Anaerobic Treatment System: A Sustainable Clean Environment and Future Hope of Renewable Energy Production

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Abstract Anaerobic digestion (AD) of organic wastes is a popular biological treatment method. It is a useful technology in waste management and environmental health especially for mitigating greenhouse gases (GHSs). It is an economic process that treats a wide range of low- to high-strength organic materials for the production of value-added products such as feed biobased products and bioenergy through a diverse group of microorganisms. Several anaerobic digestion systems have been widely employed to treat both domestic and industrial wastes before they are discharged into the environment. The application of anaerobic technologies is considered a significantly viable economically sustainable system for treatment of both solid and liquid wastes. Its benefits include removal of organic matter, high

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Mahmoud Nasr and Abdelazim M. Negm (eds.),
Cost-efficient Wastewater Treatment Technologies: Engineered Systems,
Hdb Env Chem (2023) 118: 169–198, DOI 10.1007/698_2021_792,

treatment efficiency, pathogens removal, production of renewable energy, capable of power generation at a low cost, and less biomass production. Nonetheless, this chapter is a review of the following: different anaerobic digestion systems in the treatment of waste products; the bioeconomic and social importance of using anaerobic reactor for biofuel production and methods of identification and quantification of microbial consortia in an anaerobic reactor. The review further highlights the role of different methanogens as the major group of archaea for biogas production. Other ways to increase biofuel generation are also explored. The chapter concludes that environmental and economic challenges in waste management and energy resource scarcity could be alleviated sufficiently using an anaerobic digestion system.

Keywords Anaerobic reactor, Bioeconomic, Biogas, Methanogens, Wastes management

1 Introduction

Increase in energy demand, persistent interruption in power supply, and emission of greenhouse gases (GHGs) have led to quest for alternative energy sources in recent years. The use of traditional fossils fuels for energy production is non-renewable and the causes of many environmental issues, like GHGs emissions, global warming, and oil spillage [1, 2]. These environmental issues have led to the need for alternative energy production strategies with less adverse environmental and health effects. Therefore, anaerobic production of bioenergy is a sustainable process for converting organic waste into energy and value-added chemicals through various conversion pathways and treatment methods. For many years, biotechnological production of biofuels such as biomethane, biohydrogen, biodiesel, and bioethanol has been under examination [3]. To this end, the production of bioenergy has been identified as an alternate energy source to substitute fossil fuel with little negative effects on the environment and health [1].

2 Overview of Anaerobic Technologies for Waste-to-Energy Management

Anaerobic digestion technology has proven to be an established oldest biotechnological tool for bioconversion of complex organic wastes produced by human societies. This process takes place in a warm and airtight container (the reactor) where, thorough mixing during treatment creates the ideal conditions for microorganisms to ferment the organic matter into biofuel and useful slurry that can be used as fertilizers [4, 5]. Anaerobic systems' benefits make this method a better alternative

Table 1 Comparison of aerobic and anaerobic digestion [6]

Parameter	Aerobic digestion	Anaerobic digestion
Start-up	Short start-up period	Long start-up period
Space requirement	Large space required for the reactor.	Compact reactor with small area requirements
State of development	Established technology	Still under development for specific applications
Process	Integrated nitrogen and phosphorus removal possible High sludge formation Large reactor volume necessary High nutrient requirement	No significant nitrogen or phosphorus removal. Post-treatment is required for nutrients removal Less sludge formation (5–20%) Small reactor volume and simple configuration Low nutrient requirement
Carbon balance	50–60% conversion of organic materials degradation into CO_2 40–50% conversion of organic materials degradation into biomass	95% conversion to biogas 5% into microbial biomass
Energy balance	Requires a large amount of process energy 60% of available energy is used in new biomass; 40% lost as process heat	Requires less energy instead produces bioenergy 90% retained as CH_4, 3–5% is lost as heat, and 5–7% is used in new biomass formation
Residuals	Excess sludge production No need for post-treatment	Biogas, nitrogen mineralized to ammonia Post-treatment required for removal of remaining organic matter and malodorous compounds
Costs	High operating costs for aeration, additional nutrient, and sludge removal, and maintenance	Often moderate investment costs Low operating costs due to low power consumption and additional nutrients hardly required

for waste treatment and generation of bioeconomic by-products when compared with the aerobic treatment of waste materials (Table 1) [7, 8].

2.1 *Anaerobic Reactor Types*

In the last three decades, different reactor technologies have been developed and installed to stabilize the sludge and degrade organic matter present in wastewater and solid wastes [9]. Among these technologies are up-flow anaerobic sludge bed (UASB) reactor [10–12], up-flow anaerobic solid-state reactor (UASS), anaerobic plug-flow reactor (APFR), expanded granular sludge bed (EGSB) [13], membrane bioreactor (MBR) [14], and hybrid upflow anaerobic sludge-filter bed (UASFB) [15]. Among other reactors that have been widely used for wastes treatment are anaerobic contact reactor (ACR), anaerobic baffled reactor (ABR), anaerobic sequencing batch reactor (ASBR) [16], continuous stirred tank reactors (CSTR) [2, 17, 18], anaerobic fixed-bed reactors (AFBR), and membrane technology [19, 20]. These reactors have been reported to treat different types of low- and high-strength wastewater such as brewery, cheese whey, palm oil mill, pharmaceutical compounds, and hospital wastewaters among other industrial wastes [2, 13, 21, 22]. However, the EGSB, CSTRs, and UASB reactors are the most widely used to treat high-rate anaerobic reactor and biogas production [23].

2.1.1 Continuous Stirred Tank Reactors (CSTRs)

Lagoons and continuous stirred tank reactors (CSTRs) are the simplest anaerobic systems [18]. The CSTR is also known as a vat- or back mix reactor, [24, 25] and its runs at the steady-state condition with a continuous and uniform feed of substrates and products' removal. Lagoon system that has no unique sludge retention because hydraulic retention time (HRT) is equal to the sludge retention time (SRT); thus, reducing the suspended biomass concentration and limiting the biological treatment capacity [26]. Unlike lagoon system, when sludge is introduced into a CSTR, an impeller or biogas blowers stir the liquor to ensure proper mixing while the outflow from the CSTR is moved into the settler. In the settler, the treated liquid separates from the biomass then returns the biomass to the CSTR (Fig. 1). This reactor is very efficient in treating different organic-rich wastewater [27]. However, it has limitations such as high operational costs, labor-intensive, and operates at relatively low volumetric loading rates due to the flocculent. It also dilutes the nature of the

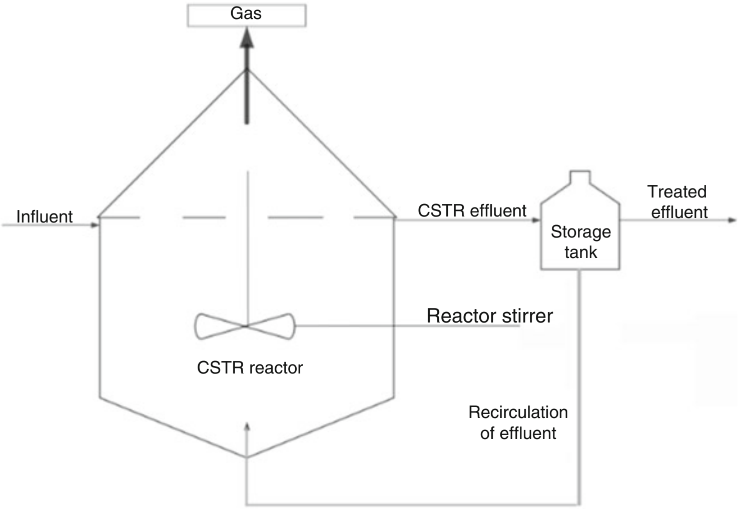


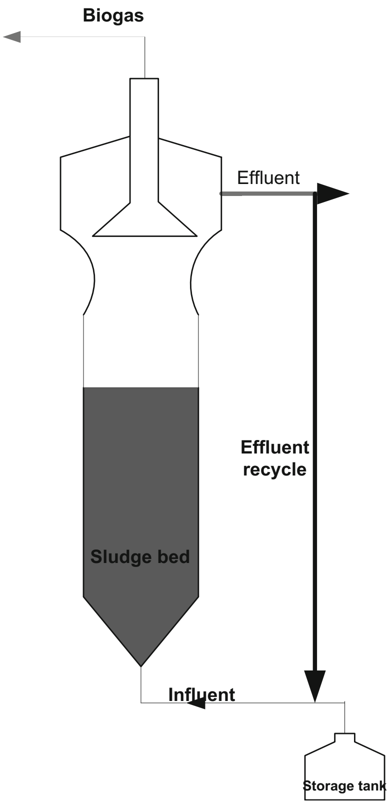
Fig. 1 Schematic diagram of a continuous stirred tank reactor (CSTR)

anaerobic sludge [18, 28]. Although, the CSTR is simple to operate, but less efficient in effluent quality than other reactor technologies [7].

