New York, pp 23–29
- WHO/UNICEF (2019) *Progress on household drinking water, sanitation and hygiene I 2000-2017*. World Health Organisation, Geneva
- Wu Y, Wang S, Liang D, Li N (2020) Conductive materials in anaerobic digestion: from mechanism to application. *Bioresour Technol* 298:122403
- Yang P, Peng Y, Tan H, Liu H, Wu D, Wang X, Li L, Peng X (2021) Foaming mechanisms and control strategies during the anaerobic digestion of organic waste: a critical review. *Sci Total Environ* 779:146531

- Yaradua SA, Muhammad BG (2016) Potential biogas energy production using human faeces. *Kats J Nat Appl Sci* 5(2):1–11
- Yasar A, Nazir S, Rasheed R, Tabinda AB, Nazar M (2017) Economic review of different designs of biogas plants at household level in Pakistan. *Renew Sust Energ Rev* 74:221–229
- Zamri MF, Hasmady S, Akhiar A, Ideris F, Shamsuddin AH, Mofijur M, Fattah IMR, Mahlia TMI (2021) A comprehensive review on anaerobic digestion of organic fraction of municipal solid waste. *Renew Sust Energ Rev* 137:110637
- Zhang J, Zhao W, Zhang H, Wang Z, Fan C, Zang L (2018) Recent achievements in enhancing anaerobic digestion with carbon-based functional materials. *Bioresour Technol* 266:555–567

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## Part II

# Sustainability Approaches for Performance Enhancement and Resource Recovery



# Performance Enhancement Strategies of Anaerobic Digestion Technology: A Critical Assessment

Taysnara Simioni, Caroline Borges Agustini, Aline Dettmer, and Mariliz Gutterres

## Abstract

Anaerobic digestion is one of the most promising conversion technologies for the management of organic solid waste due to the production of methane-rich biogas and the recycling of nutrients. However, some process limitations need to be mitigated for an efficient industrial application. This chapter consolidates and summarizes research associated with the advantages/limitations of various performance-enhancing strategies to further promote development and industrial applications of the AD technology. The proposed strategies are classified into six main areas: (1) parameter optimization; (2) physical, chemical, and biological pretreatments; (3) co-digestion; (4) additives; (5) bioreactor design; and (6) genetic engineering.

## Keywords

Parameter optimization · Pretreatments · Additives · Co-digestion · Bioreactor design · Metagenomic

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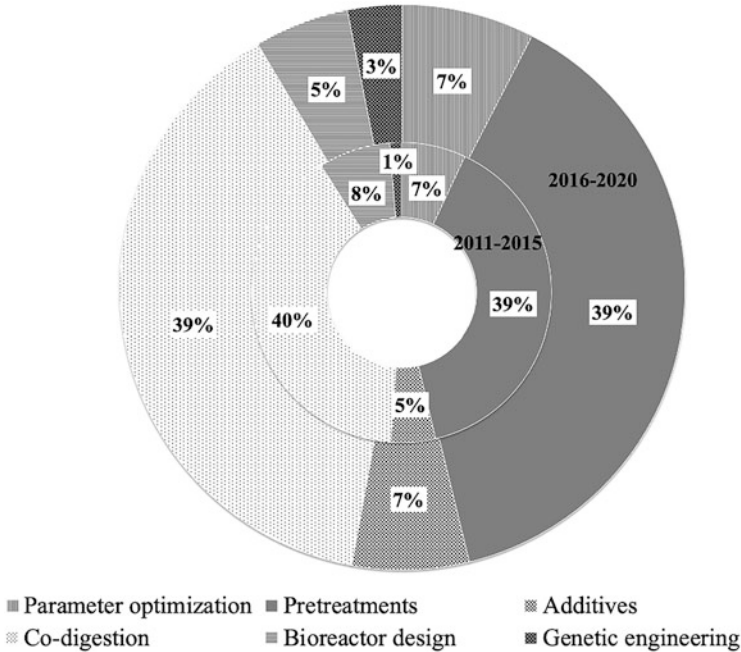
## 9.1 Strategies to Improve Anaerobic Digestion Technology

Anaerobic digestion (AD) is a suitable and efficient technology for organic materials management, and it is also predicted to play a vital role in the future of renewable energy production. However, AD is a complex and susceptible process involving numerous microorganisms with ultimate operational environmental conditions (Hagos et al. 2017), so knowing the main parameters that affect the process is essential for better decision-making on the configuration of the reaction (Cremonez et al. 2021).

Some strategies have been reported in the literature for enhancing AD performance with regard to biogas production and process stability. The proposed strategies can be classified into six major areas: optimization of operating parameter, pretreatments, additives, co-digestion, bioreactor design and optimization, and genetic technology. In order to analyze the relevance of each of these strategies, as well as to establish a trend for future research, a quantitative review of the literature published in the last decade was carried out, separated into two time intervals: 2011–2015 and 2016–2020. The articles counted in this analysis were found in the Scopus database, including title, abstract, and keyword. The terms entered in the search were “anaerobic” and “digestion” plus the term corresponding to the strategy being searched. The percentages corresponding to each strategy and for each time interval are shown in Fig. 9.1.

Data presented in Fig. 9.1 provides a good representation of the progress of the research in the past decade, indicating popularity, as well as future developmental potential. During the first time period considered, the main research focuses were almost equally placed on co-digestion and pretreatments. As characterization techniques and information on substrate influence on AD became available, the advantages of mixing different types of waste began to be studied, mainly as a strategy for nutrient balance. Furthermore, the recognition of the abundance of lignocellulosic biomass as a possible substrate for AD gave rise to research associated with pretreatment strategies. During 2016–2020, while co-digestion strategies and pretreatment techniques remain popular, genetic technology increased attention, and the use of additives was highlighted. With respect to genetic technology, the increase is associated with the greater availability of highly efficient and easy-to-use biomolecular tools for genetic analysis and manipulation. The strategy of using additives to improve the performance of AD, in turn, has gained significance because of advancements in scientific knowledge and application of new materials, such as nanoparticles.

It is also worth mentioning that considering the research as a whole, the number of published works more than doubled considering the time interval of 2016–2020 compared to 2011–2015, which confirms the interest of the scientific community in the AD of waste and consolidates biogas as a renewable energy of extreme importance and potential for the near future.



**Fig. 9.1** Percentage of published literature on strategies for improving biogas production during 2011–2015 and 2016–2020

### 9.1.1 Optimization of Operating Parameters

Operational parameters, including pH, temperature, organic loading rate (OLR), solid/hydraulic retention time (SRT/HRT), moisture content, and mixing/agitation condition, besides substrate characteristics (particle size, nutrients, and C/N ratio), are key factors that determine the operational efficiency of biogas production and the process stability of AD. Optimal configuration and manipulation of the operational parameters of AD can enhance the activity and growth of key anaerobic microorganisms, potentially resulting in significantly improved process stability and biogas yield (Wu et al. 2021).

Advanced monitoring methods can enable monitoring of key parameters in AD systems, which allows early detection of process disturbances. Ideal monitoring methods should be in situ, online, automated, and continuous, which means that the parameter detection process is directly carried out during plant operation, so that no delay or temporal dislocation is introduced in the process monitoring scheme. However, monitoring AD in biogas plants has proven to be extremely difficult because of a lack of robust and feasible online measurement systems and the high non-linearity of AD processes (Wu et al. 2019).

### 9.1.1.1 pH

pH is a crucial parameter in the dynamic detection and regulation of the AD process, as operating stability and bacterial activity are significantly affected by changes in pH (Zhang et al. 2019b). In addition, each group of microorganisms has a different range of optimum pH (Appels et al. 2008). Methanogenic archaea are extremely sensitive to pH, with an optimal value in the range of 6.5–8.2 (Kainthola et al. 2019; Zamri et al. 2021). For microorganisms acting in the steps of hydrolysis and acidogenesis, the optimum pH value is in the range of 5.5 and 6.5, respectively (Khalid et al. 2011; Mao et al. 2015). This is one of the important reasons why engineers prefer to disconnect the hydrolysis/acidification and acetogenesis/methanogenesis processes in two-stage AD reactors (Panigrahi and Dubey 2019).

Treating the process as a whole, the pH range from 6 to 8 seems to be the most suitable for AD (Xu et al. 2018), with neutral pH being the ideal (Panigrahi and Dubey 2019; Kumar and Samadder 2020). It is noteworthy that methane formation is considerably suppressed at pH values below 6.0 or at values above 8.5 (Khalid et al. 2011; Mirmohamadsadeghi et al. 2019).

Volatile fatty acids (VFAs) produced during the acidogenesis stage tend to reduce the pH. This reduction in pH is counterbalanced by the activity of methanogens, which produce alkalinity in the form of CO<sub>2</sub> (in the gas phase), ammonia, and bicarbonate (in the liquid phase) (Appels et al. 2008; Panigrahi and Dubey 2019). Low buffering capacity can be mitigated by reducing OLR, by adding salts for conversion of CO<sub>2</sub> to bicarbonate, or by direct addition of bicarbonate (Panigrahi and Dubey 2019).

### 9.1.1.2 Temperature

Temperature is one of the most important operational parameters of AD because it directly affects the thermodynamic equilibrium of the biochemical reactions, the activity of various enzymes and co-enzymes, microbial growth rate, diversity of the microorganisms, bioavailability of metals, sludge quality, stability, and consequently the production of biogas (Panigrahi and Dubey 2019; Zamri et al. 2021). Thermodynamic shows that endergonic reactions, such as the decomposition of propionate into acetate, CO<sub>2</sub>, and H<sub>2</sub>, would become energetically more favorable at higher temperatures, while exergonic reactions (hydrogenotrophic methanogenesis) are less favored at higher temperatures (Appels et al. 2008).

AD microorganisms can typically operate in three different temperature ranges: psychrophilic (below 20 °C), mesophilic (35–40 °C), and thermophilic (50–65 °C). From the point of view of biochemical reactions, a higher performance of AD can be achieved at a higher temperature within the limit of the established range. A very low-temperature digester may not facilitate the enzyme catalytic efficiency, while a too high-temperature digester may denature sensitive enzymes and subsequently lead to process failure (Zhang et al. 2019b). Compared with the mesophilic AD process, thermophilic conditions typically exhibit many advantages, including greater capacity of biogas generation, higher specific growth rate of microbes, lower HRT, higher reduction of pathogens and degradation of cell walls in feed-stock, and better separation of digestate into solids and liquid phases (Panigrahi and



Dubey 2019; Pan et al. 2021). On the other hand, the high reaction rate of acidogenesis in thermophilic process results in accumulation of VFAs in the digester, which may inhibit the activities of the methanogens. Another concern in thermophilic AD is the high energy requirement and process instability, which may negatively affect energy balance and the whole digestion process (Panigrahi and Dubey 2019). The ideal condition would be to operate at thermophilic temperature in the hydrolysis and acidogenesis steps and at mesophilic temperature in the methanogenesis step, which would be consistent with a two-phase AD reactor (Mao et al. 2015; Kainthola et al. 2019).

Generally speaking, the selection of an operating temperature depends on multiple factors, including the characteristics of feedstock, energy demand, financial support, and sanitization requirements. However, the primary concern during the construction and operation of any AD system should be temperature management and control (Wu et al. 2021). It is important to maintain a stable operating temperature in the digester as frequent fluctuations in temperature affect bacteria, especially the methanogenics (Appels et al. 2008).

### 9.1.1.3 Solid and Hydraulic Retention Time

The retention time, usually expressed in days, is the time required for the complete degradation of organic matter by microorganisms (Mao et al. 2015; Siddique and Wahid 2018; Zhang et al. 2019b). There are two types of retention time discussed: the SRT, which is defined as the average time that solids remain in the digester, and the HRT, which is defined as the average time interval that the sludge remains in the digester (Khadaroo et al. 2019; Pan et al. 2021). Generally, a retention time of 15–30 days is required to treat organic solid waste under mesophilic AD conditions and of 10–20 days for thermophilic conditions (Wu et al. 2021). However, this parameter must be specifically optimized according to process temperature, organic load, and substrate composition (Panigrahi and Dubey 2019; Pan et al. 2021).

The use of a relatively long retention time can mitigate process instability to a certain degree, yield a higher cumulative methane production, as well as lead to a greater reduction of the total volatile solid (VS), but the initial capital cost is significantly increased by the large investment required to build high-volume digesters. In contrast, too low HRT may not provide sufficient time for the multiplication of anaerobic microorganisms, and the discharging procedure may also cause “wash out” of microorganisms and smaller aggregates through the discharging procedure, eventually resulting in VFA accumulation in a manner similar to organic overload, which can cause bacterial inhibition and even process failure (Zhang et al. 2019b; Pan et al. 2021; Wu et al. 2021).

### 9.1.1.4 Organic Loading Rate

The OLR can be considered as the amount of organic matter fed to the AD process per unit of time and volume (Siddique and Wahid 2018), normally expressed as  $\text{kg}_{\text{COD}}/\text{m}^3/\text{day}$  or  $\text{kg}_{\text{VS}}/\text{m}^3/\text{day}$  (Wu et al. 2021). OLR varies depending on raw material characteristics, operating temperature, and HRT. Very low values can cause malnutrition of the fermentative microorganisms, resulting in a low efficiency of the

process (Zhang et al. 2019b). On the other hand, high values can lead to the accumulation of VFAs in the digester, to the imbalance of nutrients, and, consequently, to the inhibition of bacteria and even to the failure of the AD process (Panigrahi and Dubey 2019). Generally, biogas yield increases with increasing OLR (Wu et al. 2021), but this ratio has a limit. The maximum tolerable value of OLR is determined according to the type of organic solid waste and the operating conditions of the digester (Zhang et al. 2019b).

#### **9.1.1.5 Moisture Content**

The metabolism and activities of microorganisms require moisture. AD can be operated with a total solid (TS) content ranging from 5% to 35%. Depending on the TS content, AD can be divided into three different categories: wet (<10% TS), semi-dry (10–20% TS), and dry (>20% TS) (Kumar and Samadder 2020). Both the processes (wet and dry) have their own advantages and disadvantages. The wet process has the advantages of lower inoculum requirement, shorter retention time, higher methane production, and greater reduction of VS. In contrast, the advantages of the dry (or solid state) process include high OLR, smaller digester volume, less energy expenditure for system heating and agitation, and easier digestate handling (Mirmohamadsadeghi et al. 2019). The literature indicates that the highest methane production rates occur for cases with moisture in the range of 60–80% (Khalid et al. 2011).

#### **9.1.1.6 Mixing/Agitation Condition**

Proper agitation of the digesters favors the supply of nutrients to the microorganisms, in addition to removing products of metabolism (especially the H<sub>2</sub> blocking layer), avoiding the formation of foam and temperature gradients, and homogenizing the solution, eliminating any division into layers (Kainthola et al. 2019; Mirmohamadsadeghi et al. 2019). There are currently three major systems to accomplish agitation in the anaerobic digesters and mechanical, pneumatic, and hydraulic mixers, among which mechanical agitators are the most dominant systems being used. However, experimental results have demonstrated that aside from the type of substrate treated and agitation equipment applied in the AD process, the duration and intensity of mixing have a significant effect on the process stability and biogas yield. Vigorous mixing intensity may inhibit the process stability by disrupting microbial flocks as well as reducing the extracellular polymeric substances that affects the adhesion of microbial cells to each other as well as to other surfaces. Regarding the mixing duration, intermittently or minimally mixed systems appear to have similar or even better outcomes in terms of biogas production and process stability than the continuous mixing regime while reducing energy requirements and maintenance costs of biogas-producing systems (Alavi-borazjani et al. 2020).

#### **9.1.1.7 Particle Size**

The particle size of the substrates directly influences the biodegradation rate and the stability of AD (Siddique and Wahid 2018; Mirmohamadsadeghi et al. 2019; Zhang

et al. 2019b). Very large particles would lead to the clogging of the digester as well as difficulty in the digestion function for the responsible microbes, while reducing the particle size of the substrate could increase the specific surface area accessible to microbial attacks, thus allowing faster reaction rates and increased biogas yield (Alavi-borazjani et al. 2020). However, the excessive reduction in particle size could over-quicken the hydrolysis of substrate, resulting into build-up of VFA and ammonia which could destabilize the reactor (Kumar and Samadder 2020).

#### 9.1.1.8 Nutrients and C/N Ratio

Some micro- and macronutrients are necessary for the survival and growth of microorganisms involved in the AD process (Mirmohamadsadeghi et al. 2019). Carbon and nitrogen are the essential sources for energy and development of new cell structure (Zamri et al. 2021). Also, phosphorus is a key element for capturing and transferring energy by energy carriers in cellular activities of microorganism, and sulfur is needed as a nutrient for the growth of methanogens (Mirmohamadsadeghi et al. 2019).

In mono-digestion, a single substrate is either carbon-rich or nitrogen-rich, such that it is difficult to maintain a balanced C/N ratio. The imbalance between nutrients has a negative effect on microbial activity, being a limiting factor in the AD of organic waste (Neshat et al. 2017). At high C/N ratio, excessive acidification occurs due to rapid degradation of substrate during the initial stage of the digestion, resulting into the process instability. The excess carbon content will slow down the degradation process, as more time will be taken by the microorganisms to consume the available carbon. On the contrary, low C/N ratio limit microbial growth due to lack of carbon, which can lead to accumulation of ammoniacal nitrogen and VFAs in the digester (Kumar and Samadder 2020). Relatively high C/N ratios are found in oat straw (47–51), wheat straw (51–151), sugar cane waste (139–151), or sawdust (199–501). At the same time, relatively low C/N ratios are observed for pig manure (7–15) and food wastes (2–18) (Siddique and Wahid 2018). For the success of the DA process, the literature recommends that the C/N ratio be in the range of 20:1 to 30:1 (Panigrahi and Dubey 2019). Modulation of the C/N ratio can be done by mixing different substrates (Khalid et al. 2011; Xu et al. 2018; Zhang et al. 2019b).

Besides essential macronutrients, the anaerobic process requires microelements at a relatively lower concentration. Iron, cobalt, nickel, zinc, molybdenum, manganese, copper, selenium, and tungsten are the main metal microelements with a recommended concentration between  $1 \times 10^{-6}$  and  $1 \times 10^{-15}$  M (Rasapoor et al. 2020). Micronutrients are crucial cofactors in numerous enzymatic reactions involved in the biochemistry of methane formation (Romero-güiza et al. 2016). Iron, acting as a growth factor, plays an important role as a stimulating agent in the formation of ferredoxins and cytochromes, vital components in cell metabolism. In addition, the iron may react with  $H_2S$  to precipitate sulfur and iron sulfide (II) and reduce the effects of corrosion of  $H_2S$  in the biogas. As a growth factor for acetogenic microorganisms, cobalt assists in the stability of the AD process. Nickel is necessary for the growth of methanogenic bacteria, especially methanogenic archaea. Zinc is required for the synthesis of carbonic anhydrase by methanogens.

Depending on the methanogenic pathway (acetoclastic or hydrogenotrophic), several metalloenzymes are involved in the AD process, and, consequently, different micronutrients are needed. Generally, micronutrient supplementation, or rather the right combination of multinutrients, can improve the performance of AD. On the other hand, high concentrations of micronutrients can inhibit the AD process (Mirmohamadsadeghi et al. 2019).

### 9.1.1.9 Inhibitor Compounds

Inhibitory compounds can either be present in the substrate or intermediate compounds generated during the AD process that inhibit the process at high concentrations. The main inhibitory compounds are VFAs, ammonia, metals, and toxic compounds (Panigrahi and Dubey 2019; Kumar and Samadder 2020).

VFAs (acetic, propionic, butyric, and valeric acid) are intermediate products of the initial stage of AD process and act as indicators of the correct balance between hydrolysis, acidogenesis, and methanogenesis, since the produced VFAs should get converted into  $\text{CH}_4$  and  $\text{CO}_2$  by the active microorganisms. In a stable anaerobic digester, the concentration of VFAs is about 50–250 mg/L. On the other hand, high concentrations of VFAs decrease the pH of the medium, which can inhibit methanogenesis (Kumar and Samadder 2020). The inhibitory concentration of VFAs has been reported to be about 1500 mg/L (Neshat et al. 2017).

Ammonia has advantages and disadvantages for AD. It can act as a pH neutralizer as well as being a valuable nitrogen source for methanogenic bacteria (Neshat et al. 2017). On the other hand, high concentrations of ammonia can poison microorganisms and inhibit AD. Ammonia inhibition occurs mainly during the AD of protein-rich solid waste. This behavior occurs because the digestion of these residues results in the hydrolysis and solubilization of the protein into amino acids through proteolytic bacteria. Amino acids, in turn, are further hydrolyzed by hydrolytic/hydrogenic bacteria to release ammonia,  $\text{H}_2$ ,  $\text{CO}_2$ , and fatty acids (Agustini and Gutterres 2017). Ammonia-inhibiting concentration in the anaerobic digester mainly depends on the pH, temperature, C/N ratio, type of substrate, and inoculum (Kumar and Samadder 2020).

Metallic elements, including light metals (sodium, potassium, magnesium, calcium, and aluminum) and heavy metals (chromium, cobalt, copper, zinc, and nickel), are among the micronutrients necessary for the survival of microorganisms. However, at high concentrations, they can cause inhibition of AD by interrupting the function of enzymes (Mirmohamadsadeghi et al. 2019). Phenolic compounds, furans, cyanides, and sulfur oxides are also toxic to microorganisms and inhibit the AD process when in high concentrations (Neshat et al. 2017).

### 9.1.1.10 Chemical Composition of Substrates

Substrates contain the full range of simple and complex organic materials that can be used in the AD process, being carbohydrates, proteins, and lipids, as the main ones. Depending on their sources (agricultural, urban, food, industrial waste), specific organic compounds may predominate, although in most cases the exact composition of substrates is difficult obtaining (Rasapoor et al. 2020). A comprehensive

understanding of the types and biochemical characteristics of feedstock plays a critical role in preventing process instability and optimizing AD systems. Commonly used parameters for monitoring feeding substrates are TS, VS, C/N ratio, macro- and microelements, particle size, pH, easily degradable compounds (e.g., soluble sugar, protein, carbohydrate, lipids), low degradable or undigested compounds (e.g., hemicelluloses, cellulose, and lignin), and impurities and inhibitors (e.g., ammonia, heavy metals). Unfortunately, it remains difficult for most commercial biogas plants to achieve online/in situ monitoring and analysis of feedstock with equipment and methods that are currently available at an acceptable cost (Wu et al. 2021).

**Carbohydrate-Rich Biomass Waste** Carbohydrates are considered the most important organic component in solid waste for the production of biogas (Khalid et al. 2011). Carbohydrates (commonly sugars) are present in all substrates, in different proportions. AD from organic wastes with high levels of simple sugars can result in rapid accumulation of VFAs in the reactor, a decrease in pH, and consequent suppression of methanogenesis. For the balanced operation of anaerobic reactors, it is recommended to mix a substrate containing large amounts of simple carbohydrates with residues with a lower content of easily biodegradable organic components (Hagos et al. 2017; Siddique and Wahid 2018). Residues composed of lignocellulosic carbohydrates, by contrast, show slow degradability, considerably increasing the HRT of digesters and consequently reducing biogas production rates (Cremonese et al. 2021). A pretreatment step may be essential to improve the digestibility of these residues.

**Protein-Rich Biomass Waste** As carbohydrates, proteins are also present in the majority of organic substrates. Protein-rich substrates can produce biogas with high methane content. The microbial degradation of proteins results in the release of the ammonium ion, which can be a strong inhibitor of methanogenic bacteria, as well as ammonia, when in high concentration. Ammonia and ammonium are in balance with each other to maintain the process stability, which depends heavily on operating factors (pH, temperature). Choosing suitable co-substrates and adjusting the C/N ratio can minimize this problem (Hagos et al. 2017; Siddique and Wahid 2018).

**Lipid-Rich Biomass Waste** Organic materials with a high fat content are readily degradable and therefore have high biogas production. However, in high concentrations, lipids cause different kinds of problems in anaerobic digesters, including blocking, adsorption to biomass (causing mass transfer problems), and microbial inhibition. Mixing carbohydrate-rich with fat-rich materials (slowly and rapidly degradable, respectively) is advantageous in nutritional balance, enriches microorganisms, reduces inhibitor accumulation, and increases stability, resulting in high efficiency in biogas production and methane yield (Hagos et al. 2017; Siddique and Wahid 2018).

### 9.1.1.11 Inoculum

The choice of the inoculum is a fundamental step for the good performance of the AD process, as it not only provides trace elements, moisture content, and nutrients (macro and micro) but also provides the buffering capacity in the system (Kainthola et al. 2019; Cremonez et al. 2021). The use of sludge from digesters or treatment ponds for the degradation of residues of similar characteristics to the substrates of interest makes the systems more efficient and more adapted and may considerably reduce the lag phase time, especially in more complex systems (Cremonez et al. 2021).

Substrate and inoculum concentrations or substrate-to-inoculum (*S/I*) ratio are among the most important factors influencing AD performance and stability. Very high or low inoculum concentrations may disrupt the AD process by affecting the bacterial lag phase time, reactions rate, nutrient consumption, biomass growth behavior, and so on. Therefore, establishing a balance between substrate and inoculum concentrations, or in other words, optimizing the *S/I* ratio, seems to be a good strategy for successful operation of AD processes (Alavi-borazjani et al. 2020). Results showed that lower *S/I* ratios favored biogas production due to the rapid degradation of VFAs. Conversely, bioreactors operated at higher *S/I* ratios (50:50 and above) experienced excessive VFA concentrations, a sharp drop in pH, and consequently biogas production levels lower than theoretical values. In general, the accumulation of toxic intermediate products such as VFAs at higher *S/I* ratios indicates a kinetic imbalance between the microorganisms responsible for the production and consumption of acids inside the anaerobic reactor (Alavi-borazjani et al. 2020).

### 9.1.2 Pretreatment

Substrate pretreatment is a common step in raw material processing, especially for those with high lignocellulosic content. Characteristics such as the presence of lignin and cellulose, crystallinity and degree of polymerization of cellulose, accessible surface area, and degree of acetylation of hemicellulose have an impact on the biodegradability of biomass (Zheng et al. 2014). During pretreatment, difficult-to-degrade compounds present in the substrate, such as hemicellulose, cellulose, and lignin, are transformed into soluble compounds, which are more easily hydrolyzed by bacterial enzymes (Neshat et al. 2017; Zhang et al. 2019b). A successful pretreatment should be able to preserve the organic materials in biomass, develop the progress of beneficial to hydrolysis, avoid the formation of any toxic and/or inhibitory compounds, and to be environmentally friendly and economically feasible (Panigrahi and Dubey 2019).

In the literature, pretreatments are classified as physical, chemical, or biological. The choice of which pretreatment to apply depends on several factors, such as the crystallinity of the lignocellulose, the degree of polymerization, the accessible surface area, and the relative amount of acetyl groups (Zheng et al. 2014; Zhang

et al. 2019b). A combination of pretreatments can also be used and is often found in the literature.

### 9.1.2.1 Physical Pretreatment

Physical pretreatment refers to methods that do not use chemicals or microorganisms during the process. The physical pretreatment methods most commonly used in substrate preparation for AD include mechanical and thermal pretreatment (Neshat et al. 2017).

Mechanical pretreatment (Moset et al. 2018; Akbay et al. 2021) of biomass is used to reduce particle size and is typically applied before other pretreatment methods (Millati et al. 2020). The reduction in particle size can breakdown the lignin-hemicellulose complex, changing the morphology of the lignocellulosic biomass and reducing the degree of crystallinity and polymerization of cellulose, which increases the available cellulosic content. Furthermore, it increases the accessible surface area which provides better contact between the substrate and anaerobic bacteria, resulting in improved digestibility of solid waste (Ariunbaatar et al. 2014; Tian et al. 2018; Kainthola et al. 2019). However, if the mechanical pretreatment is excessive (obtained particle size is less than the optimal particle size), digestion performance will deteriorate, such as the accumulation of VFAs, and net energy output will be not positive (Cai et al. 2021). The advantages of mechanical pretreatment include no odor generation, an easy implementation, better dewaterability of the final anaerobic residue, and moderate energy consumption. Disadvantages include no significant effect on pathogen removal and the possibility of equipment clogging or scaling (Ariunbaatar et al. 2014).

Thermal treatment technique (Ennouri et al. 2016; Rajput et al. 2018) is of two types, thermal pretreatment (only temperature is controlled, like hot air oven, microwave, hot water bath) and hydrothermal pretreatment (both temperature and pressure are controlled, like autoclave and steam explosion) (Panigrahi and Dubey 2019). The optimal temperature is a wide range for the different substrates and pretreatment time. In general, for the hot air oven, microwave, autoclave, and hot water bath pretreatment, the optimal temperature ranges are 90–170 °C, 140–200 °C, 90–175 °C, and 90–100 °C, respectively (Cai et al. 2021). Although high temperature can achieve better pretreatment effect, it is worth noting that temperatures above 150 °C could cause the formation of some inhibitory compounds (such as phenolic, furfural, and hydroxyl methyl furfural) which are toxic to anaerobic microorganisms (Hashemi et al. 2021). The main effect of thermal pretreatment is the disintegration of cell membranes, thus resulting in solubilization of organic compounds. Thermal pretreatment also leads to pathogen removal and reduction of sludge viscosity (Ariunbaatar et al. 2014). The disadvantages are the formation of odorous compounds and high operation and maintenance costs (Khadaroo et al. 2019).

### 9.1.2.2 Chemical Pretreatment

Chemical pretreatment of solid organic waste is carried out using strong acids, alkalis, ionic liquids, and oxidizing agents. Compared to physical pretreatment techniques, chemical pretreatments have received more attention in recent years

from researchers due to their better performance in increasing the yield of biogas production (Zhang et al. 2019b).

The effect of chemical pretreatment depends on the type of method applied and the characteristics of the substrates. It is not suitable for easily biodegradable substrates containing high amounts of carbohydrates, due to their accelerated degradation and subsequent accumulation of VFAs, which leads to failure of the methanogenesis step. In contrast, it can have a clear positive effect on substrates rich in lignin (Ariunbaatar et al. 2014). The major effects of chemicals on lignocellulosic substrates are the removal of lignin and hemicellulose, which leads to an increase in the accessible surface area for enzymatic hydrolysis, and the reduction in cellulose crystallinity. Although chemical pretreatment methods are efficient methods for pretreatment with short substrate retention time, some concerns such as the high cost, effectiveness of chemical recovery, and chemical discharge into the environment are the most critical barriers in large-scale applications (Hashemi et al. 2021).

Acid pretreatment (Dai and Dong 2018; Syaichurrozi et al. 2019) commonly involves usage of  $H_2SO_4$ ,  $H_2O_2$ ,  $HCl$ ,  $HNO_3$ ,  $CH_3COOH$ , etc. (Kainthola et al. 2019; Cai et al. 2021). The main objective of acid pretreatment is to solubilize hemicellulose, reduce cellulose, and hydrolyze hemicellulose into respective monosaccharides by disrupting the covalent hydrogen bonds and van Der Waals forces (Panigrahi and Dubey 2019). Strong acidic pretreatment may result in the production of inhibitory by-products, such as furfural and hydroxymethylfurfural (HMF). Hence, strong acidic pretreatment is avoided, and pretreatment with dilute acids is commonly coupled with thermal methods. Other disadvantages associated with the acid pretreatment include the loss of fermentable sugar due to the increased degradation of complex substrates, a high cost of acids, and the additional cost for neutralizing the acidic conditions prior to the AD process (Ariunbaatar et al. 2014).

Alkaline pretreatment (Rahman et al. 2018; Sabeeh et al. 2020) uses bases such as  $NaOH$ ,  $Ca(OH)_2$ ,  $KOH$ , and  $NH_3 \cdot H_2O$  to modify the structure of lignocellulosic substrate components and make them more degradable to microorganisms and enzymes. By removing cross-links, alkaline pretreatment leads to an increase in porosity and internal surface area, decrease in the degree of polymerization and crystallinity, disruption of the lignin structure, and breaking of bonds between lignin and other polymers. The effectiveness of alkaline pretreatment is associated with the lignin content of the substrates. The main disadvantage of this technique is the high cost of the base (Zheng et al. 2014; Patinvoh et al. 2017; Tian et al. 2018).

Ionic liquid (Mancini et al. 2018; Pérez-Pimienta et al. 2021) pretreatment uses molten salts (like 1-ethyl-3-methylimidazolium acetate) and organic components such as *N*-methylmorpholine *N*-oxide (NMMO) in moderated temperatures to cause the dissolution of the biomass components (Hashemi et al. 2021). The cellulose dissolution mechanism in these reagents involves the oxygen and hydrogen atoms of the hydroxyl groups of the cellulose molecule, which form electron donor-receptor complexes and which interact with ionic liquids. After this interaction, hydrogen bonds are broken, leading to the opening of the hydrogen bonds between the cellulose molecular chains, resulting in dissolution of the molecule. The solubilized



cellulose can be precipitated with anti-solvents such as ethanol, methanol, acetone, or water. It was found that the recovered cellulose has the same degree of polymerization and polydispersity as the initial cellulose, but significantly different macro- and microstructures, especially with regard to decreasing crystallinity and increasing porosity (Zheng et al. 2014). Along with structural changes, the ionic liquids are capable of partial lignin removal (Hashemi et al. 2021). Despite the high cost of ionic liquids (Zheng et al. 2014), the main advantage of this method are the easy recycling of the pretreatment solvent by distillation (Mancini et al. 2018).

The oxidative pretreatment (Cesaro and Belgiorno 2013; Hodaei et al. 2021) accelerates the reaction rates by applying oxygen or air at high temperature (above 260 °C) and pressure (10 MPa) to the feedstock prior to the AD process. The oxidative methods are Fenton, peroxymonosulfate, dimethyldioxirane, and advanced oxidation process (AOP). Ozone treatment enhances hydrolysis step by solubilizing and/or breaking lignin (Panigrahi and Dubey 2019). Ozonation leaves no chemical residues compared to other chemical pretreatment methods (Ariunbaatar et al. 2014). The disadvantage of this process is the high operating cost (Khadaroo et al. 2019).

### 9.1.2.3 Biological Pretreatment

The fundamental concept of biological pretreatment is to improve the biodegradability either through the application of fungi, microbial consortium, and enzymes ensiling the biomass or by adding a biological treatment step prior to the AD (Koupaie et al. 2019). Mild operating conditions and no chemical added make biological pretreatment a less energy-demanding and more environmentally friendly process (Panigrahi and Dubey 2019). However, the slow reaction rate of biological pretreatment remains a problem (Millati et al. 2020).

Fungal pretreatment (Tisma et al. 2018; Zanellati et al. 2021) is a common microbial pretreatment method. Fungi can secrete cellulases, hemicellulases, and ligninase. Specifically, those functions of enzymes are involved in the structure change of lignocellulosic biomass mainly including modifying lignin structure (the ratio of guaiacyl/sinapyl), decreasing cellulose crystalline, increasing substrate porosity, and changing hemicellulose structure (the ratio of xylose/arabinose) (Cai et al. 2021). White-rot fungi are very popular to be used in this kind of pretreatment due to its effectiveness in degrading lignin among other fungi such as brown-, white-, and soft-rot fungi (Millati et al. 2020).

Besides pure fungi, the microbial consortium (Raut et al. 2021; Wang et al. 2021) was also developed and used for the pretreatment of lignocellulosic biomass. The microbial consortium can be obtained through restrictive culture technology, which can select an ideal microbial consortium; artificially, combining a variety of pure bacteria with the function of decomposing lignocellulosic biomass; or directly from natural sources, such as biogas slurry. Among the three types of microbial consortia, biogas slurry is the most likely to be applied due to its economic viability, environmental friendliness, and strong operability. The optimal pretreatment condition depends on the source of microorganisms. Pretreatment time is the most crucial factor that needs to be optimized to reduce organic matter loss and to obtain the best

results. In addition to the incubation time, pH range, temperature, and oxygen concentration are also vital parameters. In general, microbial pretreatment has good results in lab-scale study due to controllable conditions (Cai et al. 2021).

Enzymatic pretreatment (Domingues et al. 2015; Çakmak and Ugurlu 2020) involves the use of oxidative and hydrolytic enzymes often produced by bacteria and fungi. This pretreatment method is gaining more interest due to the relatively short reaction time, the low nutrition requirement for the enzymatic reactions, and also the fact that most of the enzymes are not affected by the presence of inhibitor and other microbial metabolisms. However, although enzymatic pretreatment does not require expensive processing equipment, the high cost of the enzyme remains an obstacle for its large-scale application (Koupaie et al. 2019). In order to achieve an effective and cost-efficient enzymatic pretreatment, several strategies have been studied, such as optimization of enzyme activity, enzyme recycle, development of genetically modified organisms that can produce high-quality enzymes, and improvement enzyme quality by genetic engineering (Millati et al. 2020).

#### **9.1.2.4 Hybrid Pretreatment Technologies**

Combined pretreatment (Patowary and Baruah 2018; Ambrose et al. 2020) incorporates two or more pretreatment techniques from the same or different categories. The coupling of different pretreatments may add the isolated advantages of each, leading to better prepare the substrate for the AD process (Zheng et al. 2014; Hashemi et al. 2021). Physical pretreatments would increase the accessible surface area and decrease the degree of polymerization and crystallinity of cellulose, while chemical and/or biological pretreatments would facilitate the accessibility of enzymes to cellulose (Zhang et al. 2019b). However, the combination of two or more individual pretreatment methods would require more energy input and higher costs of chemical or biological reagents, which could be a major obstacle to industrial applications. Thus, deciding on an appropriate combined pretreatment method requires a balanced consideration of chemical and enzyme costs, energy requirements, and potential gain in biogas production (Zheng et al. 2014; Zhang et al. 2019b).

#### **9.1.3 Co-digestion**

The use of a single substrate in the AD process can be hampered by some factors. The nutritional imbalance of the substrate used stands out as the main one (Hagos et al. 2017). Anaerobic co-digestion (AcoD), which is the AD from a mixture of two or more different substrates, appears as a promising option to overcome the drawbacks of mono-digestion and enhance the economic feasibility of AD process (Karki et al. 2021).

The main advantage of the AcoD-based process is the improvement in biogas production and methane yield. Co-digestion can increase biogas production from 25% to 400% over mono-digestion, considering the same substrates acting separately (Hagos et al. 2017; Karki et al. 2021).

AcoD of two or more substrates (Zahan et al. 2018; Ghosh et al. 2020; Simioni et al. 2021) provides better availability and balance of macro- and micronutrients (for good microbial growth), dilution of toxic or inhibitory compounds, moisture balance, and better buffering capacity. AcoD also allows positive synergistic effects on process efficiency, increase in biodegradable organic load, expansion of the microbial community involved in the process, and higher concentrations of active biomass. This leads to better process stability and greater biogas generation (Khalid et al. 2011; Hagos et al. 2017; Tyagi et al. 2018; Panigrahi and Dubey 2019). In addition, the economic advantages of sharing the AD system and treating more waste at the same time deserves to be highlighted (Hagos et al. 2017; Siddique and Wahid 2018).

