Research Article

Comparative evaluation of the biochemical methane potential of waste activated sludge acetic acid and cellulose substrates under mesophilic and thermophilic anaerobic digestion

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Received: 8 March 2020 / Accepted: 18 August 2020 / Published online: 24 August 2020

Abstract
Knowing the biochemical methane potential of a substrate to biogas production is a preliminary to possible selection of the pre-anaerobic digestion technology and identifying the optimum reaction time. Following standard method to reactor assembly, feed preparation and monitoring of the specific methane yield, the two temperature systems are compared among substrates. As a result, the rate of biochemical methane yield from the waste activated sludge anaerobic digestion in the thermophilic case is much faster than the mesophilic. The specific methane yield and volatile solid removal of the waste activated sludge batch anaerobic digestion is 74% of the theoretical and 81% for the thermophilic while it is 57% theoretical and 76% for the mesophilic, respectively. However, the best methane yield is recorded for the acetic acid followed by the cellulose and sludge substrates, signifying the importance of sludge pretreatment to enhance degradability in anaerobic digestion. Also, the optimum reaction time for thermophilic and mesophilic systems is different.

Keywords Waste activated sludge · Anaerobic digestion · Biochemical methane potential · Thermophilic · Mesophilic

1 Introduction

Conventional wastewater treatments are so far preferred to manage the huge volume of sewage in an urbanized society and in different countries. However, the energy and economic inefficiencies are challenging them. In this regard, recovering the energy and other chemicals from such biowaste is accepted by most central wastewater treatment facilities across the globe [1–3].

Anaerobic digestion (AD) of biomass remains a robust technology to a renewable energy, soil nutrient and waste stability advantages. However, the performance of AD has to be evaluated mainly based on the recovered biochemical methane potential (BMP) of the biomass feed, which are achieved based on acceptable protocol. Therefore, performing the BMP test of the feed is essential, in fact, not only for the later performance evaluation in terms of the energy recovered but also to judge the degradability status of the feed and thereby to determine the pretreatment required. For instance, degradability has been defined as the BMP divided by the theoretical methane potential which is calculated with the theoretical factor of 0.35 l of CH_4 per unit gram of the chemical oxygen demand (COD) [4–7].

Generally, pretreatments are existing practices in wastewater treatment to enhance performance. However, the advantages and disadvantages the different pretreatment techniques should be considered with respect to cost, technology selection, energy and the resulting secondary waste [8]. Regarding of AD feeds, substrate pretreatments are being preferred for their outweighing advantages over their cost implications. Among the many variables which

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affect the success and feasibility of AD is the feedstock pretreatment [6]. Since particle size of feed affects the hydrolysis stage, which is a rate limiting stage in AD, size reduction pretreatments, especially hybrid pretreatments, are normally acceptable for substrates with low biodegradability to improve performance of bioprocesses and perhaps the methane yield [9–11].

Recent studies have also reported correlating the BMP with polyhydroxyalkanoate (PHA) content and other derivatives from WAS and other biomass [4]. The PHA content, which is expressed per gram of the volatile suspended solids (VSS), in waste activated sludge (WAS) indicated the BMP yield better that protein and carbohydrate contents in feedstocks. The PHAs significantly contribute to possible cell disruption thereby affecting the hydrolysis and acidification stages in WAS AD. The detail of the mechanism is reported by Wang and co-authors [12]. In the current study, the BMP of WAS is compared with synthetic cellulose ($C_6H_{10}O_5$) and acetic acid ($C_2H_4O_2$) using a standard protocol. In related studies, the application of cellulose digestion is considered as control experimental run [13, 14].

Obviously, the differences in biodegradability of feedstocks affects the resulting AD performance. Determining such differences through comparison of substrates of extreme nature, which otherwise can be viewed as control substrates [15], in turn assist the suggestion of possible pretreatment options. Therefore, the objective of this study is to give the basis to evaluate the performance of WAS AD technologies in terms of the recovered methane, biogas and hence to direct the need for biomass pretreatments in comparison with cellulose and acetic acid AD. Further, this study reveals the effect of temperature on the possible recovery of bioenergy from the AD of WAS comparing thermophilic and mesophilic conditions.

