An Insight of Component and Typical Mechanism of Sludge Degrader Microbes in Dewatered Sludge

Muhammad Najib Ikmal Mohd Sabri, Nurul Atiqah Shamsuddin, Husnul Azan Tajarudin, Md Sohrab Hossain, and Muaz Mohd Zaini Makhtar

Abstract This book chapter provides insights into the potential of wastewater sludge and the characteristics of different types of sludge. Each type of sludge has unique characteristics, microorganism consortium, and reactions that occur within the sludge. The behavior of sludge, typical microorganism degraders, and reactions involved in the natural process transform complex substrates into simpler ones. The presence of microbial degraders is crucial for the exploitation of sludge valorization for future sustainability. The chapter explores the components and typical mechanisms of sludge degrader microbes in dewatered sludge. The understanding of the microbial degraders present in sludge is essential for the development of sustainable approaches to sludge management. The exploitation of sludge valorization has the potential to provide renewable energy sources, contribute to the circular economy, and reduce the environmental impact of sludge disposal. This book chapter highlights the importance of microbial degraders in the transformation of complex substrates into simpler ones and the need for sustainable approaches to exploit the potential of sludge valorization.

Keywords Sludge biosolid · Biomass conversion · Bioprocessing · Affordable and clean energy · Renewable energy

---

M. N. I. Mohd Sabri · N. A. Shamsuddin · H. A. Tajarudin
School of Industrial Technology, Bioprocess Technology Division, Universiti Sains Malaysia, 11800, Gelugor, Penang, Malaysia

M. S. Hossain
Department of Fundamental and Applied Sciences, HICoE-Centre for Biofuel and Biochemical Research, Institute of Self-Sustainable Building, Universiti Teknologi PETRONAS, 32610 Seri Iskandar, Perak Darul Ridzuan, Malaysia

M. Mohd Zaini Makhtar (✉)
School of Industrial Technology, Bioprocess Technology Division, Universiti Sains Malaysia, 11800 Gelugor, Penang, Malaysia
e-mail: muazzaini@usm.my

Centre for Innovation and Consultation, Universiti Sains Malaysia, 11800 Gelugor, Penang, Malaysia

M. Mohd Zaini Makhtar et al. (eds.), *Microbial Fuel Cell (MFC) Applications for Sludge Valorization*, Green Energy and Technology,
https://doi.org/10.1007/978-981-99-1083-0_3

1 Introduction

Wastewater that has been treated and refined from the wastewater treatment plant (typically consisting of preliminary, primary and secondary treatment) has the potential to be valorized into valuable bioproducts such as bioenergy and biomaterials. The end waste at the treatment site is in the form of dewatered sludge (biosolid) which is composed of a lot of nutrient composition and tonnes of microbes. Table 1 tabulates the differences between sludge, sewage sludge, activated sludge and leachate.

Table 1 Comparison of sludge, activated sludge, sewage sludge, and leachate

	Sludge	Activated sludge	Sewage sludge	Leachate
Definition	• Formed during both primary sewage treatment and secondary treatment	• Sludge form and growth in the biological treatment process that is composed of microorganisms • Agitated and aerated	• Sludge that is produced in form of residual and semi-solid for both municipal and industrial wastewater • Also known as biosolids	Liquid squeezed out from the waste as well as the water (solvent) which infiltrates into the waste and percolates through it carrying substances dissolved from the waste (solutes)
Position/ Location	• Preliminary treatment; biological, chemical and physical • Secondary treatment; further biological process (anaerobic digestion)	• After the secondary treatment; the product • Closed biological reactors are known as anaerobic sludge digesters	• Located at the effluent • Municipal and industrial wastewater	• Dump area
Benefit	• Can be converted to biogas through anaerobic digestion process	• Microorganisms are used to consume organic matter in WW • Aeration is required in this treatment	• Good source of plant nutrients (macronutrients); soil conditioner or fertilizer • Electron transfer (microbial fuel cell) • Carbonization as energy generation	Fermented leachate can be used to recover and adsorb acetic and butyric acid
References	[4]	[5, 6]	[7]	[8]

2 Overall Reaction and Type of Microbe Degraders

2.1 Sugar Degrader

The source of sugar came from lignocellulosic material which can be found in plant-derived residue and waste such as paper mill sludge [1]. Research conducted by Ducan and team [2], found that the conversion of mill sludge to sugar later can be used as either isoprene or ethanol. Based on Yildiz et al. [3, 6], microorganisms are used due to their ability to remediate the sugar industry effluent. The application of microorganisms is eco-friendly because they do not require any chemicals during the sludge treatment. Basically, lactic acid bacteria (LAB) that are used as sugar degrader reacted can be monitored by the reduction of pH. There are several types of LAB strains that are used as sugar degraders, for example, *Lactobacillus plantarum*, *Lactobacillus casei*, and *Streptoccuslactis.* The production of lactic acid in the early fermentation stage suppressing the growth of putrefying bacteria while enhancing the availability of inorganic compounds which are being used by these lactic acid bacteria for growth and reproduction.