2.1.2 Expanded Granular Sludge Bed (EGSB) Reactor

The use of effluent recirculation combined with a high height/diameter ratio reactors resulted in expanded granular sludge bed (EGSB) reactor [29, 30]. EGSB combines both characteristics of upflow sludge blanket (USB) and biofilm fluidized bed (BFB) processes [31]. It is considered to be a completely mixed tank digester in which both the EGSB and UASB processes use granular anaerobic biomass. They have the same operational principles but differ in terms of geometry and process parameters. A high superficial liquid velocity is applied in this type of reactor for wastewater to pass through the sludge bed while the biomass is present in a granular form. The upflow liquid velocity (10 m/h) causes the granular sludge bed to expand, which enabled the elimination of dead zones resulting in better sludge wastewater contact with the granules for better gas production (7 m/h) [10]. An increase in upflow velocity rate is accomplished by either tall reactor dimension or recirculation of effluent or both (Fig. 2) [32] while hydraulic condition determines the structure of biofilm in the

Fig. 2 Schematic diagram of an expanded granular sludge bed (EGSB) reactor



reactor. The high load and mass transfer also affect the microbial proliferation and matrix of the reactor granules [30]. The EGBS reactor is highly efficient for treating low strength wastewater (WW) (0.7–0.9 g COD/L) with good granular composition for biogas production [33, 34]. Likewise, EGSB can be operated as an ultra-high load anaerobic reactor (up to 30 kg COD/m^3/day) to treat effluents from chemical, biochemical, and biotechnological industries [13, 30].

Besides, there is a problem of instability of the granular conglomerates during continuous operation, washout, and granule disintegration due to high upflow velocities application in the EGSB reactor. Due to these facts, so much research had reported the application of EGSB combined with other reactors like anoxic and aerobic bioreactors [13, 33, 35]. Others have recommended the attachment of an extra dissolved air flotation (DAF) system which is often used before secondary biological processes to reduce sludge washout and treat difficult toxic streams [33, 36, 37]. Based on studies conducted by Wenta and Hartman [38], about 95% reduction in TSS concentration during the treatment of pulp and paper mill wastewater was observed when DAF method was used.

2.1.3 Upflow Anaerobic Sludge Blanket (UASB) Reactors

The UASB reactor designed by Lettinga et al. [39] has made anaerobic digestion the most competitive and favorable treatment technology for high-strength organic wastewaters [40, 41]. It has been widely employed to treat industrial and domestic wastes around the world due to its features such as simple design, easy construction, maintenance, low operating cost, high removal efficiency, short retention time, stability, temperature, and low energy demand [11, 42, 43]. Like EGSB, a UASB reactor is highly dependent on granular sludge as the core component during wastewater treatment for effective conversion of organic matter to biogas (Fig. 3) [44, 45]. Several laboratories, pilot, and full-scale reactors have been optimized to treat different types of domestic and industrial wastewaters [46–48]. Some of the industrial effluents treated by UASB reactor include slaughterhouse [49], pulp and paper, textile [50], pharmaceutical [51], sugar factories [52, 53], and brewery wastewater among others [40, 54, 55]. Despite the benefits and simplicity of the UASB reactor, maintaining a steady-state condition is still one of the difficulties faced by UASB reactor operators [6]. Descriptions and further information about other types of reactors have been reviewed in the literature [14, 16, 41].

2.2 Bioeconomy and Ecological Benefits of Energy Recovery from Wastes Using AD Technologies

Over the years, mankind have relied on various energy sources especially coal as the primary energy generator. However, in the twentieth century, waste management strategies and reduction of environmental impacts caused by waste disposal have gained more attention due to its effects on climate change and environmental health

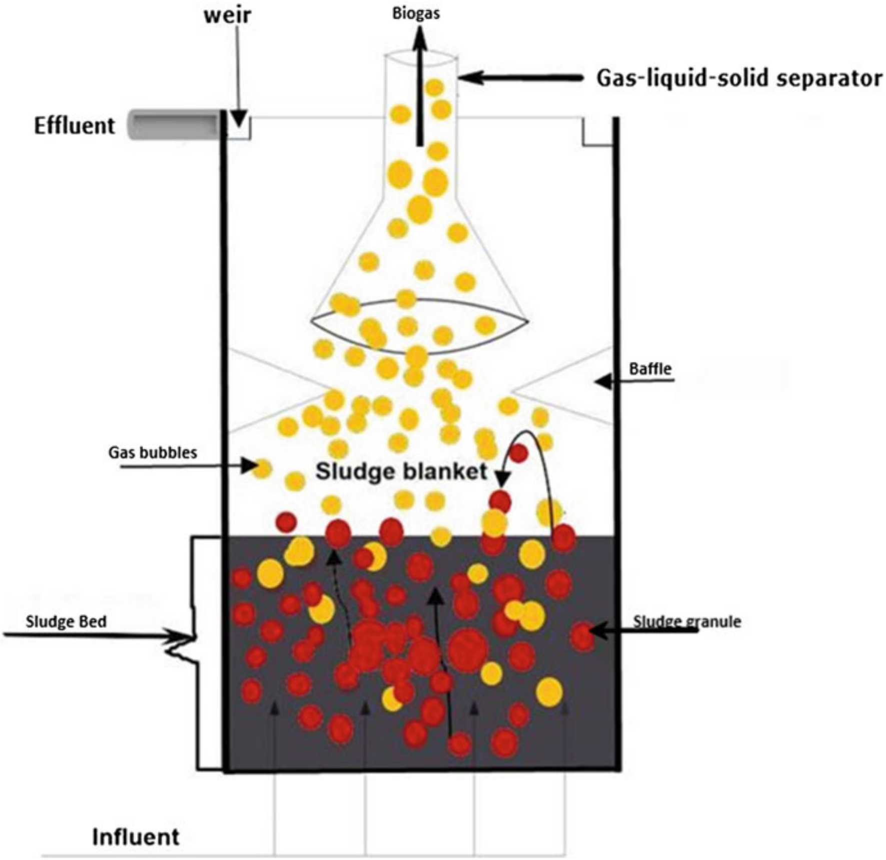
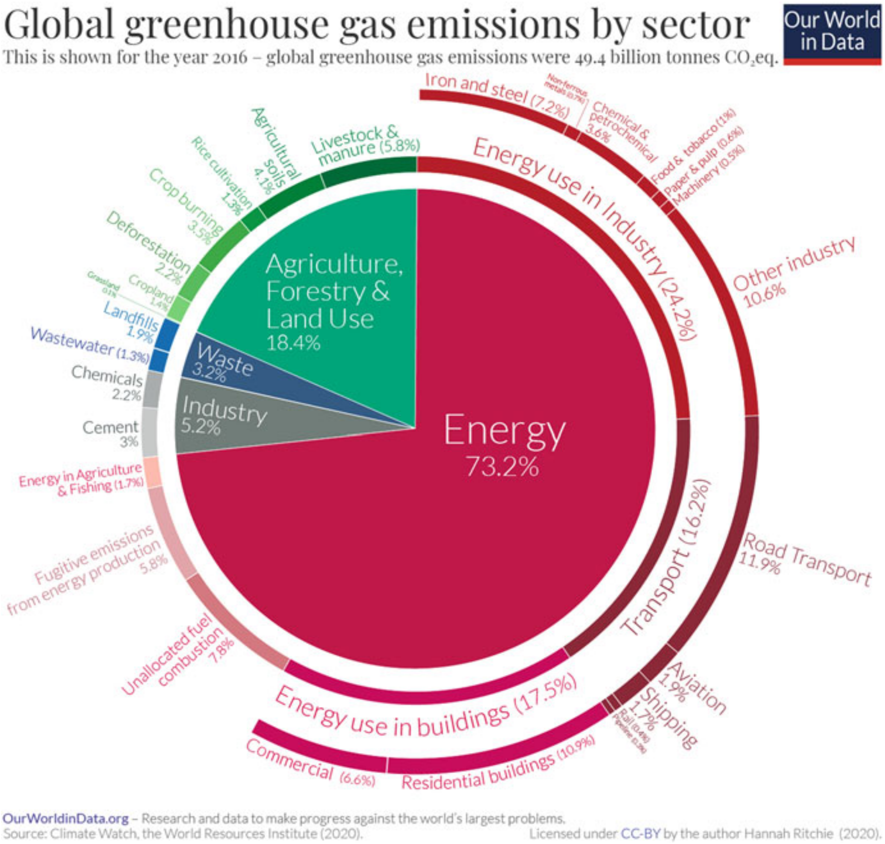