Recalling that to optimize the AcoD process, the adjustment of operational parameters (temperature, pH, OLR, etc.) of the reactor and the characterization of the substrates involved as to the C/N ratio, biodegradability, bioaccessibility, and bioavailability remain crucial (Hagos et al. 2017; Siddique and Wahid 2018).

### 9.1.4 Additives

AD depends on a set of enzymatic reactions facilitated by complex microorganisms. Many researchers argue that the application of some additives mixed with the substrate could intensify waste biodegradation and increase methane production (Zhang et al. 2019b).

Additives have been successfully used to improve methane production in anaerobic digesters by different approaches, such as (1) supplying nutrients at low concentrations, (2) adsorbing inhibitory elements at high concentrations, (3) improving digester buffer capacity, and (4) enhancing substrate biodegradability (Romero-guiza et al. 2016). The performance of additives may not be directly compared because many factors of different AD systems are not the same, such as substrate type, digester configuration, and anaerobic microbial community composition. An excess of additives may deteriorate the performance of the AD process (Liu et al. 2021).

Some studies have reported that various metal-based additives (Schmidt et al. 2014; Hassanein et al. 2019) can enhance AD performance and improve anaerobic bioconversion processes by stimulating methanogens to increase CH<sub>4</sub> production during AD. The addition of trace metals is advantageous for the growth of methanogens during enzymatic synthesis. Many enzymes include transition metals as catalytic centers at active sites or as cofactors for electron transport. Trace metals can be added to the AD process in various forms, e.g., chloride salts, metal oxides, and metal nanoparticles. The presence of trace metals in AD systems is beneficial for the hydrolysis and acidogenesis stages, and it also augments the microbial species to improve the biogas production. The optimal concentration of trace elements in an AD system depends on substrate type, especially for micronutrient-deficient substrates. The different species of methanogens have different requirements for trace element concentrations. The combined addition of the different kinds of metals

resulted in a high biogas yield in AD. In contrast, a high concentration of metals may inhibit the methanogens, thereby causing low methane production (Liu et al. 2021).

Carbon-based additives (Tian et al. 2017; Chen et al. 2020), such as activated carbon (AC), graphene, biochar, carbon cloth, graphite, granular activated carbon (GAC), and carbon nanotubes, have been widely employed to enhance methanogenesis through direct interspecies electron transfer (DIET) (Romero-güiza et al. 2016; Alavi-borazjani et al. 2020). Carbon-based materials, with its favorable physicochemical properties (fine pore structure, good electrical conductivity, large porosity, and surface area), promote microbial activity, electron transfer among anaerobes, and biogas production, because these materials provide a good immobilization matrix for microorganisms. Furthermore, carbon-based accelerants with conductive capacity can enhance AD performance by building bioelectrical relations between the methanogens and acetogens, which is beneficial for relieving VFA accumulation (Liu et al. 2021).

Most of the goals achieved by inorganic additives can also be reached through biological additives (Romero-güiza et al. 2016). Bioaugmentation technique (Pessuto et al. 2016; Akyol et al. 2019; Mlaik et al. 2019) consists of adding specifically selected microorganisms into biogas digesters to improve the performance of the AD process (Alavi-borazjani et al. 2020). The addition of bacterial consortia or cellulolytic bacteria can promote the hydrolysis rate and increase the CH<sub>4</sub> yield (Liu et al. 2021). The addition of anaerobic fungi resulted in an increase in VFA degradation, which would be favorable to alleviate the accumulation of these acids in anaerobic digesters and, consequently, increase biogas production (Alavi-borazjani et al. 2020). Enzymes can also be directly dosed into AD systems as they are capable of acting in the presence of various toxic and recalcitrant substrates, and under a wide range of environmental conditions (e.g., pH, temperature, and salinity) remaining active even if these conditions quickly change, they can work in the presence of microorganisms and inhibitors of microbial metabolism and, due to their smaller size, higher solubility, and mobility, have easier access to the substrates than microbes do (Romero-güiza et al. 2016). Bioaugmentation with a mixture of different species of anaerobic fungi or with a different enzymatic composition is more efficient, since each enzyme degrades only a few specific substrates (Liu et al. 2021).

Supplementation of mineral-based additives (Kotsopoulos et al. 2008) has shown to be a cost-effective approach to control the accumulation of undesired VFAs and to enhance the biogas production from the AD process. Among a variety of minerals existing in nature, silicate minerals like wollastonite (CaSiO<sub>3</sub>) appear to be able to react effectively with H<sup>+</sup> ions provided by VFA dissociation, thus neutralizing the pH inside the AD reactors. Aluminosilicate minerals like zeolite have also been widely used, as the ion exchanger and adsorbent for the removal of organic molecules as well as supplying the trace elements in AD (Alavi-borazjani et al. 2020).

In recent years, numerous research efforts have been made to use inorganic wastes as cost-effective supplements for dealing with VFA inhibition in AD processes. Among them, ashes (Lo 2010) from the thermochemical processing of

biomass have shown good results mainly via providing alkalis and trace metals required for balancing the AD process (Alavi-borazjani et al. 2020).

### 9.1.5 Bioreactor Design and Optimization

The design and configuration of the bioreactor used for the AD of organic solid waste have a strong influence on the performance of the process. Several types of bioreactors can be used to perform AD processes, continuous stirred-tank reactor (CSTR), upflow anaerobic sludge bioreactor (UASB), anaerobic baffled reactors (ABR), anaerobic sequencing batch biofilm reactor (AnSBBR), anaerobic packed-bed reactor (APBR), anaerobic structured-bed reactor (ASTBR), expanded granular bed reactor (EGBR), expanded granular sludge blanket (EGSB), sequential batch reactor (SBR), and leachate bed reactor (LBR) (Cremones et al. 2021). About the configuration mode, the three main groups include batch reactors, one-stage continuously fed reactors, and two- or multi-stage continuously fed system.

The operation of reactors in batch mode is quite simple: the reactor is fed with raw material, which is degraded for a certain period of time (HRT). After this time, the bioreactors are emptied and a new batch is fed. Although they are equipment simple to build and operate and of lower cost, batch reactors have some limitations. Low quality, fluctuations in biogas production, and loss of biogas during the depletion of bioreactors are among them (Khalid et al. 2011).

Regarding the other two configurations of bioreactors mentioned, systems fed continuously from one or more stages, the only difference is that in the first, all biochemical reactions take place in one bioreactor, and in the second, the stages of DA (hydrolysis, acidogenesis, acetogenesis, and methanogenesis) occur separately. The system with two or more stages is considered a promising method for the treatment of organic waste with high efficiency in terms of waste degradation and biogas production yield. This type of system allows the selection and enrichment of different bacteria in each stage, in addition to increasing the stability of the process by optimizing the HRT to avoid overloading and toxic material accumulation (Khalid et al. 2011; Hagos et al. 2017). Temperature, pH, organic load, and other conditions can be independently optimized for each stage in order to favor the specific microbial reactions of the AD step taking place there (Cremones et al. 2021).

### 9.1.6 Genetic Technology

The efficiency of methane production from the AD of waste is related to the number of species and the physiological behavior of the microbial consortium involved. Therefore, the description of these two aspects can be used not only to characterize an AD process but also to improve its efficiency. The development of genetic engineering techniques, such as gene sequencing technologies, metagenome technology, and synthetic biology, allows not only to change a DNA sequence also but to

build entirely new sequences and put them into operation in microbial cells in order to make AD faster and more efficient (Zhang et al. 2019b).

Over the past decade, the development of high-throughput sequencing technology and the decrease in its cost facilitated the application of bioinformatics tools in the studies of metagenomics data of microbial communities in anaerobic digesters. For instance, it is now known that there is a clear correlation between taxonomic and functional gene patterns of anaerobic microorganisms in biogas-producing digesters. Additionally, based on the analysis of functional genes by metagenomics studies and network-based approaches, corresponding metabolic pathways can be estimated, consequently pointing to the identification of the actual dominant metabolic pathways and mechanisms in the biogas digesters (Zhang et al. 2019a). Moreover, it has been established that both taxonomic and functional patterns can be influenced by environmental variables such as digester configuration, operational parameters, and feedstock characteristics (Luo et al. 2016).

The ongoing development of high-throughput molecular tools and bioinformatics allows sequencing of the bulk DNA instead of only 16S rRNA genes and thereby provides both taxonomic and functional information of microbiomes to an extent that was unimaginable even a few years ago (Luo et al. 2016). For instance, metagenomics analysis and functional characterization of the biogas microbiome using high-throughput shotgun sequencing and a novel binning strategy were performed to disclose nearly one million genes and extract 106 microbial genomes. As a result, several key microbial genomes encoding enzymes involved in metabolic pathways including amino acid fermentation, fatty acid degradation, carbohydrate utilization, and syntrophic acetate oxidation were identified (Zhang et al. 2019a). It is noteworthy that traditional microbiological methodologies (e.g., isolation and cultivation of pure strains) continue to be used in order to study the physiology and metabolism of new isolates derived from biogas reactors, which could not be accomplished by metagenomic sequencing. Therefore, the combination of the new molecular technologies with traditional microbiological methodologies is necessary for future studies (Luo et al. 2016).

The application of genetic engineering to improve biogas production is done through the manipulation of genes in specific pathways and/or incorporation of specific DNA fragments into target species. Current and future research trends are directed toward the development and applications of genetically modified organisms to address the challenges encountered from naturally occurring conventional strains (Christy et al. 2014). For example, a super microorganism could be created to degrade recalcitrant waste such as lignocellulosic structures, converting cellulose and hemicellulose into biogas, and making the AD of these wastes more efficient. Of course, cost-effectiveness will be an important factor to consider even if the genetic engineering approach proves effective in the biogas industry (Zhang et al. 2019b).

Knowledge obtained from the modeling of microbial communities provides the possibility of optimizing the AD process by regulating the microorganisms. Feedstock composition, digester configuration, operating parameters, and environment conditions are the leading driving factors for community structure changes (Zhang et al. 2019a).

## 9.2 Conclusions

Anaerobic co-digestion is a promising technology for effective waste management and resource recovery while promoting economic and environmental sustainability. However, AD is a complex process and depends on several factors, so some strategies to improve AD performance with respect to biogas production and process stability have been studied in this chapter. Some of the key findings related to these strategies include the following: (1) Optimal configuration and manipulation of the operational parameters of AD (pH range of 6–8, stable operating temperature, appropriate moisture content, and proper agitation; C/N ratio between 20 and 30) can enhance the activity and growth of key anaerobic microorganisms, resulting in significantly improved process stability and biogas yield. (2) Different pretreatment methods (physical, chemical, biological, or hybrid) have been applied to eliminate physical and chemical barriers, by transforming recalcitrant compounds into soluble ones, which are more easily hydrolyzed by bacterial enzymes. The selection of the optimum pretreatment technique depends on multiple factors such as the characteristics of the biomass, the capital and operating cost of the pretreatment, and the ease of the operation. (3) Co-digestion of two or more substrates appears as a promising option to overcome the drawbacks of mono-digestion (mainly with regard to nutritional imbalance) and enhance the economic feasibility of AD process. (4) Supplementation of metal-, carbon-, and mineral-based or biological additives has shown to be an effective approach to enhance AD performance and improve anaerobic bioconversion processes by stimulating methanogens to increase CH<sub>4</sub> production during AD. (5) Two- or more stages of AD bioreactors could be used to optimize and synchronize the rates of reaction of the multi-step AD process, with the selection and enrichment of different bacteria in each stage. (6) The development of genetic engineering techniques allows the description of species and physiological behavior of the microbial consortium involved in AD, which can be used not only to characterize the process but also to make it faster and more efficient. As a general conclusion, it is clear that the challenge regarding this topic is not only the optimization of each strategy separately but also to combine and synergize the various enhancement strategies, seeking to optimize the AD process and the sustained conversion of waste into sustainable bioenergy.

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## References

- Agustini CB, Gutierrez M (2017) The range of organic solid wastes from anaerobically digested tanneries. In: Vic A, Artemio N (eds) *Biogas: production, applications and global developments*, 1st edn. Nova Science Publishers, New York, NY
- Akbay HEG, Dizge N, Kumbur H (2021) Enhancing biogas production of anaerobic co-digestion of industrial waste and municipal sewage sludge with mechanical, chemical, thermal, and hybrid pretreatment. *Bioresour Technol* 340:125688. <https://doi.org/10.1016/j.biortech.2021.125688>
- Akyol Ç, Ince O, Bozan M et al (2019) Fungal bioaugmentation of anaerobic digesters fed with lignocellulosic biomass: what to expect from anaerobic fungus *Orpinomyces* sp. *Bioresour Technol* 277:1–10

- Alavi-borazjani SA, Capela I, Tarelho LAC (2020) Over-acidification control strategies for enhanced biogas production from anaerobic digestion: a review. *Biomass Bioenergy* 143: 105833
- Ambrose HW, Chin CT, Hong E et al (2020) Effect of hybrid (microwave-H<sub>2</sub>O<sub>2</sub>) feed sludge pretreatment on single and two-stage anaerobic digestion efficiency of real mixed sewage sludge. *Process Saf Environ Prot* 136:194–202. <https://doi.org/10.1016/j.psep.2020.01.032>
- Appels L, Baeyens J, Degrève J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog Energy Combust Sci* 34:755–781. <https://doi.org/10.1016/j.pecs.2008.06.002>
- Ariunbaatar J, Panico A, Esposito G et al (2014) Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Appl Energy* 123:143–156
- Cai Y, Zheng Z, Schafer F et al (2021) A review about pretreatment of lignocellulosic biomass in anaerobic digestion: achievement and challenge in Germany and China. *J Clean Prod* 299: 126885
- Çakmak EK, Ugurlu A (2020) Enhanced biogas production of red microalgae via enzymatic pretreatment and preliminary economic assessment. *Algal Res* 50:101979. <https://doi.org/10.1016/j.algal.2020.101979>
- Cesaro A, Belgiorno V (2013) Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste. *Ultrason Sonochem* 20:931–936. <https://doi.org/10.1016/j.ultsonch.2012.10.017>
- Chen J, Yun S, Shi J et al (2020) Role of biomass-derived carbon-based composite accelerants in enhanced anaerobic digestion: focusing on biogas yield, fertilizer utilization, and density functional theory calculations. *Bioresour Technol* 307:123204. <https://doi.org/10.1016/j.biortech.2020.123204>
- Christy PM, Gopinath LR, Divya D (2014) A review on anaerobic decomposition and enhancement of biogas production through enzymes and microorganisms. *Renew Sust Energy Rev* 34:167–173
- Cremonese PA, Teleken JG, Meier RTW, Alves HJ (2021) Two-stage anaerobic digestion in agroindustrial waste treatment: a review. *J Environ Manag* 281:111854
- Dai B, Dong G (2018) Comparison of different pretreatments of rice straw substrate to improve biogas production. *Waste Biomass Valoriz* 9:1503–1512. <https://doi.org/10.1007/s12649-017-9950-9>
- Domingues RF, Sanches T, Silva GS et al (2015) Effect of enzymatic pretreatment on the anaerobic digestion of milk fat for biogas production. *Food Res Int* 73:26–30. <https://doi.org/10.1016/j.foodres.2015.03.027>
- Ennouri H, Miladi B, Zahedi S et al (2016) Effect of thermal pretreatment on the biogas production and microbial communities balance during anaerobic digestion of urban and industrial waste activated sludge. *Bioresour Technol* 214:184–191. <https://doi.org/10.1016/j.biortech.2016.04.076>
- Ghosh P, Kumar M, Kapoor R et al (2020) Enhanced biogas production from municipal solid waste via co-digestion with sewage sludge and metabolic pathway analysis. *Bioresour Technol* 296: 122275. <https://doi.org/10.1016/j.biortech.2019.122275>
- Hagos K, Zong J, Li D et al (2017) Anaerobic co-digestion process for biogas production: progress, challenges and perspectives. *Renew Sust Energy Rev* 76:1485–1496. <https://doi.org/10.1016/j.rser.2016.11.184>
- Hashemi B, Sarker S, Lamb JJ, Lien KM (2021) Yield improvements in anaerobic digestion of lignocellulosic feedstocks. *J Clean Prod* 288:125447
- Hassanein A, Lansing S, Tikekar R (2019) Impact of metal nanoparticles on biogas production from poultry litter. *Bioresour Technol* 275:200–206. <https://doi.org/10.1016/j.biortech.2018.12.048>
- Hodaiei M, Ghasemi S, Khosravi A, Vossoughi M (2021) Effect of the ozonation pretreatment on biogas production from waste activated sludge of Tehran wastewater treatment plant. *Biomass Bioenergy* 152:106198. <https://doi.org/10.1016/j.biombioe.2021.106198>



- Kainthola J, Kalamdhad AS, Goud VV (2019) A review on enhanced biogas production from anaerobic digestion of lignocellulosic biomass by different enhancement techniques. *Process Biochem* 84:81–90
- Karki R, Chuenchart W, Surendra KC et al (2021) Anaerobic co-digestion: current status and perspectives. *Bioresour Technol* 330:125001
- Khadaroo SNBA, Poh PE, Gouwanda D, Grassia P (2019) Applicability of various pretreatment techniques to enhance the anaerobic digestion of Palm oil Mill effluent (POME): a review. *J Environ Chem Eng* 7:103310
- Khalid A, Arshad M, Anjum M et al (2011) The anaerobic digestion of solid organic waste. *Waste Manag* 31:1737–1744. <https://doi.org/10.1016/j.wasman.2011.03.021>
- Kotsopoulos TA, Karamanlis X, Dotas D, Martzopoulos GG (2008) The impact of different natural zeolite concentrations on the methane production in thermophilic anaerobic digestion of pig waste. *Biosyst Eng* 99:105–111. <https://doi.org/10.1016/j.biosystemseng.2007.09.018>
- Koupaie EH, Dahadha S, Lakeh AAB et al (2019) Enzymatic pretreatment of lignocellulosic biomass for enhanced biomethane production-a review. *J Environ Manag* 233:774–784
- Kumar A, Samadder SR (2020) Performance evaluation of anaerobic digestion technology for energy recovery from organic fraction of municipal solid waste: a review. *Energy* 197:117253
- Liu M, Wei Y, Leng X (2021) Improving biogas production using additives in anaerobic digestion: a review. *J Clean Prod* 297:126666
- Lo HM, Kurniawan TA, Sillanpää MET et al (2010) Modeling biogas production from organic fraction of MSW co-digested with MSWI ashes in anaerobic bioreactors. *Bioresour Technol* 101:6329–6335. <https://doi.org/10.1016/j.biortech.2010.03.048>
- Luo G, Fotidis IA, Angelidaki I (2016) Comparative analysis of taxonomic, functional, and metabolic patterns of microbiomes from 14 full - scale biogas reactors by metagenomic sequencing and radioisotopic analysis. *Biotechnol Biofuels* 9:1–12. <https://doi.org/10.1186/s13068-016-0465-6>
- Mancini G, Papirio S, Lens PNL, Esposito G (2018) Increased biogas production from wheat straw by chemical pretreatments. *Renew Energy* 119:608–614. <https://doi.org/10.1016/j.renene.2017.12.045>
- Mao C, Feng Y, Wang X, Ren G (2015) Review on research achievements of biogas from anaerobic digestion. *Renew Sust Energ Rev* 45:540–555. <https://doi.org/10.1016/j.rser.2015.02.032>
- Millati R, Wikandari R, Ariyanto T et al (2020) Pretreatment technologies for anaerobic digestion of lignocelluloses and toxic feedstocks. *Bioresour Technol* 304:122998
- Mirmohamadsadeghi S, Karimi K, Tabatabaei M, Aghbashlo M (2019) Biogas production from food wastes: a review on recent developments and future perspectives. *Bioresour Technol Rep* 7:100202. <https://doi.org/10.1016/j.biteb.2019.100202>
- Mlaik N, Khoufi S, Hamza M et al (2019) Enzymatic pre-hydrolysis of organic fraction of municipal solid waste to enhance anaerobic digestion. *Biomass Bioenergy* 127:105286. <https://doi.org/10.1016/j.biombioe.2019.105286>
- Moset V, Xavier CAN, Feng L et al (2018) Combined low thermal alkali addition and mechanical pre-treatment to improve biogas yield from wheat straw. *J Clean Prod* 172:1391–1398. <https://doi.org/10.1016/j.jclepro.2017.10.173>
- Neshat SA, Mohammadi M, Najafpour GD, Lahijani P (2017) Anaerobic co-digestion of animal manures and lignocellulosic residues as a potent approach for sustainable biogas production. *Renew Sust Energ Rev* 79:308–322. <https://doi.org/10.1016/j.rser.2017.05.137>
- Pan S, Tsai C, Liu C et al (2021) Anaerobic co-digestion of agricultural wastes toward circular bioeconomy. *ISC Rev* 24:102704
- Panigrahi S, Dubey BK (2019) A critical review on operating parameters and strategies to improve the biogas yield from anaerobic digestion of organic fraction of municipal solid waste. *Renew Energy* 143:779–797
- Patinvoh RJ, Osadolor OA, Chandolias K et al (2017) Innovative pretreatment strategies for biogas production. *Bioresour Technol* 224:13–24. <https://doi.org/10.1016/j.biortech.2016.11.083>

- Patowary D, Baruah DC (2018) Effect of combined chemical and thermal pretreatments on biogas production from lignocellulosic biomasses. *Ind Crop Prod* 124:735–746. <https://doi.org/10.1016/j.indcrop.2018.08.055>
- Pérez-Pimienta JA, García-López RM, Méndez-Acosta HO et al (2021) Ionic liquid-water mixtures enhance pretreatment and anaerobic digestion of agave bagasse. *Ind Crop Prod* 171:113924. <https://doi.org/10.1016/j.indcrop.2021.113924>
- Pessuto J, Scopel BS, Perondi D et al (2016) Enhancement of biogas and methane production by anaerobic digestion of swine manure with addition of microorganisms isolated from sewage sludge. *Process Saf Environ Prot* 104:233–239
- Rahman MA, Møller HB, Saha CK et al (2018) Anaerobic co-digestion of poultry droppings and briquetted wheat straw at mesophilic and thermophilic conditions: influence of alkali pretreatment. *Renew Energy* 128:241–249. <https://doi.org/10.1016/j.renene.2018.05.076>
- Rajput AA, Zeshan, Visvanathan C (2018) Effect of thermal pretreatment on chemical composition, physical structure and biogas production kinetics of wheat straw. *J Environ Manag* 221:45–52. <https://doi.org/10.1016/j.jenvman.2018.05.011>
- Rasapoor M, Young B, Brar R et al (2020) Recognizing the challenges of anaerobic digestion: critical steps toward improving biogas generation. *Fuel* 261:116497
- Raut MP, Pandhal J, Wright PC (2021) Effective pretreatment of lignocellulosic co-substrates using barley straw-adapted microbial consortia to enhanced biomethanation by anaerobic digestion. *Bioresour Technol* 321:124437. <https://doi.org/10.1016/j.biortech.2020.124437>
- Romero-güiza MS, Vila J, Mata-alvarez J et al (2016) The role of additives on anaerobic digestion: a review. *Renew Sust Energ Rev* 58:1486–1499
- Sabeeh M, Liaquat R, Maryam A (2020) Effect of alkaline and alkaline-photocatalytic pretreatment on characteristics and biogas production of rice straw. *Bioresour Technol* 309:123449. <https://doi.org/10.1016/j.biortech.2020.123449>
- Schmidt T, Nelles M, Scholwin F, Pröter J (2014) Trace element supplementation in the biogas production from wheat stillage - optimization of metal dosing. *Bioresour Technol* 168:80–85. <https://doi.org/10.1016/j.biortech.2014.02.124>
- Siddique MNI, Wahid ZA (2018) Achievements and perspectives of anaerobic co-digestion: a review. *J Clean Prod* 194:359–371. <https://doi.org/10.1016/j.jclepro.2018.05.155>
- Simioni T, Agustini CB, Dettmer A, Gutterres M (2021) Anaerobic co-digestion of tannery wastes and untreated/pretreated oat straw. *Bioenergy Res* 15:589. <https://doi.org/10.1007/s12155-021-10285-1>
- Syaichurrozi I, Villta PK, Nabilah N, Rusdi R (2019) Effect of sulfuric acid pretreatment on biogas production from *Salvinia molesta*. *J Environ Chem Eng* 7:102857
- Tian T, Qiao S, Li X et al (2017) Nano-graphene induced positive effects on methanogenesis in anaerobic digestion. *Bioresour Technol* 224:41–47. <https://doi.org/10.1016/j.biortech.2016.10.058>
- Tian SQ, Zhao RY, Chen ZC (2018) Review of the pretreatment and bioconversion of lignocellulosic biomass from wheat straw materials. *Renew Sust Energ Rev* 91:483–489. <https://doi.org/10.1016/j.rser.2018.03.113>
- Tisma M, Planinic M, Bucic-Kojic A et al (2018) Corn silage fungal-based solid-state pretreatment for enhanced biogas production in anaerobic co-digestion with cow manure. *Bioresour Technol* 253:220–226. <https://doi.org/10.1016/j.biortech.2018.01.037>
- Tyagi VK, Fdez-Güelfo LA, Zhou Y et al (2018) Anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW): progress and challenges. *Renew Sust Energ Rev* 93:380–399. <https://doi.org/10.1016/j.rser.2018.05.051>
- Wang S, Han Y, Lu X et al (2021) Microbial mechanism underlying high methane production of coupled alkali-microwave e H<sub>2</sub>O<sub>2</sub> e oxidation pretreated sewage sludge by in-situ bioelectrochemical regulation. *J Clean Prod* 305:127195
- Wu D, Li L, Zhao X et al (2019) Anaerobic digestion: a review on process monitoring. *Renew Sust Energ Rev* 103:1–12. <https://doi.org/10.1016/j.rser.2018.12.039>

- Wu D, Peng X, Li L et al (2021) Commercial biogas plants: review on operational parameters and guide for performance optimization. *Fuel* 303:121282
- Xu R, Zhang K, Liu P et al (2018) A critical review on the interaction of substrate nutrient balance and microbial community structure and function in anaerobic co-digestion. *Bioresour Technol* 247:1119–1127. <https://doi.org/10.1016/j.biortech.2017.09.095>
- Zahan Z, Georgiou S, Muster TH, Othman MZ (2018) Semi-continuous anaerobic co-digestion of chicken litter with agricultural and food wastes: a case study on the effect of carbon/nitrogen ratio, substrates mixing ratio and organic loading. *Bioresour Technol* 270:245–254. <https://doi.org/10.1016/j.biortech.2018.09.010>
- Zamri MFMA, Hasmady S, Akhiar A et al (2021) A comprehensive review on anaerobic digestion of organic fraction of municipal solid waste. *Renew Sust Energ Rev* 137:110637
- Zanellati A, Spina F, Poli A et al (2021) Fungal pretreatment of non-sterile maize silage and solid digestate with a *Cephalotrichum stemonitis* strain selected from agricultural biogas plants to enhance anaerobic digestion. *Biomass Bioenergy* 144:105934. <https://doi.org/10.1016/j.biombioe.2020.105934>
- Zhang L, Loh K, Wei J, Zhang J (2019a) Bioinformatics analysis of metagenomics data of biogas-producing microbial communities in anaerobic digesters: a review. *Renew Sust Energ Rev* 100:110–126
- Zhang L, Loh K, Zhang J (2019b) Enhanced biogas production from anaerobic digestion of solid organic wastes: current status and prospects. *Bioresour Technol Rep* 5:280–296. <https://doi.org/10.1016/j.biteb.2018.07.005>
- Zheng Y, Zhao J, Xu F, Li Y (2014) Pretreatment of lignocellulosic biomass for enhanced biogas production. *Prog Energy Combust Sci* 42:35–53. <https://doi.org/10.1016/j.pecs.2014.01.001>



# Sewage Sludge Pretreatment Strategies for Methane Recovery and Sanitization

# 10

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**Abstract**

Sludge, a by-product or residue of wastewater treatment facilities, has considerably increased the generation over the years. Due to its large amount and content, organic matter, metals, and pathogens, sludge poses an environmental and health risk if not properly managed. Furthermore, stabilization and management of this residue maintain affordable costs on wastewater treatment plants (WWTPs). Anaerobic digestion (AD) is a promising technology to sludge valorization; however, it needs to be made more effective because this waste leads to low degradability and consequently low energy production. Pretreatments can be used to hydrolyze sludge and consequently improve biogas production, solid removal, and sludge quality after digestion, increasing the applicability of AD. Different technologies are being studied by physical-chemical and biological methods. This chapter addresses an overview of different technologies for pretreatment, focusing on thermal, ultrasonic, and enzymatic processes, discussing their effects on sludge properties and anaerobic digestion. Concerns related to pretreatment implementation, pathogen distribution, and directives around the world are also addressed.

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**Keywords**

Anaerobic digestion · Thermal pretreatment · Enzymes · Ultrasonic · Pathogen

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## 10.1 Sewage Sludge

Biological wastewater treatment processes have been widely used to treat municipal wastewater, as a result of efficient organic removal, despite the large amounts of sludge produced (Wang et al. 2017). Municipal wastewater treatment plants (WWTPs) generate sludge as a by-product of the physical and biological processes used (Appels et al. 2008). The adequate destination of biosolids is a task of great importance for growing populations and pollution reduction efforts aimed to limit the harmful by-product generation and spread (Praspaliauskas and Pedišius 2017). Nowadays, the treatment and disposal of sludge have become one of the major challenges faced by WWTPs (Xu et al. 2017), due to high costs to manage and dispose.

Sludge can be classified into primary and secondary sludge (or **activated sludge**). The primary wastewater treatment involves screening to remove large constituents, after by gravity sedimentation of the screened wastewater or by physical-chemical processes (i.e., coagulation, flocculation, flotation) with a solid diverted to a different stream (Elalami et al. 2019; Tyagi and Lo 2011). The residue from this process is a concentrated suspension, called primary sludge, which is further treated to become a biosolid; this step removes about 40–50% of solids in wastewater (Demirbas et al. 2017; Elalami et al. 2019). Secondary sludge is produced during biological process, consisting mainly of bacteria growing on organic and **inorganic substances**,

extracellular polymeric substances (EPS), and recalcitrant organics from wastewater or formed during bacterial decay (Wang et al. 2017), being composed of 59–88% (w/v) organic matter, which is decomposable and produces the offensive odors and 95% is water (Tyagi and Lo 2011).

Sludges have higher pathogen concentration, such as bacteria, viruses, protozoa, and other parasitic helminths, as well as organic matter can create potential hazards to humans and animal health, needing additional treatments to ensure a product can be safely integrated back in the product chain (Neumann et al. 2016). Also, sludges are often contaminated by non-biological components such as heavy metals, polycyclic aromatic hydrocarbons, polychlorinated biphenyls, pharmaceuticals, and pesticides, among other contaminants (Wiśniowska 2019). The WWTPs developed over the years the concern about sludge treatment, the amount of sludge increased more and more, and with that treatment technologies are improved and change together (Praspaliauskas and Pedišius 2017).

The first directive created to standardize utilization of treated sludge in agriculture or soils in European Commission dates from 1986 (86/271). The estimative directive denotes the production of 25 kg/(P.E × year) and 68 g/(P.E × day) of dry matter (DM) in 15 member states (Kelessidis and Stasinakis 2012; Milieu 2010).

There is a huge variation in values in the world: Brazil and China possess the minimum values, counting with 5.4 and 6.2 g dry matter/(P.E × day). On the other hand, the countries with maximum values are Germany, the United Kingdom, Slovenia, Finland, and the Netherlands: 66.5 g DM/(P.E × day), 67.8 g DM/(P.E × day), 77.7 g DM/(P.E × day), 78.6, and 249 g DM/(P.E × day), respectively (LeBlanc et al. 2008). The discrepancy about the established values of minimum and maximum production of sludge per capita is based on the volume treated by person and involved level of treatment. There are other influences in production per capita, such as the type of sewerage system with capability of separation of rainwater from wastewater. Large cities possess an underground system of drainage to collect wastewater, causing dilution of the volume and diminishing COD removal efficiency as well as sludge production (Mininni et al. 2015).

Some countries are developing stringent limits to directive use of sludge, as, for example, Belgium, Austria, Finland, Denmark, Germany, Slovenia, Sweden, the Netherlands, the Czech Republic (European Union 2008). Several WWTPs in Europe don't possess the technical equipment necessary to process sludge, making it suitable for other destinations. Due to the infrastructure dated from 1980s or even earlier, these plants are incapable of dovetail in new directives to dispose of landfill or via incineration (Mininni and Dentel 2013). Among this, WWTP aims to reduce sludge production turning to more feasible disposal costs via incineration or landfilling. At the same time, there are innovative processes that allow reduction of pathogens and diminish odor.

Among the contaminants there are some minerals that can be harmful when in high concentrations (mg/kg) in sludge with agricultural purposes, such as Se, Co, Zn, and Mo, that are not standardized by sludge directive (European Union 1998). Otherwise, there are concentrations allowed to potential toxic elements (PTE) in sludge with agriculture destination. Low limits are established in Finland, Latvia,

Flanders, Belgium, Denmark, Sweden, the Netherlands, Malta (for pH 5–6), and Carinthia (for pH 5.0–5.5).

Other countries allow limits like the sludge directive. Variation of pH in soil is utilized to determine the concentration limit of PTE in some places (Bulgaria, Spain, Portugal, Malta, and Carinthia). The amount of heavy metals is also limited during a period that can vary between 3 and 10 years in places such as Hungary, Luxembourg, Italy, France, Sweden, Finland, the Netherlands, Flanders, and Three Lander in Austria (Mininni et al. 2015). The procedures for agricultural use of sludge in Brazil are established by CONAMA Resolution 375/2006; criteria includes the determination of pathogen control (fecal coliform <3 MPN/100 g; *Salmonella* sp. absence in 10 g TS; viable helminths eggs <0.25 egg/g TS; virus <0.25 PFU g TS), bacteriological and inorganic substances, and the monitoring of 34 organic substances in sewage sludge, specifying maximum concentration maximum heavy metal contents in sludge for agriculture (as it follows: As = 41, Ba = 1300, Cd = 39, Pb = 300, Cu = 1500, Cr = 1000, Hg = 17, Mo = 50, Ni = 420, Se = 100, and Zn = 2800 mg/kg SS (dry matter basis)) (CONAMA 2006).

The aim of these regulations is to protect the environment, and different sludge treatment and disposal are studied, as composting, landfill, land application, drying-incineration, and anaerobic digestion (AD). Anaerobic digestion is commonly used in WWTP for degradation of sludge, being transformed into methane and carbon dioxide and some smaller amounts of biosolids as the final residue. The methane generation is an attractive feature because it can be used as energy. However, how to maximize methane production has been a subject of special consideration.

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## 10.2 Pretreatments Applied to Improve Biodegradability During Anaerobic Digestion

The AD requires strict anaerobic conditions to proceed and depends on the successive activity of a complex microbial association to transform organic material into methane (CH<sub>4</sub>). However, hydrolysis is generally considered as rate-limiting step (Appels et al. 2008). The low efficiency of the microorganisms (hydrolysis stage) is due to sludge characteristics, mainly flocs, EPS aggregates, recalcitrant compounds of lipids and proteins, and cell walls/membrane that form strong barriers to degradation. These compounds also are responsible for increased hydraulic retention time of biodigester, once it spends more time to hydrolysis, and therefore methane production is slow (Abelleira-Pereira et al. 2015; Anjum et al. 2016).

As a result of sludge characteristics, various pretreatment methods have been developed over time to maximize biogas production. If properly designed, pretreatment process is recommended to (1) modify the physical and chemical structure of sludge, (2) solubilize organic matter, (3) increase the surface area and accelerate hydrolysis step, and (4) consequently improve methane generation (Elalami et al. 2019; Hu et al. 2019; Zhen et al. 2017). Pretreatment technology

involving mechanical, chemical, physicochemical, and biological methods and their combinations have been tested in treating residual sludge.

### 10.2.1 Thermal Hydrolysis

Thermal pretreatment technology is a well-established, spread, and commercially implemented technology used to increase the degradability of sludge, being a process where the temperature of sludge is raised to a desired temperature to significantly increase the disintegration and solubilization of sludge solids (Pilli et al. 2015). Thermal pretreatment in the temperature range from 60 to 180 °C and is considered two types of thermal treatment process: low temperature (<120 °C) and high temperature (>120 °C). Normally, high-temperature treatment is associated with pressure in a range between 600 and 2500 kPa (Tyagi and Lo 2011; Pilli et al. 2015; Kor-Bicakci and Eskicioglu 2019).

The main advantages of thermal pretreatment includes the following: (1) increases biogas/methane yield; (2) improves sludge degradability; (3) allows increase organic loading rate, decreasing the size of biodigesters; (4) reduces sludge viscosity; (5) reduces odor and pathogens; and (6) reduces scum and foaming generation (Alfaro et al. 2014; Barber 2016; Xue et al. 2015). As other pretreatments, thermal pretreatment has some disadvantages, including increase in ammonia concentration, due to protein degradation, and costs with energy demand (Oosterhuis et al. 2014; Xue et al. 2015). Table 10.1 shows examples of thermal pretreatment effect on methane production, using different sludge types and different conditions.

The above studies of thermal pretreatment application, in general, show the increment on biogas/methane production, although in some studies this increase was not significant. The approach demonstrated that thermal pretreatment is conditioning between two variables: the exposure time temperature and pressure.

Biogas increment is linked to solubilization of organic matter (proteins, lipids, and carbohydrates) improved by higher temperatures and longer treatment times (Xue et al. 2015). As temperature increase, pretreatment is more efficient. However, temperatures above 180 °C lead to solubilization of recalcitrant and toxic organic compounds (melanoidins) reducing biodegradability (Pilli et al. 2015; Wilson and Novak 2009).

The viability of thermal pretreatment implementation in WWTPs is a crucial point, which must be analyzed, and it is necessary that energy demand of pretreatment does not exceed the biogas energy generation (Cano et al. 2015). Different pretreatment combination can be a promising alternative, generation one extra increment, and in this way contribute to viability implementation of pretreatment and consequently anaerobic digestion (Kor-Bicakci and Eskicioglu 2019).