Besides, *Saccharomyces cerevisiae* is the most useful microorganism for ethanol production through alcoholic fermentation by metabolizing sugar in the absence of oxygen which leads to the production of ethanol and carbon dioxide [9].

The metabolic reaction of sugar degradation is further described below:

$$C_6H_{12}O_6 \rightarrow 2CH_3CH_2OH + 2CO_2 \quad (1)$$

where $C_6H_{12}O_6$ is glucose, CH_3CH_2OH is ethanol and CO_2 is carbon dioxide.

2.2 Amino Acid Degrader

Amino acid is a soluble monomer from a breakdown of complex organic matter dependent upon syntropic interaction of a consortium of microorganism in anaerobic digestion [10]. Amino acids vary significantly in size and structure and are fermented via different pathways to a range of products where these products are built up by amphoteric substances that contain amino and carboxyl groups. These amino acids are comprised of a four-step process of hydrolysis; amino acid fermentation, acid production and methanation of the anaerobic degradation process of proteins.

The degradation of amino acids produces organic compounds such as ammonia, carbon dioxide and small amounts of hydrogen and sulphur compounds. Amino acids are degraded in two ways that include deamination through a Stickland reaction; injection of two types of amino acids. One side of the amino acid (containing the majority of the carbon atoms) acts as an electron accepter, while the other (containing one or only a few carbon atoms) acts as an electron donor.

The reaction that takes place is the deamination by bacteria within the *Clostridium* species (obligatory species). The second type of amino acid decomposition occurs through the general fermentation process of single amino acids that requires the presence of hydrogen-utilizing bacteria. The fermentation of amino acids by the Stickland reaction; a chemical reaction that involves the coupled oxidation and reduction of amino acids to organic acids, is known to be the dominant reaction among these two types [11, 12].

Based on Table 2, there are five classifications of the bacteria based on their involvement in Stickland reactions and the amino acids that they typically utilize [11]. Group I bacteria are organisms that carry out the Stickland reactions. Fermentation process intermediately utilize proline and produce δ-aminovalerate, α-aminobutyrate or γ-aminobutyrate by these enzymes were accumulated with *Clostridial* species. While Groups II, III, IV and V do not carry Stickland reactions but ferment amino acids. These classifications mainly form obligate spore-formers (*Clostridial* species) and some non-sporing obligate anaerobes, for example, *Peptostreptococcus* (*Micrococcus*) *spp.*

Table 3 summarizes the amino acid metabolic degradation. All of the reactions are described either as Stickland or non-Stickland where there are five amino acids involved in Stickland reaction. These reactions can act either as an electron donor or electron acceptor.

2.3 Long-Chain Fatty Acid (LCFA) Degrader

Long-chain fatty acid (LCFA) is generated from the hydrolysis of lipids in sludge [13]. Fatty acids are organic molecules composed of a hydrophilic head, a carboxyl group and a hydrophobic aliphatic tail. The absence or presence of double bonds in the fatty acid aliphatic chain makes them saturated or unsaturated. Saturated and unsaturated LCFA are palmitate and oleate, respectively, thus they become the most abundant constituents [14]. The Prime way to identify the differences between saturated and unsaturated LCFAs are the presence of double bond in the fatty acid aliphatic chain, respectively. Table 4 showed the common unsaturated and saturated LCFA found in wastewater.

Hydrogen transfer between microorganisms plays a central role in LCFA degradation in methanogenic environments. This degradation through obligation syntrophic communities of proton-reducing acetogenic bacteria, converting LCFA to acetate and hydrogen/formate, *acetoclastic methanogenic archaea*, and hydrogen/formate-consuming methanogenic archaea as shown in Table 5.