Fig. 3 Schematic diagram of an upflow anaerobic sludge bed (UASB) reactor with red balls indicating granules and yellow balls indicating evolved biogas

concerns. For instance, in 2016 only, the reported data for GHGs produced worldwide from various industrial sectors and processes was about 50 billion tonnes (Fig. 4) [56]. Hence, high measures are being put in place to comply and reduce the biodegradable liquid and solid wastes flowing into water bodies and landfills in order to reduce the amount of GHGs emissions into the environment.

Since methane and carbon dioxide are considered potent GHGs, approximately 18% of global warming is thought to be caused by anthropogenic derived methane emissions. The carbon dioxide released through natural mineralization is considered neutral in terms of GHGs. For example, the uptake of carbon from the atmosphere by plants and its return to the atmosphere as part of the carbon cycle is considered a green approach to mitigate environmental pollution [5]. Therefore, environmental and economic aspects of waste management should be maintained as a circular relationship for facing existing environmental problems and resource scarcity [57]. Treatment of wastes through AD technology is an economic process that



OurWorldinData.org – Research and data to make progress against the world's largest problems.
Source: Climate Watch, the World Resources Institute (2020). Licensed under CC-BY by the author Hannah Ritchie (2020).

Fig. 4 Global greenhouse gas emission data with 49.4 billion tonnes CO_2 eq. produced from industrial sectors in 2016. Source: Our World in Data, (2020) licensed permission to reuse under the Creative Commons Attribution License – By the author Hannah Ritchie [56]

involves converting waste streams into value-added products, such as feed, biobased products, and bioenergy [3, 57]. Hence, anaerobic treatment of wastes or biomass before being discharged into the environment or municipal sewers is considered an essential aspect of waste management. Such process not only reduces topsoil and freshwater pollution, but also helps in cleaning the atmospheric air, thus prevents the emission of greenhouse gases into an open environment and reduces the coal usage for energy generation [21, 58].

2.2.1 Anaerobic Reactor for Value-Added Products Recovery

Bioenergy (biogas, bioethanol, etc.) production through anaerobic digestion of wastes is a worldwide promising energy source which offers many environmental and socio-economic advantages. The benefits are multifaceted and the process of using anaerobic digestion for the treatment of both solid and liquid wastes are without side effects (Fig. 5). These significant benefits include:

(a) Fermentation of animal/human wastes: Fermentation of wastes produced by animal and humans helps to mitigate environmental issues that could arise due to improper discharge or runoff into the water bodies. This ultimately prevents the spread of pathogens.

(b) Provision of alternative material to unsustainable deforestation: Anaerobic treatment of wastes and biogas production with high CH_4 content is an excellent alternative to fossil fuel because human and industrial activities produce sufficient amounts of wastes [59]. It is interesting to note that if biogas produced during wastes treatment contains more than 50% of CH_4, it could be used as fuel energy; for heating, cooking, lighting, or to generate electricity for domestic and broader industrial activities [60], hence mitigate the act of deforestation [14, 61].

(c) Improves air quality: As earlier mentioned, AD technology combines the treatment of industrial wastes and energy production to reduce environmental pollution. It prevents methane, a GHGs, from entering into the atmosphere by confining the degradation processes in a closed environment. It also helps to control gas flaring leading to production of carbon neutral carbon dioxide back into the carbon cycle. It lowers carbon dioxide production that is not part of the recent carbon cycle [5]. It also cuts down the impacts of emissions during energy generation from coal.

(d) Alternative energy source: Biofuel is a veritable alternative energy source to fossil fuel. Its produce can be used to generate electricity through internal engine combustion and instead of flaring the gas, the resulting biogas is combusted for boiler heating and energy to operate distillation column (see Fig. 6) [2, 62, 63]. Thus, the problems of residual stillage treatment are solved by conversion into biofuel gas and thus mitigate the problems with energy supply and spending [64]. Similarly, biodiesel from fermented animal fats and crops, bioethanol from starch crops, and sugar have shown that AD system, when used properly, could be an efficient and sustainable biofuel generator [65, 66].

(e) Production of by-products: Furthermore, an anaerobic reactor is also beneficial for producing treated sludge that farmers can use as fertilizer. Due to inconsistency in price and environmental pollutions, there are more reasons for more clean and sustainable by-products like biofertilizers. With these, global demands for fertilizers can be met because the effluent from bioreactor has proven to be very rich in nitrogen, phosphorus, and potassium. This suggests that they are useful for agricultural application. Therefore, AD system is effective for waste recycling, production of high-quality manure, and biofuel generation with zero discharge into the environment.

2.2.2 Social-Economic Benefits of AD Technology and Human Empowerment

Bioenergy is an emerging industry. Its development provides several opportunities both economically and socially. For economic and social benefits example, the use