Considering the advantages and researches over the time, the thermal pretreatment of sludge already implemented in full-scale WWTPs and is a commercial pretreatment technology, as described by Han et al. (2017), Kepp et al. (2000), Pérez-Elvira et al. (2008), and Záborská et al. (2006), proving an increase on



**Table 10.1** Biogas and/or methane production increase using thermal pretreatment method

Sludge type	Thermal pretreatment conditions	Anaerobic digestion conditions	Biogas or methane production increment	References
Activated sludge	80 °C for 6 h + mixed alkali (NaOH: Ca(OH) <sub>2</sub> molar ratios of 1:4, 2:3, 1:1, 3:2, and 4:1)	Batch, 30 days—35 °C	Cumulative methane production increased until 308.7%, compared to the control group	Zou et al. (2020)
Activated sludge	60, 80, 100, and 120 °C for 30 min	Batch, 35 days—37 °C	Increase of methane production by 13.7%, 27.0%, 29.0%, and 29.6% when treated at 60 °C, 80 °C, 100 °C, and 120 °C, respectively	Kumar Biswal et al. (2020)
Activated sludge	130–170 °C for 30 min	Batch, 25 days—36 °C	Increase in methane potential of activated sludge (17–27%), increase in refractory sCOD in return load (3.9–8.4%), and dewaterability enhancement (12–30%)	Toutian et al. (2020)
Primary sludge	70 and 90 °C for 30 min	Batch, 12 days—36 °C	The pretreatment at 90 °C for 0.5 h was much more effective and increased the productivity of methane by 58.52% compared to untreated sewage sludge. While thermal pretreatment at 70 °C showed an improvement of only 12.70% in methane productivity	Mirmasoumi et al. (2018)
Activated sludge	70, 80, and 90 °C for 3 h/70 °C for 15 h	Batch, 20 days—35 °C	The pretreatment of 80 °C and 90 °C for 3 h showed an increase of 29.2% and 31.2%, respectively. As for the pretreatment at 70 °C for 3 h and 15 h, it showed an increase of 21.0% and 18.9% in methane production, respectively	Ruffino et al. (2015)

(continued)

**Table 10.1** (continued)

Sludge type	Thermal pretreatment conditions	Anaerobic digestion conditions	Biogas or methane production increment	References
Activated sludge	170 °C	Continuous	Biogas production increased 40–50%	Yang et al. (2010b)
	30 min, 7 bar	HRT: 12 days		
Activated sludge	70 °C for 10, 20, and 30 min	Batch, 35 days—35 °C	Thermal pretreatment presented a methane potential similar with the untreated sludge	Ruiz-Hernando et al. (2014)
	80 °C for 10, 15, and 30 min			
Secondary sludge	120, 150, 170, and 200 °C at 237 rpm for 1 h	Batch, 53 °C	The amount of gas produced increased with the temperature between 120 and 170 °C. However, at 200 °C, the gas production decreased 33% in comparison to 170 °C	Abe et al. (2013)
Activated sludge	1 reactor: 160 ± 1 °C and 0.55 MPa for 30 min	Semi-continuous, 92 days of operation with variation of HRT (100, 50, and 20 days)—37 °C	The methane productions and VS removals of two reactors were similar 151.02–22–151.02 mL methane/L/day and 22.54–23.15%, respectively	Xiao et al. (2020)
	2 reactors: thermally pretreated at 60 ± 1 °C for 30 min with pH adjustment to 12			
Primary and secondary sludge	60, 70, and 80 °C exposure time varied from 15 to 90 min	Semi-continuous, 175 days with variation of HRT (22 and 15 days)—37 °C	The biogas production can be increased more than 10%, and the digestion time can be shortened significantly (thermal pretreatment at 70 °C)	Liao et al. (2016)
Activated sludge	135 °C and 190 °C, for 30 min and 15 min, respectively	Semi-continuous, HRT was fixed at 20 days—35 °C	Thermal treatment allowed an increase in biogas production, around 12% for treatment at 135 °C and around 25% for a treatment at 190 °C	Bougrier et al. (2007)

(continued)

**Table 10.1** (continued)

Sludge type	Thermal pretreatment conditions	Anaerobic digestion conditions	Biogas or methane production increment	References
Primary and secondary sludge	70 °C for 9, 24, and 48 h	Semi-continuous, HRT was fixed at 20 days—55 °C	Biogas yield was around 30% higher with pretreated sludge (0.28–0.30 L/g VS <sub>add</sub> ) when compared to raw sludge (0.22 L/g VS <sub>add</sub> ). Methane content in biogas was also higher after sludge pretreatment, around 69% vs. 64% with raw sludge	Ferrer et al. (2008)
Activated sludge	Continuous thermal 170 °C, HRT: 40 min, 7.6 bar followed by steam explosion	Pilot scale—CSTR (HRT 10 days)	Methane production increase until 82%	Souza et al. (2013)

biogas/methane production, reduction on hydraulic retention time, and sludge proved to have high fertilizer value.

### 10.2.2 Ultrasound

Ultrasound is a mechanical process of propagation of acoustic waves at a frequency higher than 16 kHz. Several phenomena are related to ultrasound depending on the applied frequency. On frequencies around 20 kHz, the most explored effect is cavitation which consist of a combined sequence of formation, growth, and collapse of microbubbles that occur in a very small interval of time (milliseconds) and realizing large amounts of energy locally. This realization of energy results in high pressure (in the range of 100–5000 bar) and temperatures (in the range of 1000–10,000 K), and these effects are observed at millions of locations in the reactor (Suslick 1990). At these conditions, •OH free radicals and H<sup>+</sup> ions are formed due to homolytic cleavage of water molecules. The recombination of these chemical species forms H<sub>2</sub>O<sub>2</sub>, a strongly oxidant compound, and therefore no selective chemical reactions can also occur in liquid media (Suslick 1990).

In sludge, the energy realized during cavitation disrupts bacterial cells by extreme shear forces, rupturing the cell wall and membranes (Bundhoo and Mohee 2018; Zou et al. 2016). The high temperature and pressure impact on physical and chemical

characteristics of biomass or waste materials such as particle size, surface area, lignin, hemicellulose and cellulose content, and organic matter solubilization, among others. Sonication process for sludge treatment was studied by various researchers in laboratory and full-scale systems (Houtmeyers et al. 2014; Tyagi et al. 2014).

Dhar et al. (2012) showed the benefits of ultrasound on protein and carbohydrate solubilization on municipal waste-activated sludge. Pretreatment condition with ultrasound of 10,000 kJ/kg TSS for 10 min was used, and the temperature was maintained below 40 °C during the experiments. After pretreatment an increase in insolubilization of carbohydrate and protein of 730% and 764%, respectively, compared with the control (without the use of ultrasound) was observed. Besides, it promoted a sludge biochemical methane potential (BMP) increase of 24%. Na et al. (2007) studied the sonication and recognize a decrease in particle size of sewage sludge, due to floc disintegration, and the sludge dewater ability was improved.

The efficiency of ultrasonic disintegration is dependent on sludge characteristics, including type of sludge, primary or activated sludge, TS content and particle size, and sonification conditions (time, intensity, temperature, pH, amplitude, and power input) (Khanal et al. 2007; Tyagi et al. 2014). Based on kinetic models, ultrasonic disintegration was impacted in the order of the following: sludge pH > sludge concentration > ultrasonic intensity > ultrasonic density (Khanal et al. 2007). On the other hand, the opinion of many researchers is that the effect of ultrasonic density is supposed to be more vital than the sonication time to the acceleration of conversion of complex organics to biodegradable substrate (Pilli et al. 2011).

Numerous studies demonstrate the benefits and impact of ultrasound pretreatment on biogas/methane production using sewage sludge as substrate (Table 10.2), as well as the combination with other pretreatment types, like alkali (Bao et al. 2020; Zhang et al. 2017), low temperature (Neumann et al. 2017), and CaO<sub>2</sub> (Li et al. 2019).

In addition to the impacts on biogas/methane production, studies evidenced changes on the methanogenic pathway after ultrasound pretreatment. Li et al. (2018) observed through microbial diversity analysis that hydrolytic and acidification bacteria were abundant in the reactors treating waste-activated sludge. *Methanocorpusculum* and *Methanosaeta* were the alternating dominant methanogens in the anaerobic reactors, with addition of sludge after different ultrasonic treatment times. As the ultrasonic time increased, the relative abundance of *Methanocorpusculum*, which can grow by using hydrogen as substrate, increased from 55.9% (control) to 80.0%, after 40 min of ultrasound, and decreased rapidly to 5.7% of abundance after 100 min of ultrasound. However, the trend in the change of the relative abundance of *Methanosaeta* was the opposite to that of *Methanocorpusculum*. *Methanosaeta* was 27.0% in the controlled reactor but as low as 0.9% after 40 min treatment, with the maximal value of 67.7% after 100 min. The authors observed that the dominant substrate for anaerobic methanogenesis changed from hydrogen to acetic acid.

According to Pilli et al. (2011), sludge ultrasound pretreatment is one of the emerging technologies for increasing the biodegradability, but optimizing the

**Table 10.2** Studies that evaluated the increase in the biogas and/or methane production in systems with ultrasound pretreatment

Sludge type	Pretreatment conditions	Anaerobic digestion conditions	Biogas or methane production increment	References
Municipal sludge	Time 5, 10, 15, 20, and 25 min. 19.1, 38.2, 57.3, 76.4, 95.5 kJ/g TS	BMP	Increase of 13%, 28%, and 35% on methane production, for respective times of 5, 10, and 15 min. However on 20 and 25 min, no increase on methane production was observed	Çelebi et al. (2020)
Mixed sewage sludge	Ultrasound-specific energy of 2000 kJ/kg TS. Thermal: 55 °C during 8 h and 70 rpm	Semi-continuous, variation of 30, 15, and 7.5 days SRT—37 °C	Sequential ultrasound-thermal pretreatment resulted in 19.1–29.9% increase in methane yield during sewage sludge anaerobic digestion	Neumann et al. (2018)
Waste-activated sludge	Frequency of 20 kHz, at different times (0, 20, 40, 60, 80, and 100 min) and at ultrasound densities of 0.5 W/mL	Semi-continuous stirred reactors (semi-CSTRs), HRT 20 days—37 °C	The gas production rate of each ultrasonic pretreated group was higher than the maximum of the control group	Li et al. (2018)
Sewage sludge	15 min in an ice bath, 20 kHz, 50 W (353 J/g TS)	BMP	Increased 34% of methane production	Mirmasoumi et al. (2018)
Activated sludge	3380 kJ/kg TS	BMP assay (35 °C)	Increment of 42% methane production and 13% VS removal	Riau et al. (2015)
Waste-activated sludge	100 W, 8 min, 96 kJ/kg TS	Semi-continuous, 37 °C, HRT 20 days	Increment of 27% biogas production	Houtmeyers et al. (2014)

methane yield (net energy yield is more than energy input) is necessary for full-scale implementation.

Xie et al. (2007) evaluated full-scale pretreatment using ultrasound for treating mixed sludge (primary and secondary sludge) and showed an increase in biogas daily production, up to 45% compared without pretreatment. Barber (2005) presented data of full-scale part-stream ultrasound pretreatment plants (Germany, Austria, Switzerland, Italy, and Japan) and showed biogas increased by 20–50% (volume/kg fed), and VS reduction improved on previous performance between 20%

and 50%. Tyagi et al. (2014) suggested one payback period of 2–3 years for a full-scale ultrasound installation.

High capital and operating costs of the ultrasonic system with high energy consumption and equipment maintenance are the main limitations of this technology (Elalami et al. 2019; Khanal et al. 2007; Tyagi et al. 2014). However, the use of ultrasound presents several advantages, like (1) no odor generation, (2) complete process automation, (3) easy maintenance, (4) potential to control filamentous bulking and foaming in the digester, (5) improved VS destruction, (6) biogas production and the quality of biosolids, (7) compact design and easy retrofit, (8) better digester stability, (9) low exposure time, and (10) a significant reduction in the size of digesters and the ultimate amount of sludge to be disposed (improved on dewater ability) (Elalami et al. 2019; Khanal et al. 2007; Tyagi et al. 2014; Pilli et al. 2011). Mass and energy balance on full-scale studies showed that 1 kW of ultrasonic energy used generates about 7 kW of electrical energy after losses (Pilli et al. 2011) which can overcome the limitations described above in a well-designed treatment plant.

### 10.2.3 Enzymes and Microorganisms

Biological pretreatment of sewage sludge offers an alternative to hydrolyze its structure by a cleaner and environmentally sustainable method by using enzymes and microorganisms as process catalysts. The use of biotechnologies is the focus of this type of pretreatment. In this scenario, enzymes and microorganisms encompass a multitude of possible relevant applications for the generation of bioenergy (Treichel et al. 2020; Zhen et al. 2017).

The increase in biogas production and higher volumes of gas recovery is directly related to pretreatment capable of breaking cell membranes of pathogens in order to reduce competitiveness with the microorganisms involved in the AD process and increase the availability of compounds that are used as substrates by these microorganisms (Zhen et al. 2017). In biological pretreatment processes, the approach comprises the application of enzymatic hydrolysis by the use of a single enzyme or enzyme cocktail or by the use of microorganisms or by thermophilic AD that consists of the pre-digestion of sludge in two stages of different temperatures (Bolzonella et al. 2012; Zhen et al. 2017).

Biological pretreatment offers some advantages over other treatments such as (1) no addition of chemical compounds during the process, ensuring greater environmental sustainability in the process; (2) increased biodegradation of the complex structure that makes up the sludge, releasing compounds that will serve as a substrate for microorganisms responsible for AD; (3) reduction of pathogens by cell membrane rupture; and (4) reduction in energy and thermal expenditure, enabling self-sufficiency in the process (Agabo-García et al. 2019; Treichel et al. 2020). Biological pretreatment presents some advantages in full scale (Ge et al. 2010; Recktenwald et al. 2008), but it still faces challenges. Mainly in terms of operation and optimization of the project due to the limitation by the complex hydrolysis mechanisms

involved in the system that can vary with the characteristics of the biomass and negatively affect the efficiency of the process (Ding et al. 2017; Zhen et al. 2017).

The biological pretreatment based on the use of enzymes for sludge hydrolysis can be carried out by enzymatic cocktails, purified commercial enzymes, or enzymatic production in situ using microorganisms with a high production potential of the enzymes of interest (Yu et al. 2013). Proteases and glycosidases are the main enzymes used in sludge pretreatments, considering that the major components of this biomass are proteins and complex carbohydrates (Bonilla et al. 2018). Furthermore, due to the presence of other compounds in the sludge structural matrix, it is possible to apply different enzymes with different specificities such as lipases due to the presence of fatty acids and peroxidases for the oxidation of other compounds (Agabo-García et al. 2019; Elalami et al. 2019).

The application of a single enzyme in the pretreatment process can reduce the efficiency of the process due to the complexity of the sludge composition, with specificity for action on different substrates being important, as reported in the study of (Yang et al. 2010a) where the enzymatic pretreatment with a cocktail of amylases and proteases increased more than the application of each enzyme separately. This factor is related to the specificity of enzymes for different structural chains. Hydrolysis of sludge by enzymatic cocktails can be facilitated due to the synergistic action of enzymes, which may disintegrate through the action of some enzymes, the outermost matrix of the sludge. This process results in the solubilization of these compounds and exposing more internal compounds previously protected from enzymatic attack, increasing process efficiency (Yang et al. 2010a; Zhou et al. 2009).

The action of hydrolytic enzymes in the sludge occurs through the cleavage of specific substrates, releasing lower-molecular-weight products into the medium. This process causes the structure of the flakes to be reduced and proteins, peptides, and carbohydrates to be released for use by microorganisms in AD, inducing a greater biogas production (Recktenwald et al. 2008; Watson et al. 2004). Since it is already biologically active, sewage sludge has enzyme activity profiles that may vary according to the microbial population present in the environment, with enzyme activities such as  $\alpha$ -glucosidase,  $\beta$ -glucosidase, alanine-aminopeptidase, esterase, dehydrogenase, proteases, phosphatases, and cellulases (Goel et al. 1998; Nybroe et al. 1992; Watson et al. 2004). The enzymatic activities present in the sludge are key elements to understand the profile of enzymes essential for greater efficiency of biological pretreatment.

The process of AD of sewage sludge depends on microorganisms' action to metabolize and stabilize the sludge. However, the microbial community can also be inserted in the process as a form of pretreatment aiming at increasing the product generated. About 50% of the organic material present in the sludge refers to proteins released during hydrolysis by proteolytic enzymes or by the action of microorganisms capable of producing these enzymes (Li et al. 2009). Strains of microorganisms such as *Penicillium* sp., *Serratia marcescens*, *Streptomyces* sp., *Rhizopus oryzae*, *Pseudomonas*, *Bacillus* sp., *Vibrio*, *Brevibacillus* sp., *Methanobrevibacter*, *Methanobacterium*, *Methanoculleus*, and *Methanocorpusculum* in addition to fungi species called white-rot fungi were

studied, found in sludge, and considered to be good sludge-hydrolyzing agents, in addition to producing proteases that can increase the pretreatment yield (Ben Rebah and Miled 2013; Treichel et al. 2020; Ventrino et al. 2018).

During pretreatment using hydrolytic microorganisms, the networks of the complex chains that make up the sludge structure and the cell walls of pathogens are depolymerized and result in the release of lower-molecular-weight compounds that are easily digested as a substrate for AD (Guo et al. 2014). This process usually occurs through the excretion or intracellular enzyme production, being advantageous mainly by dispensing the continuous addition of enzymes, reducing energy, and economic expenses (Ding et al. 2017). In addition, in contact with the sludge structural complex, microorganisms produce efficient enzymatic cocktails for degrading different parts of the structure, which act synergistically and can result in efficient solubilization processes.

Another type of biological pretreatment that has been treated as viable biotechnology mainly for full-scale application is the two-stage AD process (temperature-phased anaerobic digestion (TPAD)) (Zhen et al. 2017). This process consists of a pre-hydrolysis of the sludge before AD. It applies different temperatures to the system, aiming to separate the hydrolysis + acidogenesis and acetogenesis + methanogenesis in the reactors. This process is resulting in the enrichment of different groups of microorganisms in each reactor, increasing the efficiency of solubilization of the substrates for biogas production (Bolzonella et al. 2012; Elalami et al. 2019; Schievano et al. 2012). The enrichment of specific microorganisms for each stage of digestion will maximize the system's overall reaction rate and improve the reduction in COD (Schievano et al. 2012).

One of the significant challenges of TPAD systems is associated with the high capacity to solubilize the compounds present in the sludge, which can result in the inhibition of the methanogenic phase, mainly due to the high sensitivity of this community to volatile fatty acids (Schievano et al. 2010). As it is a complex system with many different biochemical pathways, the AD system's balance between controlling the biogas production in two stages still limits the development with the high efficiency of this system. Because it can lead to substantial changes in biochemical pathways and in the formation of metabolites, strongly influencing the population and subpopulations present in the environment (Chen et al. 2008; Schievano et al. 2012).

In the single-stage AD process, the main challenge remains the slow rates of hydrolysis for complex biomass, such as sewage sludge, and biological disintegration methods that focus on using cleaner and economical technologies are increasingly being explored in recent research. As commonly reported approaches, the use of commercial enzymes and protease-producing microorganisms in batch systems followed by single-stage AD has often been reported (Table 10.3).

The biological pretreatment is relevant considering the scenario of sewage sludge recovery to produce biogas by improving sludge biodegradability through efficient technologies and ecological sustainability. The advancement of studies is based on the use of biological pretreatment, and the challenges of this technology must be solved, such as the high cost of enzyme cocktails, the reaction time of enzymes and



**Table 10.3** Studies that evaluated the increase in the biogas and/or methane production in systems using enzymatic or microorganism pretreatment of sewage sludge

Sludge type	Pretreatment conditions	AD conditions	Increase of biogas or methane production	References
Sewage sludge	(a) <i>Bacillus licheniformis</i> (37 °C, 12 day, 150 rpm)	Batch, 23 days—37 °C	Increase of biogas production from 3.65 times and 5.77 times by treatment with <i>B. licheniformis</i> and proteases, respectively	Agabo-García et al. (2019)
	(b) Isolated commercial proteases (0.3% v/v)			
Activated sludge	(a) Amylase cocktail by <i>Bacillus subtilis</i>	Batch, 27 days—37 °C	Increase of biogas from 18.6%, 15.6%, and 20.2% by treatments, respectively  Enzyme pretreatment reduces size particle sludge	Yu et al. (2013)
	(b) Protease cocktail by <i>Aeromonas hydrophila</i>			
	(c) Cocktail combination			
Primary sludge	Proteases and lipases from <i>Bacillus amyloliquefaciens</i> DSM7T and <i>Burkholderia vietnamiensis</i> LMG 10929T, respectively	Batch, 30 days—37 °C	Increase of biogas production from 84.1% and methane production from 89.8%	Tongco et al. (2020)
Primary sludge and activated sludge	Commercial glycosidic enzymes (add in digester chamber at 40–65 °C)	Continuous reactor—full scale, 24 days—35 °C	Increase of biogas production by 10–20% in comparison to the reference digester	Recktenwald et al. (2008)

microorganisms to affect the hydrolysis of sludge, the efficient inactivation of pathogens, the need of a robust process with operational stability and low loss of efficiency due to biological inactivation of the microorganisms and enzymes involved, and moreover, finally, the main challenge of expanding scale for industrial applications (Treichel et al. 2020).

### 10.3 Pathogens and Antibiotic Resistance in Sludge and the Pre- and Post-treatment as the Controller in WWTPs

The rise of antibiotic administration to the population and animals naturally leads to its accumulation, especially in residues. Human residues are concentrated in WWTPs, being in general not efficiently treated and consequently reaching the sludge after treatment. The battle against resistant bacteria is one of the biggest

world concerns of our century. In 2015 an estimate demonstrated that antibiotic resistance was responsible for more than 23,000, 25,000, and 38,000 deaths every year in the United States, the European Union, and Thailand, respectively (Berglund 2015). Developed countries face the addendum of facilitated antibiotic handling, being a concern by self-medication and lack of education about the use of antibiotics, resulting in exaggerated use (Planta 2007; Wellington et al. 2013). Beyond that, globalization carries resistant bacteria of specific regions to different areas, via travels around the world.

Classical antibiotics intervene in biochemistry and physiology of bacteria, culminating in cell death or cessation, which diminishes or stops cell replication. There are five targets of antibiotics from out to inside: bacteria cell wall, cell membrane, protein synthesis, DNA and RNA synthesis, and folic acid metabolism. The efficiency of antibiotics depends exclusively on the non-existence of these targets on the eukaryotic cells, or different compositions when there is a similarity, being relatively non-toxic, only in situations of exacerbated use. An excellent example is the  $\beta$ -lactam antibiotics such as cephalosporins, penicillins, and carbapenems, and their activity consists in blocking the synthesis of bacteria cell wall, which is a fundamental structure to bacteria but absent in eukaryotic cells (Wright 2010).

The selective pressure exercised in bacteria to the strong exposition to antibiotics has selected resistant microorganisms. The resistance can be acquired in horizontal dissemination, being distributed into the same species and genera by means of incorporation of dispersed plasmids on the environment. Resistance can also be reached vertically through generations of microorganisms due to mutations resulting from successive challenges with antibiotics (Martinez 2009).

A strong evidence is the comparison between bacteria in the pre-date of the antibiotic era and in our days. Nonetheless, in the dynamic nature of microorganisms, the resistance is forthcoming. Emergence of resistance is related for decades occurring in parallel between clinic cases and bacteria that produce antibiotics. In recent years, studies demonstrated that most of the non-pathogenic soil bacteria are multidrug resistant. This reinforces the difference between bacteria which evolved in an environment being challenged with small bioactive compounds and a variety of toxins plentiful. On the other hand, pathogens with more virulent forms compared to commensal bacteria have not been exposed to toxins and compounds that challenge their existence (Wright 2010).

In addition, LaPara et al. (2011) relate the rise of antibiotic resistance genes (ARG) and resistant bacteria (ARB) in effluent of wastewater, considering that classical WWTPs were not designed for removal of ARG and ARB, ever after the process of disinfection of mixed filtration due to the wastewater compile the residues of city dwellers and concentrate at WWTPs (Calero-Cáceres et al. 2014; Su et al. 2015). Characteristics of sewage sludge such as microbial diversity with high density can facilitate horizontal gene transfer (HGT) by plasmids, known as mobile genetic elements (MGE) (Gaze et al. 2011; Sentchilo et al. 2013; Zhang et al. 2011). The techniques utilized to identify the presence of ARG and ARB are quantitative PCR and metagenomic investigation (Yang et al. 2013; Zhang and Zhang 2011). The

incorrect treatment of sludge can lead to the input of ARG, ARB, and antibiotics such as fluoroquinolones, macrolides, and tetracyclines into the soil (Kinney et al. 2006; Rahube et al. 2014; Sabourin et al. 2012).

Degradation of antibiotics and ARGs is related to the process applied to manure composting (Qiao et al. 2012; Sharma et al. 2009; Wang et al. 2012). Nevertheless, few studies evaluated the effect of methods of digestion of sludge, specifically tetracyclines, sulfonamides, macrolides, and resistance genes (Ma et al. 2011). The focus on sludge as mentioned is related due to the rich reservoir of ARGs and variety commonly found in sludge (Andrés et al. 2011; Rahube et al. 2014). Consequently, the post-treatment is evidenced as necessary.

In this sense, the control of pathogens is most important in WWTPs. Pretreatment methods, like ultrasound and thermal, may also impact sludge hygienization and could be used as both pretreatment and post-treatment, depending on the requirements of the WWTP (Ruiz-Hernando et al. 2014). According to studies mesophilic anaerobic digestion is inactive around  $2 \log_{10}$  of pathogens and sludge containing up to  $7 \log_{10}$  (Lizama et al. 2017).

The inactivation of three microbial indicators at  $80^\circ\text{C}$ , for 30 min, behaved differently: there was a slight reduction for SSRC (spores of sulfite-reducing clostridia) ( $0.84 \log_{10}$  of reduction), approximately  $5 \log_{10}$  of reduction for SOMCPH (somatic coliphage) and a high hygienization for *E. coli* ( $>4.01 \log_{10}$  of reduction) (Ruiz-Hernando et al. 2014). According to Yin et al. (2016), thermal pretreatment ( $70^\circ\text{C}$ ) is highly efficient to inactivate pathogens and the complete inactivation (approximately 6 log) of fecal coliform, *Salmonella* spp., and fecal streptococcus. The pretreatment effect was evaluated at different times (20, 40, 60, 80, 100, and 120 min) for different TS concentrations of fecal sludge (between 1% and 12%). Considering the results of ultrasound pretreatment, a reduction of pathogens was observed where the concentration of fecal coliforms and *Salmonella* spp. decreased by 4 (99.99%) and 3 (99.9%) log units, respectively, at 35,000 kJ/kg. The authors tested TS concentration without continuous stirring and did not achieve the same inactivation, so these two conditions interfere on ultrasound pathogen inactivation capability (Lizama et al. 2017). According to Kumar (2011), the pathogen concentration decreased as sonication time and frequency increased, and reduction is mainly caused by the effects of cavitation and decreased the bacterial cells showing ruptured shapes.

During ultrasonic treatment of sewage sludge, using 22 kHz, the load of *Giardia lamblia* cysts and *Cryptosporidium parvum* oocysts was reduced to non-detectable levels (control parasite density, 12–17 no./g of *Cryptosporidium* and 22–32 no./g of *Giardia*), at 15 min of sonication time for following applied amplitudes (10, 12, 14, 16  $\mu\text{m}$ ). The hydrodynamic shear force was considered as a factor responsible for the damaged oocyst (Graczyk et al. 2008).

Besides the treatment and pretreatment efficiency, some matrices may need addition of a post-treatment for reaching the standard established in directives for sludge use. There are some well-known options, such as the use of polishing ponds, which is common in developing countries such as India, Brazil, and China (Ali et al. 2013). This system requires large land areas, being also quite slow; however it can

reduce helminth eggs and reach the discharge standards for urban wastewaters from the European Community for nitrogen (von Sperling and Mascarenhas 2005). Constructed wetlands are another example of natural post-treatment, being especially efficient on phosphorus and nitrogen removal (Ali et al. 2013). One of the advantages of this technique is the use of natural organic matter degradation processes, associated with the macrophytes' biologic filtration, being able to reduce coliforms and even viruses (Platzer et al. 2016; Stefanakis et al. 2014).

Coagulation and flocculation are other examples of a post-treatment process; however their efficacy must be increased using disinfectants such as chlorine (Jaya Prakash et al. 2007). Down-flow hanging sponge (DHS) is a reactor developed in Japan, composed of sponge cubes diagonally linked through nylon string, providing vast areas for microbiological growth under non-submerged conditions, while the effluent passage provides the nutrients for the resident microorganism development (Agrawal et al. 1997). Down-flow hanging sponges enable the recovery of dissolved methane and thus the removal of 3.5  $\log_{s_{10}}$  of fecal contaminants (Machdar et al. 2000). Another post-treatment method for fecal contaminant removal is the use of moving bed biofilm reactors, showing a 2.3  $\log_{s_{10}}$  removal (Tawfik et al. 2008). In these systems the predation by protozoa and metazoan along with adsorption into the media was the main inactivation mechanisms responsible for pathogen reduction.

Slow sand filtration systems show great sanitization power for anaerobic digester treatment, being able to reduce 4  $\log_{s_{10}}$  of fecal contaminants, reaching most of the directives for effluent reuse (Tyagi et al. 2009). There are other techniques focused on mineral element removal, involving aeration processes, varying from micro-aeration, i.e., flash aeration, to high rate aerobic methods, such as sequential batch reactors. Micro-aeration is a great option for sulfides' biological oxidation into elemental sulfur, which can be easily recovered and commercialized (Chen et al. 2010; Khan et al. 2011). These techniques can be applied for a greater effluent sanitization and thus safer agricultural use, land application, or discharge, being all feasible options. However, the most suitable sanitization option depends on the effluent and treatment plant characteristics.

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## 10.4 Final Remarks

The WWTP's sludge is a by-product that contains a large amount of organic matters, heavy metals, and pathogens and may represent an environmental risk. In this sense, AD is a promising technology for the recovery of sludge, dependent on physical, chemical, and biological pretreatments to promote the increase in biogas production and increase the sanitary quality of the digested, aiming at valuing and recycling the final product.

## References

- Abe N, Tang YQ, Iwamura M, Morimura S, Kida K (2013) Pretreatment followed by anaerobic digestion of secondary sludge for reduction of sewage sludge volume. *Water Sci Technol* 67: 2527–2533. <https://doi.org/10.2166/wst.2013.154>
- Abelleira-Pereira JM, Pérez-Elvira SI, Sánchez-Oneto J, de la Cruz R, Portela JR, Nebot E (2015) Enhancement of methane production in mesophilic anaerobic digestion of secondary sewage sludge by advanced thermal hydrolysis pretreatment. *Water Res* 71:330–340. <https://doi.org/10.1016/j.watres.2014.12.027>
- Agabo-García C, Pérez M, Rodríguez-Morgado B, Parrado J, Solera R (2019) Biomethane production improvement by enzymatic pre-treatments and enhancers of sewage sludge anaerobic digestion. *Fuel* 255:115713. <https://doi.org/10.1016/j.fuel.2019.115713>
- Agrawal LK, Ohashi Y, Mochida E, Okui H, Ueki Y, Harada H, Ohashi A (1997) Treatment of raw sewage in a temperate climate using a uasb reactor and the hanging sponge cubes process. *Water Sci Technol* 36:433–440. [https://doi.org/10.1016/S0273-1223\(97\)00552-0](https://doi.org/10.1016/S0273-1223(97)00552-0)
- Alfaro N, Cano R, Fdz-Polanco F (2014) Effect of thermal hydrolysis and ultrasounds pretreatments on foaming in anaerobic digesters. *Bioresour Technol* 170:477–482. <https://doi.org/10.1016/j.biortech.2014.08.013>
- Ali A, Zahid R, Ahmad A, Lew B (2013) Sustainable post treatment options of anaerobic effluent. In: Chamy R, Rosenkranz F (eds) *Biodegradation - engineering and technology*. InTechOpen, London. <https://doi.org/10.5772/56097>
- Andrés P, Mateos E, Tarrasón D, Cabrera C, Figuerola B (2011) Effects of digested, composted, and thermally dried sewage sludge on soil microbiota and mesofauna. *Appl Soil Ecol* 48:236–242. <https://doi.org/10.1016/j.apsoil.2011.03.001>
- Anjum M, Al-Makishah NH, Barakat MA (2016) Wastewater sludge stabilization using pre-treatment methods. *Process Saf Environ Prot* 102:615–632. <https://doi.org/10.1016/j.psep.2016.05.022>
- Appels L, Baeyens J, Degre J, Dewil R (2008) Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog Energy Combust Sci* 34:755–781. <https://doi.org/10.1016/j.peccs.2008.06.002>
- Bao H, Yang H, Zhang H, Liu Y, Su H, Shen M (2020) Improving methane productivity of waste activated sludge by ultrasound and alkali pretreatment in microbial electrolysis cell and anaerobic digestion coupled system. *Environ Res* 180:108863. <https://doi.org/10.1016/j.envres.2019.108863>
- Barber WP (2005) The effects of ultrasound on sludge digestion. *Water Environ J* 19:2–7. <https://doi.org/10.1111/j.1747-6593.2005.tb00542.x>
- Barber WPF (2016) Thermal hydrolysis for sewage treatment: a critical review. *Water Res* 104:53–71. <https://doi.org/10.1016/j.watres.2016.07.069>
- Ben Rebah F, Miled N (2013) Fish processing wastes for microbial enzyme production: a review. *3 Biotech* 3:255–265. <https://doi.org/10.1007/s13205-012-0099-8>
- Berglund B (2015) Environmental dissemination of antibiotic resistance genes and correlation to anthropogenic contamination with antibiotics. *Infect Ecol Epidemiol* 5:28564. <https://doi.org/10.3402/iee.v5.28564>
- Bolzoniella D, Cavinato C, Fatone F, Pavan P, Cecchi F (2012) High rate mesophilic, thermophilic, and temperature phased anaerobic digestion of waste activated sludge: a pilot scale study. *Waste Manag* 32:1196–1201. <https://doi.org/10.1016/j.wasman.2012.01.006>
- Bonilla S, Choolaei Z, Meyer T, Edwards EA, Yakunin AF, Allen DG (2018) Evaluating the effect of enzymatic pretreatment on the anaerobic digestibility of pulp and paper biosludge. *Biotechnol Rep* 17:77–85. <https://doi.org/10.1016/j.btre.2017.12.009>
- Bougrier C, Delgenès JP, Carrère H (2007) Impacts of thermal pre-treatments on the semi-continuous anaerobic digestion of waste activated sludge. *Biochem Eng J* 34:20–27. <https://doi.org/10.1016/j.bej.2006.11.013>

- Bundhoo ZMA, Mohee R (2018) Ultrasound-assisted biological conversion of biomass and waste materials to biofuels: a review. *Ultrason Sonochem* 40:298–313. <https://doi.org/10.1016/j.ultrasonch.2017.07.025>
- Calero-Cáceres W, Melgarejo A, Colomer-Lluch M, Stoll C, Lucena F, Jofre J, Muniesa M (2014) Sludge As a Potential Important Source of Antibiotic Resistance Genes in Both the Bacterial and Bacteriophage Fractions. *Environ Sci Technol* 48:7602–7611. <https://doi.org/10.1021/es501851s>
- Cano R, Pérez-Elvira SI, Fdz-Polanco F (2015) Energy feasibility study of sludge pretreatments: a review. *Appl Energy* 149:176–185. <https://doi.org/10.1016/j.apenergy.2015.03.132>
- Çelebi EB, Aksoy A, Sanin FD (2020) Effects of anaerobic digestion enhanced by ultrasound pretreatment on the fuel properties of municipal sludge. *Environ Sci Pollut Res* 27:17350–17358. <https://doi.org/10.1007/s11356-020-08230-4>
- Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: a review. *Bioresour Technol* 99:4044–4064
- Chen C, Ren N, Wang A, Liu L, Lee DJ (2010) Enhanced performance of denitrifying sulfide removal process under micro-aerobic condition. *J Hazard Mater* 179:1147–1151. <https://doi.org/10.1016/j.jhazmat.2010.02.065>
- Conama. CONAMA – Conselho Nacional do Meio Ambiente. Ministério do Meio Ambiente, Brasil (2006) Resolução No. 375
- Demirbas A, Edris G, Alalayah WM (2017) Sludge production from municipal wastewater treatment in sewage treatment plant. *Energy Sources Part A Recover Util Environ Eff* 39:999–1006. <https://doi.org/10.1080/15567036.2017.1283551>
- Dhar BR, Nakhla G, Ray MB (2012) Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. *Waste Manag* 32:542–549. <https://doi.org/10.1016/j.wasman.2011.10.007>
- Ding HH, Chang S, Liu Y (2017) Biological hydrolysis pretreatment on secondary sludge: enhancement of anaerobic digestion and mechanism study. *Bioresour Technol* 244:989–995. <https://doi.org/10.1016/j.biortech.2017.08.064>
- Elalami D, Carrere H, Monlau F, Abdelouahdi K, Oukarroum A, Barakat A (2019) Pretreatment and co-digestion of wastewater sludge for biogas production: recent research advances and trends. *Renew Sust Energ Rev* 114:109287. <https://doi.org/10.1016/j.rser.2019.109287>
- European Union (1998) Directive 2008/98/EC of the European Parliament and of the council of 19 November 2008 on waste and repealing certain Directives. EC, Brussels
- European Union (2008) Regulation (EC) No 1272/2008 of the European Parliament and of the Council of 16 December 2008. EC, Brussels
- Ferrer I, Ponsá S, Vázquez F, Font X (2008) Increasing biogas production by thermal (70°C) sludge pre-treatment prior to thermophilic anaerobic digestion. *Biochem Eng J* 42:186–192. <https://doi.org/10.1016/j.bej.2008.06.020>
- Gaze WH, Zhang L, Abdousslam NA, Hawkey PM, Calvo-Bado L, Royle J, Brown H, Davis S, Kay P, Boxall ABA, Wellington EMH (2011) Impacts of anthropogenic activity on the ecology of class 1 integrons and integron-associated genes in the environment. *ISME J* 5:1253–1261. <https://doi.org/10.1038/ismej.2011.15>
- Ge H, Jensen PD, Batstone DJ (2010) Pre-treatment mechanisms during thermophilic–mesophilic temperature phased anaerobic digestion of primary sludge. *Water Res* 44:123–130. <https://doi.org/10.1016/j.watres.2009.09.005>
- Goel R, Mino T, Satoh H, Matsuo T (1998) Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor. *Water Res* 32:2081–2088. [https://doi.org/10.1016/S0043-1354\(97\)00425-9](https://doi.org/10.1016/S0043-1354(97)00425-9)
- Graczyk TK, Kacprzak M, Neczaj E, Tamang L, Graczyk H, Lucy FE, Girouard AS (2008) Occurrence of *Cryptosporidium* and *Giardia* in sewage sludge and solid waste landfill leachate and quantitative comparative analysis of sanitization treatments on pathogen inactivation. *Environ Res* 106:27–33. <https://doi.org/10.1016/j.envres.2007.05.005>