The degradation of saturated LCFA follows the classic β-oxidation pathway while the unsaturated LCFA may require a preliminary step of hydrogenation or an alternative degradation pathway. The coculture of *Syntrophomonas and Methanospirillumhungatei* can degrade palmitate in LCFA [15, 16]. There are 14 fatty-acid-degrading syntrophic bacteria that have been obtained in pure culture and coculture with hydrogen-consuming microorganisms, all belong to *Syntrophomonadaceae* and

Table 2 Classification of anaerobic bacteria which degrade amino acids [11]

Group species		Enzyme production	Amino acids utilized	Characteristics
I	*C. bifermentans*	proteo/saccharolytic	proline, serine, arginine, glycine	organisms that carry out Stickland reaction
	C. sordellii	proteo/saccharolytic	leucine, isoleucine, valine	reaction
	C. botulinum types A, B, F	proteo/saccharolytic	ornithine, lysine, alanine,	prolineutilised by all species
	C. caloritolerans	–	cysteine, methionine, aspartate	δ–aminovalerate
	C. sporogenes	proteo/saccharolytic	threonine, phenylalanine	α-aminobutyrate and γ-aminobutyrate are produced
	C. cochlearium– one strain	specialist	tyrosine, tryptophan and glutamate	
	C. difficile	saccharolytic		
	C. putrificum	proteo/saccharolytic		
	C. sticklandii	specialist		
	C. ghoni	proteolytic		
	C. mangenotii	proteolytic		
	C. scatologenes	saccharolytic		
	C. lituseburense	proteo/saccharolytic		
	C. aerofoetidum	–		
	C. butyricum	saccharolytic		
	C. caproicum	–		
	C. carnofoetidum	–		
	C. indolicum	–		
	C. mitelmanii	–		
	C. saprotoxicum	–		
	C. valerianicum	–		
II	*C. botulinum types C*	proteo/saccharolytic	glycine, arginie, histidine and lysine	glycine is used by all species; δ-aminovalerate not produced
	C. histolyticum	proteolytic		

(continued)

Table 2 (continued)

Group species		Enzyme production	Amino acids utilized	Characteristics
	C. cochlearium– one strain	specialist		
	C. subterminale	proteolytic		
	C. botulinum types G	–		
	P. anaerobius	–		
	P. variabilis	–		
	P. micros	–		
III	*C. cochlearium– one strain*	Specialist	glutamate, serine, histidine,	δ–aminovalerate not produced;
III	*C. tetani*	Proteolytic	arginine, aspartate, threonine	histidine, serine and glutamate
	C. tetanomorphum	Saccharolytic	tyrosine, tryptophan and	used by all species
	C. lentoputrescens	–	cysteine	
	C. limosum	proteolytic		
	C. malenomenatum	specialist		
	C. microsporum	–		
	C. perfringens	proteo/saccharolytic		
	C. butyricum	saccharolytic		
	P. asaccharolyticus	–		
	P. prevotii	–		
	P. activus	–		
IV	*C. putrefaciens*	proteolytic	serine and threonine	δ-aminovalerate not produced
V	*C. propionicum*	specialist	alanine, serine, threonine, cysteine and methionine	δ-aminovalerate not produced

Syntrophaceae within the phyla *Firmicutes* and *Deltaproteobacteria*, respectively. During fatty acid degradation, these syntrophic bacteria are working together with hydrogenotrophicarchaea or hydrogen-consuming sulphate-reducing bacteria [14].

Table 3 Stoichiometry for amino acid fermentation (catholic reactions only) [11]

No	Reaction	Type
1	$C_6H_{13}O_2N$ (Leu) + 2 H_2O → $C_5H_{10}O_2$ (3-methylbutyrate) + NH_3 + CO_2 + $2H_2$ + ATP	Stickland
2	$C_6H_{13}O_2N$ (Leu) + H_2 → $C_6H_{12}O_2$ (4-methylvalerate) + NH_3	Stickland
3	$C_6H_{13}O_2N$ (Ile) + $2H_2O$ → $C_5H_{10}O_2$ (2-methylbutyrate) + NH_3 + CO_2 + $2H_2$ + ATP	Stickland
4	$C_5H_{11}O_2N$ (Val) + $2H_2O$ → $C_4H_8O_2$ (2-methylpropionate) + NH_3 + CO_2 + $2H_2$ + ATP	Stickland
5	$C_9H_{11}O_2N$ (Phe) + $2H_2O$ → $C_8H_8O_2$ (phenylacetate) + NH_3 + CO_2 + $2H_2$ + ATP	Stickland
6	$C_9H_{11}O_2N$ (Phe) + H_2 → $C_9H_{10}O_2$ (phenylpropionate) + NH_3	Stickland
7	$C_9H_{11}O_2N$ (Phe) + $2H_2O$ → C6H6 (phenol) + $C_2H_4O_2$ (acetate) + NH_3 + CO_2 + H_2 + ATP	Non-Stickland

2.4 Valerate and Butyrade Degrader

Butyrate and valerate are two compositions which can be found in a typical volatile fatty acid of an acidic anaerobic digestion reactor of sludge [17]. The degradation kinetics of normal and branched chain butyrate and valerate are important in protein-fed anaerobic systems, as a number of amino acids degrade to these organic acids.