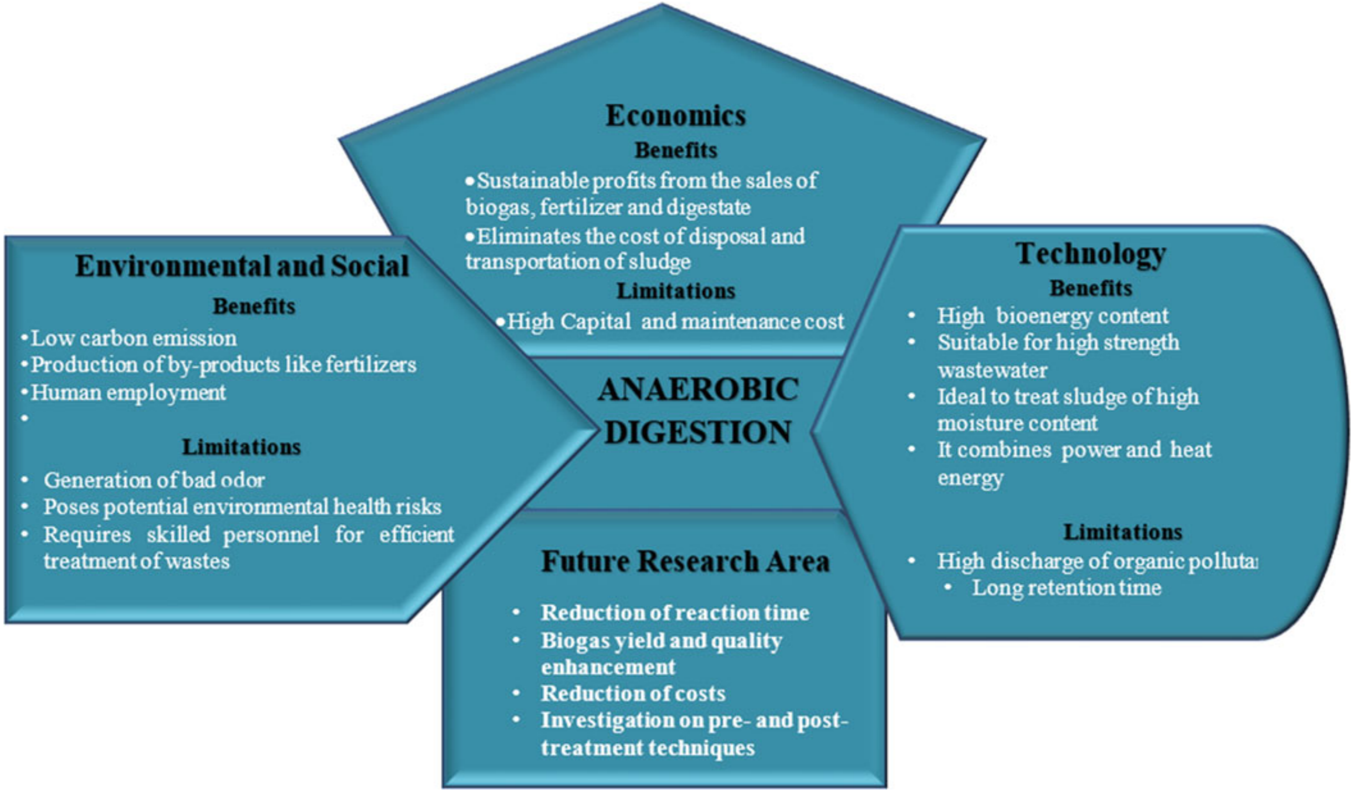


Fig. 5 Environmental, socio-economic, and technological evaluation of anaerobic digestion for wastes treatment

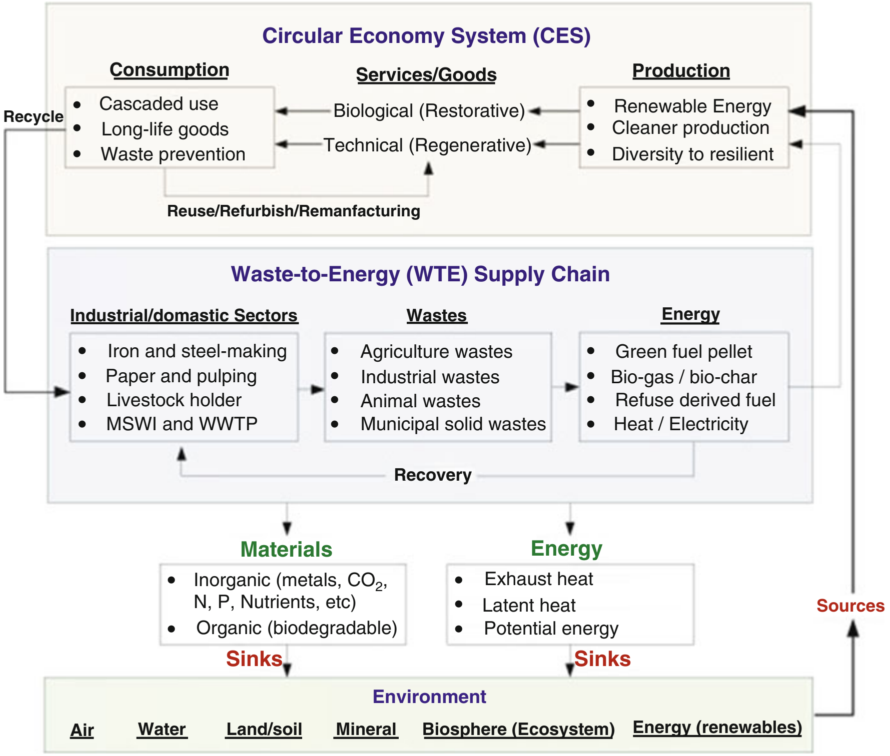


Fig. 6 Conceptual framework of the relationship between environment, circular economy system (CES), waste-to-energy (WTE), and supply chains [56]

of AD system for wastes treatment engenders human empowerment, integrated agriculture through fertilizer production, carbon emissions reduction, and national policy incentives (Fig. 5) [67]. It enhances job creation through the manufacturing of small-scale biogas digesters. More importantly, the use of biogas could assist the female folks to concentrate less on the utilization of wood and charcoal in their cooking activities. In 2016, about 41.6 million rural households in China used biogas [67, 68], which shows that the biogas industry is empowering people, creating jobs, and reducing air pollutants like particulate matters that could be inhaled by people. This is aiding the prevention of health-related issues and thus mitigates the effects of GHGs on the environment and its impact on climate change [69].

3 Development of Biorefinery for Bioenergy Production

From a Clean Development Mechanism (CDM) point of view, mitigating CH_4 emissions is fascinating since the Global Warming Potential (GWP) of Methane is 21 times higher than that of CO_2 [70]. Under anaerobic conditions, CH_4, CO_2, nitrogen (N_2), hydrogen (H_2), hydrogen sulfide (H_2S), and oxygen (O_2) called "biogas" are produced [71] with calorific values of 21–24 Mj/m^3, an equivalent value of 6 KWh/m^3 of CH_4 [9, 72]. Energy generation from biomass is classified as a "carbon neutral" process because CO_2 released during this process is balanced by the CO_2 absorbed by plants during their growth [70]. Electricity production from renewable energy is increasing through the fermentation of sludge produced from sewerage treatment plants into liquid fuels [73]. Tons of biosolids are converted into crude oil that can be used to replace oil extraction from beneath the earth's surface. Based on the US Department of Energy, bioenergy technologies treating municipal wastewater have been described as promising bioenergy production sources [74]. Methane gas from AD as a renewable energy source has been adopted as one of the CDM to obtain a certified emission reduction (CER) credit under the Kyoto Protocol. The ignition of biogas burns cleanly without soot or foul smell as compressed natural gas (CNG) and liquefied petroleum gas (LPG). It, therefore, facilitates biogas promotion for the reduction of greenhouse effect by cutting down methane emissions into the atmosphere.

Biogas generation has been widely adopted in Asia, particularly in places like Bangladesh, China, India, and Nepal for energy production [75, 76]. This offers such developing countries the advantage of foreign investments in sustainable renewable energy projects [76, 77]. Between 2007 and 2015, for instance, implementing national biomass energy from industrial wastes was passed in China with over 118 biomass CDM projects approved by the National and Local Development and Reform Commissions (NDRC and LDRC) [70, 76, 78]. In 2009, the Danish Carbon Fund (DCF) signed six emission reduction purchase agreements (ERPA) (1) with the Thailand Sapthip Wastewater Management project, (2) two China Baotou Energy Efficiency projects, and (3) one Mexico Monterrey II LFG. The worth of these projects was valued at €53.8 million ($77 million) with total emission reductions of 6.6 million tons of CO_2. The Sapthip Wastewater Management Project in Thailand aimed at reducing the emissions at the Sapthip Company's bioethanol plant as part of a clean development mechanism to generate and capture methane-containing biogas produced during the treatment of wastewater in anaerobic reactors. The biogas produced can be used as fuel to operate two 20-ton-per-hour-capacity boilers that supply steam to the ethanol plant's backup capacity [79].

Excluding household biogas plants, approximately 113,000 biodigesters were built throughout China [80], among which 6, 737 are large scale and 34 are super large scale. About 306 reactors treat industrial wastes, 458 plants utilize a straw, and approximately 99.6% of these plants use animal manure as feedstock [67]. In 2015, upgrade of biogas to bio-natural gas (BNG) projects was for the first time carried out at the central government level in China with a total of 65 BNG projects between 2015 and 2017. Meanwhile, 197 projects are estimated to be functioning by the end of 2020 [69]. Yet, it was estimated that fossil energy would gradually be replaced by hydroelectric power and nuclear energy. By 2035, more than half of China's demands will be filled by renewable energy [67]. The Indian waste-to-energy market is presently 750 MW, which is expected to reach a whopping 3 GW by 2050 [81]. In

Brazil, on the other hand, renewable energy was converted to electricity with the capacity of 30 kW microturbine [67].