- Guo L, Lu M, Li Q, Zhang J, Zong Y, She Z (2014) Three-dimensional fluorescence excitation–emission matrix (EEM) spectroscopy with regional integration analysis for assessing waste sludge hydrolysis treated with multi-enzyme and thermophilic bacteria. *Bioresour Technol* 171: 22–28. <https://doi.org/10.1016/j.biortech.2014.08.025>
- Han D, Lee CY, Chang SW, Kim DJ (2017) Enhanced methane production and wastewater sludge stabilization of a continuous full scale thermal pretreatment and thermophilic anaerobic digestion. *Bioresour Technol* 245:1162–1167. <https://doi.org/10.1016/j.biortech.2017.08.108>
- Houtmeyers S, Degrève J, Willems K, Dewil R, Appels L (2014) Comparing the influence of low power ultrasonic and microwave pre-treatments on the solubilisation and semi-continuous anaerobic digestion of waste activated sludge. *Bioresour Technol* 171:44–49. <https://doi.org/10.1016/j.biortech.2014.08.029>
- Hu K, Chen W, Jia S, Wang W, Han F (2019) Enhanced degradation of waste activated sludge in microbial electrolysis cell by ultrasonic treatment. *Front Microbiol* 10:00128. <https://doi.org/10.3389/fmicb.2019.00128>
- Jaya Prakash K, Tyagi VK, Kazmi AA, Kumar A (2007) Post-treatment of UASB reactor effluent by coagulation and flocculation process. *Environ Prog* 26:164–168. <https://doi.org/10.1002/ep.10192>
- Kelessidis A, Stasinakis AS (2012) Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. *Waste Manag* 32:1186–1195. <https://doi.org/10.1016/j.wasman.2012.01.012>
- Kepp U, Machenbach I, Weisz N, Solheim OE (2000) Enhanced stabilisation of sewage sludge through thermal hydrolysis - three years of experience with full scale plant. *Water Sci Technol* 42:89–96. <https://doi.org/10.2166/wst.2000.0178>
- Khan AA, Gaur RZ, Lew B, Mehrotra I, Kazmi AA (2011) Effect of aeration on the quality of effluent from UASB reactor treating sewage. *J Environ Eng* 137:464–471. [https://doi.org/10.1061/\(ASCE\)EE.1943-7870.0000348](https://doi.org/10.1061/(ASCE)EE.1943-7870.0000348)
- Khanal SK, Grewell D, Sung S, van Leeuwen J (2007) Ultrasound applications in wastewater sludge pretreatment: a review. *Crit Rev Environ Sci Technol* 37:277–313. <https://doi.org/10.1080/10643380600860249>
- Kinney CA, Furlong ET, Zaugg SD, Burkhardt MR, Werner SL, Cahill JD, Jorgensen GR (2006) Survey of organic wastewater contaminants in biosolids destined for land application. *Environ Sci Technol* 40:7207–7215. <https://doi.org/10.1021/es0603406>
- Kor-Bicakci G, Eskicioglu C (2019) Recent developments on thermal municipal sludge pretreatment technologies for enhanced anaerobic digestion. *Renew Sust Energy Rev* 110:423–443. <https://doi.org/10.1016/j.rser.2019.05.002>
- Kumar K (2011) Influence of ultrasonic treatment in sewage sludge. *J Waste Water Treat Anal* 2:2. <https://doi.org/10.4172/2157-7587.1000115>
- Kumar Biswal B, Huang H, Dai J, Chen GH, Wu D (2020) Impact of low-thermal pretreatment on physicochemical properties of saline waste activated sludge, hydrolysis of organics and methane yield in anaerobic digestion. *Bioresour Technol* 297:122423. <https://doi.org/10.1016/j.biortech.2019.122423>
- LaPara TM, Burch TR, McNamara PJ, Tan DT, Yan M, Eichmiller JJ (2011) Tertiary-treated municipal wastewater is a significant point source of antibiotic resistance genes into duluth-superior harbor. *Environ Sci Technol* 45:9543–9549. <https://doi.org/10.1021/es202775r>
- LeBlanc RJ, Matthews P, Richard RP (2008) Global atlas of excreta, wastewater sludge, and biosolids management: moving forward the sustainable and welcome uses of a global resource. United Nations Human Settlements Programme (UN-HABITAT). <https://unhabitat.org/sites/default/files/download-manager-files/Global%20Atlas%20of%20Excreta%2C%20Wastewater%20Sludge%2C%20and%20Biosolids%20Management.pdf>
- Li X, Ma H, Wang Q, Matsumoto S, Maeda T, Ogawa HI (2009) Isolation, identification of sludge-lysing strain and its utilization in thermophilic aerobic digestion for waste activated sludge. *Bioresour Technol* 100:2475–2481. <https://doi.org/10.1016/j.biortech.2008.12.019>

- Li X, Guo S, Peng Y, He Y, Wang S, Li L, Zhao M (2018) Anaerobic digestion using ultrasound as pretreatment approach: changes in waste activated sludge, anaerobic digestion performances and digestive microbial populations. *Biochem Eng J* 139:139–145. <https://doi.org/10.1016/j.bej.2017.11.009>
- Li X, Liu Y, Xu Q, Liu X, Huang X, Yang J, Wang D, Wang Q, Liu Y, Yang Q (2019) Enhanced methane production from waste activated sludge by combining calcium peroxide with ultrasonic: performance, mechanism, and implication. *Bioresour Technol* 279:108–116. <https://doi.org/10.1016/j.biortech.2019.01.115>
- Liao X, Li H, Zhang Y, Liu C, Chen Q (2016) Accelerated high-solids anaerobic digestion of sewage sludge using low-temperature thermal pretreatment. *Int Biodeterior Biodegradation* 106:141–149. <https://doi.org/10.1016/j.ibiod.2015.10.023>
- Lizama AC, Figueiras CC, Herrera RR, Pedreguera AZ, Ruiz Espinoza JE (2017) Effects of ultrasonic pretreatment on the solubilization and kinetic study of biogas production from anaerobic digestion of waste activated sludge. *Int Biodeterior Biodegradation* 123:1–9. <https://doi.org/10.1016/j.ibiod.2017.05.020>
- Ma Y, Wilson CA, Novak JT, Riffat R, Aynur S, Murthy S, Pruden A (2011) Effect of various sludge digestion conditions on sulfonamide, macrolide, and tetracycline resistance genes and class I integrons. *Environ Sci Technol* 45:7855–7861. <https://doi.org/10.1021/es200827t>
- Machdar I, Sekiguchi Y, Sumino H, Ohashi A, Harada H (2000) Combination of a UASB reactor and a curtain type DHS (downflow hanging sponge) reactor as a cost-effective sewage treatment system for developing countries. *Water Sci Technol* 42:83–88. <https://doi.org/10.2166/wst.2000.0362>
- Martinez JL (2009) The role of natural environments in the evolution of resistance traits in pathogenic bacteria. *Proc R Soc B Biol Sci* 276:2521–2530. <https://doi.org/10.1098/rspb.2009.0320>
- Milieu L (2010) Environmental, economic and social impacts of the use of sewage sludge on land. [https://ec.europa.eu/environment/archives/waste/sludge/pdf/part\\_ii\\_report.pdf](https://ec.europa.eu/environment/archives/waste/sludge/pdf/part_ii_report.pdf)
- Mininni G, Dentel SK (2013) State of sewage sludge management in EU members states and in the United States. In: IWA Holistic Sludge Management Conference Proceedings, Sweden
- Mininni G, Blanch AR, Lucena F, Berselli S (2015) EU policy on sewage sludge utilization and perspectives on new approaches of sludge management. *Environ Sci Pollut Res* 22:7361–7374. <https://doi.org/10.1007/s11356-014-3132-0>
- Mirmasoumi S, Ebrahimi S, Saray RK (2018) Enhancement of biogas production from sewage sludge in a wastewater treatment plant: evaluation of pretreatment techniques and co-digestion under mesophilic and thermophilic conditions. *Energy* 157:707–717. <https://doi.org/10.1016/j.energy.2018.06.003>
- Na S, Kim YU, Khim J (2007) Physiochemical properties of digested sewage sludge with ultrasonic treatment. *Ultrason Sonochem* 14:281–285. <https://doi.org/10.1016/j.ultsonch.2006.06.004>
- Neumann P, Pesante S, Venegas M, Vidal G (2016) Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. *Rev Environ Sci Biotechnol* 15:173–211. <https://doi.org/10.1007/s11157-016-9396-8>
- Neumann P, González Z, Vidal G (2017) Sequential ultrasound and low-temperature thermal pretreatment: process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. *Bioresour Technol* 234:178–187. <https://doi.org/10.1016/j.biortech.2017.03.029>
- Neumann P, Barriga F, Álvarez C, González Z, Vidal G (2018) Process performance assessment of advanced anaerobic digestion of sewage sludge including sequential ultrasound–thermal (55 °C) pre-treatment. *Bioresour Technol* 262:42–51. <https://doi.org/10.1016/j.biortech.2018.03.057>
- Nybroe O, Jørgensen PE, Henze M (1992) Enzyme activities in waste water and activated sludge. *Water Res* 26:579–584. [https://doi.org/10.1016/0043-1354\(92\)90230-2](https://doi.org/10.1016/0043-1354(92)90230-2)
- Oosterhuis M, Ringoot D, Hendriks A, Roeleveld P (2014) Thermal hydrolysis of waste activated sludge at Hengelo Wastewater Treatment Plant, The Netherlands. *Water Sci Technol* 70:1–7. <https://doi.org/10.2166/wst.2014.107>



- Pérez-Elvira SI, Fernández-Polanco F, Fernández-Polanco M, Rodríguez P, Rouge P (2008) Hydrothermal multivariable approach. Full-scale feasibility study. *Electron J Biotechnol* 11: 1–10. <https://doi.org/10.2225/vol11-issue4-fulltext-14>
- Pilli S, Bhunia P, Yan S, LeBlanc RJ, Tyagi RD, Surampalli RY (2011) Ultrasonic pretreatment of sludge: a review. *Ultrason Sonochem* 18:1–18. <https://doi.org/10.1016/j.ultsonch.2010.02.014>
- Pilli S, Yan S, Tyagi RD, Surampalli RY (2015) Thermal pretreatment of sewage sludge to enhance anaerobic digestion: a review. *Crit Rev Environ Sci Technol* 45:669–702. <https://doi.org/10.1080/10643389.2013.876527>
- Planta MB (2007) The role of poverty in antimicrobial resistance. *J Am Board Fam Med* 20:533–539. <https://doi.org/10.3122/jabfm.2007.06.070019>
- Platzer C, Hoffmann H, Miglio RM (2016) Long term experiences with dimensioning and operation of vertical flow constructed wetlands in warm climate regions of South America. In: *Proceeding of the IWA special conference on Wetland Systems for water pollution control*. [http://brasil.rotaria.net/wp-content/uploads/2017/08/IWA-2016-wetland\\_vertical\\_experiences.pdf](http://brasil.rotaria.net/wp-content/uploads/2017/08/IWA-2016-wetland_vertical_experiences.pdf)
- Praspaliauskas M, Pedišius N (2017) A review of sludge characteristics in Lithuania's wastewater treatment plants and perspectives of its usage in thermal processes. *Renew Sust Energ Rev* 67: 899–907. <https://doi.org/10.1016/j.rser.2016.09.041>
- Qiao M, Chen W, Su J, Zhang B, Zhang C (2012) Fate of tetracyclines in swine manure of three selected swine farms in China. *J Environ Sci* 24:1047–1052. [https://doi.org/10.1016/S1001-0742\(11\)60890-5](https://doi.org/10.1016/S1001-0742(11)60890-5)
- Rahube TO, Marti R, Scott A, Tien YC, Murray R, Sabourin L, Zhang Y, Duenk P, Lapen DR, Topp E (2014) Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. *Appl Environ Microbiol* 80:6898–6907. <https://doi.org/10.1128/AEM.02389-14>
- Recktenwald M, Wawrzynczyk J, Dey ES, Norrlöw O (2008) Enhanced efficiency of industrial-scale anaerobic digestion by the addition of glycosidic enzymes. *J Environ Sci Health Part A* 43: 1536–1540. <https://doi.org/10.1080/10934520802293693>
- Riau V, De la Rubia MA, Pérez M (2015) Upgrading the temperature-phased anaerobic digestion of waste activated sludge by ultrasonic pretreatment. *Chem Eng J* 259:672–681. <https://doi.org/10.1016/j.cej.2014.08.032>
- Ruffino B, Campo G, Genon G, Lorenzi E, Novarino D, Scibilia G, Zanetti M (2015) Improvement of anaerobic digestion of sewage sludge in a wastewater treatment plant by means of mechanical and thermal pre-treatments: performance, energy and economical assessment. *Bioresour Technol* 175:298–308. <https://doi.org/10.1016/j.biortech.2014.10.071>
- Ruiz-Hernando M, Martín-Díaz J, Labanda J, Mata-Alvarez J, Llorens J, Lucena F, Astals S (2014) Effect of ultrasound, low-temperature thermal and alkali pre-treatments on waste activated sludge rheology, hygienization and methane potential. *Water Res* 61:119–129. <https://doi.org/10.1016/j.watres.2014.05.012>
- Sabourin L, Duenk P, Bonte-Gelok S, Payne M, Lapen DR, Topp E (2012) Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. *Sci Total Environ* 431:233–236. <https://doi.org/10.1016/j.scitotenv.2012.05.017>
- Schievano A, D'Imporzano G, Malagutti L, Fragali E, Ruboni G, Adani F (2010) Evaluating inhibition conditions in high-solids anaerobic digestion of organic fraction of municipal solid waste. *Bioresour Technol* 101:5728–5732. <https://doi.org/10.1016/j.biortech.2010.02.032>
- Schievano A, Tenca A, Scaglia B, Merlino G, Rizzi A, Daffonchio D, Oberti R, Adani F (2012) Two-stage vs single-stage thermophilic anaerobic digestion: comparison of energy production and biodegradation efficiencies. *Environ Sci Technol* 46:8502–8510. <https://doi.org/10.1021/es301376n>
- Sentchilo V, Mayer AP, Guy L, Miyazaki R, Green Tringe S, Barry K, Malfatti S, Goessmann A, Robinson-Rechavi M, van der Meer JR (2013) Community-wide plasmid gene mobilization and selection. *ISME J* 7:1173–1186. <https://doi.org/10.1038/ismej.2013.13>

- Sharma R, Larney FJ, Chen J, Yanke LJ, Morrison M, Topp E, McAllister TA, Yu Z (2009) Selected antimicrobial resistance during composting of manure from cattle administered sub-therapeutic antimicrobials. *J Environ Qual* 38:567–575. <https://doi.org/10.2134/jeq2007.0638>
- Souza TSO, Ferreira LC, Sapkaite I, Pérez-Elvira SI, Fdz-Polanco F (2013) Thermal pretreatment and hydraulic retention time in continuous digesters fed with sewage sludge: assessment using the ADM1. *Bioresour Technol* 148:317–324. <https://doi.org/10.1016/j.biortech.2013.08.161>
- von Sperling M, Mascarenhas LCAM (2005) Performance of very shallow ponds treating effluents from UASB reactors. *Water Sci Technol* 51:83–90. <https://doi.org/10.2166/wst.2005.0432>
- Stefanakis A, Akkratos CS, Tshirintzis VA (2014) Vertical flow constructed wetlands. Elsevier Science, Philadelphia, PA. <https://doi.org/10.1016/C2012-0-01288-4>
- Su JQ, Wei B, Ou-Yang WY, Huang FY, Zhao Y, Xu HJ, Zhu YG (2015) Antibiotic resistome and its association with bacterial communities during sewage sludge composting. *Environ Sci Technol* 49:7356–7363. <https://doi.org/10.1021/acs.est.5b01012>
- Suslick KS (1990) The chemical effects of ultrasound. *Science* 247:1439–1445. <https://doi.org/10.1126/science.247.4949.1439>
- Tawfik A, El-Gohary F, Ohashi A, Harada H (2008) Optimization of the performance of an integrated anaerobic–aerobic system for domestic wastewater treatment. *Water Sci Technol* 58:185–194. <https://doi.org/10.2166/wst.2008.320>
- Tongco JV, Kim S, Oh BR, Heo SY, Lee J, Hwang S (2020) Enhancement of hydrolysis and biogas production of primary sludge by use of mixtures of protease and lipase. *Biotechnol Bioprocess Eng* 25:132–140. <https://doi.org/10.1007/s12257-019-0302-4>
- Toutian V, Barjenbruch M, Unger T, Loderer C, Remy C (2020) Effect of temperature on biogas yield increase and formation of refractory COD during thermal hydrolysis of waste activated sludge. *Water Res* 171:115383. <https://doi.org/10.1016/j.watres.2019.115383>
- Treichel H, Fongaro G, Scapini T, Frumi Camargo A, Spitz Stefanski F, Venturin B (2020) Waste biomass pretreatment methods. In: Treichel H, Fongaro G, Scapini T, Camargo AF, Stefanski FS, Venturin B (eds) *Utilising biomass in biotechnology*. Springer International Publishing, Cham, pp 19–48. [https://doi.org/10.1007/978-3-030-22853-8\\_3](https://doi.org/10.1007/978-3-030-22853-8_3)
- Tyagi VK, Lo SL (2011) Application of physico-chemical pretreatment methods to enhance the sludge disintegration and subsequent anaerobic digestion: an up to date review. *Rev Environ Sci Biotechnol* 10:215–242. <https://doi.org/10.1007/s11157-011-9244-9>
- Tyagi VK, Khan AA, Kazmi AA, Mehrotra I, Chopra AK (2009) Slow sand filtration of UASB reactor effluent: a promising post treatment technique. *Desalination* 249:571–576. <https://doi.org/10.1016/j.desal.2008.12.049>
- Tyagi VK, Lo SL, Appels L, Dewil R (2014) Ultrasonic treatment of waste sludge: a review on mechanisms and applications. *Crit Rev Environ Sci Technol* 44:1220–1288. <https://doi.org/10.1080/10643389.2013.763587>
- Ventorino V, Romano I, Pagliano G, Robertiello A, Pepe O (2018) Pre-treatment and inoculum affect the microbial community structure and enhance the biogas reactor performance in a pilot-scale biodigestion of municipal solid waste. *Waste Manag* 73:69–77. <https://doi.org/10.1016/j.wasman.2017.12.005>
- Wang L, Oda Y, Grewal S, Morrison M, Michel FC, Yu Z (2012) Persistence of resistance to erythromycin and tetracycline in swine manure during simulated composting and lagoon treatments. *Microb Ecol* 63:32–40. <https://doi.org/10.1007/s00248-011-9921-9>
- Wang Q, Wei W, Gong Y, Yu Q, Li Q, Sun J, Yuan Z (2017) Technologies for reducing sludge production in wastewater treatment plants: state of the art. *Sci Total Environ* 587–588:510–521. <https://doi.org/10.1016/j.scitotenv.2017.02.203>
- Watson SD, Akhurst T, Whiteley CG, Rose PD, Pletschke BI (2004) Primary sludge floc degradation is accelerated under biosulphidogenic conditions: enzymological aspects. *Enzym Microb Technol* 34:595–602. <https://doi.org/10.1016/j.enzmictec.2004.01.004>
- Wellington EM, Boxall AB, Cross P, Feil EJ, Gaze WH, Hawkey PM, Johnson-Rollings AS, Jones DL, Lee NM, Otten W, Thomas CM, Williams AP (2013) The role of the natural environment in


- the emergence of antibiotic resistance in Gram-negative bacteria. *Lancet Infect Dis* 13:155–165. [https://doi.org/10.1016/S1473-3099\(12\)70317-1](https://doi.org/10.1016/S1473-3099(12)70317-1)
- Wilson CA, Novak JT (2009) Hydrolysis of macromolecular components of primary and secondary wastewater sludge by thermal hydrolytic pretreatment. *Water Res* 43:4489–4498. <https://doi.org/10.1016/j.watres.2009.07.022>
- Wiśniowska E (2019) Sludge activation, conditioning, and engineering. In: Vara Prasad MN, de Campos Favas PJ, Vithanage M, Mohan SV (eds) *Industrial and municipal sludge emerging concerns and scope for resource recovery*. Elsevier Science, Philadelphia, PA, pp 181–199. <https://doi.org/10.1016/B978-0-12-815907-1.00009-X>
- Wright GD (2010) Q & A: Antibiotic resistance: where does it come from and what can we do about it? *BMC Biol* 8:123. <https://doi.org/10.1186/1741-7007-8-123>
- Xiao B, Tang X, Yi H, Dong L, Han Y, Liu J (2020) Comparison of two advanced anaerobic digestions of sewage sludge with high-temperature thermal pretreatment and low-temperature thermal-alkaline pretreatment. *Bioresour Technol* 304:122979. <https://doi.org/10.1016/j.biortech.2020.122979>
- Xie R, Xing Y, Ghani YA, Ooi K, Ng S (2007) Full-scale demonstration of an ultrasonic disintegration technology in enhancing anaerobic digestion of mixed primary and thickened secondary sewage sludge. *J Environ Eng Sci* 6:533–541. <https://doi.org/10.1139/S07-013>
- Xu Q, Li X, Ding R, Wang D, Liu Y, Wang Q, Zhao J, Chen F, Zeng G, Yang Q, Li H (2017) Understanding and mitigating the toxicity of cadmium to the anaerobic fermentation of waste activated sludge. *Water Res* 124:269–279. <https://doi.org/10.1016/j.watres.2017.07.067>
- Xue Y, Liu H, Chen S, Dichtl N, Dai X, Li N (2015) Effects of thermal hydrolysis on organic matter solubilization and anaerobic digestion of high solid sludge. *Chem Eng J* 264:174–180. <https://doi.org/10.1016/j.cej.2014.11.005>
- Yang Q, Luo K, Li X, Wang D, Zheng W, Zeng G, Liu J (2010a) Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes. *Bioresour Technol* 101:2924–2930. <https://doi.org/10.1016/j.biortech.2009.11.012>
- Yang X, Wang X, Wang L (2010b) Transferring of components and energy output in industrial sewage sludge disposal by thermal pretreatment and two-phase anaerobic process. *Bioresour Technol* 101:2580–2584. <https://doi.org/10.1016/j.biortech.2009.10.055>
- Yang Y, Li B, Ju F, Zhang T (2013) Exploring variation of antibiotic resistance genes in activated sludge over a four-year period through a metagenomic approach. *Environ Sci Technol* 47:10197–10205. <https://doi.org/10.1021/es4017365>
- Yin F, Li Z, Wang D, Ohlsen T, Dong H (2016) Performance of thermal pretreatment and mesophilic fermentation system on pathogen inactivation and biogas production of faecal sludge: initial laboratory results. *Biosyst Eng* 151:171–177. <https://doi.org/10.1016/j.biosystemseng.2016.08.019>
- Yu S, Zhang G, Li J, Zhao Z, Kang X (2013) Effect of endogenous hydrolytic enzymes pretreatment on the anaerobic digestion of sludge. *Bioresour Technol* 146:758–761. <https://doi.org/10.1016/j.biortech.2013.07.087>
- Zábranská J, Dohányos M, Jeníček P, Kutil J (2006) Disintegration of excess activated sludge – evaluation and experience of full-scale applications. *Water Sci Technol* 53:229–236. <https://doi.org/10.2166/wst.2006.425>
- Zhang XX, Zhang T (2011) Occurrence, abundance, and diversity of tetracycline resistance genes in 15 sewage treatment plants across china and other global locations. *Environ Sci Technol* 45:2598–2604. <https://doi.org/10.1021/es103672x>
- Zhang T, Zhang XX, Ye L (2011) Plasmid metagenome reveals high levels of antibiotic resistance genes and mobile genetic elements in activated sludge. *PLoS One* 6:e26041. <https://doi.org/10.1371/journal.pone.0026041>
- Zhang B, Ji M, Wang F, Li R, Zhang K, Yin X, Li Q (2017) Damage of EPS and cell structures and improvement of high-solid anaerobic digestion of sewage sludge by combined (Ca(OH)<sub>2</sub> + multiple-transducer ultrasonic) pretreatment. *RSC Adv* 7:22706–22714. <https://doi.org/10.1039/C7RA01060E>

- Zhen G, Lu X, Kato H, Zhao Y, Li YY (2017) Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: current advances, full-scale application and future perspectives. *Renew Sust Energy Rev* 69:559–577. <https://doi.org/10.1016/j.rser.2016.11.187>
- Zhou J, Wang YH, Chu J, Luo LZ, Zhuang YP, Zhang SL (2009) Optimization of cellulase mixture for efficient hydrolysis of steam-exploded corn stover by statistically designed experiments. *Bioresour Technol* 100:819–825. <https://doi.org/10.1016/j.biortech.2008.06.068>
- Zou S, Wang X, Chen Y, Wan H, Feng Y (2016) Enhancement of biogas production in anaerobic co-digestion by ultrasonic pretreatment. *Energy Convers Manag* 112:226–235. <https://doi.org/10.1016/j.enconman.2015.12.087>
- Zou X, Yang R, Zhou X, Cao G, Zhu R, Ouyang F (2020) Effects of mixed alkali-thermal pretreatment on anaerobic digestion performance of waste activated sludge. *J Clean Prod* 259: 120940. <https://doi.org/10.1016/j.jclepro.2020.120940>



# Toward a Circular Economy of Sewage Sludge Anaerobic Digestion: Relevance of Pre-treatment Processes and Micropollutant Presence for Sustainable Management

# 11

Gladys Vidal , Patricio Neumann, and Gloria Gómez

## Abstract

Circular economy is defined as a system in which the value of products, materials, and resources is maintained for as long as possible, minimizing their consumption and the generation of waste. Anaerobic digestion (AD) represents an attractive technology, as it uses waste to produce biogas as renewable energy and stabilizes the sewage sludge for land application. However, these potential benefits may be limited by negative impacts related to the land disposal of the stabilized sewage sludge due to the presence of organic micropollutants (OMPs) in the input sludge, and the inability of current AD methods to remove them is a recognized potential risk for human health and for the environment. However, as the conversion of sewage sludge during AD is limited by the low rate of hydrolysis of solids and complex organic compounds, the degradation of OMPs is also limited, as most pharmaceuticals, industrial additives, fragrances, and other synthetic compounds exhibit low biodegradability as a result of the high stability of most commercially available substances. While the effect of pre-treatments on the performance of anaerobic digestion has been studied mainly with the aim of increasing biogas production and reducing the volume of solids, the solubilization caused by pre-treatments can also increase the bioavailability of OMPs for anaerobic microorganisms, increasing its rate of degradation during the AD process.

The selection of the most suitable sewage sludge management, in terms of their environmental sustainability, scenario for every particular scenario needs the

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usage of decision-making tools like life cycle assessment (LCA) that allow for the assessment of the potential environmental impacts of proposed strategies. The most relevant environmental aspects and impacts associated with sewage sludge generation and management could be found at the global, regional, and local level. The eutrophication, in terrestrial and aquatic ecosystems, or acidification is an environmental aspect at the regional level due to the sewage sludge disposal. However, human toxicity or infectious disease propagation and decreased quality of life could environmental aspects found at the local level.

The aim of this chapter of this book is to show the circular economy of sewage sludge anaerobic digestion considering the relevance of pre-treatments and micropollutant presence for sustainability.

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**Keywords**

Sewage sludge · Anaerobic digestion · Pre-treatment · Organic micropollutants · Circular economy · Life cycle assessment

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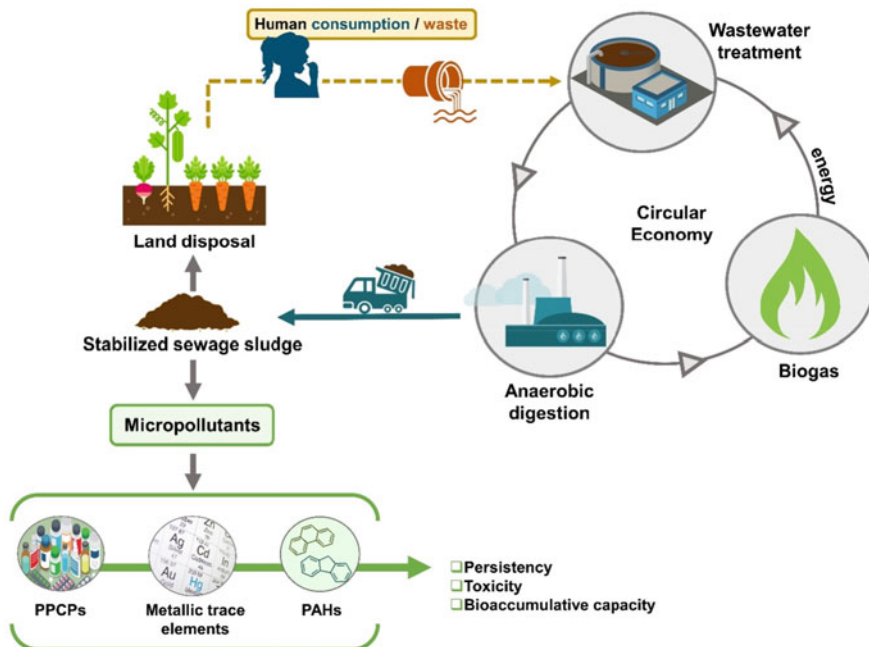
## 11.1 Introduction

Wastewater treatment plants (WWTPs) play a fundamental role in the adequate purification of wastewater to maintain the health of ecosystems and human beings. The most widespread wastewater treatment technology is by activated sludge (Vera et al. 2013). In this type of WWTP, around 60% of the organic matter present in the activated sludge will be transferred to the sanitary sludge, and the management of these implies a cost between 30% and 65% of the operation cost of the WWTP.

Sewage sludge (SS) generated during activated sludge processes is characterized by high concentrations of solids (2–12% total solids for liquid sludge and 12–40% for dehydrated sludge), organic matter (55–85% of volatile solids in dry basis), pathogens (109 fecal coliforms/100 mL; 2500–70,000 virus/100 mL; 200–1000 helminth/100 mL), and nutrients (>8 mg P/kg, >30 mg N/kg, >3 mg K/kg) (De Maria et al. 2010; EPA 1995).

Sewage treatment represents a fundamental mainstay for public health protection. Through successive physical, chemical, and biological processes, efficient removal of solids, organic matter, nutrients, and pathogens can be achieved. However, removal of organic micropollutants (OMPs) that enter in the sewage system from household and industrial wastewaters is not always efficient, being influenced by the physicochemical characteristics of the pollutant and the treatment technology used (Luo et al. 2014). Furthermore, in most conventional treatment processes, the fate of a significant fraction of the different OMPs is the sludge generated during the depuration process (Martín et al. 2012; Reyes-Contreras et al. 2020).

Within the circular economy (CE) framework, anaerobic digestion (AD) represents an attractive technology, as it uses waste to produce biogas as renewable energy and stabilizes the sewage sludge for land application. In this way, this technology contributes to “closing the loop” between energy consumption,



**Fig. 11.1** The sewage sludge considering the anaerobic digestion as a technology of stabilization. (Adapted from Venegas et al. 2021)

food production, and the disposal of the subsequent waste. However, these potential benefits may be limited by negative impacts related to the land disposal of the stabilized sewage sludge. For example, the presence of micropollutants (MPs) in the input sludge and the inability of current AD methods to remove them are recognized potential risks for human health and for the environment (Venegas et al. 2021), as show, Fig. 11.1.

On the other hand, the selection of most suitable sewage sludge management, in terms of their environmental sustainability, scenario for every particular scenario needs the usage of decision-making tools, i.e., life cycle assessment (LCA), that allow for the assessment of the potential environmental impacts of proposed strategies (Kacprzak et al. 2017).

The objective of the chapter of this book is to show the circular economy of sewage sludge anaerobic digestion considering the relevance of pre-treatments and micropollutant presence for sustainability.

## 11.2 Anaerobic Digestion

Anaerobic digestion (AD) is a biological process in the absence of oxygen, which involves a series of complex biochemical reactions that occur in four main consecutive stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, respectively.

AD is one of the most widely used stabilization technologies. During AD, biogas (gaseous mixture composed principally of  $\text{CO}_2$  and  $\text{CH}_4$ ) is produced by biological activity from stabilization of sewage sludge. Due to this, sludge may be considered with commercial value as a bioenergy source (Vidal et al. 2001a).

However, the presence of high-molecular-weight compounds and complex organic matter in sludge limits the hydrolysis step of AD (Vidal et al. 2001b), requiring large reactor volumes and long retention times to achieve adequate stabilization prior to disposal or reuse. This also limits the applicability of AD as an energy recovery strategy based on the circular economy principles, as the amount of biogas produced from biogas is directly related to the efficiency of the overall process to convert organic matter during the successive steps of digestion. One strategy to overcome this limitation is to subject sludge to a pre-hydrolysis process before AD (a *pre-treatment*), with the objective of increasing the rate and extent of sludge transformation in  $\text{CH}_4$  during the biodegradation process.

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## 11.3 Sewage Sludge Pre-treatment to Improve Methane Production

The application of pre-treatment allows reducing both energy and economic costs that the waste treatment process demands within the WWTP. Various types of pre-treatments have been described in the literature, among which physical, chemical, and biological processes stand out. Pre-treatment involves thermal, physical, chemical, or biological means to disrupt the floc structure of sludge and hydrolyze organic matter. This provides significant enhancements in terms of solid reduction, biogas production, and digested sludge properties (Neumann et al. 2016, 2017).

Most pre-treatment studies have been oriented toward biogas production improvements, which have been observed to be related to the efficiency of solubilization achieved through thermal hydrolysis, cavitation, oxidation, and other phenomena depending on process configuration (Neumann et al. 2017).

In general, the methods are aimed at disintegrating the floccular structure and rupture of the cell membrane, which results in the lysis or disintegration of the bacteria that mainly make up the secondary sludge. In this way, it is achieved that the organic matter that is slowly biodegradable is transformed into compounds of lower molecular weight and rapidly biodegradable by the biomass in the AD process (Patil et al. 2016).

Most of the research associated with the pre-treatment of organic waste involve the use of mechanical, thermal, and chemical processes, with an abundance of 33%, 24%, and 21% of the total of reports, respectively (Mata-Alvarez et al. 2014). Such



results agree with that reported by Neumann et al. (2016), highlighting that a third of all the pre-treatment methods found in the literature correspond to mechanical and thermal. Most of the studies direct their objectives toward evaluating the effect of pre-treatment of sludge to be stabilized by AD in mesophilic conditions in temperature ranges between 35 and 37 °C (Neumann et al. 2016).

Indeed, the various pre-treatments show better results before AD in mesophilic than thermophilic conditions. This is probably due to the unnecessary effect of a pre-treatment before thermophilic AD, since under such a condition a favoring of the hydrolysis stage is achieved. Among the pre-treatments that offer an increase in the solubilization of organic matter and increased AD performance, there are physical pre-treatments such as ultrasound (US) and thermal (Cesaro and Belgioirno 2014).

Physical pre-treatments such as US and thermal can induce considerable changes on the physical, chemical, and biological properties of the sludge. One of the representative physical properties of the sludge is associated with the high-water content it contains, which makes its handling and subsequent disposal difficult, leading to drainage constituting a critical stage (Ruiz-Hernando et al. 2015) in the line of management and handling of sludge in the WWTP.

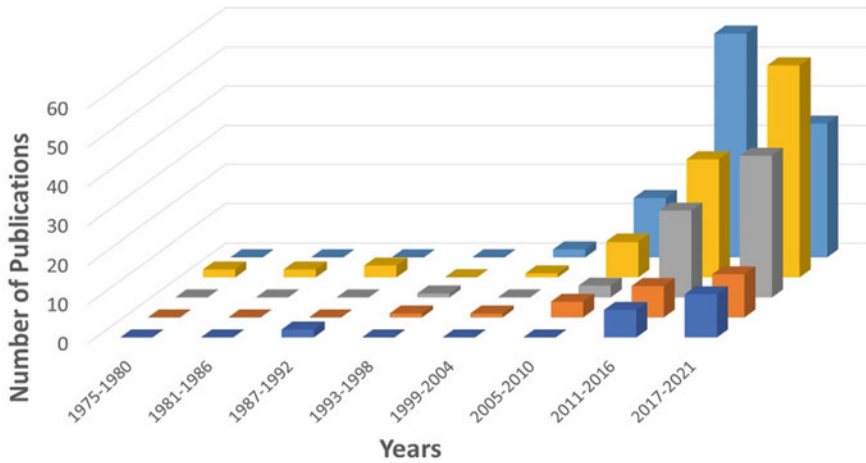
The existence of extracellular polymeric substances (EPS) constitutes one of the major causes of the low dewatering capacity of the sludge, because its components lead to the interstitial union of water. The dehydratability of the sludge can be improved through several pre-treatments, among which methods such as US (Ruiz-Hernando et al. 2015) and incubation stand out. The main objective of the application of any pre-treatment is based on partially disintegrating the sludge, which triggers the release of interstitial water, leading to an increase in the dehydratability of the sludge (Neyens et al. 2004).

There are several ways to quantify the sludge dewatering capacity. One of the most widely used methods is the determination of capillary suction time (CST). This parameter is defined as the time involved in the advance of filtering between two electrodes (APHA 1998), so that the lower the determined CST, the greater the dewatering capacity of the sludge.

Another method for evaluating dehydratability consists of determining the specific resistance to filtration (SRF), which indicates the ease with which the sludge is drained through a filtration process (Foladori et al. 2010). In accordance with the above, the decrease in the SRF value indicates an increase in filtration, which corresponds to an improvement in the dewatering capacity of the sludge.

Pre-treatment technologies have been studied since the late 1970s. The first applications of sludge pre-treatment were based on thermal processes. Figure 11.2 shows the research trend regarding sludge pre-treatment from 1975 to 2021.

The main research in sludge pre-treatment since the 2000s to date has been in physical and thermal technology. Moreover, a significant growth in the last years has shown the combined sludge pre-treatment treatments. The most common pre-treatment combinations are physical/chemical and thermo-/chemical. Processes based on the integration of mechanical or chemical disruption and dual digestion or thermally phased anaerobic digestion have also received attention. The growing number on reports of combined processes reflects the need to overcome the



**Fig. 11.2** Number of papers on sludge pre-treatment for AD published between years 1975 and 2021. ■ chemical, ■ biological, ■ combined, ■ thermal, ■ physical

limitations of single processes and achieve more significant improvements in AD through synergistic effects. The decrease in references related to thermal and chemical pre-treatments in the last 5 years could also be related to a shift in focus to combined thermo-/chemical processes.