Based on Table 6, the degradation for both *n*-buytrate and *n*-valerate is via β-oxidation to acetate and acetate + propionate, respectively. The organisms that are capable to degrade butyrate are *Syntrophaceae* sp, *Tepidanaerobacter* sp. and *Clostridium* spp. Typically, if one of these substrates can be degraded by these organisms then it may potentially degrade the others. *I*-butyrate is also oxidized by the same organisms, and reciprocal isomerism between the two forms of butyrate has been well established [18, 19]. Both *neo*-valerate and *i*-valerate are more complex and difficult to access in environmental situations, as they are lumped in gas chromatography measurements.

Clostridium bryantiisp. can oxidize *neo*-valerate to acetate and propionate via β-oxidation while *i*-valerate degrades to acetate as the only organic acid product [18].

.

2.5 Propionate Degrader

Abundance of *Smithella* spp. among Syntrophotbacterales indicates syntrophic degradation of propionate and butyrate. The syntrophy of bacteria (illustrated in Fig. 1) is responsible for carrying out degradation of amino acids, aromatic

Table 4 Saturated and unsaturated LCFA commonly found in wastewaters (shown as % of total LCFA) [14]

	LCFA common name (structure*)						
	Saturated LCFA				Unsaturated LCFA		
Wastewaters	Laureate (C12:0)	Myristate (C14:0)	Palmitate (C16:0)	Stearate (C18:0)	Palmitoleate (C16:1)	Oleate (C18:1)	Linoleate (C18:2)
Domestic sewage		2.2	16.4	8.1	0.9	30.5	29.2
Dairy wastewater			27.0	7.0		37.0	13.0

Table 5 Gibbs free energy changes for some of the acetogenic and methanogenic reactions (presumably) involved in syntrophic conversion of different fatty acids [14]

Reactant	Equation
Fatty acids oxidation reactions	
Linoleate (C18: 2)	Linoleate + $16H_2O \rightarrow$ 9acetate + $14H_2 + 8H^+$
	$CH_3(CHCH)COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$
Oleate (C 18:1)	Oleate + $16H_2O \rightarrow$ 9acetate + $15H_2 + 8H^+$
Stearate (C 18: 0)	Stearate + $16H_2O \rightarrow$ 9acetate + $16H_2 + 8H^+$
Palmitate (C 16: 0)	Palmitate + $14H_2O \rightarrow$ 8acetate + $14H_2 + 7H^+$
Butyrate (C 4: 0)	Butyrate + $2H_2O \rightarrow$ 2acetate + $2H_2 + H^+$
Methanogenic reactions	
Hydrogen	$H_2 + 1/4HCO_3^- + 1/4H^+ \rightarrow 1/4CH_4 + 3/4H_2O$
Acetate	Acetate + $H_2O \rightarrow HCO_3^- + CH_4$

Table 6 Butyrate and valerate degradation reactions [18]

Reaction	Substrate	Reaction
1	*n*-buytrate	$CH_3CH_2CH_2OOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$
2	*i*-butyrate	$CH_3(CHCH)COOH + 2H_2O \rightarrow 2CH_3COOH + 2H_2$
3	*n*-valerate	$CH_3CH_2CH_2CH_2COOH + 2H_2O \rightarrow CH_3COOH + CH_3CH_2COOH + 2H_2$
4	*neo*-valerate	$CH_3CH_2(CHCH_3)COOH + 2H_2O \rightarrow CH_3COOH + CH_3CH_2COOH + 2H_2$
5	*i*-valerate	$CH_3(CHCH_3)CH_2COOH + CO_2 + 2H_2O \rightarrow 3CH_3COOH + H_2$
6	*i*-valerate	$CH_3(CHCH_3)CH_2COOH + 4H_2O \rightarrow 2CH_3COOH + CO_2 + 5H_2$

compounds and propionate and butyrate which ultimately leads to the formation of CH_4 [20].