2 Materials and methods

2.1 Experimental setup

Generally, the method applied in the current BMP test is based on published sources [13, 16]. The temperature of the AD is set at two levels, which is 35 ± 1 °C for mesophilic and 55 ± 1 °C for thermophilic systems. Glass bottles of 120 ml volume consisting of 80 ml slurry and a 40 ml gas space are used. The inoculation of the thermophilic and mesophilic inocula into those bottles at 50 ml volume is done first.

Following, substrates formulated into slurry are poured at a dosage of 0.5 g of the total COD (CODt) per g-VSS of the inoculum (Table 1). After filling the slurry, the bottles are sealed with a rubber stopper and aluminum lid. The inside of each bottle is then flushed with nitrogen gas using a needle pricking through the rubber stopper for 2 min before they are placed into their respective thermal chamber. During the reaction time, the biogas yield from those serum bottles was measured using the liquid displacement system while the biogas composition and hence its quality is determined using the gas chromatography (GC) Fisons GC8000 2014 (Fison Instruments, Italy). The GC with a thermal conductivity detector assembled capillary metal tube round chromatography column (PORAPAK Q) and a hydrogen carrier gas have been applied during gases detection and quantification. Injector, column, and detector temperatures were set at 80, 60 and 90 °C, respectively, and the gas flow rate was 30 ml/min. One ml of gas sample is obtained using syringes and needle for analysis, which is shortly injected to GC.

The inocula for the current work are brought from working large-scale anaerobic digesters. By the same protocol, a blank or endogenous and control cellulose as well as acetic acid substrates were run side by side in triplicates for a retention time of 44 days (Fig. 1). For all digesters, the pH of the inocula was around eight, which did not require the adjustment in favor of the methanogens.

2.2 Sampling and sample preparation

For the solids and Nammon analysis, the sludge sample volume of 10 ml is used while proper sample volume of centrifuged and diluted sludge is used to both the soluble chemical oxygen demand (CODsol) and the CODt. Measurement of CODt is made by diluting the samples by a factor of 50. The DS and the CODsol samples are centrifuged first at an angular speed of 1300 revolutions per minute for

Table 1 Substrate portion of the biochemical methane potential assay fed to both temperature bottles

Parameters	Mesophilic				Thermophilic			
	Inoculum	Substrates			Inoculum	Substrates		
		WAS	Cellulose	Acetic acid		WAS	Cellulose	Acetic acid
CODt (g/l)	9.5	28.4	1190	23.1	19	28.4	1190	23.1
Volume (ml)	50	11.5	0.3	14	50	11	0.3	13
VSS (g/l)	13.1	49.0			12.3	49.0		

Fig. 1 Setup of the biochemical methane potential assay

10 min. After centrifugation, 10 ml of the expressed water is pipetted into the drying cups to do the dissolved solid (DS). For the CODsol, 2.5 ml of sample is pipetted after dilution into the digestion glass vials. To do the COD different dilution rates; 5 times for the mesophilic and 10 times for the thermophilic sludge samples are applied because of their variations in VS concentration with respect to the detection limit of the spectrophotometer. Whereas the WAS is analyzed without dilution. All the three samples are analyzed around 19 °C.

2.3 The solids analysis

Solids in the sludge samples are determined according to the standard methods outlined in the American Public Health Association's standard [17]. The total solid (TS) is analyzed by drying the sludge sample in an oven at 105 ± 2 °C. This temperature is kept for 3 h, which will evaporate all the water in the sample and it is latter weighed to constant weight. The volatile solid (VS) is determined by combusting the solid sample after the oven drying in a furnace kept at a temperature of 550 ± 2 °C and run for 2 h. Because of its complete mineralization, the organic content in the sample with an insignificant inorganic matter volatilizes during ignition. Whereas, the total dissolved solid (TDS) and the volatile dissolved solid (VDS) are estimated using a centrifuged sample and after the separation of its suspended fraction. The suspended solids (SSs) are then obtained from the calculation of the differences between the total and the dissolved part. The residue obtained after the mineralization of the sample at 550 ± 2 is the inorganic fraction, also called fixed solid (FS).