#### 11.4 Environmental Performance of Sludge Anaerobic Digestion Including Pre-treatments

As described previously, pre-treatments have the capability to increase the methane production rate and yield during sludge AD, leading to potential increases in the energy recovery of the overall stabilization process. However, in order to do so, pre-treatments require energy and/or material inputs, and therefore, the energy balance of the process is particularly important in order to determine the viability of a particular pre-treatment configuration (Cano et al. 2015).

Furthermore, pre-treatments require the implementation of new equipment and can significantly modify important parameters of quality in the sludge, such as the concentration of pollutants and elements (metals, organic pollutants, MPs, N, P, K, and others), the presence of pathogens, and the dewaterability of the digested sludge (Carballa et al. 2008; Neumann et al. 2018; Reyes-Contreras et al. 2020). All these factors are relevant from an environmental management perspective, as these not only influence the operations inside the wastewater treatment plant but also the environmental impacts related to the full life cycle of sludge. Table 11.1 shows a summary of the most relevant environmental aspects and impacts associated with sewage sludge generation and management.

**Table 11.1** Summary of relevant environmental aspects and impacts associated with sewage sludge generation and management

Environmental impacts	Scale	Environmental aspects	Main processes and management stages involved
Climate change	Global	Electricity and fossil fuel consumption	Pumping, thickening, stabilization processes, dewatering, transport
		Direct CH <sub>4</sub> emission	Storage, anaerobic stabilization, dewatering
		Direct N <sub>2</sub> O emission	Storage, disposal of sludge (in soil or landfill)
		Replacement of fossil fuels and electricity <sup>a</sup>	Combined heat and power generation from biogas
		Replacement of commercial fertilizers <sup>a</sup>	Application of stabilized sludge to crops
Mineral and fossil resource depletion	Global	Electricity and fossil fuel consumption	Pumping, thickening, stabilization processes, dewatering, transport
		Chemical reagent consumption	Thickening, dewatering, chemical stabilization processes
		Material consumption	Infrastructure, landfill disposal of sludge
		Replacement of fossil fuels and electricity <sup>a</sup>	Combined heat and power generation from biogas
		Replacement of commercial fertilizers <sup>a</sup>	Application of stabilized sludge to crops
Eutrophication (in terrestrial and aquatic ecosystems)	Regional	Direct NH <sub>3</sub> emission	Stabilization processes, disposal of sludge (in soil or landfill)
		Lixiviation/runoff of N and P from soils	Disposal of sludge (in soil or landfill)
		Accumulation of N and P in soils	Disposal of sludge (in soil or landfill)
		Electricity and fossil fuel consumption	Pumping, thickening, stabilization processes, dewatering, transport
Acidification	Regional	Direct NH <sub>3</sub> emission	Stabilization processes, disposal of sludge (in soil or landfill)
		Electricity and fossil fuel consumption	Pumping, thickening, stabilization processes, dewatering, transport
Ecotoxicity	Local	Lixiviation/runoff of heavy metals and organic pollutants from soils	Disposal of sludge (in soil or landfill)

(continued)

**Table 11.1** (continued)

Environmental impacts	Scale	Environmental aspects	Main processes and management stages involved
		Accumulation of heavy metals and organic pollutants in soils	Disposal of sludge (in soil or landfill)
		Electricity and fossil fuel consumption	Pumping, thickening, stabilization processes, dewatering, transport
Human toxicity	Local	Direct contact with heavy metals and organic pollutants in soils	Disposal of sludge (in soil or landfill)
		Translocation of heavy metals and organic pollutants to crops	Disposal of sludge (in soil)
		Electricity and fossil fuel consumption	Pumping, thickening, stabilization processes, dewatering, transport
Infectious disease propagation and decreased quality of life	Local	Propagation of pathogenic organisms (virus, bacteria, parasites)	Operations during treatment, transportation, and disposal of sludge
		Odor propagation and attraction of infectious vectors	Operations during treatment, transportation, and disposal of sludge

<sup>a</sup> Associated with positive impacts; table based on Carballa et al. (2011), Yoshida et al. (2013), and Harder et al. (2014)

Life cycle assessment (LCA) is a methodology used to evaluate the potential environmental impacts of a product, process, service, or organization with an integral perspective. The strengths of LCA lies both in the potential evaluation of the full life cycle of products (including the extraction and processing of natural resources, the transportation and distribution of raw materials and products, the use of the products, and the management of wastes) and the quantification of a wide array of indicators related to different environmental issues, including climate change, eutrophication, resource depletion, and ecotoxicity, among others (Hauschild et al. 2017).

LCA has been widely applied to the assessment of sludge management alternatives, which generally shows favorable results for AD compared to other alternatives (Hospido et al. 2005; Suh and Rousseaux 2002; Yoshida et al. 2013). However, the literature associated with the assessment of pre-treatment processes prior to AD is more limited. In one of the most extensive studies, Carballa et al. (2011) concluded that mechanical (i.e., pressurize-depressurize) and chemical (acid or alkaline) pre-treatments showed better environmental performance than technologies such as thermal, freeze-thaw, and ozonation processes. On another relevant study, Mills et al. (2014) reported that thermal hydrolysis improved the environmental performance of sludge digestion, while Gianico et al. (2015) reported

that while the energetic cost of implementing sludge wet oxidation and thermal pre-treatment processes surpassed the benefits of the combined heat and power (CHP) produced from biogas, the environmental performance of the upgraded facility was similar to that of the conventional plant. In a more recent study, Cartes et al. (2018) compared conventional AD with an advanced AD process that includes a sequential ultrasound-thermal pre-treatment, concluding that the overall environmental performance of both systems was similar. The main differences were a decrease in the climate change potential (from  $-5.4$  to  $-12.6$  kg CO<sub>2</sub> eq/ton of sludge) and an increase in the potential depletion of abiotic resources (from  $-9.3$  to  $3.6$  kg Sb eq/ton of sludge), related to the effects of the pre-treatment over energy recovery, sludge transport requirements, and nutrient loads in the stabilized sludge. In particular, one of the most significant drivers of these changes was the increased transport requirements of the sludge after including the pre-treatment, associated with an observed 4.2% decrease in its dewaterability. Overall, both the conventional and advanced AD scenarios showed substantially lower environmental impacts than *business-as-usual* scenarios based on alkaline stabilization and landfill disposal (e.g.,  $-12.6$  to  $-5.4$  compared to  $677.5$ – $713.9$  kg CO<sub>2</sub> eq/ton of sludge), highlighting the relevance of energy and nutrient recovery from a circular economy perspective. Moreover, these results illustrate the relevance of the life cycle perspective in order to properly assess the environmental sustainability of sludge valorization strategies.

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## 11.5 Relevance of Micropollutants for Closing the Loop During Sewage Sludge Management

However, even though AD followed by land application appears as a sustainable management strategy for stabilized sludge, there is a rising concern related to the presence of pollutants that are not removed during the stabilization process and could represent a source of contamination for soils and water bodies. While the concentration of heavy metals and pathogens is normally regulated in the directives developed for land application of sludge (e.g., EPA 1995), other relevant pollutants that can be present in sludge in low concentrations (i.e., micropollutants) are not included. Moreover, as the conversion of sewage sludge (SS) during AD is limited by the low rate of hydrolysis of solids and complex organic compounds, the degradation of OMPs is also limited, as most pharmaceuticals, industrial additives, fragrances, and other synthetic compounds exhibit low biodegradability as a result of the high stability of most commercially available substances. While the effect of pre-treatments on the performance of anaerobic digestion has been studied mainly with the aim of increasing biogas production and reducing the volume of solids, the solubilization caused by pre-treatments can also increase the bioavailability of OMPs for anaerobic microorganisms, increasing its rate of degradation during the AD process (Reyes-Contreras et al. 2019).

Therefore, there is a knowledge gap on the quality of biosolids, the associated risk of contamination, and the possible human exposure to OMPs present in the SS

digestate, a fundamental question to promote its beneficial use or to devise new treatment strategies.

### 11.5.1 Organic Micropollutants

OMPs are anthropogenic or natural compounds present in the environment at trace concentrations ranging from ng/L to µg/L, for which negative effects on the environment or human health are either confirmed or suspected. The relatively low concentrations of MPs in wastewaters not only complicate their detection and quantification but also create challenges for water and wastewater treatment processes (Luo et al. 2014). As the toxicological and environmental impacts of these substances have been only noticed during the last few decades, some of these compounds are also referred to as Contaminants of Emerging Concern (CEC), which includes pharmaceuticals and personal care products (PPCPs), polycyclic aromatic hydrocarbons (PAHs), plasticizers, flame retardants, and surfactants. The described toxic effects of OMPs cover a wide range of deleterious effects, from DNA damage and mutagenesis to dysfunctions in the endocrine system, reproductive toxicity, immunological impairment, and developmental defects, both over humans and wildlife (De Jesus et al. 2015). Another OMPs very common in sewage sludge are per- and polyfluoroalkyl substances (PFAS) which are a family of human-made chemicals found in a wide range of everyday products used in paper, cookwares, carpet, clothes, cosmetics, and food packaging (Yu et al. 2020). Regarding the concentrations reported in sewage sludge, Eriksson et al. (2017) determined values of several PFAS in sludge from municipal wastewater treatment plants between 0.8 and 20 ng/g. Similar results were observed by Venkatesan and Halden (2013) which determined 13 PFAS in US biosolids with mean concentrations between 2 and 21 ng/g. Lakshminarasimman et al. (2021) evaluated the behavior of different sludge treatment systems which include pelletization, alkaline stabilization, and aerobic and anaerobic digestion processes for removing several types of PFAS. Regarding the performance of AD technology, three of the five of AD systems showed removal efficiencies of PFAS until 40%.

The concentrations of these compounds in sewage sludge are greatly dependent on their physicochemical properties, including water solubility, octanol-water partition coefficient ( $\text{Log } K_{ow}$ ), and soil adsorption coefficient ( $\text{Log } K_{oc}$ ).  $K_{ow}$  is frequently used to predict adsorption of MPs on solids (Luo et al. 2014), and according to Rogers (1996),  $\text{Log } K_{ow} < 2.5$  indicates low sorption potential,  $2.5 < \text{Log } K_{ow} < 4$  indicates medium sorption potential, and  $\text{Log } K_{ow} > 4$  indicates high sorption potential. Similarly,  $K_{oc}$  is a measure of the tendency of compounds to bind to soils. Higher  $\text{Log } K_{oc}$  values correlate to less mobile organic chemicals, while lower  $\text{Log } K_{oc}$  values correlate to more mobile organic chemicals (USEPA 2002). Moreover, sludge characteristics (pH, organic matter, cation's concentration, and others) and the operational parameters of wastewater treatment plants, such as the hydraulic retention time and organic loading rates (OLRs), can also influence the concentration of MPs both in sludge and treated sewage (Neumann et al. 2016).

### 11.5.2 PPCP in Sewage Sludge

As an important group of OMPs, PPCPs have received growing attention in recent years for their possible negative effects to the environment and human health (Ebele et al. 2017). They include a diverse group of organic compounds used for the daily personal care, like soaps, lotions, fragrances, and sunscreens (Liu and Wong 2013; Reyes-Contreras et al. 2019). Table 11.2 shows the physicochemical properties and concentrations of PPCPs detected in sewage sludge and biosolids from AD. Regarding fragrances, the most common compounds were galaxolide and tonalide, with values that varied between 13 and 427,000  $\mu\text{g}/\text{kg}$  in sewage sludge. These substances have hydrophobic characteristics, with  $\text{Log } K_{ow}$  values above 6, and therefore present a tendency to be found in the solid phase (sewage sludge) rather than in the liquid phase (Venegas et al. 2021).

Pharmaceutical drugs are also present in sewage emissions. The most prevalent compound in sewage sludge is ibuprofen, with values that fluctuate between 1.9 and 950  $\mu\text{g}/\text{kg}$ . For this anti-inflammatory compound,  $\text{Log } K_{ow}$  ranges between 2.5 and 4, being therefore present in the solid and liquid phases (Gonzalez-Gil et al. 2016). Hormones and hormone-like compounds are also commonly found in sludges, either from natural sources (estriol, estrone, and estradiol) or from hormonal regulation pharmaceuticals, like ethynyl estradiol or progesterone (Venegas et al. 2021). These compounds are extremely potent drugs as they are able to interact with the endocrine system, altering it and affecting the reproductive functions of humans and other animals, a possibly causing effect known as endocrine disruption (ED). The best-known ED effect is the feminization caused by many natural or synthetic estrogens, which are found both in sewage and sludge at concentrations high enough to elicit physiological responses in fish and other vertebrates (Chamorro et al. 2010).

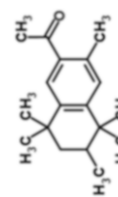
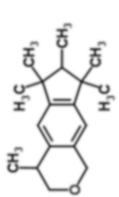
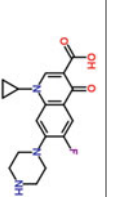
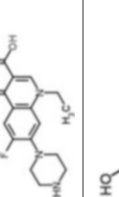
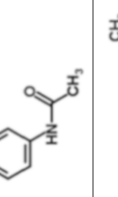
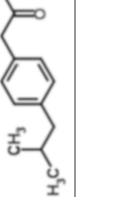
A particular category of pharmaceuticals also present in sewage sludge are antibiotics, widely used in both human and livestock antibacterial treatments. Although their toxicity for humans and animals is intrinsically very low, there is a growing concern that their persistence in sludges and soils may favor the emergence of antibiotic-resistant bacteria that can ultimately result in untreatable bacterial illnesses in humans or livestock (Piña et al. 2020). One example is sulfamethoxazole, found in sewage sludge at concentrations around 100  $\mu\text{g}/\text{kg}$ , and with a high potential to migrate to the liquid phase, given its  $\text{Log } K_{ow}$  value of 0.89 (Gonzalez-Gil et al. 2018).

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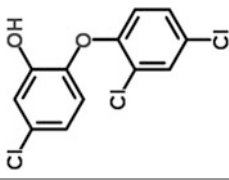
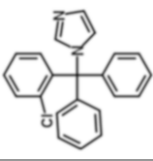
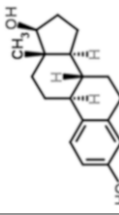
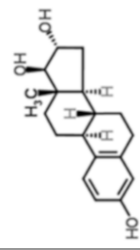
## 11.6 Conclusions

Under the concept of circular economy, AD is an attractive technology to “close the loop” because this system is focused on maximizing the reuse of resources and minimizing their depreciation with the reuse of biogas and the land disposal of biosolids. Despite benefits for soil properties, this sewage sludge contains different OMPs which are associated with negative effects for the human health and the environment. These compounds are found in biosolids from AD with concentrations

**Table 11.2** Physical-chemical properties and concentrations of PPCPs detected in sewage sludge (adapted from Venegas et al. 2021)

Classification	Analyte	Log $K_{ow}$	Log $K_{oc}$	Molecular structure	Matrix	Concentration ( $\mu\text{g}/\text{kg}$ )
Fragrances	Tonalide	6.35	4.72		Digested sludge	4000
				Biosolid	78–427,000	
				Secondary sludge	400–11,200	
				UASB sludge	803–2407	
Antibiotics	Galaxolide	6.26	4.10		Digested sludge	26,000
				Biosolid	13–177,000	
				Secondary sludge	4200–36,000	
				UASB sludge	3842–12,304	
	Ciprofloxacin	0.28	1.55		Digested sludge	1000–2400
				Secondary sludge	960	
Drugs	Norfloxacin	-1.03	1.96		Digested sludge	1500–2400
				Biosolid	1400	
				Secondary sludge	111	
	Acetaminophen	0.46	1.79			
	Ibuprofen	3.97	2.59		Secondary sludge	1.9–950



Personal care products	Triclosan	4.76	4.26		Secondary sludge Biosolids	620–17,500 190–9850
	Clotrimazole	6.26	6.21		Secondary sludge	50
Hormones	$\beta$ -Estradiol	4.01	4.20		Secondary sludge	17–50
	Estriol	2.45	2.90		Secondary sludge	80

Notes: Digested sludge is the product of anaerobic digestion; biosolids are stabilized organic solids derived from sewage treatment processes which can be managed safely to be used beneficially for land application; secondary sludge is the biomass resulting from biological treatment; UASB sludge, sludge from upflow anaerobic sludge bed;  $K_{ow}$ , octanol-water partition coefficient;  $K_{oc}$ , soil adsorption coefficient. Both values were obtained from ChemSpider®

that depend on the physicochemical properties of OMPs. Because of their persistency, toxicity, and bioaccumulative capacity, it is necessary to evaluate the potential risks of their presence on the biosolids. Owing to the detection and treatment difficulties of OMPs, concentration reduction at sources needs to be emphasized through legislation, imposing restricted environmental release of the compounds, and public awareness.

Future research must obtain biosolids with physical and chemical properties for reusability as soil amendment but without biological activity due to the OMPs, in order to end the cycle sustainably. The application of LCA will be key to evaluate the environmental aspects and impacts at different levels of scales (global, regional, and local) of the generated sludge and of the applied technologies.

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## References

- APHA - American Public Health Association (1998) Standard methods for the examination of wastewater, 20th edn. APHA, Washington, DC
- Cano R, Pérez-Elvira SI, Fdz-Polanco F (2015) Energy feasibility study of sludge pretreatments: a review. *Appl Energy* 149:176–185
- Carballa M, Omil F, Lema JM (2008) Influence of different pretreatments on anaerobically digested sludge characteristics: suitability for final disposal. *Water Air Soil Pollut* 199:311–321
- Carballa M, Duran C, Hospido A (2011) Should we pretreat solid waste prior to anaerobic digestion? An assessment of its environmental cost. *Environ Sci Technol* 45:10306–10314
- Cartes J, Neumann P, Hospido A, Vidal G (2018) Life cycle assessment of sludge management alternatives for sewage treatment plants in Chile: does advanced anaerobic digestion improve environmental performance compared to current practices? *J Mater Cycles Waste Manag* 20(3): 1530–1540
- Cesaro A, Belgiorno V (2014) Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions. *Chem Eng J* 240:24–37
- Chamorro S, Hernández V, Monsalvez E, Becerra J, Mondaca MA, Piña B, Vidal G (2010) Detection of estrogenic activity from kraft mill effluents by the yeast estrogen screen. *Bull Environ Contam Toxicol* 84:165–169
- De Jesus GV, Almeida CMM, Rodrigues A, Ferreira E, Benoliel MJ, Cardoso VV (2015) Occurrence of pharmaceuticals in a water supply system and related human health risk assessment. *Water Res* 72:199–208
- De Maria IC, Chiba MK, Costa A, Berton RS (2010) Sewage sludge application to agricultural land as soil physical conditioner. *Rev Bras Ciência do Solo* 34:967–974
- Ebele AJ, Abou-Elwafa Abdallah M, Harrad S (2017) Pharmaceuticals and personal care products (PPCPs) in the freshwater aquatic environment. *Emerg Contam* 3:1–16
- EPA (1995) Process design manual: land application of sewage sludge and domestic septage. Environmental Protection Agency (EPA), Washington, DC
- Eriksson U, Haglund P, Kärman A (2017) Contribution of precursor compounds to the release of per- and polyfluoroalkyl substances (PFASs) from waste water treatment plants (WWTPs). *J Environ Sci* 61:80–90
- Foladori P, Andreottola G, Ziglio G (2010) Sludge reduction technologies in wastewater treatment plants. IWA Publishing, London, p 361
- Gianico A, Bertanza G, Braguglia CM, Canato M, Laera G, Heimersson S, Svanström M, Mininni G (2015) Upgrading a wastewater treatment plant with thermophilic digestion of thermally

- pre-treated secondary sludge: techno-economic and environmental assessment. *J Clean Prod* 102:353–361
- Gonzalez-Gil L, Papa M, Feretti D, Ceretti E, Mazzoleni G, Steimberg N, Pedrazzani R, Bertanza G, Lema JM, Carballa M (2016) Is anaerobic digestion effective for the removal of organic micropollutants and biological activities from sewage sludge? *Water Res* 102:211–220
- Gonzalez-Gil L, Mauricio-Iglesias M, Serrano D, Lema JM, Carballa M (2018) Role of methanogenesis on the biotransformation of organic micropollutants during anaerobic digestion. *Sci Total Environ* 622–623:459–466
- Harder R, Heimersson S, Svanström M, Peters GM (2014) Including pathogen risk in life cycle assessment of wastewater management. 1. Estimating the burden of disease associated with pathogens. *Environ Sci Technol* 48:9438–9445
- Hauschild M, Rosenbaum R, Olsen S (eds) (2017) *Life cycle assessment: theory and practice*, 1st edn. Springer, Berlin. [https://doi.org/10.1007/978-3-319-56475-3\\_33](https://doi.org/10.1007/978-3-319-56475-3_33)
- Hospido A, Moreira MT, Martín M, Rigola M, Feijoo G (2005) Environmental evaluation of different treatment processes for sludge from urban wastewater treatments: anaerobic digestion versus thermal processes. *Int J Life Cycle Assess* 10:336–345
- Kacprzak M, Neczaj E, Fijałkowski K, Grobelak A, Grosser A, Worwag M, Singh BR (2017) Sewage sludge disposal strategies for sustainable development. *Environ Res* 156:39–46
- Lakshminarasimman N, Gewurtz SB, Parker WJ, Smyth SA (2021) Removal and formation of perfluoroalkyl substances in Canadian sludge treatment systems – a mass balance approach. *Sci Total Environ* 754:142431
- Liu JL, Wong MH (2013) Pharmaceuticals and personal care products (PPCPs): a review on environmental contamination in China. *Environ Int* 59:208–224
- Luo Y, Guo W, Ngo HH, Faisal DN, Hai FI, Zang J, Liang S, Wang XC (2014) A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. *Sci Total Environ* 473–474:619–641
- Martín J, Camacho-Muñoz MD, Santos JL, Aparicio I, Alonso E (2012) Distribution and temporal evolution of pharmaceutically active compounds alongside sewage sludge treatment. Risk assessment of sludge application onto soils. *J Environ Manag* 102:18–25
- Mata-Alvarez J, Dosta J, Romero-Guiza MS, Fonoll X, Peces M, Astals S (2014) A critical review on anaerobic co-digestion achievements between 2010 and 2013. *Renew Sust Energy Rev* 36:412–427
- Mills N, Pearce P, Farrow J, Thorpe RB, Kirkby NF (2014) Environmental & economic life cycle assessment of current & future sewage sludge to energy technologies. *Waste Manag* 34:185–195
- Neumann P, Pesante S, Venegas M, Vidal G (2016) Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge. *Rev Environ Sci Biotechnol* 15(2):173–211
- Neumann P, González Z, Vidal G (2017) Sequential ultrasound and low temperature thermal pretreatment: process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. *Bioresour Technol* 234:178–187
- Neumann P, Barriga F, Álvarez C, González Z, Vidal G (2018) Process performance assessment of advanced anaerobic digestion of sewage sludge including sequential ultrasound – thermal (55 °C) pre-treatment. *Bioresour Technol* 262:42–51
- Neyens E, Baeyens J, Dewil R, De Heyder B (2004) Advanced sludge treatment affects extracellular polymeric substances to improve activated sludge dewatering. *J Hazard Mater* 106:83–92
- Patil PN, Gogate PR, Csoka K, Dregelyi-Kiss A, Horvath M (2016) Intensification of biogas production using pretreatment based on hydrodynamic cavitation. *Ultrason Sonochem* 30:79–86
- Piña B, Bayona JM, Christou A, Fatta-Kassinos D, Guillon E, Lambropoulou D, Michael C, Polesel F, Sayen S (2020) On the contribution of reclaimed wastewater irrigation to the potential exposure of humans to antibiotics, antibiotic resistant bacteria and antibiotic resistance genes - NEREUS COST Action ES1403 position paper. *J Environ Chem Eng* 8(1):102131

- Reyes-Contreras C, López D, Leiva AM, Domínguez C, Bayona JM, Vidal G (2019) Removal of organic micropollutants in wastewater treated by activated sewage sludge and constructed wetlands: a comparative study. *Water* 11:2515
- Reyes-Contreras C, Neumann P, Barriga F, Venegas M, Domínguez C, Bayona JM, Vidal G (2020) Organic micropollutants in sewage sludge: influence of thermal and ultrasound hydrolysis processes prior to anaerobic stabilization. *Environ Technol* 41(11):1358–1365
- Rogers HR (1996) Sources, behaviour and fate of organic contaminants during sewage treatment and in sewage sludges. *Sci Total Environ* 185:3–26
- Ruiz-Hernando M, Cabanillas E, Labanda J, Llorens J (2015) Ultrasound, thermal and alkali treatments affect extracellular polymeric substances (EPSs) and improve waste activated sludge dewatering. *Process Biochem* 50:438–446
- Suh YJ, Rousseaux P (2002) An LCA of alternative wastewater sludge treatment scenarios. *Resour Conserv Recycl* 35:191–200
- USEPA (2002) Supplemental Guidance for developing soil screening levels for Superfund sites. Office of Solid Waste and Emergency Response (OSWER), US-EPA, Washington, DC, pp 1–187. <https://nepis.epa.gov/Exe/ZyPDF.cgi/91003IJK.PDF?Dockey=91003IJK.PDF>
- Venegas M, Leiva AM, Reyes-Contreras C, Neumann P, Piña B, Vidal G (2021) Presence and fate of micropollutants during anaerobic digestion of sewage and their implications for the circular economy: a short review. *J Environ Chem Eng* 9(1):104931
- Venkatesan AK, Halden RU (2013) National inventory of perfluoroalkyl substances in archived U.S. biosolids from the 2001 EPA National Sewage Sludge Survey. *J Hazard Mater* 252–253: 413–418
- Vera I, Sáez K, Vidal G (2013) Performance of 14 full-scale sewage treatment plants: comparison between four aerobic technologies regarding effluent quality, sludge production and energy consumption. *Environ Technol* 34(15):2267–2275
- Vidal G, Navia R, Levet L, Mora ML, Diez MC (2001a) Kraft mill anaerobic effluent color enhancement by a fixed-bed adsorption system. *Biotechnol Lett* 23:861–865
- Vidal G, Videla S, Diez MC (2001b) Molecular weight distribution of *Pinus radiata* kraft mill wastewater treated by anaerobic digestion. *Bioresour Technol* 77(2):183–191
- Yoshida H, Christensen TH, Scheutz C (2013) Life cycle assessment of sewage sludge management: a review. *Waste Manag Res* 31:1083–1101
- Yu J, Nickerson A, Li Y, Fang Y, Strathmann TJ (2020) Fate of per- and polyfluoroalkyl substances (PFAS) during hydrothermal liquefaction of municipal wastewater treatment sludge. *Environ Sci Water Res Technol* 6:1388–1399



# Inoculum Optimization Strategies for Improving Performance of Anaerobic Biodigester: Current Trends and Future Perspectives

# 12

Mukesh Kumar Meghvansi, Richa Arya, N. K. Tripathi, Vijai Pal, and A. K. Goel

## Abstract

Burgeoning global population has posed several challenges before the policy-makers among which the issues concerning public health, hygiene and sanitation have been receiving greater attention in the present time. Sanitation has been included as one of the Sustainable Development Goals (SDG6) in 2030 agenda of UN Sustainable Development Goals. For the purpose of minimizing environmental pollution caused due to unscientific disposal of human waste, various off-site and on-site sanitation technologies/solutions have been developed among which anaerobic biodigesters have gained the currency owing to their several unique advantages over the other conventional strategies. Anaerobic microbial inoculum (AMI) is one of the cardinal components of anaerobic digesters. In order to reduce the startup time of an anaerobic biodigester and its sustained operation for the desired performance, it is important to make an optimal use of the inoculum. There are a multitude of digester process variables which are influenced by the inoculum source, type, concentration and substrate-to-inoculum ratio. Therefore, inoculum optimization and improvement strategies are crucial for enhancing the overall efficiency of anaerobic biodigesters. The present chapter deals with various aspects of inoculum optimization strategies to improve anaerobic biodigester performance. Through in-depth and critical analysis of the latest scientific literature, the chapter also provides future perspectives where concerted research efforts are warranted.

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**Keywords**

Inoculum · Anaerobic biodigester · Human waste · Biofilm carrier media · Attached growth systems

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## 12.1 Introduction

It is estimated that global human population has been increasing by around 1.1% per year. According to a recent report of a United Nations agency, there has been more than three times increase in population size rising from approximately 2.5 billion in 1950 to around 7.9 billion in 2021 (UN DESA 2021). This report also provides a projection that by the end of this century, the population could rise to around 11 billion. The population growth at an unprecedented pace has posed several challenges including those related to public health, hygiene and sanitation before the policy-makers globally. Considering the global average amount of 350 g of wet human faeces produced per capita per day (Wignarajah et al. 2006; Torondel 2010), the total generation of human faeces by 7.9 billion strong population is a staggering figure of 2.765 billion kg day<sup>-1</sup>. Disposal of such a large amount of human waste without proper treatment can pose a serious environmental threat and public health risk. These challenges thus underpin the requirement of urgent attention of all the stakeholders because the implications of environmental pollution due to poor sanitation are multifarious. Inadequate sanitation has been identified as a serious public health concern because it is one of the key contributory factors associated with diarrhoea leading to diseases and even deaths, particularly among the children below the age of 5 years. This problem is much severe in the third-world countries due to poor sanitation infrastructure. A study has found that maladies related with improper water, sanitation and hygiene conditions are one of the major contributors to the environmental encumbrance of diseases globally accounting for 6–7% of deaths in less developed nations (Jeuland et al. 2013). Also, Global Burden of Disease Study (2016) has highlighted the diarrhoea as the eight leading cause of deaths accounting for more than 1.6 million mortality cases out of which 26.93% diarrhoeal deaths reported in the children having age less than 5 years and about 90% of diarrhoeal deaths were from South Asia and sub-Saharan Africa only (GBD 2016). Therefore, it has also been recognized that failure to meet the targets of SDG6 may jeopardize the entire 2030 agenda for sustainable development because of its integrated nature (United Nations 2018).

In a bid to mitigate environmental pollution caused due to unscientific disposal of human waste, in the past few decades, the concerted efforts have been made by the sanitation researchers yielding a variety of off-site and on-site sanitation technologies/solutions ranging from conventional latrines to advanced treatment systems which are suitable for different conditions. Human waste being rich in high-strength organic material is suitable for anaerobic digestion resulting into methane production and has been considered as a viable option for renewable energy purposes also (Lu et al. 2017). Therefore, among various technological options

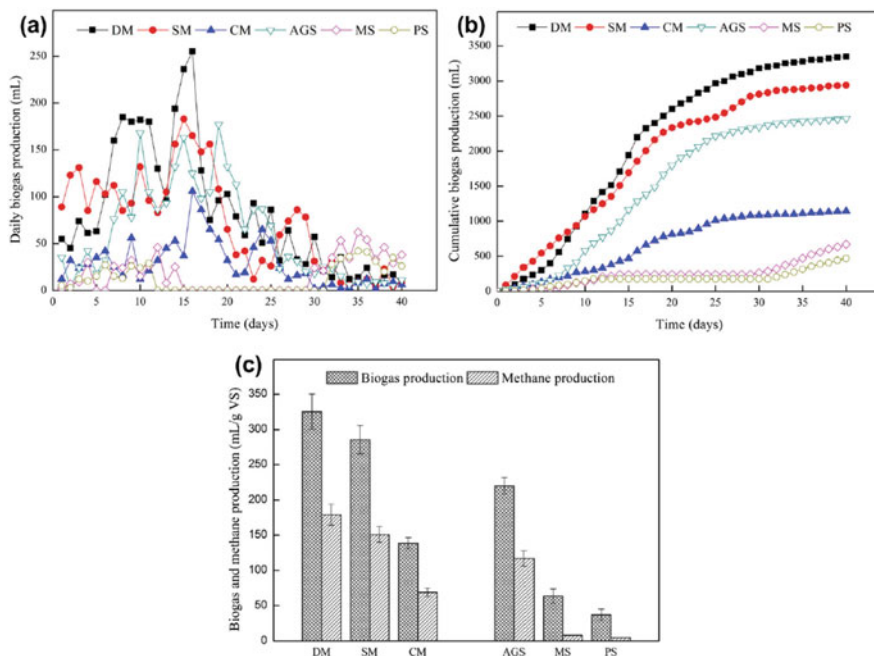
available, anaerobic biodigester technology has shown a considerable potential for treatment of human faecal matter with dual advantages of minimizing environmental pollution and simultaneous production of methane (Meghvansi et al. 2018). This technology works on the principles of anaerobic biodegradation and equalization of complex organic substrate in the absence of oxygen with the help of microbial consortium leading to production of biogas. Biogas comprises of methane (~60%) and carbon dioxide (~35%). The various sequential steps of methane production include hydrolysis, acidogenesis, acetogenesis and methanogenesis (Charles et al. 2009) which are mediated by different microorganisms.

Various studies conducted worldwide have acknowledged that inoculum plays a pivotal role not only for the biodigester startup by poisoning the populations of methanogens and syntrobacter rendering syntrophic metabolism plausible from thermodynamics viewpoint (Mir et al. 2016) but for the desired sustained operational performance also. Several researchers have investigated the role of inoculum from diverse perspectives for the purpose of optimal biodigester performance (Xing et al. 2020; Ferraro et al. 2020). Inoculum origin has been found to influence the initial microbial community structure and function which ultimately tends to have a significant effect on the biodigester performance (Suksong et al. 2019; Obata et al. 2020). Xing et al. (2020) while studying the effect of long-term adaptation on the appropriate food-waste-to-cow-manure ratio and substrate-to-inoculum ratio ( $S/I$ ) in anaerobic co-digestion highlighted the necessity of periodical optimization of  $S/I$  for enhancing the co-digestion efficiency for the purpose of production of biogas. Other studies reported that the quantity of inoculum and percolate recirculation strategies can be useful for enhancing digester performance (Li et al. 2018; Rico et al. 2020). It has also been postulated that when the relative quantity of inoculum is enhanced, the digestion process becomes more stable, thereby leading to the achievement of higher methane yields. However, this strategy has implications in terms of the cost due to the increase in the size of digester (Di Maria et al. 2012). Similarly, when the digester is inoculated with solid digestate, it reduces overall volume capacity of the digester (Qian et al. 2017). These perplexing factors necessitate more in-depth investigations on the exploration of inoculum optimization for the enhanced digester performance. The scope of present chapter therefore includes critical discussion on various inoculum optimization strategies concerning inoculum source and type, substrate-to-inoculum ( $S/I$ ) ratio, inoculum enrichment using various types of additives, inoculum encapsulation and the role of attached growth systems/biofilm carrier media with a view to achieve augmented performance of anaerobic biodigester. Through critical analysis of recently published literature, we further attempt to identify gaps in existing knowledge and provide directions for the future research in the realm of optimal usage of inoculum taking into consideration various aspects associated with it.

## 12.2 Inoculum Source and Type

Successful startup and subsequent operation of anaerobic digester necessitate a highly effective inoculum having well-balanced microbial consortium which gets acclimatized over a period of time. Therefore, a good inoculum source should be capable of providing better anaerobic biodegradability and process stability with minimal lag phase. Due to performance variability, diverse inoculum sources and types have been explored suiting digestion of specific substrates. Hidalgo and Martín-Marroquín (2014) evaluated two different inocula obtained from an industrial digester fed with organic waste from hospitality sector (called HORECA) and from municipal wastewater treatment plant (mWWTP) for the treatment of refuge vegetable oils. The experimental results of this study suggested that HORECA inoculum exhibited greater methanogenic activity as compared to mWWTP inoculum. Furthermore, the resistance to waste vegetable oil (WVO) residue toxicity was noticed higher for the HORECA inoculum than for the mWWTP inoculum. In another empirical study comparing the performance of six different digestates (three types of manures from dairy, swine/piggery and poultry sources and three types of sludge of municipal, anaerobic granular and paper mill origins) as inoculum in batch reactors for anaerobic digestion of rice straw, it was observed that greater biogas production and enzymatic activities leading to better lignocellulose degradation could be achieved in the reactors which were inoculated with the digested manures (Gu et al. 2014; Fig. 12.1). Similarly, a more recent study which investigated the influence of inoculum obtained from different sources such as natural wetland, lab-level and full-capacity anaerobic reactors on the anaerobic digestion of wheat straw noted that the digesters seeded with inoculum from full-scale reactors performed better as evident from the higher methane production and faster startup in the setup (Li et al. 2019). In these digesters, acetoclastic methanogens, including *Methanosaeta* and *Methanosarcina*, were found to be dominant microorganisms, while the hydrogenotrophic methanogenic bacteria were dominant in other reactors implying that inoculum origin and process conditions could be the important factors which govern the microbial communities in the highly effective reactors (Li et al. 2019). Interestingly, some recent studies have explored the local resources as source of inoculum in the absence of availability of mature digestate from full-scale anaerobic digester. For instance, Obata et al. (2020) examined the performance of thalassic sediments as inoculum for anaerobic degradation of three marine macroalgae (*Laminaria digitata*, *Fucus serratus* and *Saccharina latissima*) and non-marine biomass. The study findings revealed that maximum methane yield was recorded in both *L. digitata* and *S. latissima* cultures when seeded with thalassic sediment. When digested sludge was used as inoculum, poor digestion was reported in case of *F. serratus*. This study suggests that marine sediment may offer an effective alternative as an inoculum for anaerobic biodegradation of selected marine biomass. Another study treating dairy manure and waste-activated sludge in an anaerobic digester with a granular form of the sludge as inoculum concluded that granular biomass out-competed suspended biomass as inoculum in the biomethane potential (BMP) assay as evident from the overall



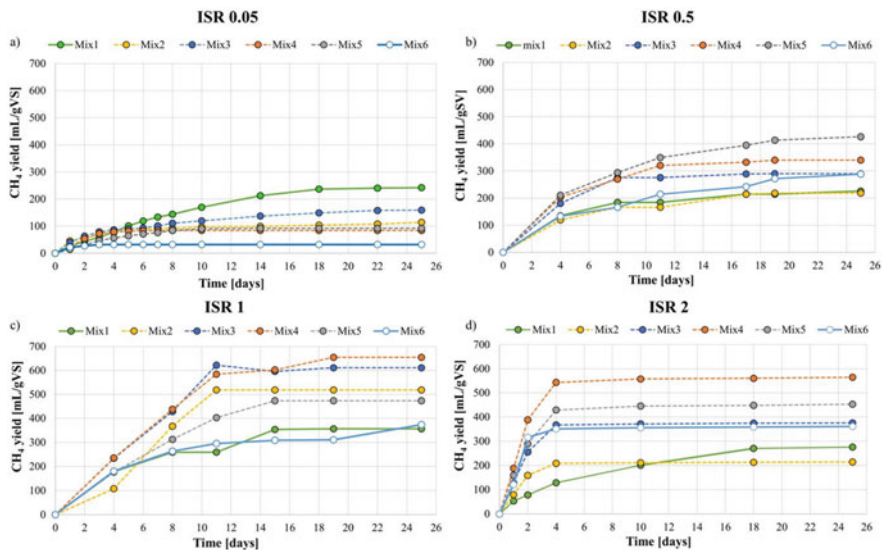


**Fig. 12.1** (a–c) Comparative performance of six different inocula for anaerobic digestion of rice straw at I/S ratio 0.5 (DM, digested dairy manure; SM, digested swine manure; CM, digested chicken manure; MS, digested municipal sludge; AGS, anaerobic granular sludge; PS, paper mill sludge). (Figure source: Gu et al. 2014; reproduced with permission from Elsevier, Ref. 501726364)

higher BMP and faster degradation kinetics (Posmanik et al. 2020). These observations highlight that the inoculum source and types should be evaluated from all possible scenarios so as to identify the most suitable inoculum for the anaerobic degradation of a given substrate.