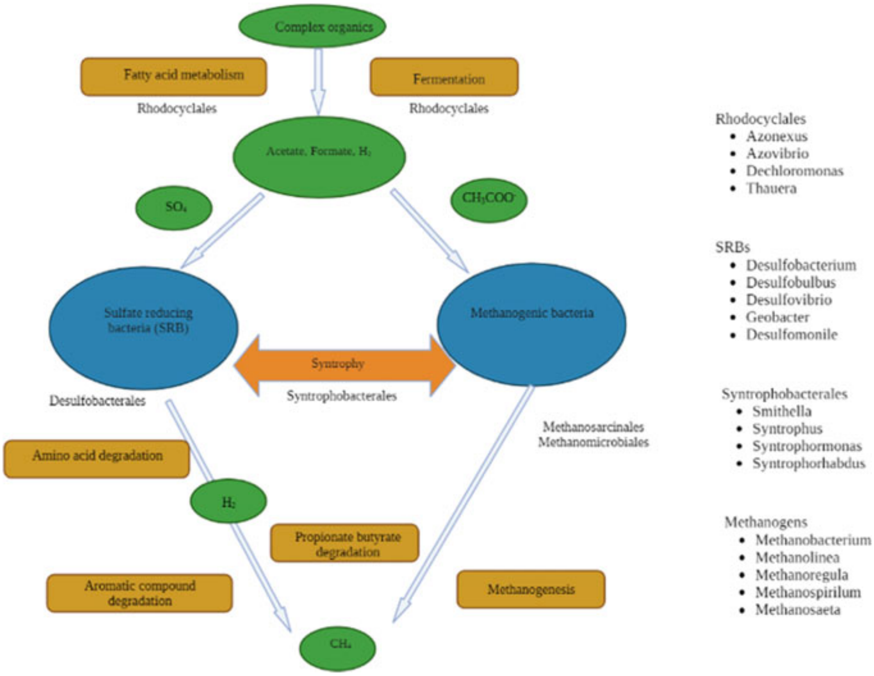

Fig. 1 Schematic representation of anaerobic carbon mineralization in sewage sludge with the microbial communities. Adapted from [20] (Created with Biorender)

2.6 Acetate Degrader

The source of acetate in sludge is originated from the conversion of volatile fatty acid in dark fermentation: acetogenesis [17]. Acetotrophic is a condition in which methyl groups are reduced by *Methanosarcinales* genus which uses simple compounds (acetate) for their growth. Acetotrophic methanogens are obligatory anerobes that transform acetate to methane and carbon dioxide. It was found that, during the anaerobic processing of sewage sludge and manure, the number of *Methanosaeta* genus increased with decreasing acetate in environment, simultaneously intensive growth of bacteria which are acetotrophic methanogens [21, 22]. Research conducted by Detman et al. [23] highlighted that *Methanosaeta* genus can be evaluated based on MAGs phylogenetic tree which shows *Methanothrix soehngenii* had the most abundant (12.1%) [23].

Stoichiometry reaction degradation of acetate:

$$\text{Acetate} + H_2O \rightarrow HCO_3^- + CH_4 \tag{2}$$

where H_2O is water, HCO_3^- is bicarbonate and CH_4 is methane.

$$CH_3COO^- + SO_4^{2-} \rightarrow 2HCO_3^- + HS^- \quad (3)$$

where CH_3COO^- is acetate and SO_4^{2-} is sulphate.

2.7 Hydrogen Degrader

The anaerobic microorganisms produce hydrogenase enzyme which is capable to evolve and taking up hydrogen (H_2 [24]. Hydrogen production by fermentative microorganisms is an expectable method compared with photosynthetic bacteria due to its high utilization of organic compounds or wastes as substrate to produce hydrogen day and night. The production of molecular hydrogen (fermentation process) is generally associated with intracellular iron–sulphur protein, ferredoxin, which is an electronegative electron carrier [24]. The electrons transfer from ferredoxin to H^+ is catalyzed by hydrogenase enzyme. Two classes of fermentative bacteria are capable of producing hydrogen at a high rate and yield, including strictly anaerobic and facultative anaerobic bacteria. First *Clostridium butyricum* largely utilized in the biotechnological hydrogen production and secondly *Klebsiella pneumonia* typically a facultative anaerobic bacteria as nitrogen fixing [24].

Reaction 4summarized stoichiometry for both *Sporomusasphaeroides* and *Woliniela* for reduction of CO_2 to acetate.

Metabolism degradation of hydrogen:

$$H_2 + \text{fumarate} \leftrightarrow \text{Succinate} \quad (4)$$

Clostridium butyricum strict anaerobic bacterium, is known as a classical acid producer and usually ferments carbohydrates to butyrate, acetate, carbon dioxide, and molecular hydrogen [25, 26]. Based on Fig. 2, there are two pathways to produce hydrogen, one is via the cleavage of pyruvate to acetyl-CoA and the other to NAD^+ to generate $NADH_2$.