3.1 Utilization of Anaerobic Reactor for Conversion of Wastes to Bioenergy in South Africa

In Africa, bioenergy generation from wastes is still at the infant stage. Most developing countries like South Africa have paid little attention to implementing national biomass energy from wastewater than the world's implementation of AD technology [82]. The first anaerobic digester in the countries was installed by John Fry to treat wastes produced by a pig farm in 1957. By 1958, the first bioelectricity was generated from the same plant to power pumps [83]. Many digesters have since been installed in the country, although at a slow rate compared to market penetration in the aforementioned countries. South Africa, among other African countries, have been using anaerobic reactors to treat industrial wastes [6, 84]. There was a report on four full-scale reactors treating abattoir, brewery, egg processing, and petrochemical wastes to generate biogas that can be converted to electricity. In another survey, four leading international companies (ADI, Biothane, Paques, and Enviroasia) installed anaerobic treatment plants with typical application of UASB reactors to wastewater from different industries [85]. About 700 installed reactors are currently being employed to treat various South African wastes as shown in Table 2 [83, 87].

For energy sustainability, more development in renewable and sustainable energy was developed by different sectors. For instance, Talbot & Talbot installed four on-site anaerobic digesters to treat food and beverage wastes to produce biogas that can be harvested to power boilers for fuel production which could replace fossil fuel usage [82, 88]. Based on a literature survey, most industries are still flaring or venting the biogas produced from the on-site anaerobic reactor into the atmosphere [86]. It has been shown that biogas produced during the anaerobic treatment process is 10% to 11% of the total energy required to safeguard the power supply for many industries [88]. This demonstrates that the usage of bioenergy from AD system has poorly been integrated into the energy sector, and the opportunity to mitigate greenhouse gas (GHG) emissions has not been fully embraced in many countries.

However, few on-site digesters use biogas to power their reactor, heat boiler, and building spaces [86]. For example, Cape Flats wastewater treatment plant in Cape Town, PetroSA's gas-to-liquids refinery in Mossel Bay, SA breweries Prospecton, Durban, and some isolated communities, households, and small-scale industrial anaerobic digesters are a few exceptions where biogas plants have been adopted for energy generation [82, 86, 89]. Cape Flats wastewater treatment plant in Cape Town installed a reactor to treat dry and pellets wastewater sludge for biogas production while reducing environmental contamination and sludge disposal costs. This plant serves as an energy source while the pellets were reported to have ~16.6 MJ/kg helping a local cement factory as an additional energy source to

Table 2 Energy potentials of wastewaters from various South Africa sectors [86]

Wastewater type	Volume produced	Approach to calculation	Energy potential: thermal power (MWt)	Area
Brewery		Distributed. Seven breweries	17	
Distillery	–	Distributed. Grain, grape, and sugar-cane (molasses) are considered. Compared to grain and grape, molasses have the most significant energy potential; they are not seasonal and less distributed (three significant plants, all in KwaZulu-Natal)	70	KwaZulu-Natal
Winery	0.7 and 3.8 m3/ton of grapes processed (0.8–4.4 L/L of wine produced) COD = 6 g/L; 1,000 ML/year of wastewater	Distributed and seasonal	3	
Fruit processing	20% of 2,100,000 ton citrus fruit (2005) was used For juice, wastewater COD = 15 g/L; 205,000 ton deciduous in 1999/2000 for which the wastewater COD averages 5 g/L	Distributed and seasonal. Only the wastewaters from canning and juicing in Western cape are considered (pulp and pomace excluded). Operates 4 months of the year	68	Western cape
Petrochemical waste	PetroSA electricity plant produces 12 MW electricity using biogas as the raw material	Sasol is assumed to produce 3× more based on plant size. PetroSA and Sasol. Four refineries and one gas to liquid fuel refinery	48	
Textile industry	–	Distributed	22	
Pulp and paper		17 mills	45–100	
Olive production	100 g/L; 89ML/year	Distributed and seasonal	4	
Animal husbandry		Cattle in feedlots. Mixed solid and liquid wastes slurries. They represent point sources that can be accessed through on-site energy recovery. Nine	79–215	

(continued)

Table 2 (continued)

Wastewater type	Volume produced	Approach to calculation	Energy potential: thermal power (MWt)	Area
		feedlots represent more than half the total cattle in feedlots		
		Red meat and poultry abattoirs consider liquid wastes only	1–55	
		Piggeries mixed solid and liquid waste slurries	18–715	
		Poultry farms considers solid waste only	940–2,976	
		Rural cattle considers solid waste only that are collected at night in kraals. Only a small percentage of this energy is realistically recoverable	1,271–3,445	
		Dairies mixed solid and liquid waste slurries collected including washing and milk spills	117–121	
Domestic Blackwater (human feces)	200 L/day wastewater per person. Population of SA = 48.5 million, hence 9,70 × 10^9 L/day is generated. COD estimated at 0.860 g/L, Energy content = 15 MJ	Municipal treatment plants serve only 60% of the population, therefore only 60% of human feces are currently captured. These plants are distributed, approximately 968 WWT plants exist in SA. The majority of these plants are small at <0.5 ML/day, with larger plants of 2.5 ML/day. Treatment plants also receive domestic urine, greywater, and industrial load, not considered here	509–842	
Abattoir	4 t of wastes/day	–	100 kW	Bredasdorp
Abattoir	20 t of wastes/day	–	500 kW	Cavatter

power their kilns [86]. In 2018, a waste-to-energy generator was launched in Cape Town as a sustainable power production with a capacity of 220 kw using a combined heat and power unit (CHP) system for the treatment of abattoir waste with the capacity to produce electrical power and thermal energy.

South Africa's first independent State-owned power plant, PetroSA's gas-to-liquids refinery near Mossel Bay in South Africa was commissioned in 2007 and funded by carbon credits generated under the CDM of the Kyoto Protocol [90]. It is a combined heat and power plant to utilize the biogas production from wastewater treatment plant (WWTP). The electrical output replaces 4.2 MW of grid-based electricity and the plant was expected to produce approximately 33, 000 t per year of certified emissions' reductions (CERs). Along with receiving debt financing from the Development Bank of South Africa, the sale of emissions credits has contributed to the PetroSA project's economic viability [86, 90]. Stafford et al. [86] and Mutungwazi et al. [87] reviewed the treatment of different types of wastewater using an anaerobic reactor (industries and domestic blackwater) (Table 2). Anaerobic reactor was reported to have the potential to recover 3,200–9,000 MWth of energy equating about 7% of current electrical power supply in South Africa with approximately 140,000 MWth or 42,000 MWe energy demand [86].

Anaerobic treatment technology has helped in multifaceted areas by providing solutions to social, economic, and environmental issues with robust commercial viability in biofuel production that can generate heat, electricity, transportation fuel, and mitigation of GHGs [81]. However, implementation of the full potential of bioenergy at the commercial level is facing challenges in South Africa due to limitations such as insufficient skills by anaerobic reactor operators, lack of awareness about AD biogas technology, and non-implementation of research findings among many other factors (Fig. 7) [86].