### 12.3 Substrate-to-Inoculum (S/I) Ratio

Previous literature has demonstrated that the substrate-to-inoculum ratio is one of the major parameters that can govern the overall performance of anaerobic biogas production through change in occurrence and duration of lag phase, methanogenesis, VS/COD reduction as well as susceptibility of the microbial biomass towards the inhibitory effects (Raposo et al. 2011; Xu et al. 2013). Depending upon the type and complexity of the substrate, different S/I ratios have been extensively investigated and proposed for the field use. Fagbohunge et al. (2015) studied the effect of different S/I ratios ranging from 0.5 to 4 on the rate and magnitude of methane production in an anaerobic digester treating human faecal material. The study results revealed that the greatest amount of methane production (i.e. as high as  $254.4 \pm 12.6 \text{ mL CH}_4 \text{ g VS}^{-1}$ ) added and pathogen elimination with a value of



**Fig. 12.2** Cumulative methane yield curves of OPMSW and SS at (a) ISR equal to 0.05, (b) ISR equal to 0.5, (c) ISR equal to 1, and (d) ISR equal to 2. (Source/courtesy: Corsino et al. 2021; reproduced with permission of copyright owner)

$2.7 \times 10^4 \pm 40$  CFU mL<sup>-1</sup> and  $2.5 \times 10^3 \pm 0.5$  CFU mL<sup>-1</sup>, respectively, for *E. coli* and faecal coliform bacteria) was obtained at S/I ratio of 0.5. Corsino et al. (2021) examined the effect of the inoculum-to-substrate ratio (ISR) and the mixture ratio between organic part of municipal solid waste (OPMSW) and sewage sludge (SS) on the total methane production potential attainable from the anaerobic co-digestion under mesophilic milieu. The findings of this study revealed that the ISR and the ratio OPMSW/SS in the co-digestion mixture had a significant impact on the methane yield, the production rate and the synergistic effect produced during the biodegradation process with ISR1 leading to production of the highest methane yield (655 mL g VS<sup>-1</sup>) and synergistic effects (+40%) and ISR2 resulting in the highest methane production rate (207 mL g VS<sup>-1</sup> day<sup>-1</sup>) (Fig. 12.2). Dastyar et al. (2021) while carrying out experimental interventions on high solid anaerobic digestion of municipal waste at varying S/I ratios (1–3) observed that although S/I ratio of 1 yielded maximum methane recovery yet from practical considerations (i.e. clogging-related issues, etc.), it was more prudent to have S/I ratio of 2 for such systems as it enabled around 45% more organic processing capacity in terms of volatile solids. In view of this, it is important to highlight that on the one hand, higher amount of inoculum may speed up the startup process leading to an increase in specific methane production rate by providing a greater number of methanogenic count initially, and on the other hand, it needs more space, thereby limiting the availability of overall working volume in terms of substrate uptake. Therefore, it is paramount to carefully calibrate the requirement of anaerobic digester treating a specific substrate with respect to S/I ratio.

## 12.4 Inoculum Enrichment Through the Use of Additives

In recent years, the research interest has been renewed in inoculum enrichment strategies for enhancing the performance of biodigester with a focus on improving key functional roles of microbial communities through bioaugmentation. Some studies have highlighted the utility of direct addition of specific microbial cultures aimed at increasing the hydrolysis rate and extent (Wei et al. 2016; Tsapekos et al. 2017), while others have focused on the use of enzyme supplementation (Parawira 2012). For instance, a study demonstrated that the inoculation of *Coprothermobacter proteolyticus* could improve the hydrolysis and fermentation of the left-over proteins and carbohydrates in the effluent of a digester using waste-activated sludge (Lü et al. 2014). It has been established that the startup phase of anaerobic biodigester is very critical because the probability of imbalance of microbial community at this stage is very high. Therefore, the efforts have been made to attain process stability at the startup phase of the anaerobic digestion through bioaugmentation. For instance, the DRDO-biodigester technology developed at Defence Research and Development Establishment, Gwalior (India), which is presently being used in Indian Railway passenger-coaches for on-board treatment of human waste as well as in individual households uses a specially developed anaerobic microbial inoculum (AMI) that was developed through enrichment of bacteria obtained from various biogas plants functioning in hilly and low-temperature areas of India (Singh and Kamboj 2018; Kamboj et al. 2020). Lins et al. (2014) noted the importance of using inoculum rich in *Methanosarcina* for enhancing the overall process in acetate-dominant setup, especially in the startup phase. Moreover, bioaugmentation strategy has been successful in some of the cases pertaining to biodigester troubleshooting. It is well-established that ammonia inhibition is among the most frequently encountered problems linked with digester failure because of a strong negative influence of ammonia on acetoclastic methanogens. Nevertheless, recovery of anaerobic digestion process has successfully been achieved through the use of specific bacteria. In a continuously stirred tank reactor (CSTR) which operated under compromised steady state at considerably high ammonia concentration (i.e. 5000 mg of ammoniacal nitrogen L<sup>-1</sup>), biofortification with *Methanoculleus bourgensis* could provide 31% increase in methane yield (Fotidis et al. 2014). This trend was further correlated with microbial community analysis, in which a fivefold enhancement in the abundance of *Methanoculleus* bacteria was noticed in the inoculated reactors.

Several studies have investigated the role of addition of nutrients (reviewed by Romero-Güiza et al. 2016) for performance enhancement and stable operation of anaerobic digester. It is well-established that macro-nutrients not only provide the required buffering in the digester but also are indispensable components of microbial biomass. Likewise, micronutrients such as molybdenum (Mo), nickel (Ni), iron (Fe) and cobalt (Co) serve as critical cofactors for various enzymatic reactions associated with biochemical pathways of anaerobic digestion (Table 12.1). A study conducted under mesophilic milieu using digested maize silage as substrate observed that Ni and Co deficiency (i.e. <0.1 mg Ni<sup>2+</sup> kg<sup>-1</sup> and <0.02 mg Co<sup>2+</sup> kg<sup>-1</sup> on wet basis) had adversely affected the process stability of digester at an organic

**Table 12.1** Commonly used micronutrients in anaerobic digester and their role in methanogenesis

Micronutrient	Role in biochemistry of methanogenesis	References
Co	Co is present in cobalamides which are intermediates between methyl-H4MPT and coenzyme M. Cobalamides act as methyl carriers in methanogenesis from methylated compounds	DiMarco et al. (1990)
	cob(I)amide prosthetic group is present in methyl-H4MPT:CoM-SH methyltransferase which is considered important for the enzymatic function	Hedderich and Whitman (2006)
Fe	Fe is associated with various enzymes such as formylmethanofuran dehydrogenase, carbon monoxide dehydrogenase/acetyl-CoA synthase (CODH/ACS) and hydrogenases. CODH/ACS participate in Wood-Ljungdahl pathway used by methanogens	Worm et al. (2009)
Mo	Mo is known to be associated with format-dehydrogenase and formylmethanofuran dehydrogenase enzymes	Worm et al. (2009)
Ni	Ni is also known to be associated with CODH, methylreductase and hydrogenases	Ma et al. (2009)
Se	Se is also known to be associated with format-dehydrogenase, formyl-MF-dehydrogenase as well as CODH/ACS	Worm et al. (2009)

loading rate of  $2.6 \text{ g TS L}^{-1} \text{ day}^{-1}$  as evident from accumulation of VFA which was reversed when Co and Ni concentrations were increased to  $0.6 \text{ mg kg}^{-1}$  and  $0.05 \text{ mg kg}^{-1}$ , respectively (Pobeheim et al. 2011). On the contrary, a more recent study concluded that dosing of Co (at  $0.012\text{--}0.302 \text{ mg L}^{-1}$ ) and Ni (at  $0.066\text{--}0.356\text{--} \text{mg L}^{-1}$ ) did not exhibit any statistically significant influence (either increase or decrease) on methane produced from the anaerobic degradation of domestic sewage in batch reactors tested with a Plackett-Burman experimental design (Alves da Silva et al. 2021). In addition, the dosing of micronutrients in digesters has been reported to influence microbial community dynamics, thereby changing the metabolic pathways adopted by the microorganisms. For instance, a study carried out by Banks et al. (2012) with OPMSW in anaerobic biodigester running at  $3\text{--}5 \text{ g VS L}^{-1} \text{ day}^{-1}$  supplemented with Se and Co micronutrients observed the acetotrophic pathway to be the primary methanogenic pathway instead of acetoclastic methanogenesis which was attributed to differences in trace element requirements. It is also important here to emphasize that excessive concentration of macro- and micronutrients may be detrimental to anaerobic digestion. Therefore, optimal dosing of the nutrients as additives is crucial for achieving the desired results. Moreover, the digester temperature is also reported to govern the nutrient requirements. As a general trend, higher micronutrient requirements have been noticed for thermophilic conditions as compared to mesophilic conditions owing to their implications on nutrient bioavailability. Zitomer et al. (2008) while investigating the effect of Ni, Co and Fe ( $25 \text{ mg L}^{-1}$ ) addition on mesophilic and thermophilic groups in five full-scale digesters treating sewage sludge found considerable differences in the extent of increase in uptake rates of propionate and acetate

following micronutrient addition either individually or all three micronutrients along with propionate utilization rates being more frequently augmented by micronutrient addition as compared to acetate, particularly in thermophilic milieu. The foregoing discussion amply suggests the potential of bioaugmentation for enhanced digester performance and troubleshooting; however, there are still many concerns which need to be addressed for implementation of these strategies in full-scale field biodigesters. These concerns include economic considerations also apart from technical considerations as because most of the data generated on these aspects is from lab-scale digesters and the interactive effects of nutrient supplementation in inoculum on process variable may be very complex. Optimal dose, mode of use and efficacy issues are very important in this regard which need to be investigated adequately. Furthermore, it is a challenging task to monitor microbial community behaviour in a digester under the influence of bioaugmentation.

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## 12.5 Inoculum Encapsulation

One of the major challenges associated with anaerobic digester startup is proper acclimatization of microbial consortium during the initial phase when it may encounter diverse types of shocks/stress conditions. To mitigate this, extensive research endeavours have been made to use encapsulated bacteria. This strategy has several advantages. It renders the bacteria comparatively less prone to biomass washout during the startup phase. In addition, the methanogens get minimal exposure to oxygen which is otherwise toxic to them. Moreover, immobilization/encapsulation strategy is more favourable for the conversion of substrate to intermediates and their subsequent transfer for further digestion owing to aggregation of various microbial groups in a relatively smaller area (Baloch 2011). Using one-step liquid-droplet-forming technique, Youngsukkasem et al. (2012) successfully encapsulated the methane-producing bacteria in spherical capsules of an average diameter of 1.3 mm with membrane thickness of 0.2 mm. These capsules were prepared from alginate, calcium ions and carboxymethylcellulose. Further they were filled in PVDF sachets which were used subsequently in the anaerobic digestion process for holding the bacteria. The results indicated successful biogas production in digestion experiment and its diffusion through the membrane indicating the potential utility of encapsulation strategy for inoculum improvement. Another study evaluated the application of reverse membrane bioreactor to retain the cells using PVDF membrane. In this setup, microbial cells are enclosed in PVDF membrane which remains immersed into the bioreactor facilitating the diffusion of a substrate through the membrane and of metabolic products back to the medium (Youngsukkasem et al. 2015). Using this strategy, biomethanation of syngas could be successfully achieved implying its potential application in anaerobic digester. Similarly, Zhu et al. (2018) carried out a detailed investigation on selective encapsulation of hydrogen-producing biomass in alginate-based polymer gel in order to attain high-rate recovery of hydrogen from anaerobic digester-treating wastewater. These researchers also evaluated various aspects such as the effect of cross-linking agents, composite

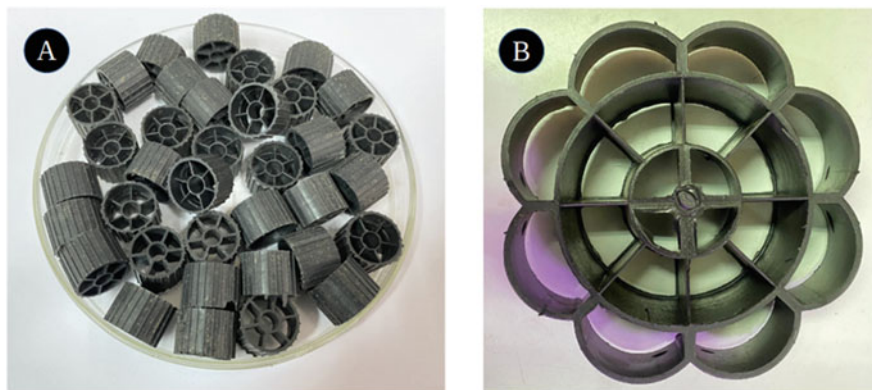


**Fig. 12.3** Algininate beads prepared from anaerobic microbial inoculum

coating on the beads and multiple layering using different materials on the differential ability to retain the encapsulated biomass. This study clearly supported the idea that encapsulation strategy holds an immense potential for use in anaerobic digestion systems. Figure 12.3 shows the algininate beads prepared from anaerobic microbial inoculum which are being investigated by the authors of this chapter. Nevertheless, it is also evident that encapsulation process and the performance of encapsulated inoculum in digester are influenced by several factors, thereby necessitating development of an optimized process for a given treatment system using various substrates.

## 12.6 Attached Growth Systems

In the attached growth system, the microorganisms of the inoculum are immobilized onto inert carrier with solid surface/supporting media where they form dense colonies over a period of time. The microorganisms produce extracellular polymeric compounds which help them get stabilized there. It has been observed that the number of attached bacteria could exceed as high as 200-fold in sewage effluent (McLean et al. 1994), thereby digesting the organic substrate more efficiently as compared to suspended microorganisms (Cohen 2001). By virtue of this, the anaerobic digesters with attached growth systems are capable of handling greater organic load with less hydraulic retention time as compared to the suspended growth systems (Polprasert 1989). Moreover, the suspended growth systems are known to produce sparse sludge which favours the growth of filamentous microorganisms causing the problem of bulking and foaming in the treatment systems (Wanner 1994). On the contrary, sludge generated by attached growth system is more densified which



**Fig. 12.4** Biofilm carrier media: (a) Kaldnes type (~21 mm dia). (b) Flower-shaped media (~180 mm dia)

minimizes settling issues and obviates foaming and bulking issues (Droste 1997). Harnessing these attributes, various researchers have evaluated a diverse range of attached growth systems based on artificial solid materials such as polypropylene, polyurethane and polyethylene. PVC immobilization matrix attached to the partition walls of fermentation tank (Biodigester) has been provided in DRDO-biodigester technology used for human waste digestion as well as in individual households (Singh and Kamboj 2018; Kamboj et al. 2020). Besides, natural solid materials like various plant parts, clay, zeolites, volcanic rocks, ceramics and activated carbon have also been investigated (Fig. 12.4). For instance, Mshandete et al. (2008) evaluated biofilm carrier material consisting of sisal fibre waste, pumice stone and porous glass beads for anaerobic degradation of waste leachate from sisal leaf. The results reported the maximum COD elimination of 80–93% at an organic loading range of 2.4–25 g COD L<sup>-1</sup> day<sup>-1</sup> for the digester having sisal fibre waste as carrier material. In a bid to decipher mechanistic aspects of anaerobic microbial population immobilized on zeolite through an extensive use of advanced microscopic techniques, Weiß et al. (2013) convincingly established that activated zeolites could serve as natural biofilm carrier material with immense potential to stabilize and augment the biogas production process from recalcitrant plant biomass through anaerobic digestion. More recently, Liu et al. (2017) evaluated that performance of four different types of fibrous biofilm carriers (i.e. polyester, polyamide, polypropylene and polyurethane) in an anaerobic degradation system with corn straw. The results revealed significant enhancement in biogas as well as methane production with addition of biofilm carriers in the system. Overall, the use of polypropylene fibre as biofilm carrier could provide maximum biogas and methane production which were 44.80% and 49.84% greater, respectively, as compared to the control. This strategy also yielded the greatest removal efficiency in terms of total solid, volatile solid and COD removal (Liu et al. 2017). The important considerations for selection of suitable biofilm carrier include bioadsorption properties,

biocompatibility, porosity and roughness of the material surface, density, specific surface area, wettability, resistance to degradation and high mechanical strength which need to be optimized for a desired application in anaerobic biodigester. These factors contribute to the efficiency of biofilm formation leading to more effective degradation. Another crucial point to be taken into account is the fact that microbial colony is negatively charged, thus necessitating selection of biofilm carrier material having cationic solid surface. It is well-known that pure polyethylene and polypropylene have negatively charged surface which hampers the adhesion of bacteria on their surface owing to repulsive force. Therefore many studies have focused on modification of surface properties to overcome this limitation with an emphasis on reduction of water contact angle. Shen et al. (2007) through modification of the surface of polysulfone hollow fibre membrane employing grafting hydrophilic acrylamide chain were able to lower the water contact angle from  $70^\circ$  to  $48^\circ$  which resulted into increased surface biomass and in turn greater COD removal efficiency indicating that this surface modification strategy could also be useful in anaerobic digester. Similarly, Mao et al. (2017) observed that the surface of modified HDPE carriers with two different types of positively charged polymers (i.e. polyquaternium-10 and cationic polyacrylamides) had considerably less water contact angle ( $58.8^\circ$ ), as compared to that of unmodified HDPE ( $94.3^\circ$ ) which resulted into enhanced biofilm growth. Apart from this, another strategy that has gained the currency is to harness the ability of magnetic field to change the permeability of bacterial cell membrane leading to enhanced metabolism and growth. In addition, magnetic biofilm carriers can prevent biomass washout in CSTRs which contribute to enhanced solid retention time and reduced hydraulic retention time. A recent study noticed that a magnetic biofilm carrier based on carbon fibre loaded with  $\text{Fe}_3\text{O}_4$  was able to produce significantly greater (i.e. more than threefold) dry weight of biofilm per unit as compared to nonmagnetic carbon fibre. It was also observed that under the influence of magnetic biofilm carrier, the wastewater treatment efficiency enhanced considerably (Xu and Jiang 2018). Some studies have also noted that the biofilm carriers can act as redox mediators that can be oxidized and reduced reversibly. As the redox mediators can speed up reactions by decreasing the activation energy of the overall reaction (van der Zee and Cervantes 2009), they have been explored for degrading recalcitrant compounds in wastewater treatment plants (Lu et al. 2010).

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## 12.7 Concluding Remarks and Future Perspectives

Appropriate disposal of human waste has become inevitable in the present scenario from the sanitation and hygiene viewpoints. Anaerobic biodigestion is one of the most widely used strategies for the efficient degradation of human waste. The selection of suitable inoculum for anaerobic digestion of human waste is one of the critical prerequisites for proper startup and subsequent attainment of the desired performance of the digester. Several strategies have been explored by various researchers across the globe for the purpose of inoculum optimization which have



been analysed comprehensively in this chapter. As discussed in this chapter, inoculum source and type can influence digester performance. There have been reported instances where even the conventional matured activated sludge obtained from running waste treatment plant was not able to produce the desired results indicating that more research work is required to identify the factors responsible for such behaviour of activated sludge. Critical traversal of published literature with respect to *S/I* ratio earmarks the requirement not only for the optimization of *S/I* ratio but also for its monitoring for the sustained performance. In addition, varying *S/I* ratio has direct implication on the working volume of the biodigester which needs to be optimized with greater precision. Various inoculum enrichment strategies have convincingly established that they hold immense potential for performance enhancement of anaerobic digester. Nevertheless, the variability reported in the efficiency of these studies allows us to suggest that microbial community dynamics in anaerobic digestion may be far more complex than perceived which warrants development of appropriate standard operating procedures for the field deployment of such strategies. Inoculum encapsulation is another promising approach which primarily facilitates the anaerobic bacteria to withstand the shock loads. More research work is required in this area particularly for development of suitable recipe for inoculum encapsulation taking into account various aspects such as polymerization, bacterial entrapment and diffusion. Further, the cost-effective protocols need to be developed for large-scale production of encapsulated inoculum and its storage at the commercial scale. In the last few decades, considerable progress has been made in the realm of utilization of attached growth systems for increasing solid retention time of the anaerobic digester. As discussed in this chapter, there are a lot of challenges associated with selection of suitable biocompatible material for the attached growth systems. In this regard, the recent work conducted in the direction of modification of surface properties of the biofilm carrier media holds immense possibilities which needs to be validated at larger-scale operations. Nevertheless, the biofilm formation is a complex process and is influenced by several factors. Therefore, apart from physico-chemical attributes of the supporting media, the behaviour of the biofilm and its overall contribution for the enhanced digestion efficiency should be investigated in holistic and integrated manner in order to derive maximum benefits from such systems.

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## References

- Alves da Silva J, Braga AFM, Feroso FG, Zaiat M, Silva GHR (2021) Evaluation of the influence of trace metals on methane production from domestic sewage, using the Plackett-Burman experimental design. *J Environ Manag* 294:113002
- Baloch MI (2011) Methanogenic granular sludge as a seed in an anaerobic baffled reactor. *Water Environ J* 25:171–180

- Banks CJ, Zhang Y, Jiang Y, Heaven S (2012) Trace element requirements for stable food waste digestion at elevated ammonia concentrations. *Bioresour Technol* 104:127–135
- Charles W, Walker L, Cord-Ruwisch R (2009) Effect of pre-aeration and inoculum on the start-up of batch thermophilic anaerobic digestion of municipal solid waste. *Bioresour Technol* 100: 2329–2335
- Cohen Y (2001) Biofiltration – the treatment of fluids by microorganisms immobilized into the filter bedding material: a review. *Bioresour Technol* 77:257–274
- Corsino SF, Torregrossa M, Viviani G (2021) Biomethane production from anaerobic co-digestion of selected organic fraction of municipal solid waste (OFMSW) with sewage sludge: effect of the inoculum to substrate ratio (ISR) and mixture composition on process performances. *Int J Environ Res Public Health* 18:1304
- Dastyar W, Mohammad MS, Dhadwal M, Dhar BR (2021) High-solids anaerobic digestion of organic fraction of municipal solid waste: effects of feedstock to inoculum ratio and percolate recirculation time. *Bioresour Technol* 337:125335
- Di Maria F, Sordi A, Micale C (2012) Optimization of solid state anaerobic digestion by inoculum recirculation: the case of an existing mechanical bio- logical treatment plant. *Appl Energy* 97: 462–469
- DiMarco AA, Bobik TA, Wolfe RS (1990) Unusual coenzymes of methanogenesis. *Annu Rev Biochem* 59:355–394
- Droste RL (1997) Theory and practice of water and wastewater treatment. Wiley, New York, NY
- Fagbohunge MO, Herbert BMJ, Li H, Ricketts L, Semple KT (2015) The effect of substrate to inoculum ratios on the anaerobic digestion of human faecal material. *Environ Technol Innov* 3: 121–129
- Ferraro A, Massini G, Mazzurco Miritana V, Rosa S, Signorini A, Fabbicino M (2020) A novel enrichment approach for anaerobic digestion of lignocellulosic biomass: process performance enhancement through an inoculum habitat selection. *Bioresour Technol* 313:123703
- Fotidis IA, Wang H, Fiedel N, Gang L, Karakashev D, Angelidaki I (2014) Bioaugmentation as a solution to increase methane production from ammonia- rich substrate. *Environ Sci Technol* 48:7669–7676
- GBD (2016) GBD 2015 mortality and causes of death collaborators. Global, regional, and national life expectancy, all-cause mortality, and cause-specific mortality for 249 causes of death, 1980–2015: a systematic analysis for the global burden of disease study 2015. *Lancet* 8 388(10053): 1459–1544. [https://doi.org/10.1016/S0140-6736\(16\)31012-1](https://doi.org/10.1016/S0140-6736(16)31012-1)
- Gu Y, Chen X, Liu Z, Zhou X, Zhang Y (2014) Effect of inoculum sources on the anaerobic digestion of rice straw. *Bioresour Technol* 158:149–155
- Hedderich R, Whitman WB (2006) Physiology and biochemistry of the methane-producing archaea. In: Dworkin M, Falkow S, Rosenberg E, Schleifer KH, Stackebrandt E (eds) *Prokaryotes*, vol 2, 3rd edn. Springer, New York, NY, pp 1050–1079
- Hidalgo D, Martín-Marroquín JM (2014) Effects of inoculum source and co-digestion strategies on anaerobic digestion of residues generated in the treatment of waste vegetable oils. *J Environ Manag* 142:17–22
- Jeuland MA, Fuente DE, Ozdemir S, Allaire MC, Whittington D (2013) The long-term dynamics of mortality benefits from improved water and sanitation in less developed countries. *PLoS One* 8(10):e74804
- Kamboj DV, Singh L, Chauhan RS, Meghvansi MK (2020) *Biodigester: a new horizon for sanitation technology*. New Delhi Publishers, New Delhi. ISBN:9789388879354
- Li W, Loh KC, Zhang J, Tong YW, Dai Y (2018) Two-stage anaerobic digestion of food waste and horticultural waste in high-solid system. *Appl Energy* 209:400–408
- Li K, Yun J, Zhang H, Yu Z (2019) Full-scale anaerobic reactor samples would be more suitable than lab-scale anaerobic reactor and natural samples to inoculate the wheat straw batch anaerobic digesters. *Bioresour Technol* 293:122040

- Lins P, Reitschuler C, Illmer P (2014) *Methanosarcina* spp., the key to relieve the start-up of a thermophilic anaerobic digestion suffering from high acetic acid loads. *Bioresour Technol* 152: 347–354
- Liu Y, Zhu Y, Jia H, Yong X, Zhang L, Zhou J et al (2017) Effects of different biofilm carriers on biogas production during anaerobic digestion of corn straw. *Bioresour Technol* 244:445–451
- Lu H, Zhou J, Wang J, Si W, Teng H, Liu G (2010) Enhanced biodecolorization of azo dyes by anthraquinone-2-sulfonate immobilized covalently in polyurethane foam. *Bioresour Technol* 101:7196–7199
- Lü F, Li T, Wang T, Shao L, He P (2014) Improvement of sludge digestate biodegradability by thermophilic bioaugmentation. *Appl Microbiol Biotechnol* 98:969–977
- Lu JW, Zhang JR, Zhu ZB, Zhang YH, Zhao Y, Li RR, Watson J, Li BM, Liu ZD (2017) Simultaneous production of biocrude oil and recovery of nutrients and metals from human feces via hydrothermal liquefaction. *Energy Convers Manag* 134:340–346
- Ma J, Mungoni LJ, Verstraete W, Carballa M (2009) Maximum removal rate of propionic acid as a sole carbon source in UASB reactors and the importance of the macro- and micro-nutrients stimulation. *Bioresour Technol* 100:3477–3482
- Mao Y, Quan X, Zhao H, Zhang Y, Chen S, Liu T et al (2017) Accelerated startup of moving bed biofilm process with novel electrophilic suspended biofilm carriers. *Chem Eng J* 315:364–372
- McLean RJC, Caldwell DE, Costerton JW (1994) Biofilms, naturally occurring communities of immobilized cells. In: Veliky IA, McLean RJC (Eds), *Immobilized biosystems*. Chapman & Hall, London
- Meghvansi MK, Kumar P, Vasudevan V, Tomar A, Kamboj DV, Singh L (2018) Biodigester technology for effective and ecofriendly decomposition of nightsoil. In: Varjani SJ et al (eds) *Waste bioremediation, energy, environment and sustainability*. Springer Nature, Singapore, pp 353–372
- Mir MA, Hussain A, Verma C (2016) Design considerations and operational performance of anaerobic digester: a review. *Cogent Eng* 3:1181696
- Mshandete A, Björnsson L, Kivaisi A, Rubindamayugi M, Mattiasson B (2008) Performance of biofilm carriers in anaerobic digestion of sisal leaf waste leachate. *Electron J Biotechnol* 11:93–100
- Obata O, Ditchfield A, Hatton A, Akunna J (2020) Investigating the impact of inoculum source on anaerobic digestion of various species of marine macroalgae. *Algal Res* 46:101803
- Parawira W (2012) Enzyme research and applications in biotechnological intensification of biogas production. *Crit Rev Biotechnol* 32:172–186
- Pobeheim H, Munk B, Lindorfer H, Guebitz GM (2011) Impact of nickel and cobalt on biogas production and process stability during semi-continuous anaerobic fermentation of a model substrate for maize silage. *Water Res* 45:781–787
- Polprasert C (1989) *Organic waste recycling*. Wiley, New York, NY
- Posmanik R, Kim AH, Labatut RA, Usack JG, Angenent LT (2020) Granular sludge is a preferable inoculum for the biochemical methane potential assay for two complex substrates. *Bioresour Technol* 309:123359
- Qian M, Zhang Y, Li R, Nelles M, Stinner W, Li Y (2017) Effects of percolate recirculation on dry anaerobic Co-digestion of organic fraction of municipal solid waste and corn straw. *Energy Fuel* 31:12183–12191
- Raposo F, Fernández-Cegrí V, De la Rubia M et al (2011) Biochemical methane potential (BMP) of solid organic substrates: evaluation of anaerobic biodegradability using data from an international interlaboratory study. *J Chem Technol Biotechnol* 86(8):1088–1098
- Rico C, Montes JA, Lobo A (2020) Dry batch anaerobic digestion of food waste in a box-type reactor system: inoculum preparation and reactor performance. *J Clean Prod* 251:119751
- Romero-Güiza MS et al (2016) The role of additives on anaerobic digestion: a review. *Renew Sustain Energy Rev* 58:1486–1499

- Shen Y-j, Wu G-X, Fan Y-b, Zhong H, Wu L-l, Zhang S-l et al (2007) Performances of biological aerated filter employing hollow fiber membrane segments of surface-improved poly (sulfone) as biofilm carriers. *J Environ Sci* 19:811–817
- Singh L, Kamboj DV (2018) Biodigester: an innovative technology for on-board disposal of human waste in Indian Railways. Daya Publishing House, New Delhi, India. ISBN: 9387057534
- Suksong W, Mamimin C, Prasertsan P, Kongjan P, O-Thong S (2019) Effect of inoculum types and microbial community on thermophilic and mesophilic solid-state anaerobic digestion of empty fruit bunches for biogas production. *Ind Crop Prod* 133:193–202
- Torondel B (2010) Sanitation ventures literature review: on-site sanitation waste characteristics. London School of Hygiene and Tropical Medicine Publications, London
- Tsapekos P, Kougias PG, Vasileiou SA, Treu L, Campanaro S, Lyberatos G, Angelidaki I (2017) Bioaugmentation with hydrolytic microbes to improve the anaerobic biodegradability of lignocellulosic agricultural residues. *Bioresour Technol* 234:350–359
- UN DESA (2021) United Nations Department of Economic and Social Affairs, Population Division. Global Population Growth and Sustainable Development. UN DESA/POP/2021/TR/NO. 2
- United Nations (2018) Sustainable development goal 6 synthesis report 2018 on water and sanitation. UN, New York, NY. ISBN: 978-92-1-101370-2
- Wanner O (1994) Activated sludge bulking and foaming control, a Technomic publication. Taylor and Francis, New York, NY
- Wei Y-Q, Long R-J, Yang H, Yang H-J, Shen X-H, Shi R-F, Wang Z-Y, Du J-G, Qi X-J, Ye Q-H (2016) Fiber degradation potential of natural cocultures of *Neocallimastix frontalis* and *Methanobrevibacter ruminantium* isolated from yaks (*Bos grunniens*) grazing on the Qinghai Tibetan Plateau. *Anaerobe* 39:158–164
- Weiß S, Lebuhn M, Andrade D, Zankel A, Cardinale M, Birner-Gruenberger R et al (2013) Activated zeolite—suitable carriers for microorganisms in anaerobic digestion processes? *Appl Microbiol Biotechnol* 97(7):3225–3238
- Wignarajah K, Litwiller E, Fisher J, Hogan J (2006) Simulated human feces for testing human waste processing technologies in space systems. *SAE Trans* 115:424
- Worm P, Feroso F, Lens PN, Plugge C (2009) Decreased activity of a propionate degrading community in a UASB reactor fed with synthetic medium without molybdenum, tungsten and selenium. *Enzym Microb Technol* 45:139–145
- Xing B-S, Han Y, Cao S, Wang XC (2020) Effects of long-term acclimatization on the optimum substrate mixture ratio and substrate to inoculum ratio in anaerobic codigestion of food waste and cow manure. *Bioresour Technol* 317:123994
- Xu S, Jiang Q (2018) Surface modification of carbon fiber support by ferrous oxalate for biofilm wastewater treatment system. *J Clean Prod* 194:416–424
- Xu F, Shi J, Lv W, Yu Z, Li Y (2013) Comparison of different liquid anaerobic digestion effluents as inocula and nitrogen sources for solid-state batch anaerobic digestion of corn stover. *Waste Manag* 33:26–32
- Youngsukkasem S, Rakshit KS, Taherzadeh MJ (2012) Biogas production by encapsulated methane producing bacteria. *Bioresources* 7(1):56–65
- Youngsukkasem S, Chandolia K, Taherzadeh MJ (2015) Rapid bio-methanation of syngas in a reverse membrane bioreactor: membrane encased microorganisms. *Bioresour Technol* 178:334–340
- van der Zee FP, Cervantes FJ (2009) Impact and application of electron shuttles on the redox (bio)transformation of contaminants: a review. *Biotechnol Adv* 27:256–277
- Zhu K, Arnold W, Sakkos J, Davis C, Novak P (2018) Achieving high-rate hydrogen recovery from wastewater using customizable alginate polymer gel matrices encapsulating biomass. *Environ Sci Water Res Technol* 4:1867
- Zitomer DH, Johnson CC, Speece RE (2008) Metal stimulation and municipal digester thermophilic/mesophilic activity. *J Environ Eng* 134:42–47



# Efficient Biogas Production Through Syntrophic Microbial Partnerships in the Presence of Conductive Materials in Anaerobic Digesters Treating Organic Waste Streams: A Critical Assessment

# 13

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## Abstract

The limited availability of resources is nowadays the main driving force of changing our societal focus from conventional waste treatment and disposal toward resource recovery from organic waste streams. One possibility for wastewater treatment that generates net energy is anaerobic digestion (AD), where microorganisms break down complex organic matter anaerobically into a variety of volatile organic acids, which are subsequently converted into biogas mainly methane ( $\text{CH}_4$ ) by methanogens. Since methanogens can only consume mono- and/or di-carbon organic compounds, such as acetate, they have to build syntrophic partnerships with other microorganisms (e.g., fermenting bacteria) for  $\text{CH}_4$  production from more complex substrates, including food wastewater. These syntrophic partnerships involve interspecies electron transfer via electron carriers, where methanogens use  $\text{H}_2$  and/or formate as electron shuttles to scavenge electrons from bacterial electron donors to bacterial electron acceptors, which results in the reduction of  $\text{CO}_2$  to methane. However, recent studies suggested that a specific type of electroactive bacteria could use the advantage of conductive materials to transfer electrons directly to methanogens without the need for the indirect  $\text{H}_2$ /formate pathway. This unique intercellular electron transfer route—which is known as “direct interspecies electron transfer (DIET)” —allows more efficient  $\text{CH}_4$  production from organic matter in a metabolically and thermodynamically more efficient manner, enabling higher  $\text{CH}_4$  production rate and shorter start-up time. This book chapter will provide a critical assessment of the DIET mechanism driven by conductive materials that enable

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value-added resource recovery from different types of organic waste streams, their current limitations, and their potential scaling-up opportunities.

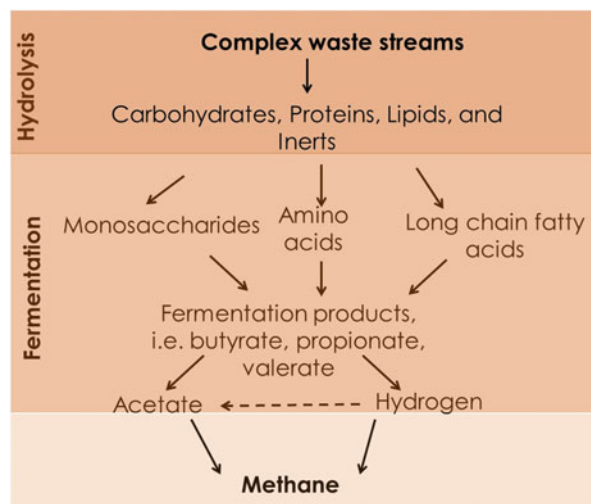
### Keywords

Anaerobic biotechnology · Circular economy · Resource recovery · Conductive materials · Direct interspecies electron transfer · Methanogenesis · Wastewater treatment

## 13.1 Introduction

Anaerobic digestion (AD) is a commonly used process for converting waste streams into a variety of volatile fatty acids (fermentation step), which are subsequently metabolized into useful energy in the form of biogas mainly methane ( $\text{CH}_4$ ) by methanogens as shown in Fig. 13.1 (Smith et al. 2014). Owing to the complex nature of waste streams, their biodegradation involves cooperation between different microbial species (i.e., fermenting bacteria, homoacetogens, and methanogens), resulting in a highly diverse microbial community composition (Wang et al. 2021). Although AD represents a promising option for coupling wastewater treatment and renewable energy production, its application is often limited by low efficiency and poor stability (Xu et al. 2019). The high retention time and slow growth rate of the key microbial species are the main causes of low efficiency, while the poor stability results from the produced metabolites and inhibitory toxicants (Chen et al. 2008). Several strategies have been suggested to overcome these limitations by optimizing operating conditions (i.e., temperature, pH, organic loading rate, hydraulic retention time, food-to-microorganism (F/M) ratio, and inoculum

**Fig. 13.1** The anaerobic food chain of methanogenic anaerobic digestion



type) and improving the substrates' biodegradability (El-Qelish et al. 2020; Meegoda et al. 2018; Panigrahi and Dubey 2019).