The production of 2,3-butaediol, ethanol and lactate from pyruvate by $NADH_2$ as a reductant, but not for H_2 [27].While *Klebsiella pneumonia;* a facultative anaerobic and nitrogen-fixing bacteria also has the ability to produce hydrogen in high quantities. Nitrogen is mainly associated for hydrogen production by *K. pneumonia.*

2.8 Sulphate Degrader

Sulphate ion ($S0_4^{2-}$) is one of the most universal anions occurring in rainfall, especially in air masses that have encountered metropolitan areas (During anaerobic conditions, sulphate is reduced to sulphide by sulphate-reducing bacteria [SRB]).

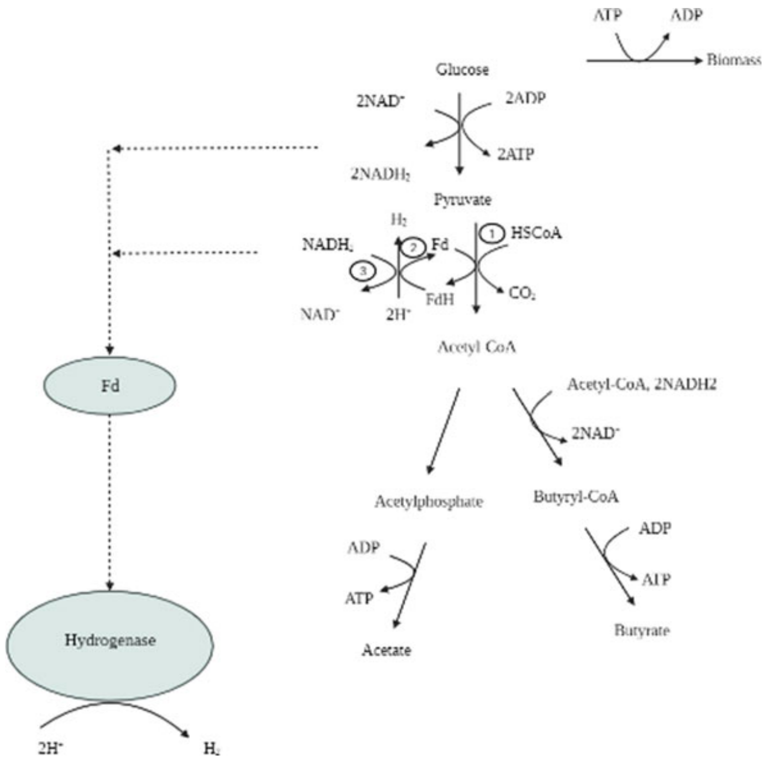

Fig. 2 Metabolic pathway of glucose by *Clostridiumbutyricum* under anaerobic conditions. (1) Pyruvate: ferredoxinoxidoreductase (PFOR); (2) Hydrogenase; (3) NADH: ferredoxinoxidoreductase. Adapted from 24 (Created with Biorender)

This SRB play a fundamental role as sulphate bioremediator through the conversion of sulphate to sulphide in the stabilization process [5]. Additionally they can compete with other anaerobic bacteria for a wide range of carbon sources and electron donors such as glucose, lactate, propionate, acetate, butyrate and ethanol. SRB found famously to grow at pH range 6–8 or called as neutrophilic condition [28]. Sulphate reducers that degrade carbon can be divided into two groups: (i) bacterial group that can completely degrade the carbon to carbon dioxide and (ii) bacterial group that catalyze partial carbon degradation to acetate which can be clearly figure in Table 7. The SRB can generate twice as much energy during the incomplete oxidation of lactate compared with its complete oxidation [29].

Table 7 Reduction of sulphate only partially oxidized [30]

Reaction
$2\textbf{Lactate}^{-} + \textbf{S}0_4^2 \leftrightarrow 2\textbf{acetate}^{-} + 2\boldsymbol{H}_2\boldsymbol{O} + 2\boldsymbol{CO}_2 + \boldsymbol{S}^{2-}$
$2\textbf{Ethanol} + 3\textbf{S}0_4^2 \leftrightarrow 6\boldsymbol{H}_2\boldsymbol{O} + 4\boldsymbol{CO}_2 + 3\boldsymbol{S}^{2-}$
$2\textbf{Malate}^{2-} + 3\textbf{S}0_4^2 + 4\boldsymbol{H}^{+} \leftrightarrow 6\boldsymbol{H}_2\boldsymbol{O} + 8\boldsymbol{CO}_2 + 3\boldsymbol{S}^{2-}$