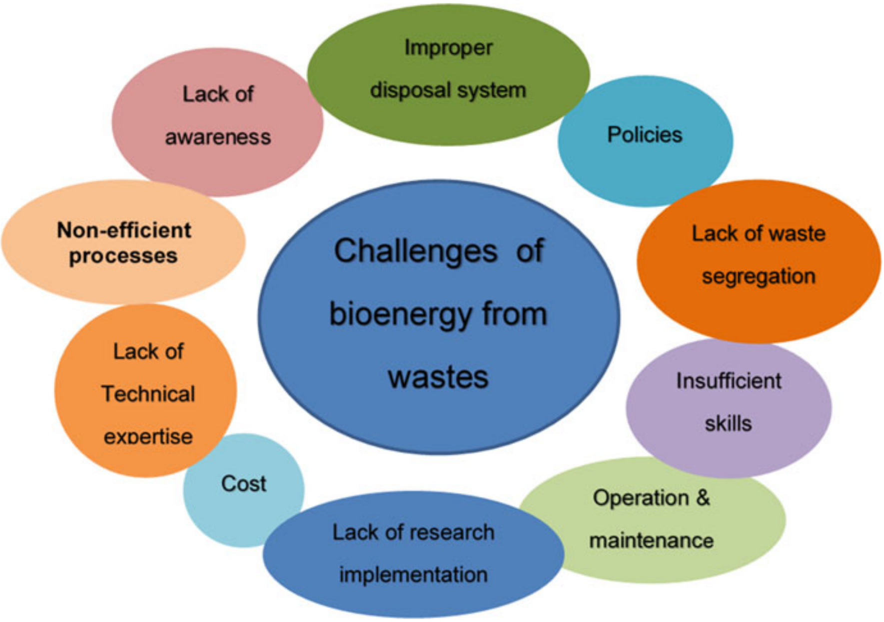


Fig. 7 Various challenges facing the future of wastes-to-bioenergy production

4 Biochemistry and Microbiology of Anaerobic Digestion Process

Understanding the overall biochemistry and microbial composition of technology is necessary to improve bioenergy recovery during the anaerobic digestion process. During this process, there are four key sequential stages, namely; hydrolysis, acidogenesis, acetogenesis, and methanogenesis. At each stage, the breakdown of feedstock in the substrates is facilitated by a group of facultative, obligate, and strictly anaerobic bacteria [91, 92]. These organisms are divided into four groups based on the biochemical processes and the metabolites they produce (Fig. 8, Table 3). Under ideal conditions, these microorganisms breakdown the complex organic compounds through a variety of intermediates into the components of biogas. Example of elements are CH_4 and CO_2 with small levels of H_2S, H_2, and N_2 based on the overall reaction shown in Eq. (1) [6, 92, 96, 97].

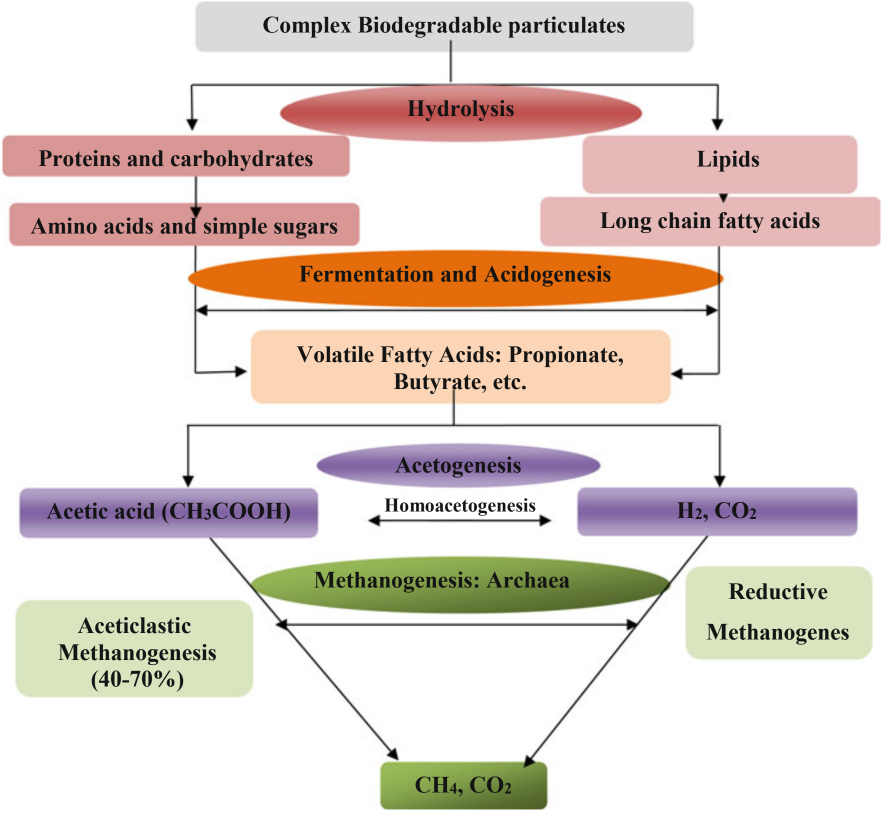


Fig. 8 The key stages of anaerobic digestion process during the treatment of wastes products [6]

Table 3 Fermentation reactions associated with the anaerobic conversion process in a successful operating bioreactor [93–95]

Reaction	ΔG (kJ mol^{-1})
Acidogenesis:	
$C_6H_{12}O_6 + 2H_2O \rightarrow$ 2 ethanol + $2HCO_3^- + 2H^+$	−225.4
$C_6H_{12}O_6 + 2H_2O \rightarrow$ butyrate + $2HCO_3^- + 3H^+ + 2H_2$	−254.4
$C_6H_{12}O_6 \rightarrow$ 2 lactate + $2H^+$	−198.1
$C_6H_{12}O_6 \rightarrow$ 3 acetate + $3H^+$	−310.6
$C_6H_{12}O_6 + HCO_3^- \rightarrow$ succinate^{2-} + acetate$^-$ + formate$^-$ + $3H^+ + H_2O$	−144.0
3 lactate$^-$ $\rightarrow$ 2 propionate$^-$ + acetate$^-$ + $HCO_3^- + H^+$	−164.8
2 lactate$^-$ + $2H_2O \rightarrow$ butyrate$^-$ + $2HCO_3^- + H^+ + 2H_2$	−56.2
Acetogenesis:	
Ethanol + $2HCO_3 \rightarrow$ acetate + 2 formate$^-$ + $H_2O + H^+$	+7.0
Ethanol + $H_2O \rightarrow$ acetate + $2H_2 + H^+$	+9.6
Lactate$^-$ + $2H_2O \rightarrow$ acetate$^-$ + $2H_2^+ + H^+$	−3.9
Butyrate$^-$ + $2H_2O \rightarrow$ 2 acetate$^-$ + $2H_2^+ + H^+$	+48.1
Benzoate$^-$ + $6H_2 \rightarrow$ 3 acetate$^-$ + $3H_2^+ + CO_2 + 2H^+$	+53.0
succinate^{2-} + $4H_2O \rightarrow$ acetate$^-$ + $2HCO_3^- + 3H_2^+ + H^+$	+56.1
Propionate$^-$ + $3H_2O \rightarrow$ acetate$^-$ + $HCO_3^- + 3H_2 + H^+$	+76.1
Homoacetogenesis:	
$4H_2^+ + 2HCO_3^- + H^+ \rightarrow$ acetate$^-$ + $4H_2O$	−104.5
Methanogenesis:	
Acetate$^-$ + $H_2O \rightarrow$ methane + HCO_3^-	−31.0
$4H_2^+ + HCO_3^- + H^+ \rightarrow$ methane + $3H_2O$	−135.6
$4HCO_2^- + H^+ + H_2O \rightarrow$ methane + $3HCO_3^-$	−130.4
4 methanol $\rightarrow$ 3 methane + $HCO_3^- + H^+ + H_2O$	−312.8

$$\text{Organic matter} \rightarrow CH_4 + CO_2 + H_2 + NH_3 + H_2S \tag{1}$$

On the one hand, about 70% of the total CH_4 production by methanogenic archaea during AD is from acetic acid. On the other hand, the remaining 30% comes from H_2 and CO_2 conversion [98]. It has been reported that about 80–90% CH_4 composition can be produced in reactors treating wastewater and more from biosolid wastes. The origin of the AD process and the biodegradable materials determine the composition of biogas produced. The percentage composition of methane is based on biodegradable feedback used in the digestion process and running conditions of the anaerobic reactor (Table 4). The stability of the microbial ecosystem in the AD process has been shown to depend on the methanogenic activity, which is characterized by slow growth rates of microorganisms. They are very sensitive to operational and environmental variations such as salinity, sludge properties, temperature, pH, mineral composition, loading rate, HRT, carbon-to-nitrogen ratio, and volatile fatty acids (VFAs). These factors influence organic matter and biogas production [12, 99].