The key parameter for an efficient anaerobic digestion process is the methanogenesis step, which depends on the syntrophic partnership between different microbial species (Baek et al. 2018). Since methanogens can only consume simple organic compounds, such as acetate and H<sub>2</sub>/formate, they have to build syntrophic partnerships with other microorganisms (e.g., fermenting bacteria) for efficient CH<sub>4</sub> production from complex substrates, including food wastewater (De Bok et al. 2004). These syntrophic partnerships involve interspecies electron transfer (IET) via electron carriers (e.g., H<sub>2</sub> and formate) between methanogens and their syntrophic partners, e.g., fermenting bacteria (Batstone et al. 2006; McInerney et al. 2009). However, the partial pressure of H<sub>2</sub> should always be kept at a low level ( $\leq 10^{-4}$  atm) in order to allow fermentation to become thermodynamically feasible with the possibility of altering the fermentation pathways and stoichiometry upon H<sub>2</sub> build-up (Angenent et al. 2004; Madigan et al. 2008; Mahmoud et al. 2017a; McInerney et al. 2008; Stams and Plugge 2009).

Many strategies have been developed for increasing the AD process efficiency and stability. Among several alternatives, direct interspecies electron transfer (DIET) has been identified as a successful strategy for improving CH<sub>4</sub> production in a metabolically and thermodynamically more efficient manner compared to the interspecies electron transfer mechanism (Lovley 2011; Xu et al. 2019). The hallmark of the DIET-based mechanism is the ability of electroactive bacteria (EAB) to exchange electrons directly into methanogens. This unique electron transfer route is achieved by establishing biologically wired connections with methanogens by producing pili or nanowire instead of being shuttled by interspecies H<sub>2</sub>/formate transfer (Morita et al. 2011; Rotaru et al. 2014). Thus, the energetics and mechanisms of DIET are completely different from those of IET since electron exchange through metabolites is not required. On the other hand, several non-biogenic materials, e.g., granular activated carbon (GAC), carbon cloth (CC), biochar, iron nanoparticles, and carbon-doped materials, were found to promote DIET in anaerobic digesters within diverse microbial communities that cannot produce conductive appendages like e-pili and *c*-cytochrome (Kato et al. 2012; Liu et al. 2012). Over the past decade, it becomes obvious that DIET is often involved in the metabolism of organic materials in natural and engineered systems (McGlynn et al. 2015; Zhao et al. 2015a).

A recent study suggested that the use of conductive carbon cloth can also relieve the toxicity resulting from high acidity due to the acidogenesis process, leading to more biogas production and higher AD stability (Zhao et al. 2017b). Coexistence of conductive materials and electroactive microorganisms (e.g., *Geobacter* spp., *Desulfobulbus* spp., *Syntrophobacter* spp., *Sphaerochaeta* spp., *Desulfovibrio* spp., and *Shewanella* spp.) in the anaerobic digester has been reported to enhance the stability and performance of the anaerobic digestion process (Lei et al. 2019; Wang et al. 2018; Xiao et al. 2020; Yee et al. 2020; Zhao et al. 2017b). The interaction between extracellular appendages of the electron donors and externally added conductive materials allows methanogens to receive electrons directly,

transporting them through intracellular reduced-enzyme carriers. The electrons received by methanogens are used to reduce  $\text{CO}_2$  into formyl, methylene, and then methyl (Welte and Deppenmeier 2014) controlled by coenzymes ferredoxin and  $\text{F}_{420}\text{-H}_2$ , while the extracellular membrane appendages control the intracellular proton balance to keep sufficient energy for other methanogenic pathways.

This chapter aims to critically evaluate the recent research advances and implications of DIET with a focus on the anaerobic digestion technology. It also discusses the main mechanisms for DIET in the engineered system and highlights its ecological advantages over conventional IET. Furthermore, we provided insight on different approaches to stimulate DIET (e.g., by adding electrically conductive materials) in anaerobic digesters fed with different types of donor substrates, which would lead to efficient recycling of wastewater and biomass for creating valuable biorefinery-based technology for a sustainable circular economy.

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## 13.2 Principles and Mechanisms of DIET

Compared with the IET, the DIET mechanism depends on physical direct contact between microbial cells through membrane-bound structures to exchange electrons instead of diffusive electron transfer, which occurs through intracellularly produced electron carriers. Over the past decade, DIET mechanisms have been extensively studied with the help of several molecular and imaging tools (McGlynn et al. 2015; Rotaru et al. 2014; Wegener et al. 2015). The mechanism of DIET has not been fully understood yet, including the microbial species participating in such a process and the electrical connections used for electron transfer. However, the findings of the pure culture assays are independently evolved from many experiments and found that the bacterial appendages which act as the electrical connections are not the only way for the DIET, as some of the microorganisms that participate in DIET do not have such electrical appendages. Recent studies suggested that cytochromes and e-pili represent the main components involved in the DIET processes.

The e-pili, which are also known as nanowires, are filamentous proteinous appendages with dimensions of 3–5 nm in diameter and 10–20  $\mu\text{m}$  long. These structures are crucial for the DIET because of their conductive properties, which have been reported at *Geobacter sulfurreducens* from 37  $\mu\text{S cm}^{-1}$  at pH 10.5 up to 188  $\text{mS cm}^{-1}$  at pH 2 (Reguera et al. 2005). E-pili motivate the DIET by assuring the direct flow of electrons from electron-donating species to methanogens. Recent studies suggested that electron transfer might be via two ways: (1) metal conduction via overlapping of the amino acids in the portentous structure, or (2) electrons travel in the pili by hopping between cytochromes (Mahmoud et al., 2017b; Feliciano et al. 2015).

Cytochromes are membrane-bound heme proteins with iron-based functional groups that are found in different types of microorganisms (Xu et al. 2019). They have been reported to be responsible for transferring electrons through oxidation and reduction reactions, especially for photosynthesis and respiration processes (Guo et al. 2021). They are classified into four types (A, B, C, and D) depending on the



formulation of iron in the functional group (Neilands 1974). They are key structures for the flow of electrons between microbial species via oscillating the iron oxidation status between the  $\text{Fe}^{3+}/\text{Fe}^{2+}$ -oxidation/reduction processes. *C*-type cytochromes transfer electrons between the cytoplasm of the donating microbial species and the extracellular electron acceptors of another species. It has been reported that electron donors, such as *Shewanella* and *Geobacter*, employ *c*-type cytochrome for electron donation through the microbial cell membranes to electron acceptors, such as bioelectrochemical electrodes, metal oxides (Fe and Mn), and soluble redox compounds. For example, electron flow between *Geobacter metallireducens* (electron donor) and *Geobacter sulfurreducens* (electron acceptor) has been identified through OmcS cytochrome along with e-pili of *Geobacter sulfurreducens* (Summers et al. 2010).

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### 13.3 Existence of DIET in Engineered Systems

Co-culture of electron-donating microbial species (such as *Geobacter metallireducens*) and electron acceptor (such as *Methanosaeta harundinacea*) has evidenced the existence of DIET (Smith and Ingram-Smith 2007). DIET is independent of the traditional mechanism of electron transfer through hydrogen/formate, where the electrons from electron donors are used to reduce protons into hydrogen and then the electron acceptors consume hydrogen to reduce  $\text{CO}_2$  into methane (Summers et al. 2010). However, the extracellular conductive appendages, e.g., e-pili and *c*-cytochromes, in the electron-donating microbial species have been identified as the key structures in the DIET process (Lovley 2017). In addition, externally added non-biogenic conductive materials, such as magnetite and carbon-based materials, have been identified to function similarly to the extracellular conductive appendages and therefore stimulate the DIET in co-cultures (Sieber et al. 2014). The coexistence of microbial species of *Geobacter* and *Methanosaeta* has been reported in the anaerobic digesters treating different kinds of waste streams, including food wastes (Morita et al. 2011; Rotaru et al. 2014). Metal-like conductivity has been detected in the anaerobic digester dominated by *Geobacter* species having the e-pili appendages (Morita et al. 2011). Co-culture of *Syntrophomonas wolfei* and *Methanospirillum hungatei* supplemented with carbon nanotubes showed an enhanced rate of butyrate oxidation and increased methane yield (Salvador et al. 2017). *Methanosarcina*, a genus of acetoclastic methanogens isolated from sediments, landfills, and anaerobic digesters (Zhao et al. 2016, 2015a, b), has been reported to accept electrons from externally added conductive materials (Salvador et al. 2017; Shrestha and Rotaru 2014). A co-culture of *Methanosarcina barkeri* and *Geobacter metallireducens* supplemented with electrically conductive granular activated carbon has shown an increase in the methane yield (Shrestha and Rotaru 2014). Similar trends have been observed by Salvador et al. (2017), in which iron was supplied as an electron mediator between the electron donors and electron acceptors in an anaerobic digester dominated by *Geobacter* and *Methanosarcina* species. Although the DIET-based AD has been extensively investigated in

**Table 13.1** Summary of substrates and methanogenic communities involved in DIET-based engineered systems

Substrate	Inoculum source	Electron donors	Electron acceptors	References
Ethanol	Co-culture	<i>Geobacter metallireducens</i>	<i>Methanosarcina barkeri</i>	Rotaru et al. (2015)
Acetate	Co-culture	<i>G. metallireducens</i>	<i>Methanosarcina barkeri</i>	Chen et al. (2014)
Propionate	Paddy soil	<i>Sedimentibacter</i> and <i>Thauera</i>	<i>Methanobacterium</i>	Yang et al. (2016)
Butyrate	Paddy soil	<i>Geobacteraceae</i>	<i>Methanosarcinaceae</i>	Li et al. (2015)
Ethanol	Anaerobic sludge	<i>Geobacter</i> and <i>Pseudomonas</i>	<i>Methanobacterium</i> and <i>Methanospirillum</i>	Lin et al. (2017)
Ethanol	Anaerobic sludge	<i>Geobacter</i>	<i>Methanosarcina</i>	Zhao et al. (2017a)
Glucose	Anaerobic sludge	<i>Bacteroidales</i>	<i>Methanosarcina</i>	Xu et al. (2015)
Dog food	Anaerobic sludge	<i>Sporanaerobacter</i>	<i>Methanosarcina</i>	Dang et al. (2016)
Food waste	Anaerobic sludge	<i>Alkaliphilus</i> and <i>Syntrophomonas</i>	<i>Methanothermobacter</i>	Capson-Tojo et al. (2018)
Waste-activated sludge	Anaerobic sludge	<i>Geobacter</i>	<i>Methanosaeta</i> and <i>Methanosarcina</i>	Yang et al. (2017)
Leachate	Anaerobic sludge	<i>Geobacter</i>	<i>Methanosarcina</i>	Lei et al. (2019)
Leachate	Anaerobic sludge	<i>Anaerolineaceae</i> and <i>Eubacteriaceae</i>	<i>Methanosarcina</i> and <i>Methanosaeta</i>	Lei et al. (2018)
Sucrose	Anaerobic sludge	<i>Clostridium sensu stricto</i>	<i>Methanosaeta</i>	Hu et al. (2017)
Sucrose	Anaerobic sludge	<i>Clostridiales</i>	<i>Methanosaeta concilii</i>	Li et al. (2015)
Glucose	Anaerobic sludge	<i>Syntrophomonas</i> spp. and <i>Clostridiaceae</i>	<i>Methanosaeta</i> and <i>Methanosarcina</i>	Luo et al. (2015)
Glucose	Anaerobic sludge	<i>Caloramator</i>	<i>Methanosaeta</i> and <i>Methanosarcina</i>	Yan et al. (2017)
Benzoate	Anaerobic sludge	<i>Bacillaceae</i> and <i>Peptococcaceae</i>	<i>Methanobacterium</i>	Zhuang et al. (2015)

well-defined co-culture systems, the occurrence of DIET-based AD in real engineered systems is not clear owing to the complexity associated with real systems. However, recent studies reveal a remarkable change in the electrical conductivity and structure of mixed-culture microbial communities in anaerobic digesters as a result of supplementing the anaerobic digester with external conductive materials (Table 13.1).

## 13.4 Conductive Additives for Boosting Anaerobic Digestion

Many additives have been implemented for stimulating anaerobic digestion to achieve maximum efficiency (Gahlot et al. 2020). Of them, the application of conductive materials has been studied, and hereby we explored the mechanism of action of these materials in enhancing the anaerobic digestion process through influencing the microbial communities' behavior. Conductive materials work by enriching the syntrophic relationship between microbial species and therefore establish an electrical connection between fermenters (electron donors) and methanogens (electron acceptors) via DIET (Table 13.2). DIET via conductive material is much more effective than IIET via hydrogen or formate (Park et al. 2018). Electrons produced extracellularly from the fermentation of organic compounds are transferred to conductive materials. The electron acceptors use those electrons to reduce  $\text{CO}_2$  to methane (Park et al. 2018). Conductive material application couples acceleration of DIET, enhancing the growth of the methanogens and reducing the lag phase time required for the adaptation of the microbes for the surrounding environment (Madigan et al. 2008).

### 13.4.1 Carbon-Based Materials

Carbon-based materials, such as biochar, carbon cloth, graphite, granular activated carbon, powdered activated carbon, graphene, and carbon nanotubes, have a particle size larger than microorganisms. They are characterized by having a high specific surface area per weight, therefore providing an attachment surface for bacterial colonization, which, in turn, favors the syntrophic relationship between DIET microorganisms. In addition to being relatively cheap, they provide many advantages, such as high electrical conductivity, chemical stability, and biocompatibility (Gahlot et al. 2020). The carbon-based materials act like rechargeable batteries, where they ingest the electrons from the donating microbes and deliver them to the methanogens for reduction of  $\text{CO}_2$  to methane. The mechanism of action of carbon-based materials for enhancing the DIET process depends on the surface area and the capacity of storing electrons and to a lower extent to the electrical conductivity (Martins et al. 2018). Granular activated carbon (GAC) has been tested for enhancing the anaerobic digestion of many feedstocks, including ethanol, propionate, and surplus sludge in both batch and continuous anaerobic digesters (Liu et al. 2012). Granular activated carbon serves as a mediator for the DIET process, where microorganisms can accept an electron from GAC directly in addition, GAC can absorb the toxic compounds, which may inhibit anaerobic digestion processes, resulting in shortening the lag phase, and consequently speeding up the overall anaerobic digestion process. Anaerobic digestion process supplemented with GAC accelerates DIET by creating a large surface area for the microbial species, preventing their aggregation, and allowing the conductive appendages to act more efficiently (Baek et al. 2018). GAC and carbon cloth addition to anaerobic digester have positively affected not only biomethane production but also treatment

**Table 13.2** Conductive materials successfully applied in DIET

Substrate	Inoculum source	Conductive material	Dose	Reactor type	References
Waste-activated sludge	Anaerobic sludge	Powdered activated carbon	0.5 g/L	AnSBR	Yan et al. (2020)
Food waste	Industrial pre-adapted anaerobic sludge	Granular activated carbon	10 g/L	Consecutive batch anaerobic digestion	Capson-Tojo et al. (2018)
Bagasse wastes	Anaerobic sludge	GAC	10, 25, 50, 75 and 100 g/L	UASB	Zhao and Zhang (2019)
Dairy waste	Anaerobic sludge	GAC	10 g/ reactor	SBR	Zhao et al. (2017a)
Glucose	Lab-scale UASB	GAC	5 g/L	UASB	Guo et al. (2021)
Dairy wastewater	Anaerobic sludge	Magnetite	20 mM	Completely mixed tank reactors	Baek et al. (2015)
Leachate	Full-scale anaerobic digester-treating food wastewater	Magnetite	10 g/L	UASB	Lei et al. (2018)
Sucrose	Lab-scale UASB	Magnetite	1.368 g/L	Expanded granular sludge bed	Wang et al. (2019)
Acetate	Full-scale expanded granular sludge bed	Magnetite	100 g/L	UASB	Chen et al. (2020)
Propionate	Thermophilic anaerobic digester	Magnetite	Not mentioned	SBR	Yamada et al. (2015)
Sucrose	Municipal wastewater treatment plant	Biochar	4 g/L	UASB	Wang et al. (2018)
Phenol	Mesophilic UASB unit of brewery wastewater plant	Biochar	15 g/L	SBR	Wang et al. (2020)
Acetate	Granular sludge from UASB	Carbon nanotube	1 g/L	SBR	Shen et al. (2020)
Oleic acid	Mixed culture of anaerobic digester sludge	Carbon nanotube	1 g/L	Batch experiment	Mostafa et al. (2020)
Ethanol	Lab digester-treating cellulose	Graphene	0.5, 1.0, 2.0 g/L	Batch experiments	Lin et al. (2017)
Glucose	Lab digester-treating cellulose	Graphene	0.5, 1.0, 2.0 g/L	Batch experiments	Lin et al. (2017)
Acetate	Rice paddy field soil	Hematite	20 mM	Batch experiments	Kato et al. (2012)
Ethanol	Rice paddy field soil	Hematite	20 mM	Batch experiments	Kato et al. (2012)

efficiencies, such as removal of suspended solids and chemical oxygen demand. Biochar has been widely applied for the removal of different contaminants from wastewaters such as heavy metals, pharmaceuticals, chlorophenols, and tylosin (Ahmad et al. 2014; Lu et al. 2017; Nasr et al. 2021). Biochar has been reported to enhance the DIET process and consequently methanogenesis. Besides, it has been also applied as a soil amendment because of its ability to retain the nutrients in the digestate (Chiappero et al. 2020).

### 13.4.2 Iron-Based Materials

Iron-based oxides are essential for many biological processes; therefore, they have been widely applied for many applications in medicine and biosensing (Wu et al. 2015). There are various types of iron oxides depending on their physical characteristics such as magnetite (conductive), hematite (semi-conductive), and ferrihydrite (insulative) (Baek et al. 2018). Naturally, these oxides are abundant; therefore, the existence of microorganisms depending on such oxides to facilitate the extracellular electron transfer takes place in surface and subsurface sediments (Liu et al. 2014). Magnetite and hematite have been successfully applied for stimulating DIET by favoring the syntrophic relationship between the electron donors and the electron acceptors (methanogens) by efficient transfer of electrons to reduce carbon dioxide to methane. Although magnetite has much higher electrical conductivity compared to hematite, they both exhibited similar enhancement effects for the DIET process (Kato et al. 2012). Application of magnetite particles has stimulated methane production from propionate (Cruz Viggi et al. 2014) and butyrate (Li et al. 2015) by accelerating electron transfer between syntrophic microorganisms taking the advantage of the electrical conductivity of magnetite.

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## 13.5 Conclusion

DIET is a novel electron transfer mechanism, which involves a cell-to-cell interaction for exchanging electrons with valuable implications in natural and engineered bioenergy-producing systems. In this manuscript, we shed the light on the recent research advances and implications of DIET with a focus on the anaerobic digestion technology. Furthermore, the main mechanisms have been extensively reviewed. We also provided insight on different approaches to stimulate DIET (e.g., by adding electrically conductive materials) in engineered systems (e.g., anaerobic digester), allowing more efficient CH<sub>4</sub> production from organic matter in a metabolically and thermodynamically more efficient manner. Despite the great promise of this approach, it is still in its early stage of development. Thus, more research is required in order to enhance the holistic understanding of DIET mechanisms, especially in engineered systems when real wastewater is used as the main donor substrates.

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## References

- Ahmad M, Rajapaksha AU, Lim JE, Zhang M, Bolan N, Mohan D, Vithanage M, Lee SS, Ok YS (2014) Biochar as a sorbent for contaminant management in soil and water: a review. *Chemosphere* 99:19–33
- Angenent LT, Karim K, Al-Dahhan MH, Wrenn BA, Domínguez-Espinosa R (2004) Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends Biotechnol* 22: 477–485
- Baek G, Kim J, Cho K, Bae H, Lee C (2015) The biostimulation of anaerobic digestion with (semi) conductive ferric oxides: their potential for enhanced biomethanation. *Appl Microbiol Biotechnol* 99:10355–10366
- Baek G, Kim J, Kim J, Lee C (2018) Role and potential of direct interspecies electron transfer in anaerobic digestion. *Energies* 11(1):107
- Batstone DJ, Picioreanu C, van Loosdrecht MCM (2006) Multidimensional modelling to investigate interspecies hydrogen transfer in anaerobic biofilms. *Water Res* 40:3099–3108
- Capson-Tojo G, Moscoviz R, Ruiz D, Santa-Catalina G, Trably E, Rouez M, Crest M, Steyer JP, Bernet N, Delgenès JP (2018) Addition of granular activated carbon and trace elements to favor volatile fatty acid consumption during anaerobic digestion of food waste. *Bioresour Technol* 260:157–168
- Chen Y, Cheng JJ, Creamer KS (2008) Inhibition of anaerobic digestion process: a review. *Bioresour Technol* 99:4044–4064
- Chen S, Rotaru AE, Liu F, Philips J, Woodard TL, Nevin KP, Lovley DR (2014) Carbon cloth stimulates direct interspecies electron transfer in syntrophic co-cultures. *Bioresour Technol* 173: 82–86
- Chen Q, Liu C, Liu X, Sun D, Li P, Qiu B, Dang Y, Karpinski NA, Smith JA, Holmes DE (2020) Magnetite enhances anaerobic digestion of high salinity organic wastewater. *Environ Res* 189: 109884
- Chiappero M, Demichelis F, Norouzi O, Berruti F, Hu M, Mašek O, Di Maria F, Fiore S (2020) Review of biochar application in anaerobic digestion processes. *Renew Sust Energ Rev* 131: 110037
- Cruz Viggì C, Rossetti S, Fazi S, Paiano P, Majone M, Aulenta F (2014) Magnetite particles triggering a faster and more robust syntrophic pathway of methanogenic propionate degradation. *Environ Sci Technol* 48:7536–7543
- Dang Y, Holmes DE, Zhao Z, Woodard TL, Zhang Y, Sun D, Wang LY, Nevin KP, Lovley DR (2016) Enhancing anaerobic digestion of complex organic waste with carbon-based conductive materials. *Bioresour Technol* 220:516–522
- De Bok FAM, Plugge CM, Stams AJM (2004) Interspecies electron transfer in methanogenic propionate degrading consortia. *Water Res* 38:1368–1375
- El-Qelish M, Chatterjee P, Dessì P, Kokko M, El-Gohary F, Abo-Aly M, Rintala J (2020) Bio-hydrogen production from sewage sludge: screening for pretreatments and semi-continuous reactor operation. *Waste Biomass Valoriz* 11:4225–4234
- Feliciano GT, Steidl RJ, Reguera G (2015) Structural and functional insights into the conductive pili of *Geobacter sulfurreducens* revealed in molecular dynamics simulations. *Phys Chem Chem Phys* 17:22217–22226
- Gahlot P, Ahmed B, Tiwari SB, Aryal N, Khursheed A, Kazmi AA, Tyagi VK (2020) Conductive material engineered direct interspecies electron transfer (DIET) in anaerobic digestion: mechanism and application. *Environ Technol Innov* 20:101056

- Guo X, Chen H, Zhu X, Xia A, Liao Q, Huang Y, Zhu X (2021) Revealing the role of conductive materials on facilitating direct interspecies electron transfer in syntrophic methanogenesis: a thermodynamic analysis. *Energy* 229:120747
- Hu Q, Sun D, Ma Y, Qiu B, Guo Z (2017) Conductive polyaniline nanorods enhanced methane production from anaerobic wastewater treatment. *Polymer* 120:236–243
- Kato S, Hashimoto K, Watanabe K (2012) Methanogenesis facilitated by electric syntrophy via (semi) conductive iron-oxide minerals. *Environ Microbiol* 14:1646–1654
- Lei Y, Wei L, Liu T, Xiao Y, Dang Y, Sun D, Holmes DE (2018) Magnetite enhances anaerobic digestion and methanogenesis of fresh leachate from a municipal solid waste incineration plant. *Chem Eng J* 348:992–999
- Lei Y, Sun D, Dang Y, Feng X, Huo D, Liu C, Zheng K, Holmes DE (2019) Metagenomic analysis reveals that activated carbon aids anaerobic digestion of raw incineration leachate by promoting direct interspecies electron transfer. *Water Res* 161:570–580
- Li H, Chang J, Liu P, Fu L, Ding D, Lu Y (2015) Direct interspecies electron transfer accelerates syntrophic oxidation of butyrate in paddy soil enrichments. *Environ Microbiol* 17:1533–1547
- Lin R, Cheng J, Zhang J, Zhou J, Cen K, Murphy JD (2017) Boosting biomethane yield and production rate with graphene: the potential of direct interspecies electron transfer in anaerobic digestion. *Bioresour Technol* 239:345–352
- Liu F, Rotaru AE, Shrestha PM, Malvankar NS, Nevin KP, Lovley DR (2012) Promoting direct interspecies electron transfer with activated carbon. *Energy Environ Sci* 5:8982–8989
- Liu S, Song H, Wei S, Yang F, Li X (2014) Bio-cathode materials evaluation and configuration optimization for power output of vertical subsurface flow constructed wetland—microbial fuel cell systems. *Bioresour Technol* 166:575–583
- Lovley DR (2011) Reach out and touch someone: potential impact of DIET (direct interspecies energy transfer) on anaerobic biogeochemistry, bioremediation, and bioenergy. *Rev Environ Sci Biotechnol* 10:101–105
- Lovley DR (2017) Syntrophy goes electric: direct interspecies electron transfer. *Annu Rev Microbiol* 71:643–664
- Lu K, Yang X, Gielen G, Bolan N, Ok YS, Niazi NK, Xu S, Yuan G, Chen X, Zhang X (2017) Effect of bamboo and rice straw biochars on the mobility and redistribution of heavy metals (Cd, Cu, Pb and Zn) in contaminated soil. *J Environ Manag* 186:285–292
- Luo C, Lü F, Shao L, He P (2015) Application of eco-compatible biochar in anaerobic digestion to relieve acid stress and promote the selective colonization of functional microbes. *Water Res* 68:710–718
- Madigan MT, Martinko JM, Dunlap PV, Clark DP (2008) Brock biology of microorganisms 12th Ed. *Int Microbiol* 11:65–73
- Mahmoud M, Torres CI, Rittmann BE (2017a) Changes in glucose fermentation pathways as a response to the free ammonia concentration in microbial electrolysis cells. *Environ Sci Technol* 51:13461–13470
- Mahmoud M, Parameswaran P, Torres CI, Rittmann BE (2017b) Electrochemical techniques reveal that total ammonium stress increases electron flow to anode respiration in mixed-species bacterial anode biofilms. *Biotechnol Bioeng* 114: 1151–1159 <https://doi.org/10.1002/bit.26246>
- Martins G, Salvador AF, Pereira L, Alves MM (2018) Methane production and conductive materials: a critical review. *Environ Sci Technol* 52:10241–10253
- McGlynn SE, Chadwick GL, Kempes CP, Orphan VJ (2015) Single cell activity reveals direct electron transfer in methanotrophic consortia. *Nature* 526:531–535
- McInerney MJ, Struchtemeyer CG, Sieber J, Mouttaki H, Stams AJM, Schink B, Rohlin L, Gunsalus RP (2008) Physiology, ecology, phylogeny, and genomics of microorganisms capable of syntrophic metabolism. *Ann N Y Acad Sci* 1125:58–72
- McInerney MJ, Sieber JR, Gunsalus RP (2009) Syntrophy in anaerobic global carbon cycles. *Curr Opin Biotechnol* 20:623–632
- Meegoda JN, Li B, Patel K, Wang LB (2018) A review of the processes, parameters, and optimization of anaerobic digestion. *Int J Environ Res Public Health* 15:2224

- Morita M, Malvankar NS, Franks AE, Summers ZM, Giloteaux L, Rotaru AE, Rotaru C, Lovley DR (2011) Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates. *MBio* 2:e00159–e00111
- Mostafa A, Im S, Song YC, Kang S, Kim DH (2020) Enhanced anaerobic digestion of long chain fatty acid by adding magnetite and carbon nanotubes. *Microorganisms* 8:333
- Nasr M, Tawfik A, Awad HM, Galal A, El-qelish M, Abdul M, Mumtaz M, Khan A, Rehan M, Nizami A, Lee M (2021) Dual production of hydrogen and biochar from industrial effluent containing phenolic compounds. *Fuel* 301:121087
- Neilands JB (1974) *Microbial iron metabolism: a comprehensive treatise*. Academic Press, New York, NY
- Panigrahi S, Dubey BK (2019) A critical review on operating parameters and strategies to improve the biogas yield from anaerobic digestion of organic fraction of municipal solid waste. *Renew Energy* 143:779–797
- Park JH, Kang HJ, Park KH, Park HD (2018) Direct interspecies electron transfer via conductive materials: a perspective for anaerobic digestion applications. *Bioresour Technol* 254:300–311
- Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. *Nature* 435:1098–1101
- Rotaru AE, Shrestha PM, Liu F, Markovaite B, Chen S, Nevin KP, Lovley DR (2014) Direct interspecies electron transfer between *Geobacter metallireducens* and *Methanosarcina barkeri*. *Appl Environ Microbiol* 80:4599–4605
- Rotaru AE, Woodard TL, Nevin KP, Lovley DR (2015) Link between capacity for current production and syntrophic growth in *Geobacter* species. *Front Microbiol* 6:1–8
- Salvador AF, Martins G, Melle-Franco M, Serpa R, Stams AJM, Cavaleiro AJ, Pereira MA, Alves MM (2017) Carbon nanotubes accelerate methane production in pure cultures of methanogens and in a syntrophic coculture. *Environ Microbiol* 19:2727–2739
- Shen N, Liang Z, Chen Y, Song H, Wan J (2020) Bioresource Technology Enhancement of syntrophic acetate oxidation pathway via single walled carbon nanotubes addition under high acetate concentration and thermophilic condition. *Bioresour Technol* 306:123182
- Shrestha PM, Rotaru AE (2014) Plugging in or going wireless: strategies for interspecies electron transfer. *Front Microbiol* 5:237
- Sieber JR, Le HM, McNerney MJ (2014) The importance of hydrogen and formate transfer for syntrophic fatty, aromatic and alicyclic metabolism. *Environ Microbiol* 16:177–188
- Smith KS, Ingram-Smith C (2007) Methanosaeta, the forgotten methanogen? *Trends Microbiol* 15: 150–155
- Smith AL, Stadler LB, Cao L, Love NG, Raskin L, Skerlos SJ (2014) Navigating wastewater energy recovery strategies: a life cycle comparison of anaerobic membrane bioreactor and conventional treatment systems with anaerobic digestion. *Environ Sci Technol* 48:5972–5981
- Stams AJM, Plugge CM (2009) Electron transfer in syntrophic communities of anaerobic bacteria and archaea. *Nat Rev Microbiol* 7:568–577
- Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley DR (2010) Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. *Science* 330:1413–1415
- Wang D, Han Y, Han H, Li K, Xu C, Zhuang H (2018) New insights into enhanced anaerobic degradation of Fischer-Tropsch wastewater with the assistance of magnetite. *Bioresour Technol* 257:147–156
- Wang C, Wang C, Jin L, Lu D, Chen H, Zhu W, Xu X, Zhu L (2019) Response of syntrophic aggregates to the magnetite loss in continuous anaerobic bioreactor. *Water Res* 164:114925
- Wang G, Gao X, Li Q, Zhao H, Liu Y, Wang XC, Chen R (2020) Redox-based electron exchange capacity of biowaste-derived biochar accelerates syntrophic phenol oxidation for methanogenesis via direct interspecies electron transfer. *J Hazard Mater* 390:121726
- Wang Z, Wang T, Si B, Watson J, Zhang Y (2021) Accelerating anaerobic digestion for methane production: potential role of direct interspecies electron transfer. *Renew Sust Energy Rev* 145: 111069



- Wegener G, Krukenberg V, Riedel D, Tegetmeyer HE, Boetius A (2015) Intercellular wiring enables electron transfer between methanotrophic archaea and bacteria. *Nature* 526:587–590
- Welte C, Deppenmeier U (2014) Bioenergetics and anaerobic respiratory chains of acetoclastic methanogens. *Biochim Biophys Acta Bioenerg* 1837:1130–1147
- Wu W, Wu Z, Yu T, Jiang C, Kim WS (2015) Recent progress on magnetic iron oxide nanoparticles: synthesis, surface functional strategies and biomedical applications. *Sci Technol Adv Mater* 16:23501
- Xiao L, Liu F, Lichtfouse E, Zhang P, Feng D, Li F (2020) Methane production by acetate dismutation stimulated by *Shewanella oneidensis* and carbon materials: an alternative to classical CO<sub>2</sub> reduction. *Chem Eng J* 389:124469
- Xu S, He C, Luo L, Lü F, He P, Cui L (2015) Comparing activated carbon of different particle sizes on enhancing methane generation in upflow anaerobic digester. *Bioresour Technol* 196:606–612
- Xu H, Chang J, Wang H, Liu Y, Zhang X, Liang P, Huang X (2019) Enhancing direct interspecies electron transfer in syntrophic-methanogenic associations with (semi)conductive iron oxides: effects and mechanisms. *Sci Total Environ* 695:133876
- Yamada C, Kato S, Ueno Y, Ishii M, Igarashi Y (2015) Conductive iron oxides accelerate thermophilic methanogenesis from acetate and propionate. *J Biosci Bioeng* 119:678–682
- Yan W, Shen N, Xiao Y, Chen Y, Sun F, Tyagi VK, Zhou Y (2017) The role of conductive materials in the start-up period of thermophilic anaerobic system. *Bioresour Technol* 239:336–344
- Yan W, Mukherjee M, Zhou Y (2020) Direct interspecies electron transfer (DIET) can be suppressed under ammonia-stressed condition—re-evaluate the role of conductive materials. *Water Res* 183:116094
- Yang Z, Guo R, Shi X, Wang C, Wang L, Dai M (2016) Magnetite nanoparticles enable a rapid conversion of volatile fatty acids to methane. *RSC Adv* 6:25662–25668
- Yang Y, Zhang Y, Li Z, Zhao Z, Quan X, Zhao Z (2017) Adding granular activated carbon into anaerobic sludge digestion to promote methane production and sludge decomposition. *J Clean Prod* 149:1101–1108
- Yee MO, Deutzmann J, Spormann A, Rotaru AE (2020) Cultivating electroactive microbes—from field to bench. *Nanotechnology* 31:174003
- Zhao Z, Zhang Y (2019) Application of ethanol-type fermentation in establishment of direct interspecies electron transfer: a practical engineering case study. *Renew Energy* 136:846–855
- Zhao Z, Zhang Y, Wang L, Quan X (2015a) Potential for direct interspecies electron transfer in an electric-anaerobic system to increase methane production from sludge digestion. *Sci Rep* 5:1–12
- Zhao Z, Zhang Y, Woodard TL, Nevin KP, Lovley DR (2015b) Enhancing syntrophic metabolism in up-flow anaerobic sludge blanket reactors with conductive carbon materials. *Bioresour Technol* 191:140–145
- Zhao Z, Zhang Y, Quan X, Zhao H (2016) Evaluation on direct interspecies electron transfer in anaerobic sludge digestion of microbial electrolysis cell. *Bioresour Technol* 200:235–244
- Zhao Z, Li Y, Quan X, Zhang Y (2017a) New application of ethanol-type fermentation: stimulating methanogenic communities with ethanol to perform direct interspecies electron transfer. *ACS Sustain Chem Eng* 5:9441–9453
- Zhao Z, Zhang Y, Li Y, Dang Y, Zhu T, Quan X (2017b) Potentially shifting from interspecies hydrogen transfer to direct interspecies electron transfer for syntrophic metabolism to resist acidic impact with conductive carbon cloth. *Chem Eng J* 313:10–18
- Zhuang L, Tang J, Wang Y, Hu M, Zhou S (2015) Conductive iron oxide minerals accelerate syntrophic cooperation in methanogenic benzoate degradation. *J Hazard Mater* 293:37–45



# Application of Anaerobic Digestion in Decentralized Faecal Sludge Treatment Plants

# 14

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## Abstract

Over 80% of the population in low- and middle-income countries (LMIC) depends on on-site sanitation, largely pit latrines and septic tanks. Anaerobic digestion (AD) can stabilize the organic fraction of faecal sludge (FS) while also generating biogas to offset some energy needs at the treatment plant. This chapter examined the technical and operational feasibility, as well as opportunities for AD of FS. FS that has spent long time in containment systems produces less gas than the fresh one. Therefore, FS from container-based sanitation facilities can boost gas production in biogas facilities receiving aged FS. In addition, co-digestion with different organic waste substrates improves the quantity and quality of biogas production. However, a system for transportation, pre-treatment and storage of organic feedstock for co-digestion with FS should be examined against the backdrop of cost and benefits to determine whether the improved gas production matches with the required resource inputs. In conclusion, biogas is not the only driving factor for AD. Other benefits such as organic matter stabilization and environmental benefits such as pathogen and odour reduction contribute to

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the driving factors for adopting AD of FS. The mineralized nutrient content in bio-slurry can be taken advantage of, although with care to avoid microbial health risks.

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**Keywords**

Anaerobic digestion · Biogas · Co-digestion · Decentralized · Faecal sludge · Organic waste · Treatment plant

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**14.1 Introduction**

There is appreciation in using anaerobic digestion (AD) as a step at treatment plants managing faecal sludge (FS) from communities or towns. Small-scale digesters are more practical for use in faecal sludge treatment plants (FSTPs) in low- and middle-income countries (LMIC) due to low complexity, low capital and maintenance costs as opposed to large-scale digesters at centralized wastewater treatment plants (Tayler 2018). Unlike on-site biodigester toilets, where fresh excreta (faeces and urine) are used as the feedstock in institutions and public places, biodigesters at decentralized scale receive FS previously stored in on-site sanitation facilities (pit latrines, public toilets, septic tanks, aqua-privies and container-based/portable toilets) and are expected to behave differently. The biodegradable characteristics of FS from these sources do not only vary with different technology options but also other geographical and environmental factors such as groundwater infiltration, emptying frequency, user habits, constituent materials, type, concentration of contaminants and location of sanitation facilities (Still and Foxon 2012). For example, biomethane potential (BMP) is very low in FS from septic tanks since it is partially stabilized but high from public and container-based toilets, where the FS retention period is short (Rose 2015). In addition, FS from septic tanks at the household level desludged after 5 years is expected to be more stabilized compared to that from septic tanks in public places such as schools and hotels, desludged in less than 1 year (Schoebitz et al. 2014). The difference in retention times causes variation in BPM of FS depending on the source. This makes AD of FS from containment technologies of varying retention times feeding the same decentralized digester technology (at treatment plant) complex. However, there is limited information on technical and operational feasibility, as well as opportunities for AD of FS at decentralized scales. This chapter presents the potential of AD technology at decentralized FS treatment plants by analysing the biodegradability characteristics of FS from different sources, BMP, co-digestion, operation and maintenance as well as management options of the produced slurry.