3 Conclusions

This book chapter provides an insight into the fundamental components of sewage sludge, including the natural microbe degraders present in the sludge. The knowledge of the microbial community in the sludge allows for the exploitation of the sludge and the isolation of suitable microbes for bioremediation purposes. The microbiological approach is a greener method for solving environmental pollution and has the potential to provide a sustainable solution.In addition to bioremediation, the chapter highlights the potential for the purification of useful chemical compounds from sewage sludge, such as expensive fatty acids that can be obtained through the isolation of certain species found in the sludge. This demonstrates the potential for the valorization of sludge in new emerging green technologies.One such technology is the microbial fuel cell (MFC), which requires a comprehensive and effective microbial degrader to accelerate the degradation process and increase the oxidation process, resulting in higher current density for energy recovery. The understanding of the microbial degraders in sewage sludge is essential for the development of effective and sustainable approaches to sludge management.Overall, this book chapter provides an insight into the components and typical mechanisms of sludge degrader microbes in dewatered sludge and highlights the potential for the exploitation of sludge valorization in sustainable approaches to sludge management. The utilization of natural microbe degraders can provide solutions to environmental pollution, produce valuable chemical compounds, and contribute to the development of new emerging green technologies such as the microbial fuel cell.

Acknowledgements The authors would like to thank the Universiti Sains Malaysia for the financial support of this study via APEX Era grant (1001/PTEKIND/881004). The authors have declared no conflict of interest for the manuscript.

References

1. Ahmed T, et al (2019) Biomass and bioenergy hydrothermal carbonization of lignocellulosic biomass for carbon rich material preparation: a review. Biomass Bioenergy 130(August):105384. https://doi.org/10.1016/j.biombioe.2019.105384
2. Duncan SM, Alkasrawi M, Gurram R, Almomani F, Wiberley-Bradfordand AE, Singsaas E (2020) Paper mill sludge as a source of sugars for use in the production of bioethanol and isoprene. Energies 13(18). https://doi.org/10.3390/en13184662

3. Doas N, Ghandour MA, Abd-alla MH (2018) Sludge reduction in wastewater of beet sugar industry using the effective microorganisms. In Abu Qurqas sugar factory. Egypt Sugar J 10:63–82
4. Oladejo J, Shi K, Luo X, Yang G, Wu T (2019) A review of sludge-to-energy recovery methods. Energies 12(1):1–38. https://doi.org/10.3390/en12010060
5. Tambo N, Kobayashi M, Thebault P, Haubry A (1982) Sludge treatment and disposal, vol 1, no 2/3. IWA
6. Yildiz BS (2012) 18—Water and wastewater treatment: biological processes. In Zeman FBT-MS (ed) Woodhead Publishing series in energy. Woodhead Publishing, Sawston, pp 406–428
7. Kumar V, Chopra AK, Kumar A (2017) A review on sewage sludge (biosolids) a resource for sustainable agriculture. Arch Agric Environ Sci 2(4):340–347. https://doi.org/10.26832/24566632.2017.020417
8. Talebi A, Razali YS, Ismail N, Rafatullah M, Azan Tajarudin H (2020) Selective adsorption and recovery of volatile fatty acids from fermented landfill leachate by activated carbon process. Sci Total Environ 707:134533. https://doi.org/10.1016/j.scitotenv.2019.134533
9. Raharja R, Murdiyatmo U, Sutrisno A, Wardani AK (2019) Bioethanol production from sugar-cane molasses by instant dry yeast. IOP Conf Ser Earth Environ Sci, 230(1). https://doi.org/10.1088/1755-1315/230/1/012076
10. Anukam A, Mohammadi A, Naqvi M, Granström K, A review of the chemistry of anaerobic digestion: methods of accelerating and optimizing process efficiency, pp 1–19.
11. Ramsay IR, Pullammanappallil PC (2001) Protein degradation during anaerobic wastewater treatment: derivation of stoichiometry. Biodegradation 12(4):247–256. https://doi.org/10.1023/A:1013116728817
12. Park J, Park S, Kim M (2015) Anaerobic degradation of amino acids generated from the hydrolysis of sewage sludge, April. https://doi.org/10.1080/09593330.2013.863951
13. Singh S, et al (2020) Enhanced methanization of long-chain fatty acid wastewater at 20°c in the novel dynamic sludge chamber–fixed film bioreactor. Front Energy Res, 8:166 [Online]. https://www.frontiersin.org/article/10.3389/fenrg.2020.00166
14. Sousa DZ, Smidt H, Alves MM, Stams AJM (2009) Ecophysiology of syntrophic communities that degrade saturated and unsaturated long-chain fatty acids. https://doi.org/10.1111/j.1574-6941.2009.00680.x
15. Roy F, Samain E, Dubourguier HC, Albagnac G (1986) Synthrophomonas sapovorans sp. nov., a new obligately proton reducing anaerobe oxidizing saturated and unsaturated long chain fatty acids. Arch Microbiol 145(2):142–147. https://doi.org/10.1007/BF00446771
16. Hatamoto M, Imachi H, Ohashi A, Harada H (2007) Identification and cultivation of anaerobic, syntrophic long-chain fatty acid-degrading microbes from mesophilic and thermophilic methanogenic sludges. Appl Environ Microbiol 73(4):1332–1340. https://doi.org/10.1128/AEM.02053-06
17. Moestedt J, Westerholm M, Isaksson S, Schnürer A (2020) Inoculum source determines acetate and lactate production during anaerobic digestion of sewage sludge and food waste. Bioengineering, 7(1). https://doi.org/10.3390/bioengineering7010003
18. Batstone DJ, Pind PF, Angelidaki I (2003) Kinetics of thermophilic, anaerobic oxidation of straight and branched chain butyrate and valerate. Biotechnol Bioeng 84(2):195–204. https://doi.org/10.1002/bit.10753
19. Hatamoto M, Imachi H, Yashiro Y, Ohashi A, Harada H (2008) Detection of active butyrate-degrading microorganisms in methanogenic sludges by RNA-based stable isotope probing. Appl Environ Microbiol 74(11):3610–3614. https://doi.org/10.1128/AEM.00045-08
20. Sidhu C, Vikram S, Pinnaka AK (2017) Unraveling the microbial interactions and metabolic potentials in pre- and post-treated sludge from a wastewater treatment plant using metagenomic studies, 8(July): 1–10. https://doi.org/10.3389/fmicb.2017.01382
21. The Scientific World Journal (2017) Retracted: microbial ecology of anaerobic digesters: the key players of anaerobiosis. Sci World J 2017:3852369. https://doi.org/10.1155/2017/3852369
22. Griffin ME, McMahon KD, Mackie RI, Raskin L (1998) Methanogenic population dynamics during start-up of anaerobic digesters treating municipal solid waste and biosolids.