2.4 The chemical oxygen demand analysis

Use of the COD instead of the VS as a parameter of an organic strength is also applicable since both parameters tell the amount of organic matter in a sample directly or indirectly whereby the former is faster to determine. To do the CODt as well as the soluble fraction the chemical digestion method is applied [18]. Hence, the sludge sample is mineralized by adding a concentrated H_2SO_4 and $K_2Cr_2O_7$ solutions into those COD vials containing the sludge sample and are oxidizers in DRB 200 digester which is set at a temperature of 150 °C for 2 h. Next, the absorbance of the sample is measured at the 600 nm, which works based on a change in color of the potassium dichromate. A spectrophotometer, DR3900 (HACH LANGE), is used to measure the absorbances. Indeed, a centrifuge is used to separate the solid from the supernatant during the testing of CODsol.

2.5 The ammonia nitrogen analysis

NH_3-N (Nammon) is determined using Kjeldahl Method or Kjeldahl digestion performed in a distillation unit, known with the trade mark K-350. In this method, the sludge sample is first heated by sulfuric acid to molder the organic matter and release the reduced nitrogen as ammonium sulfate. Decomposition is completed when the dark color of the sample is changed to a clear colorless product. The solution obtained is then distilled using a small amount of caustic soda which converts the ammonium ion into ammonia. The amount of ammonia and hence the amount of nitrogen is determined by doing the back-titration. Since the ammonia reacts with a known amount of boric acid in a flask which is connected to the end of the condenser, the excess acid is then titrated with sodium carbonate while using pH indicator. Digital pH probe (SENTRON, SI4007400-010) is used to measure the pH and the temperature of sludge sample almost every day with frequent calibration [19].

3 Results and discussion

3.1 Characterization of inocula and the waste activated sludge sample

Both inocula and the WAS that is not pretreated are characterized first for their solids, the COD and Nammon content. The various solids determined in these analyses are thus in increasing concentration in order from the WAS to mesophilic inoculum and to thermophilic inoculum (Table 2) this would be due to the location of sampling for both inocula and also the amount of biomass present in those inocula as well as degree of treatment applied at the wastewater treatment plants. The solids expressed as TS and VS as well as the ratio between these two slightly varies from what is reported by the European Commission (E.C) in this case [20]. The amount of TS for WAS is slightly higher in our case while it is lower for the inocula so their

Table 2 Result of the physicochemical characterization of the WAS, the mesophilic inoculum & the thermophilic inoculum in mean ± (standard deviation)

Sample	pH	TS (mg/l)	TDS (mg/l)	VS (mg/l)	VDS (mg/l)	TSS (mg/l)	VSS (mg/l)	VSS/TSS	COD (mg/l)	CODt (mg/l)	CODt/VS	$N\text{-}NH_4^+$ (mg/l)	FS (mg/l)
WAS	7.0	7723 (15)	710 (17)	5203 (35)	103 (15)	7013	5100	0.7	46 (9)	7033 (764)	1.4	46 (2)	2520 (26)
Mesophilic inoculum	7.7	22753 (86)	1607 (15)	13143 (156)	567 (21)	21147	12577	0.6	918 (45)	21633 (1069)	1.6	1024 (4)	9607 (65)
Thermophilic inoculum	8.0	27167 (258)	2836 (6)	15440 (629)	1863 (25)	24330	13577	0.6	3083 (107)	24100 (984)	1.5	1524 (20)	11727 (391)

ratio varies accordingly. However, given the fact that the stabilization in these inocula may not be complete and the E C report is years old and the European Union is represented of sum of countries the deviation is acceptable.

According to the result of the VSS/TSS ratio, it is expected that a WAS before the AD has a ratio which ranges between 0.7 and 0.8 (70–80%) due to more degradable organic matter expressed as VS. Accordingly the stabilized WAS and hence the inocula are expected to reach down to 0.5 of that same ratio (50%) which is based on a relative measure of stabilization. Thus, the value in the table lies within this assumption, though the stabilization requires longer time for both inocula.

The CODsol of the WAS sample falls around the lower value of the range published by Zanetti and co-worker which is 51–135 mg/l. The Nammon is lower by comparison to a case study in Germany, which is 650 mg/l for an activated sludge that was actually from a waste stream high in nitrogen content. Conversely, the CODt of the sludge sample in our case falls between the CODt of raw sewage sludge and WAS as reported by Zanetti et al. and Meyer and Wilderer [21, 22].