## 14.2 Faecal Sludge as a Feedstock

Faecal sludge treatment facilities mainly receive partially stabilized FS, which has stayed for varying durations in different types of on-site containments. However, considerable amounts of organics have been realized in various types of FS received at treatment facilities as reflected by the higher fraction of volatile solids in Table 14.1. The purpose of the AD is stabilization of the partially digested, fresh or raw FS, pathogen and odour reduction, as well as production of biogas energy and slurry.

The optimum pH for AD for high biogas yield ranges between 6.5 and 7.5 (Vögeli et al. 2014), which is generally the pH range for the various types of raw and partially digested FS. There is no need of adding chemicals to raise or lower pH for the operation of the plant. However, container-based FS exhibits low pH ranges,

**Table 14.1** Characteristics of faecal sludge from various containments

Constituent	FS feedstock source			
	Septic tank FS	Pit latrine FS	Public toilet FS	Container-based toilet FS <sup>a</sup>
Moisture (%)	95–99	83–95	88–98	80–95
Total solids (%)	<3	5.3–19	2.94–11.94	4.6–9.5
Volatile solids (% TS)	45–76	41–69	70	65–75
pH	6.7–8	7.5–7.9	7.2	6.1–6.4
Total carbon (%)	NA	24.1	40.1	50–52
Total nitrogen (%)	1.0	2.1	3.7	4.8–7.3
Carbon-to-nitrogen (C:N) ratio	19–30	11.6	11.0	8.5–10
Methane yield (mL/g VS)	NA	49–199	NA	260–405
NH <sub>4</sub> -N (mg/L)	120–1200	1853–9000	845–5000	396–5000
References	Heinss et al. (1998), Manga et al. (2016), Niwagaba et al. (2014)	Coetzee et al. (2011), Still and Foxon (2012), Rose et al. (2015), Semiyaga et al. (2017)	Heinss et al. (1998), Koné and Strauss (2004), Rose et al. (2015)	Rajagopal et al. (2013), Rose et al. (2015)

NA not available

<sup>a</sup> This is FS from container-based toilets having a younger age of less than 4 days

which necessitates mixing with FS from other containments to improve characteristics for a good biogas substrate. This can be achieved by introducing a mixing/buffer tank before the digester to aid in homogenization of FS from different containments.

Total solids (TS) for FS from most containments average >6%, which is required for unstirred fixed-dome digesters, in order to limit solids settling (Sasse 1988). In addition TS in the range of 5–10% is optimal for operating anaerobic digesters without addition of extra water (Nijaguna 2002). However, for TS > 4%, there is a need to have fixed-dome digester base slanting towards the middle for easy collection and removal of any settled sludge. The volatile solids (VS) of FS from various sources are >50% TS, which is required for application of fixed-dome digesters. FS temperature in most tropical countries is in the mesophilic range (20–40 °C), which is resistant to operational challenges since this can be achieved at minimal or no extra energy input.

The C/N ratio of FS from most containments is below the optimal requirement (16–25) for AD. This may lead to ammonia accumulation in the digester, which is toxic to methane-forming bacteria. Therefore, it may necessitate raising the C/N ratio during operation, through various ways such as co-digestion with organic feedstocks of a higher C/N ratio. FS can potentially be co-digested with other organic waste streams such as market wastes, municipal solid wastes, brewery waste or primary sludge to improve AD (Englund and Strande 2019).

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### 14.3 Biomethane Potential for Faecal Sludge

Biomethane potential (BMP) measurements provide evidence for biogas production performance from substrates based on hydrolysis and degradation rates (Raposo et al. 2012; Hagos et al. 2017). BMP test is crucial before the full-scale design of anaerobic digesters to predict biogas production. However, since biogas production in real-time conditions faces design limitations, the expected values are lower than laboratory-scale values (Rose 2015). This is because the methane gas yields are under optimal conditions (such as mixing and constant temperature), whereas actual yields are reduced due to various uncertainties in full-scale operation.

BMP experiments, on the other hand, allow comparison between different substrates, and more reliable information can be obtained through setting up pilot-scale digesters (Englund and Strande 2019). FS presents higher theoretical BMP values when degradability is not accounted for. Therefore, fresh FS from container-based sanitation technologies presents higher BMP values, which is more suitable for AD, as compared to other sources of FS. Studies of different types of FS have indicated methane production completed within 10 days, implying that sludge holding times exceeding this period don't improve the yield of biogas (Rose 2015). However, a minimum hydraulic retention time (HRT) of 20 days has been reported for odour reduction (Englund and Strande 2019; Tayler 2018).

## 14.4 Co-digestion of Faecal Sludge with Organic Waste Streams

Co-digestion involves the use of more than one waste stream in anaerobic degradation, aimed at circumventing the limitation of using single substrates, thereby improving biogas production (Hagos et al. 2017). This is a promising technology in most cities or towns of LMIC, where management of different waste streams poses a number of challenges. AD process can co-manage more waste streams, improving biodegradability and nutrient balance.

### 14.4.1 Case Studies on Co-digestion of Faecal Sludge and Organic Waste Streams

Various researchers have determined the implications of co-digesting FS with other waste streams as summarized in Table 14.2. Majority of the studies reviewed were performed in laboratory experiments, with only one that used a field-installed biodigester. The experimental reactors were all batch fed and operated mainly under mesophilic conditions. The feedstocks/substrates added to FS in these studies included organic food waste, garden waste, cattle and poultry manure, sludge and effluent from wastewater treatment plant (WWTP). The performance/dependent variable for the experiments was mainly biogas generation.

From these studies, the volume of biogas produced increased when FS was digested with other wastes than when digested alone. Hoang and Nguyen (2020) noted better biogas quality when co-digested, where a high rise in composition of CH<sub>4</sub> was observed to reach 71.5%. The optimal biogas generation/yield is linked to feedstock type, co-digestion, particle size reduction, operational temperature, C/N ratio and pH. Hoang and Nguyen (2020) obtained a biogas yield of 13 mL/g dry matter (DM) when FS was digested alone, but the yield increased to 18 mL/g DM when FS was mixed with poultry manure (PM), cow manure (CM) and sewage sludge (SS). However, mixing two substrates produced more gas than mixing four substrates. For example, FS with PM produced a biogas yield of 28 mL/g DM, FS and CM produced 25 mL/g DM and FS with SS produced 25 mL/g DM.

Anaerobic digesters operating under thermophilic temperature ranges take shorter time to produce gas compared to those in mesophilic temperature range (Burka et al. 2021). The favourable pH range for AD is near neutral; hence, an initial drop in pH, resulting from acidogenesis and acetogenic oxidation, inhibits biogas generation. However, low pH can be solved through addition of sodium bicarbonate as a buffer (Afifah and Priadi 2017). Another product of acidogenic fermentation that often causes inhibition of anaerobic degradation is ammonia.

Overall, it can be deduced that while biogas can be recovered from FS, a co-feeding substrate is necessary for enhancing AD, thereby improving the quantity and quality of biogas recovery (Table 14.2). This is more so, considering the fact that FS undergoes partial stabilization during collection in on-site containment systems, resulting into a degraded quality with decreased biogas potential. Containments filling for longer time are more affected than those which are filled for shorter

**Table 14.2** Case studies on co-digestion of faecal sludge and other waste streams

Nature of study	Inputs	Operating conditions	Key findings	References
Laboratory experiment	• Faecal sludge	• 51 L stainless steel reactor size	• Initial pH range of 5.2–6.3	Afifah and Priadi (2017)
	• Food waste	• Batch feeding, temperatures	• Ammonia was 240–504 mg/L (below the inhibition level)	
	• Garden waste	• 27–30 °C	• Gradual increase in biogas generated with higher values for higher sludge content (50% FS) 0.56 m <sup>3</sup> CH <sub>4</sub> /kg VS	
		• 25–50% FS based on VS	• High reduction in VS and COD	
		• Buffer was applied on the 10th day	• A methane yield of 10–20-fold greater than the FS digested alone	
Laboratory experiment	• FS from septic tank	• 500 mL constantly stirring reactor vessels	• Biogas yield equalled 13 mL/g DM in 14 days	Burka et al. (2021)
	• Sewage sludge (SS)	• Thermophilic conditions (55 °C)	• Biogas yield for mixed substrates (FS + PM + CM + SS) reached 18 mL/g DM and 28 mL/g for FS + PM, 22 mL/g for FS + CM and 25 mL/g for FS + SS mixtures	
	• Cow manure (CM)	• Period was 14 days		
	• Poultry manure (PM)	• 95% moisture content of substrates		
		• 1:1 feeding ratio		
Laboratory experiment	• FS from pit latrine	• 200 L of plastic drum reactors	• A total of 285 L of biogas was recovered	Madikizela et al. (2017)
	• Anaerobic digester effluent at wastewater treatment plant (WWTP)	• Mesophilic temperature conditions (29 ± 2 °C)	• Biogas can be recovered from pit latrine FS, but a co-feed was necessary for AD to improve the quantity of biogas recovery	
	• Cow manure	• 1:2 mixture FS + WWTP effluent		
		• 2:1 mixture FS + WWTP effluent		
• Cow paunch manure added in all the reactors				
Laboratory experiment	• FS from septic tank	• 4:1, 3:1 and 2:1 mix ratios (by weight) of FS to organic waste	• Highest biogas yield (514.3 L/kg VS) for 3:1 mix ratio	Phuong and Thai (2018)
	• Organic solid waste			

(continued)

**Table 14.2** (continued)

Nature of study	Inputs	Operating conditions	Key findings	References
Laboratory experiment	• FS from septic tank	• 500 mL volume, continuous stirred reactors	• FS-specific methane yield from 269.3 N mL CH <sub>4</sub> /g VS	An (2017)
	• Sewage sludge (primary sewage sludge and waste activated sludge (WAS))	• Mesophilic temperature (35 ± 0.5 °C)	• Only WAS digested	
		• Feeding ratio, WAS only; FS, WAS of 1:6, 1:3, 1:2 and 1:1 (VS content)	• Higher value 294.8 N mL CH <sub>4</sub> /g VS in case of co-digestion, with a ratio of FS:WAS of 1:1 (VS content)	
		• pH range of 7.17–7.78		
Composite digester placed 1.9 m below the ground	• Faecal sludge (public and household toilets)	• Winter temperature of 16–18 °C and summer temperature from 30 to 32 °C	• Temperature raised to 35–38.2 °C, in 0–9 days thereafter, decreased to 32–33 °C after 30 days. Summer registered high temperature noted in summer	Hoang and Nguyen (2020)
		• Organic solid waste	• pH dropped from 6.5 to 6.8 within the first 6 days and raised after to 7.4	
	• 3:1 mix ratio (FS, organic waste)	• Largest and fastest biogas generation realized at higher temperatures (summer)		
		• Maximum daily biogas production obtained during summer and ranged from 2768 to 3670 NL/day (winter) compared to 3033–3917 NL/day during summer		
		• Methanogenesis took place when conditions were suitable for AD between the 13th and 25th day. The digester heated to 35–38 °C, pH was 7–7.4 and alkalinity was 2400–2900 mg CaCO <sub>3</sub> /L		
			• CH <sub>4</sub> varied from 20.4% to 31.6% at the start to 64.4–71.5% mid-way and 67.3–69.2% towards the end	

(continued)



**Table 14.2** (continued)

Nature of study	Inputs	Operating conditions	Key findings	References
Laboratory experiment	• Faecal sludge	• Glass digesters	• About 6.2 tonnes/DM of biomass per day	Krou et al. (2021)
	• Solid waste	• Normal environmental conditions	• 122 m <sup>3</sup> of biogas, of which 113 m <sup>3</sup> of methane could be produced per day • The methane content was estimated at 65.6%	
Laboratory experiment	• FS from septic tank	• Hydrothermal pre-treatment (HTP) in a high-pressure vessel at 180 °C and 10 bars for 30 min	• Specific methane production (SMP) of STS reached 211.6 mL/g COD, ≈41.3% of the theoretical methane production (TMP, 350 mL/g COD)	Zhang and Li (2010)
	• Food waste	• 500-mL continually stirred reactor bottles	• After THP, SCOD increased from 960 to 2010 mg/L, 70% of organic matter remained in solid particles	
		• Operation temperature 35 ± 1 °C	• An increase in SMP to 250.6 mL/g COD (52.4% of the TMP) was noted	
		• Substrate ratios of FS: food waste = 1:0, 1:1, 2:1, 0:1 (based on COD)	• While screening the fine particles, the SMP increased to 274.9 mL/g COD • Overall, methane yield increased owing to THP, although methane production rate didn't improve significantly • Co-digestion of FW with filtrate after the THP of STS increased the SMP, and the values were 213.8 mL/g COD for the filtrate, 220.5 mL/g COD for the ratio of 2:1, 251.3 mL/g COD for 1:1 and 309.1 mL/g COD for only the FW	

(continued)

**Table 14.2** (continued)

Nature of study	Inputs	Operating conditions	Key findings	References
Laboratory experiment	• Untreated primary sludge	• Batch digestion tests performed in 2.5-L digester filled to 80%	• Highest yield of biogas was from AUPS/RCM and SUPS/RCM concoctions mixed in the ratio of 10:90 and 90:10	Hassan et al. (2022)
	• Biomass	• Mesophilic under 35 °C	• In all treatments, the biogas production increased suddenly within the first days of digestion, gradually decreasing thereafter	
	• Raw chicken manure	• Each glass was 2.5 L filled to 80% in all reactors	• Statistical significance was observed between biogas rates and low total coliforms ( $p$ 0.001) and faecal coliforms ( $p$ = 0.002)	
	• Fresh untreated primary sludge (UPS) collected from municipal wastewater treatment plant	• All reactors were gently mixed by hand for around 1 min/day at the start of each biogas determination	• pH was optimum at 7.0, giving the best biogas products. A typical pH range for optimal biogas production is 6.5–7.6	
	• Raw chicken manure (RCM)	• Six different mixing mass ratios of 100:0, 90:10, 50:50, 30:70, 10:90 and 0:100 were tested to obtain the best combination of untreated primary sludge		
	• South Valley University (SUPS sludge)			
Laboratory experiment	• Food waste (FW) septage were septic tanks	• Mix ratios FW and septage	• Yield in biogas from FW was 647–952 mL/g VS 89–96 mL/g VS from septage	Prabhu et al. (2015)
		• Mix ratios were (FW: septage) 1:1, 1.5:1, 2:1, 1:1.5 and 1:2	• Co-digestion studies with FW: septage at 1:2 ratio produced 2896 m <sup>3</sup> /day of biogas	
			• FW alone, which lacked zinc, cobalt and iron produced less biogas	
			• Co-digesting septage and FW improved AD of FW at limiting values of Zn, Co and Fe	

time, where FS from CBS systems preferred for biogas production in decentralized FSTPs.

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## 14.5 Anaerobic Digestion Products

The leading AD products (biogas and bio-slurry) produced from digestion and/or co-digestion of FS and other substrates such as organic solid waste, cattle, pig and buffalo dung have immense benefits. Biogas offers an alternative clean and modern energy source that can replace dirty biomass fuels, with the potential to contribute to poverty alleviation. Bio-slurry can boost agriculture production with recyclable sustainable nutrients. Utilization of both biogas and bio-slurry from AD can contribute towards alleviating climate change-induced impacts (Warnars and Oppenoorth 2014). The products of AD are discussed in the following sections.

### 14.5.1 Biogas Use Alternative

Biogas can be put to use in various ways such as cooking, lighting or driving engines of vehicles or other machineries. The latter is applicable for large-scale systems or treatment plants, where the produced heat can also be put to use. At decentralized FSTPs, biogas can be used to meet the cooking requirements of the workers at the plant and heat requirements for stabilization of equipment for the plant laboratory.

The biogas production patterns at the FSTPs do not match consumption. Gas usage mainly happens during the day, but production continues throughout the night. In cases of low biogas usage at the treatment plant, there may be a need to package the gas in gas storage bags to be used at a different location. However, biogas has a limitation of low energy density ( $6 \text{ kW h/m}^3$ ), which necessitates large storage volumes unless it is compressed. The option of storage in compressed medium to high-pressure gas cylinders is not feasible for decentralized FSTPs due to high costs involved (Vögeli et al. 2014).

In cases where packaging is not feasible and/or gas utilization patterns are interrupted such as non-functioning gas stoves, biogas needs to be flared in order to control methane release to the environment. Therefore, a gas burner for use in flaring needs to be considered in the design of FSTPs based on AD technology.

### 14.5.2 Bio-slurry Management and Uses

The effluent from the digester after gas production is known as bio-slurry or digestate. Like most digesters in developing countries, which operate under mesophilic temperature zone, the generated slurry at FSTPs still has high levels of pathogens; hence it cannot be used in the same 'as generated' state unless further treatment is done.

Bio-slurry has high water content; hence there is a need to be dewatered before use. Dewatering can be done using the common existing technologies such as sand drying beds. The liquid effluent from the drying beds percolates downwards through the filter media, while the solid fraction is retained on the beds, which is later dried. Dried bio-slurry has calorific value in the same range as FS; hence it can be put to similar uses such as production of fuel briquettes, soil conditioner, vermi-compost, animal feeds and protein-rich supplement or bricks for construction work (Semiya et al. 2015). However, cost (capital, operation and maintenance) assessment of the required technologies for various products needs to be considered, since this has been reported to be challenging in sustaining the decentralized plant operations (Massoud et al. 2009).

Where agricultural activities exist without food crops to be eaten raw, or in tree farming, bio-slurry can be applied without further treatment. It can also be applied in agriculture with deep row entrenchment. Furthermore, pathogens are not assimilated in plant material (roots, shoots, leaves or fruits). Therefore, health risks associated with the use of microbiologically contaminated bio-slurry can be averted by implementing a multi-barrier approach (WHO 2006) in combination with the sanitation safety planning approach (WHO 2016). The advantage with the use of bio-slurry in agriculture is that it contains plant nutrients, which are already mineralized and therefore readily available to the crops. This is as opposed to unstabilized organics, which, when applied in agriculture, must undergo several conversion processes before they are available to the crops. Moreover, slurry application in agriculture, as a replacement to synthetic fertilizers, not only replaces finite resources but also adds humic substances to soil. Humic substances are not present in synthetic fertilizers. The end result of using bio-slurry is to replenish soil fertility, which contributes to cycling of plant nutrients, leading to sustainable agriculture.

The liquid effluent stream after dewatering joins the liquid line of the FSTP, which is later discharged or reused after treatment. Liquid effluent is a plant nutrient-rich irrigation resource although it should not be applied on plants which are eaten raw such as vegetables and root crops such as cassava and potato, since the treatment system at most FSTPs does not eliminate viruses and bacteria. Therefore, the most practical means of managing liquid effluent stream is through disposal into the environment after treatment; hence the presence of a sink such as a wetland is crucial when locating a treatment plant, based on AD.

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## 14.6 Case Studies on Decentralized Scale Anaerobic Digesters

Although available literature indicates the viability of AD, in sanitation, solid waste management and energy recovery, only a few deals with its application in FS treatment at the decentralized scale. This section provides an overview of twelve (12) documented case studies, which are limited to geographic areas where FS management is prominent, such as sub-Saharan Africa and South Asia. Nine (9) documented cases of AD plants are in operation at the full scale, and two

(2) are pilot scale, while one (1) is an experimental digester (Table 14.3). Most FSTPs have adopted a fixed-dome biogas digester, which is preceded by a screen chamber that receives the FS from the desludging vehicles. FS collected from pit latrines is reported to have high municipal solid waste content, which is problematic to AD processes. Therefore, application of screening stage before the digester unit at the treatment plant holds the key (Zziwa et al. 2016). Digesters handling FS should be positioned after the reception and preliminary treatment facilities. Ideally, the extraneous materials such as solid wastes and grit need to be removed by screening and grit removal units, since these waste streams do not contribute to the biogas production. In addition, FS after screening has particle sizes of less than 5 mm, which is reported to be in a range required to have onset of AD (Semiyaga et al. 2017).

After screening and/or grit removal, a homogenization/feeding tank is applied in a number of treatment plants to prevent shock loading of the digesters, since FS arriving at the treatment facility has varying characteristics. For example, at the Devanahalli (India), a treatment plant receives FS from septic tanks and soak pits into a feeding tank, where settling takes place. The anaerobic digester only receives the settled solid fraction, while the liquid stream is treated in other proceeding units (Rao et al. 2020). The anaerobic digesters in this case are not stirred. On the other hand, the two case studies cited in China make use of continuously stirred tank reactors (CSTR) (Shikun et al. 2017). Stirring helps in shortening the hydraulic retention time; hence it can be ideal where large volumes of FS are to be digested. However, there is more energy involved in operation of the stirred reactors.

Most anaerobic digesters are reported to operate under mesophilic conditions, with the exception of the CSTR that operated either under mesophilic or thermophilic conditions. The biogas digesters vary in size with retention time of the FS ranging from 4 h (for the UASB) to 20–45 days in case of unstirred reactors. From the cases reported in Table 14.3, two value propositions are noted: (1) biogas that is often used for heating and lighting at the treatment plant and (2) bio-slurry that is processed to form a compost or soil conditioner. In one case (Nashik, Maharashtra, India), where FS and organic solid wastes are co-digested, the biogas is purified and used to generate electricity.

Finally, two cases reported successful operation and maintenance (O & M) of the plants by municipalities (Rao et al. 2020; Rath and Schellenberg 2020). However, some of the challenges cited from the O & M include non-operation of the plants to their full capacity owing to small volumes of FS collected from the communities. Commercialization of biogas generated presented a challenge, as its cost is found to be higher than the use of alternative energy sources. As such biogas is often used within the treatment plants. The sale of compost is also limited, which was attributed to limited farmlands within the plant location. Therefore it was opted for use in landscaping within the municipalities.

**Table 14.3** Case study faecal sludge treatment plants using anaerobic digestion as a treatment step

Digester location	Type	Core technology	Capacity	Feedstocks	HRT	Value proposition	Institutional setup and revenue base	Other finding challenges/recommendations	References
Devanahalli, Bangalore, India	Fixed-dome	Screening, feeding tank, biogas digesters, stabilization tank (ST), drying bed, co-composting unit, anaerobic baffled reactor (ABR) and filter, horizontal gravel filter (HGF) planted with macrophytes and percolation pit	6 m <sup>3</sup>	Faecal sludge from septic tanks and soak pits	12–14 days	Compost and treated water	O & M contracted to Kam-Avida Enviro Engineers Private Limited	Operating below the capacity, occasioned by low demand for FS desludging from containments	Rao et al. (2020), Rath and Schellenberg (2020)
Beijing International Airport, China	CSTR, mesophilic, 625 m <sup>3</sup> comprising of 400 m <sup>3</sup> (main digester; +225 m <sup>3</sup> digestate storage)	Pre-treatment (screens and homogenization mixer), disinfection (pasteurization batch basin) and anaerobic digester	20 tonnes (average)	Faecal sludge	20 days	200–400 m <sup>3</sup> /day of biogas, effluent and slurry use in nearby greenhouse	Owned by village committee. Revenue from sale of organic vegetables or fruits	Almost no smell reported	Shikun et al. (2017)
Beijing, China	CSTR, thermophilic, 180 m <sup>3</sup> × 2	Separation, pyrolysis, pulping, coagulation, composting, AD, liquid treatment (anaerobic, oxic and MBR)	300 tonnes of FS and 50 tonnes of kitchen waste (KW) per day	FS and kitchen waste	2 days	800–1500 m <sup>3</sup> /day of gas, biodiesel and compost	Owned and operated by the local government. Revenue from disposal fees for FW and KW and sale of biodiesel	Odour management by sealing and use of wooden bio-filter	
Chazanga, Lusaka, Zambia	Fixed-dome	Intake, anaerobic digester, settling tank, sludge receptor tank,	50 m <sup>3</sup>	Pit latrine FS (from manual emptiers)	20 days	Biogas and soil conditioner	Owned by water utility company and managed by community-based	Failure of selling biogas to nearby houses (landlords unwilling to give	Soeters et al. (2021)

(continued)

Table 14.3 (continued)

Digester location	Type	Core technology	Capacity	Feedstocks	HRT	Value proposition	Institutional setup and revenue base	Other finding challenges/recommendations	References
		unplanted drying bed, dried sludge storage, gravel filter and polishing pond with a soak pit					organization (Kanyama and Chazanga Water Trusts) Revenue from proposed sale of soil conditioner and biogas used within the FSTP	consent to tenants to get a gas connection) Higher gas proposed gas prices compared to other sources (coal and electricity)	
Upper Citarum, Indonesia	Pilot scale	Screening, equalization tank, anaerobic digester (tank prefabricated from PVC with metal frame support) and drying beds	1.4 m <sup>3</sup>	Domestic wastewater and FS (5% FS loading)					Hastuti et al. (2021)
Kanyama, Lusaka, Zambia	Fixed-dome	Intake, anaerobic digester, settling tank, sludge reception tank, unplanted drying bed, storage area for dried sludge, gravel filter and polishing pond with a soak pit	50 m <sup>3</sup>	Pit latrine FS (from manual emptiers)	20 days	Biogas and soil conditioner	Owned by water utility company and managed by community-based organization (Kanyama and Chazanga Water Trusts) Revenue from proposed sale of soil conditioner and biogas used within the FSTP		

Warangal, Telangana, India	Fixed-dome digester and geo-bag (pilot demo)	Screening and homogenization	15 m <sup>3</sup>				Biosolids use a soil conditioner	No current business model		
Brahmapuram, Kerala, India	UASB reactor	Sludge retention tank, UASBR, sludge drying beds (solid fraction)	30 m <sup>3</sup>	Faecal sludge	4–5 h		Biogas used in heat exchanger to heat and keep UASBR at 37 °C	Revenue funding from sale of compost and FS discharge fees	Revenue from reuse is not feasible; thus focus is on generation	Rao et al. (2020)
Nashik, Maharashtra, India	Fixed-dome	Biodigester, pasteurization unit, biogas scrubbing, CHP, power grid	30 TPD of waste	Faecal sludge and organic waste			Purified biogas generates 2200–3300 kW h/day of electricity and soil conditioner	Financial support from the municipal council could benefit from selling soil conditioner		
Maseru, Lesotho	Fixed-dome	Sedimentation and floatation		FS mixed with organic solid waste						Müller (2009)
Kumasi, Ghana	Geo-bag digester	Pilot scale	8 m <sup>3</sup> volume in series				Biogas			Taylor (2018)
Antananarivo, Madagascar	Geo-bag digester	Geo-bag digester, upward flow anaerobic filter	4 no. each 40 m <sup>3</sup>	Faecal sludge						
Grahamstown, South Africa	Modified plastic drums	Experimental setup	200 L	Faecal sludge, WWTP digestate, bovine paunch manure	45 days		Biogas		No recovery of biogas from only pit latrine FS	Madikizela et al. (2017)
									Biogas recovery with co-digestion	



## 14.7 Operation and Maintenance of Biodigesters at FS Treatment Stations

There is a need to develop and implement an operation and maintenance (O & M) strategy that includes a task schedule and allocation of responsibilities and having control mechanisms for proper checking of the completed duties. Some of the specific O & M considerations for anaerobic reactors at FSTPs start from feeding digesters, regular monitoring and periodic maintenance.

The anaerobic digester is fed regularly to maintain stable gas production; hence, the design has to be adequate for the routinely delivered FS quantities and co-digestion substrates (if any). The alternative organic wastes for co-digestion should be pre-treated to remove impurities (such as metals, glass and plastics) and reduce particle sizes to <5 cm. This is necessary to raise the surface area for microorganisms to access and degrade the material faster. This is relevant for AD, where the microorganisms are slow degrading.

For periodic maintenance purposes, solids that settle or accumulate at the digester bottom are not easy to remove, particularly in fixed-dome digester type. Tayler (2018) proposed periodic removal of the settled solids (sludge) using vacuum trucks, leaving the digester for several days to reduce risk from dangerous gases and then manually emptying the residual sludge. This can be achieved by considering design of two digesters operating in parallel. However, precaution should be taken by the workers to use appropriate personal protective equipment (PPE) with breathing apparatus.

Other general monitoring activities can be regularly checked, such as gas tightness of the pipes and dome, pipe blockages, slurry levels in the expansion chamber and biogas stove (where cooking is an option) (Vögeli et al. 2014).

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## 14.8 Conclusions

Faecal sludge treatment plants in LMIC have a huge AD feedstock potential. This potential is driven by the majority of the population in these areas using on-site sanitation technologies, where FS is generated. Most of the existing FS treatment plants do not make use of the available FS feedstock to recover energy in the form of biogas. The characteristics of FS such as TS, pH, temperature and BMP depict potential for biogas production. The fresher the FS, the higher is the potential to generate large quantities of FS. AD will suit the treatment of FS, if other benefits from the process such as organic matter stabilization as well as reduction of pathogen and odours are aggregated. To achieve increased biogas production from FS, its low C/N ratio should be boosted by co-digestion with other organic waste materials. The logistics of collecting source-separated organic solid wastes and delivery to where the AD plant is located should be analysed in view of the costs involved and the benefits to be realized. The use of bio-slurry in agriculture, although advantageous, since AD mineralizes nutrient content, making it readily available to crops, is riddled with its pathogenic content due to insufficient sanitization occurring, in AD. The

microbial health risks involved can be circumvented by applying in fertilizing crops not to be consumed raw, deep row entrenchment and implementation of multi-barrier approaches in combination with sanitation safety planning.

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## References

- Afifah U, Priadi CR (2017) Biogas potential from anaerobic co-digestion of faecal sludge with food waste and garden waste. *AIP Conf Proc* 1826:020032. <https://doi.org/10.1063/1.4979248>
- An VTH (2017) Bio-mathane potential test for anaerobic co-digestion of faecal sludge and sewage sludge. *Vietnam J Sci Technol* 55(4C):27
- Burka MS, Basamykina AN, Kharlamova MD (2021) Technological features of biogas production while anaerobic co-digestion of faecal sludge, sewage sludge and livestock. *IOP Conf Ser Earth Environ Sci* 666(4):042052. <https://doi.org/10.1088/1755-1315/666/4/042052>
- Coetzee MAA, Van Der Merwe MPR, Badenhorst J (2011) Effect of nitrogen loading rates on nitrogen removal by using a biological filter proposed for ventilated improved pit latrines. *Int J Environ Sci Technol* 8(2):363–372
- Englund M, Strande L (2019) Faecal sludge management: highlights and exercises. *J Chem Inf Model* 53(9):1
- Hagos K, Zong J, Li D, Liu C, Lu X (2017) Anaerobic co-digestion process for biogas production: progress, challenges and perspectives. *Renew Sust Energ Rev* 76:1485–1496. <https://doi.org/10.1016/J.RSER.2016.11.184>
- Hassan M, Aboelenin SM, Soliman MM, Attia HF, Metwally KA, Salem HM, Khalaphallah R (2022) Biogas manufacture from co-digestion of untreated primary sludge with raw chicken manure under anaerobic mesophilic environmental conditions. *Saudi J Biol Sci* 29(4): 2969–2977
- Hastuti E, Riyana R, Joy B, Supratman U, Pamekas R (2021) Integrated community onsite sanitation system for close loop faecal management. *E3S Web Conf* 249:01005. 1–10
- Heinss U, Larmie SA, Strauss M (1998) Solids separation and pond systems for the treatment of septage and public toilet sludges in the tropics – lessons learnt and recommendations for preliminary design. *EAWAG, Duebendorf*
- Hoang LP, Nguyen KT (2020) Application of composite biogas tank in anaerobic co-digestion faecal sludge and organic waste. In: *Sustainable waste management: policies and case studies*. Springer, Singapore, pp 517–526
- Koné D, Strauss M (2004) Low-cost options for treating faecal sludges (FS) in developing countries - challenges and performance. In: *IWA Specialist Group Conference on Waste Stabilization Ponds, 27 September - 1 October 2004, Avignon, France*
- Krou NM, Baba G, Akpaki O (2021) Estimation of the Amount of Electrical Energy Available from the Biogas Produced at the Faecal Sludge Treatment Plant in the City of Sokodé. *TH Wildau Eng Nat Sci Proc* 1:77–82. <https://doi.org/10.52825/thwildauensp.v1i.13>
- Madikizela P, Laubscher R, Tandlich R, Ngqwala N (2017) Optimizing biogas recovery from pit latrine faecal sludge. In: *5th International Conference on Sustainable Solid Waste Management*, pp 1–10
- Manga M, Evans BE, Camargo-Valero MA, Horan NJ (2016) Effect of filter media thickness on the performance of sand drying beds used for faecal sludge management. *Water Sci Technol* 74(12): 2795–2806. <https://doi.org/10.2166/wst.2016.451>
- Massoud MA, Tarhini A, Nasr JA (2009) Decentralized approaches to wastewater treatment and management: applicability in developing countries. *J Environ Manag* 90(1):652–659. <https://doi.org/10.1016/j.jenvman.2008.07.001>
- Müller C (2009) Decentralised co-digestion of faecal sludge with organic solid waste: case study in Maseru, Lesotho. In: *Water and sanitation in developing countries*. Government of Canada, Ottawa, ON

- Nijaguna B (2002) Biogas technology. New Age International (P) Ltd, New Delhi
- Niwagaba CB, Mbéguéré M, Strande L (2014) Faecal sludge quantification, characterisation and treatment objectives. In: Strande L, Ronteltap M, Brdjanovic D (eds) Faecal sludge management - systems approach implementation and operation. IWA Publishing, London, pp 15–42
- Puong HL, Thai NTK (2018) The potential of biogas recovery from anaerobic co-digestion of fecal sludge and organic waste. In: Achi C, Gedam V (eds) Utilization and management of bioresources. Springer, Singapore, pp 113–121. <https://doi.org/10.1007/978-981-10-5349-8>
- Prabhu M, Waigaonkar S, Dube R, Walther D, Mutnuri S (2015) Anaerobic co-digestion of food waste and septage—a waste to energy project in Nashik city. Carbon Sci Technol 7(2):87–98
- Rajagopal R, Lim JW, Mao Y, Chen CL, Wang JY (2013) Anaerobic co-digestion of source segregated brown water (feces-without-urine) and food waste: for Singapore context. Sci Total Environ 443:877–886
- Rao KC, Velidandla S, Scott CL, Drechsel P (2020) Business models for fecal sludge management in India, vol 18. IWMI, New Delhi. <https://doi.org/10.5337/2020.209>
- Raposo F, De La Rubia MA, Fernández-Cegrí V, Borja R (2012) Anaerobic digestion of solid organic substrates in batch mode: an overview relating to methane yields and experimental procedures. Renew Sust Energ Rev 16(1):861–877. <https://doi.org/10.1016/J.RSER.2011.09.008>
- Rath M, Schellenberg T (2020) Decentralized wastewater and Faecal sludge management: case studies from India, vol 4. Asian Development Bank Institute, Tokyo
- Rose C (2015) Developing a nutrient recovery process for recovering nutrients in anaerobic digestate in low income countries. Cranfield University, Cranfield
- Rose C, Parker A, Jefferson B, Cartmell E (2015) The characterization of feces and urine: a review of the literature to inform advanced treatment technology. Crit Rev Environ Sci Technol 45(17): 1827–1879. <https://doi.org/10.1080/10643389.2014.1000761>
- Sasse L (1988) Biogas plants. German Appropriate Technology Exchange (GATE) and German Agency for Technical Cooperation (GTZ), GmbH. <http://www.borda-net.org/modules/wfdownloads/viewcat.php?cid=5>
- Schoebitz L, Bischoff F, Ddiba D, Okello F, Nakazibwe R, Niwagaba C, Lohri CR, Strande L (2014) Results of faecal sludge analyses in Kampala, Uganda (Issue April). [http://www.eawag.ch/fileadmin/Domain1/Abteilungen/sandec/publikationen/EWM/Laboratory\\_Methods/results\\_analyses\\_kampala.pdf](http://www.eawag.ch/fileadmin/Domain1/Abteilungen/sandec/publikationen/EWM/Laboratory_Methods/results_analyses_kampala.pdf)
- Semiyaga S, Okure MAE, Niwagaba CB, Katukiza AY, Kansiime F (2015) Decentralized options for faecal sludge management in urban slum areas of Sub-Saharan Africa: a review of technologies, practices and end-uses. Resour Conserv Recycl 104:109–119. <https://doi.org/10.1016/j.resconrec.2015.09.001>
- Semiyaga S, Okure MAE, Niwagaba CB, Nyenje PM, Kansiime F (2017) Dewaterability of faecal sludge and its implications on faecal sludge management in urban slums. Int J Environ Sci Technol 14(1):151–164. <https://doi.org/10.1007/s13762-016-1134-9>
- Shikun C, Lei Z, Mingyue Z, Xue B, Zifu L, Mang H (2017) Assessment of two faecal sludge treatment plants in urban areas: case study Assessment of two faecal sludge treatment plants in urban areas: case study in Beijing. Int J Agric Biol Eng 10(3):237–245. <https://doi.org/10.3965/j.ijabe.20171003.3067>
- Soeters S, Mukheibir P, Willetts J (2021) Treatment technologies in practice: on-the-ground experiences of faecal sludge and wastewater treatment. SNV Netherlands Development Organisation, The Hague
- Still D, Foxon K (2012) Tackling the challenges of full pit latrines. Volume 2: How fast do pit toilets fill up? A scientific understanding of sludge. WRC, Gezina
- Taylor K (2018) Faecal sludge and septage treatment: a guide for low and middle income countries. In: Faecal sludge and septage treatment. Practical Action Publishing, Rugby. <https://doi.org/10.3362/9781780449869>

- Vögeli Y, Lohri CR, Gallardo A, Diener S, Zurbrügg C (2014) Anaerobic digestion of biowaste in developing countries: practical information and case studies. Swiss Federal Institute of Aquatic Science and Technology (EAWAG)-SANDEC, Duebendorf
- Warnars L, Oppenoorth H (2014) A study on bioslurry results and uses
- WHO (2006) WHO guidelines for the safe use of wastewater, excreta and greywater. Volume 1: Policy and regulatory aspects. WHO, Geneva
- WHO (2016) Sanitation safety planning: manual for safe use and disposal of wastewater, greywater and excreta. WHO, Geneva
- Zhang Y, Li H (2010) Enhancing biogas production from septic tank sludge via hydrothermal pre-treatment and co-digestion with food waste, p 5–6
- Zziwa A, Lugali Y, Wanyama J, Banadda N, Kabenge I, Kambugu R, Kyazze F, Kigozi JB, Tumutegyeize P (2016) Contextual investigation of factors affecting sludge accumulation rates in lined pit latrines within Kampala slum areas, Uganda. *Water SA* 42(3):490–495