Biotechnol Bioeng 57(3):342–355. https://doi.org/10.1002/(SICI)1097-0290(19980205)57:3%3c342::AID-BIT11%3e3.0.CO;2-I
23. Detman A et al (2021) Evaluation of acidogenesis products' effect on biogas production performed with metagenomics and isotopic approaches. Biotechnol Biofuels 14(1):125. https://doi.org/10.1186/s13068-021-01968-0
24. Chen X, Sun Y, Xiu Z, Li X, Zhang D (2006) Stoichiometric analysis of biological hydrogen production by fermentative bacteria. Int J Hydrogen Energy 31(4):539–549. https://doi.org/10.1016/j.ijhydene.2005.03.013
25. Zigová J, Šturdík E, Vandák D, Schlosser S (1999) Butyric acid production by Clostridium butyricum with integrated extraction and pertraction. Process Biochem 34:835–843. https://doi.org/10.1016/S0032-9592(99)00007-2
26. Vignais P, Billoud B, Meyer J (2001) Vignais PM, Billoud B, Meyer J. Classification and phylogeny of hydrogenases. FEMS Microbiol Rev 25:455–501. https://doi.org/10.1111/j.1574-6976.2001.tb00587.x
27. Kurokawa T, Tanisho S (2005) Effects of formate on fermentative hydrogen production by Enterobacter aerogenes. Mar Biotechnol 7(2):112–118. https://doi.org/10.1007/s10126-004-3088-z
28. Tran TTT, Kannoorpatti K, Padovan A, Thennadil S (2021) Sulphate-reducing bacteria's response to extreme ph environments and the effect of their activities on microbial corrosion. Appl Sci 11(5):1–19. https://doi.org/10.3390/app11052201
29. Rubio-rincón F, et al (2017) Effects of electron acceptors on sulphate reduction activity in activated sludge processes, pp 6229–6240. https://doi.org/10.1007/s00253-017-8340-3
30. Wake LV, Christopher RK, Rickard PAD, Andersen JE, Ralph BJ (1977) A thermodynamic assessment of possible substrates for sulphate-reducing bacteria. Aust J Biol Sci 30(2):155–172. https://doi.org/10.1071/BI9770155