As to the CODt/VS ratio, it is generally suggested that this ratio lies within standard deviation around the mean value of 1.5 which actually varies among different reports. The finding here for those inocula as well as the WAS (Table 2) are not far deviated according to the report by Parker et al. [23]. However, according to Kabouris and coworkers [24] the CODt/VS ratio is lower compared to what they found out on a thickened WAS, 1.94. Conversely, it is a little higher than what is reported earlier. Therefore, it is evident that various sludge sources have different outcomes as a matter of the nature of the waste itself, the treatment applied at the waste treatment plants and, if any, pretreatment applied to the sludge samples.

Compared to the WAS, the mean ammonia concentration of both inocula is far higher than what is desired for methanogenesis process which is below 200 mg/l (Table 2) however the inhibitory level is much higher than that. The thermophilic sludge is even 1.5 times higher than the mesophilic by comparison. A free ammonia concentration of 560–568 mg NH_3-N/l is claimed to reduce methane production by 50% [25]. Total inhibition can occur at a concentration of 10 g-N/l [26]. Further, the pH of the thermophilic inoculum is relatively higher.

3.2 The biochemical methane potential assay

Under the mesophilic mode of the AD, the normalized biochemical methane yield among WAS, acetic acid and cellulose digestions brought significant difference after 44 days of sludge retention time. The yields at standard temperature and pressure conditions for acetic acid, cellulose and

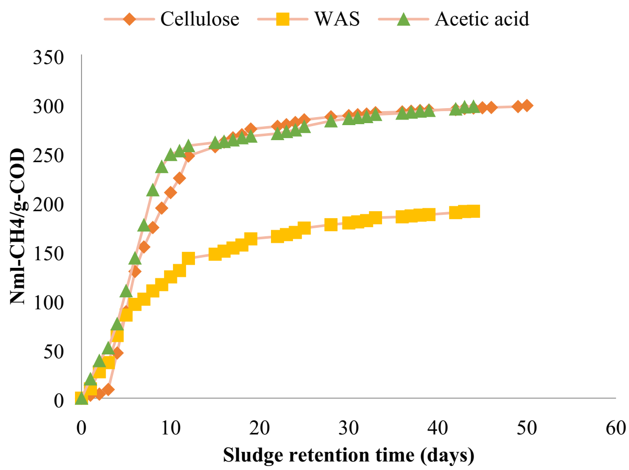

Fig. 2 Specific cumulative biochemical methane yield from the mesophilic biochemical methane potential assay

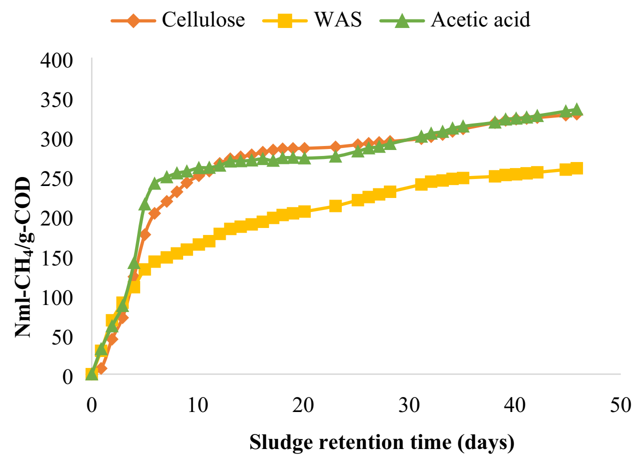

Fig. 3 Specific cumulative biochemical methane yield from the thermophilic biochemical methane potential assay

WAS were 297, 295.5, and 190.4 Nml-CH4/g-COD, respectively. However, there was continuity of production of methane by the cellulose digester even after 44 days of the mentioned sludge retention time, which may be due to its less solubility compared with the acetic acid (Fig. 2).

Based on the data collected, the methane yield in the mesophilic batch systems is over 86% of the expected theoretical yield for cellulose and the acetic acid. For the sludge, it is around 57% of the expected theoretical yield from a pure substrate that is obviously because of the nature of media and it is not equally degradable like those pure media. Compared to those pure media, it is nearly 30% less in methane yield, but it should be borne in mind that it is a secondary medium brought after an activated sludge process (Fig. 2). The BMP test yield for the WAS (> 190 Nml-CH_4/g-COD) that is digested at the mesophilic temperature is in agreement with the report by Carrere et al. [27], but it is lower than what is reported by Pontoni et al. [28, 29].