Table 4 Percentage of methane composition from different anaerobic fermented feedstocks

Feedstock	Composition of methane (%)
Barley straw	77
Beet leaves	84.8
Cattle manure	50.60
Corn silage	54.5
Grass	84
Dried leaves	58
Poultry waste	68
Pig manure	60
Sheep dung	65
Horse dung	84
Wheat straw	77

4.1 Functions of Methanogenic Archaea in Biogas Production

Unlike bacteria, methanogens are archeons. They have no typical peptidoglycan (mureinic) skeleton. Instead, several genera in archaea domain have pseudomurein. Others have walls consisting of lipids composed of isoprenoid hydrocarbons glycerol lipids with different metabolism [100, 101]. They are slow-growing archeons with a generation time between 3 days at 35 °C and 50 days at 10 °C [102]. Generally, methanogens are largely differentiated morphologically. They exhibit almost all shapes occurring in bacteria including cocci (*Methanococcus*), rods (*Methanobacterium*), short rods (*Methanobrevibacter*), spirillaceae (*Methanospirillum*), sarcina (*Methanosarcina*), and filiforms (*Methanothrix*) [6]. They are strict anaerobes and contain neither catalase nor superoxide dismutase with size ranges from 0.3 to 7.4 μm [103].

Studies have shown that three different major pathways exist for CH_4 formation depending on the source of the reducing potential and the carbon compound used as substrate (Fig. 9); which include hydrogenotrophic, aceticlastic, and the methylotrophic [100, 104]. Hydrogenotrophic methanogens are H_2 using organism. They use H_2 as an electron donor to reduce CO_2 to CH_4 (Fig. 9; Eq. (2)). This group helps in maintaining very low levels of partial pressure needed by the aceticlastic methanogens for the conversion of VFA and alcohols to acetate [93].

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{2}$$

Abundance of *Methanobacterium*, *Methanobrevibacter,* and *Methanococcus* of orders *Methanobacteriales, Methanomicrobiales, and Methanococcales* in different types of anaerobic bioreactor wastewaters treatment has been reported [18, 54, 105, 106]. The second group is the acetotrophic or aceticlastic methanogens which convert acetate to CH_4 and CO_2 [105, 107]. The overall reaction is shown in Eq. (3);

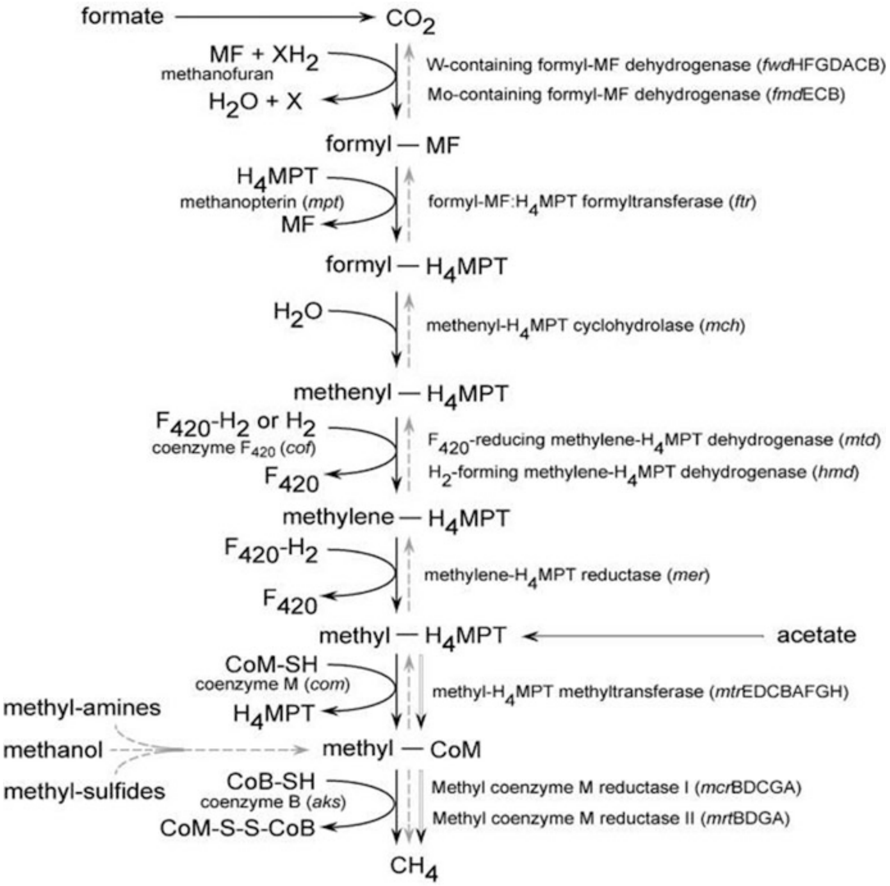


Fig. 9 Biochemical pathways of methanogenesis: hydrogenotrophic (double-lined arrows), aceticlastic (solid arrows), and methylotrophic (broken gray arrows) during anaerobic treatment. Licensed permission to reuse under the Creative Commons Attribution License (https://creativecommons.org/licenses/by/4.0/) [104]

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (3)$$

The most commonly reported aceticlastic methanogens from bioreactors belong to the genera *Methanosaeta* (coccoid bacteria) and *Methanosarcina*-sheathed rods or long filaments bacteria [106, 108]. This group of methanogens helps in the production of about 70% of the total CH_4 generated during the AD of wastewater [98]. *Methanosaeta* sp. such as *M. thermophila* and *M. concilii* belonging to genus *Methanosaeta* utilize acetate, while *Methanosarcina* strains like *M. barkeri, M. mazei,* and *M. thermophila* utilize acetate, methanol, methylamines, H_2, and CO_2 as [108]. The abundance of *Methanosarcina* sp. at high acetate levels and *Methanosaeta* sp. at low acetate concentrations was also reported [108]. An abundance of *Methanosarcina* and *Methanosaeta* sp. in granules treating different wastewaters at steady-state conditions has been reported in the literature [109, 110]. The third group is the methylotrophic methanogens belonging to order *Methanosarcinales* and *Methanobacteriales* which are directly responsible for producing CH_4 from assimilation of methyl groups (-CH_3), methylamines [$(CH_3)^3$-N], and methanol (CH_3OH) as substrate [93]. Methanol is usually found as organic pollutant in several wastewaters and is a substrate for both methanogens and acetogens [104]. Ziemiński and Frąc [100] reviewed the sensitivity of biochemistry, physiology, and ecology of methanogens to oxygen. Some of their characteristics include their sensitivity to changes in pH and temperature, inhibiting their growth by a high level of H_2, sulfur, NH_3, VFAs, and other compounds in the environment or in the anaerobic bioreactor [100, 111]. For sustainable and economic development using renewable energy and maximization of biofuel yield during anaerobic digestion, operational parameters must be properly optimized to encourage the growth of microorganisms required to produce biofuel of interest [112]. These, therefore influence biogas production during anaerobic digestion of solids and liquid wastes. For more details on this, see Sawyerr et al. [113] and Tabatabaei et al. [112].

5 Determination of Microbial Fingerprint in an Anaerobic Reactor Using Molecular Techniques

The main aims of studying microbial ecology include identifying, classifying, and determining microbial activity in an anaerobic reactor [100]. In the past, traditional methods of identification used to determine the morphology and phenotypic characteristics [114] were time-consuming and limited. Many microorganisms, especially the methanogens, are difficult to culture using such traditional methods because they are slow-growing organisms with restricted environmental conditions and particular nutritional requirements [115]. The development of advanced molecular techniques (Fig. 10) to study the complex microbial populations in environmental samples by targeting the 16S rRNA gene has eliminated the use of more elaborate traditional methods of culturing microorganisms. It has increased our understanding of the microbial communities present in anaerobic bioreactors and contributed to development of an improved process that encourages the growth of desirable microorganisms and enhances bioenergy yield.