Under the thermophilic mode of the AD, the normalized biochemical methane yield among WAS, acetic acid and cellulose digestions brought significant difference after 45 days of sludge retention time. The yields at standard temperature and pressure conditions for acetic acid, cellulose and WAS were 334.6, 328.6, and 260.2 Nml-CH4/g-COD, respectively (Fig. 3).

The biochemical methane yield in the thermophilic batch AD assay is greater than the mesophilic systems. It was about 95% of the theoretical yield per unit gram of COD for the pure substrates; cellulose and the acetic acid. Still better yield in the WAS thermophilic batch AD is recorded at a little over 74% of the theoretical yield (Fig. 3) for pure substrate. However, similar to the mesophilic sludge digestion, the biochemical methane yield of the WAS thermophilic batch assay is lower than those pure media that happened due to the less degradability of WAS. The BMP of WAS is even proved to be significantly lower than that of the mixture of primary and secondary sludge [30].

In both temperatures of digestion, the acetic acid and cellulose showed a slight difference in degradation rates. For the acetic acid reaching early peak, which is due to the solubility of the substrate compared to both the sludge and cellulose. Conversely, the production of methane becomes slightly higher for the cellulose after the 15th day of digestion, which is still due to the difference in the degradability of the substrate since cellulose takes longer time to degrade relative to acetic acid and hence there is the availability of the substrate is prolonged in the system. In the case of cellulose and sludge BMP assay the rate of methane production is faster by the thermophilic bottles which is due to the faster hydrolysis as a result of the higher temperature of digestion [31].

The rate of biochemical methane yield from the WAS AD in the thermophilic case is much faster than the mesophilic with the disparity widening with increase in time (Fig. 4). The reason for that to happen is due to the fast hydrolysis in the thermophilic case.

These findings that the less degradable cellulose media's methane is tapped better than the WAS indicating that the physical state of the media and hence the solubility and efficient degradability matters. Therefore, proper pretreatment of the sludge in terms of disintegrating the

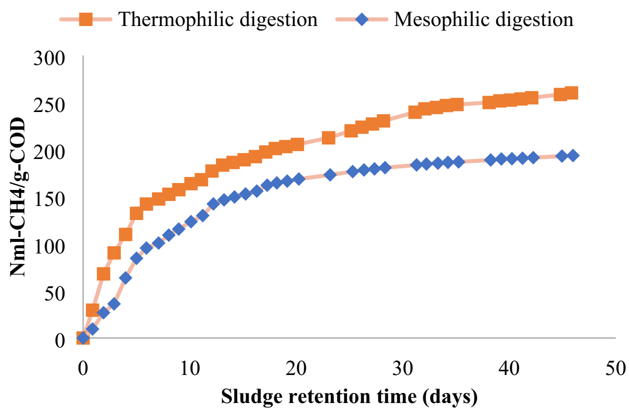

Fig. 4 The specific cumulative biochemical methane yields as compared between mesophilic and thermophilic waste activated sludge biochemical methane potential assay

particles in the WAS would enhance its BMP. Though several pretreatments for WAS AD are available, recent studies are revealing the humus and lignocellulose solubilization effect of CaO_2 application. The optimum breakdown of humus and lignocelluloses in WAS using CaO_2 to enhance methane yield as well as its fundamental mechanism are also elucidated [32].

After digestion is ceased the sludge is analyzed for its pH, Nammon, CODsol, and solids (Table 3). Overall, the pH values of all digesters are well in the optimum range. Relatively the pH for the thermophilic bottles is little higher compared to the mesophilic pH readings. The highest Nammon by the WAS fed digesters is due to the nature of the substrate that obviously contains protein compounds.

The CODsol compared between the mesophilic and the thermophilic sludge is higher for thermophilic in all kinds of substrates and the control. This over twofold concentration of CODsol by the thermophilic sludge is attained due to high background of thermophilic inocula and the effect of temperature on hydrolysis and the subsequent dissolution of compounds. The effect of dissolution is also evident from the TDS and VDS determined after digestion. Conversely, the TSS and VSS are higher for the mesophilic sludge. The VSS removal by the thermophilic digester is higher than by the mesophilic WAS batch AD which are 81% and 76%, respectively.