Molecular techniques are grouped into two main groups; quantitative and qualitative. Qualitative methods include polymerase chain reaction (PCR), PCR-based denaturing gradient gel electrophoresis (PCR-DGGE) [116, 117], temperature gradient gel electrophoresis (TGGE), and terminal restriction fragment length polymorphism (TRFLP). The most recent technique is next-generation sequencing such as Illumina platform, Pyrosequencing, and Ion Torrent platforms [105, 116, 118]. Microbial profiling techniques involve the amplification of isolated nucleic acids, sequencing and identification by comparing the generated sequences with

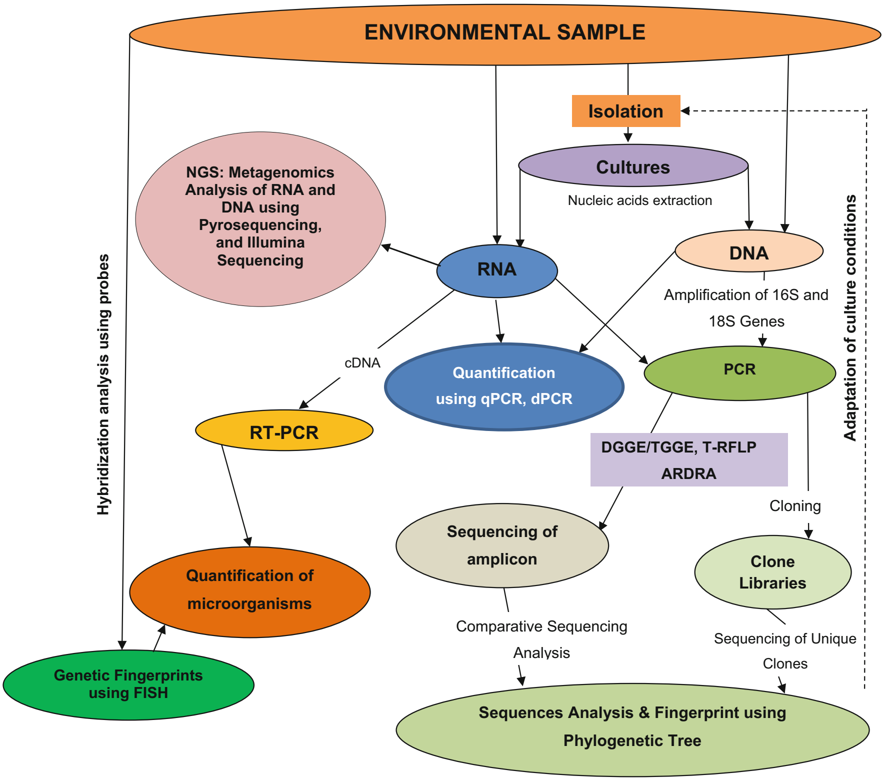


Fig. 10 Flow diagram of different steps used in studying microbial communities' structure and functions in an environmental sample

known sequences in the database. Next-generation sequencing (NGS) has emerged as a powerful tool for studying microbial communities because it is less costly and time-consuming and produces a greater yield of sequence data [119]. These methods have successfully been used to determine the shift and microbial populations in the laboratory- and industrial-scale bioreactors [105, 119, 120]. Due to the high cost of reagents and the need for technical skills to interpret the data generated, NGS's application remains at infant stage to determine the anaerobic organisms. A number of reports are however available on applying next-generation sequencing to understand microbial composition of full-scale bioreactors treating solid and liquid wastes [105, 116, 118, 121].

On the other hand, fluorescence in-situ hybridization (FISH) and quantitative real-time PCR (QPCR) are quantitative techniques that have been adopted for surveying microbial ecologies [52, 106, 122–124]. Fluorescence in-situ hybridization technique is based on the hybridization of whole cells with specific probes and microscopic analysis of dyed hybridized cells using epifluorescence microscopy, flow cytometry, or scanning electron microscopy [106, 122, 125, 126]. Fluorescence in-situ hybridization has been used to analyze and understand microorganisms' spatial distribution [106, 115]. Quantitative real-time PCR, on the other hand, can be used to amplify and simultaneously quantify targeted DNA sequences by employing a PCR-based technique that enables one to quantify the number of gene copies or the relative number of gene copies in a given sample. The reliability and amplified gene copy number of QPCR results strongly depend on the extracted genomic DNA and reflect the relative abundance of the sample's microbial population [123]. The amplification principle of QPCR is similar to that of PCR. It monitors the amplified target's concentration after each PCR cycle using a fluorescent dye or probe change in fluorescence intensity that reflects the amplified gene's concentration in the real-time assay [123]. Either absolute or relative quantification can be used to determine the concentration of DNA or RNA in an extracted sample. The technique has been widely used to quantify the microbial population and dynamics in anaerobic reactors in their natural environments [124, 127].

However, it is challenging to monitor specific groups or a domain using only one technique as each technique has its own merits and demerits. Therefore, a combination of qualitative and quantitative methods, including PCR-DGGE, QPCR, and microarrays could be used to overcome the limitations of using one technique [128]. A combination of different molecular methods such as electron microscopy, PCR-based DGGE, cloning, and FISH to gain insight into the physical appearance, function, and structure of microbial diversity of methanogenic granules in a bioreactor treating wastewater has been explored in the past [18, 129]. The PCR-based DGGE and FISH analysis identify the microbial populations in a full-scale UASB reactor treating brewery wastewater. *Delta* and *Gammaproteobacteria, Methanosaeta concilii,* and *Methanobacterium formicicum* were reported as some of the dominant bacterial and Archaea bands detected in the full-scale UASB reactor [129]. Likewise, PCR-DGGE was used to fingerprint and identify the microbial consortium present in different types of granules collected from the UASB reactor treating brewery wastewater [108] and wastewater polluted with organic solvents [129]. Methanogens such as *Methanosarcina, Methanosaeta, Methanobacterium,* and uncultured bacteria in the Archaea domain were identified and fingerprinted using PCR-DGGE [108]. FISH and PCR also detected the microbial community present in a full-scale reactor treating industrial wastewater [106]. Similarly, microbial fingerprints in a full-scale reactor treating agricultural and industrial wastes were compared using TRFLP and Illumina platforms [130]. Further reviews of the molecular detection of anaerobes that enhances biogas-rich reactors performance are found in the literature [65, 66].

6 Conclusions

As discussed, anaerobic digestion technology is an economic process that uses a biological treatment method to treat a wide range of low- to high-strength organic materials to produce value-added products, such as feed, biobased products, and bioenergy through a diverse group of microorganisms. Due to these benefits, the AD process is considered a significantly viable competent and economically sustainable system in treating organic wastes at a low cost with less biomass production and high treatment efficiency. It has also been shown that the technology is efficient in the removal of suspended solids, chemical and biological oxygen demand notwithstanding the digester temperature with the recovery of bio-nutrients and renewable energy in an enclosed system. Such processes will also reduce topsoil and freshwater pollution; help to clean the atmospheric air; prevent greenhouse gas emissions into an open environment; and reduce coal usage for energy generation. In essence, environmental and economic problems facing waste management and resource scarcity could be tackled using an anaerobic digestion system.

7 Recommendation and Future Perspectives

The principal aims of the anaerobic reactors are to reduce greenhouse gas and improve methane-biogas content for energy generation by treating different types of wastes to produce millions of megawatt-hours of electricity per year. However, there is a need to optimize the digesters for proper functioning and increase the methane content for energy generation by operating the reactors in optimum conditions to avoid formidable technological and dissemination challenges. In addition to the future research focus mentioned in Fig. 5, more research on the mechanisms of action by different microbial groups in the AD reactor for biogas production needs to be investigated. Collaboration and information-sharing between research groups, government agencies, and municipal practitioners on bioenergy production should be encouraged. Furthermore, there should be more emphasis on generating clean and renewable energy by giving incentives such as feed-in tariffs, green energy tariffs, or peak tariffs at the local level. Offer on the certified emission reductions and the production of other secondary products such as fertilizer that could tip the balance of economic feasibility when implementing energy from a wastewater project should be available.

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