Since the extent of mineralization and hence the biochemical methane yield in all the substrates is better by the thermophilic batch systems so does is the solid removal which can be understood from the TS and VS determined after digestion for both temperature sludge. The TS and VS concentrations are low after digestion in the thermophilic sludge in all the substrate as well as the control cases.

4 Conclusions

Determining the BMP of any AD substrates is essential to identify the optimum energy recovery in a certain reaction time whereby maximizing the energy self-sufficiency of existing and emerging central waste management systems and ultimately ensure economic efficiency and sustainability. The BMP of WAS is relatively lower compared to the laboratory grade cellulose and acetic acid as a substrate, which is mainly due to its nondegradable complex nature formed after an activated sludge process. However, knowing this untapped energy potential can lead to the possible pretreatment so that its conversion into bioenergy can be enhanced. Thermophilic systems can recover the bioenergy from such substrates better and faster compared to the mesophilic systems. Despite the least recovery of the BMP of WAS, it can still be a good source of substrate for AD after a suitable pretreatment which is suggested for further study.

Table 3 Post-anaerobic digestion characterization of the biochemical methane potential assay

Parameter	Mesophilic				Thermophilic			
	Inoculum	Cellulose	WAS	Acetic acid	Inoculum	Cellulose	WAS	Acetic acid
pH	7.6	7.3	7.5	7.6	7.8	7.8	7.7	7.9
Nammon (mg/l)	1160	1146	1473	1123	1146	1156	1478	1185
CODsol (mg/l)	845	785	1115	1005	2105	1910	2905	2850
TS (g)	17.6	17.7	22.4	20.7	15.9	15.5	20.5	18.9
TDS (g)	1.3	1.3	1.7	4.8	2.01	1.94	2.8	5.9
TSS (g)	16.3	16.4	20.7	15.8	13.9	13.6	17.7	13
VS (g)	9.7	9.9	12.6	10.6	8.5	8.3	11.1	9.5
VDS (g)	0.7	0.7	0.9	0.81	1.44	1.35	2.0	2.0
VSS (g)	9	9.2	11.7	9.8	7.0	6.9	9.1	7.5

Data availability Experimental data are available in excel based on request.

Compliance with ethical standards

Conflict of interest The author declares that there is no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. 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. https://doi.org/10.3389/fenrg.2019.00019
2. Maktabifard M, Zaborowska E, Makinia J (2018) Achieving energy neutrality in wastewater treatment plants through energy savings and enhancing renewable energy production. Rev Environ Sci Biotechnol 17(4):655–689. https://doi.org/10.1007/s11157-018-9478-x
3. Bachmann N, la Cour JJ, Bochmann G, Montpart N (2015) Sustainable biogas production in municipal wastewater treatment plants. IEA Bioenergy Massongex, Switzerland
4. Chan C, Guisasola A, Baeza JA (2020) Correlating the biochemical methane potential of bio-P sludge with its polyhydroxyalkanoate content. J Clean Prod 242:118495. https://doi.org/10.1016/j.jclepro.2019.118495
5. Braguglia CM, Mininni AG (2009) Effect of ultrasound on particle surface charge andfilterability during sludge anaerobic digestion. Water Sci Technol J Int Assoc Water Pollut Res 60:8. https://doi.org/10.2166/wst.2009.505
6. Gallipoli A, Gianico A, Montecchio D, Pagliaccia P, Braguglia C (2019) Exploring the complex role of pre-treatments in anaerobic digestion: from batch to continuous mode. https://www.researchgate.net/publication/336717402_Exploring_the_complex_role_of_pretreatments_in_anaerobic_digestion_from_batch_to_continuous_mode
7. Braguglia CM, Gianico A, Gallipoli A, Mininni G (2015) The impact of sludge pre-treatments on mesophilic and thermophilic anaerobic digestion efficiency: role of the organic load. Chem Eng J 270:362–371. https://doi.org/10.1016/j.cej.2015.02.037
8. Crini G, Lichtfouse E (2019) Advantages and disadvantages of techniques used for wastewater treatment. Environ Chem Lett 17(1):145–155. https://doi.org/10.1007/s10311-018-0785-9
9. Tyagi VK, Lo S-L (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(3):215. https://doi.org/10.1007/s11157-011-9244-9
10. 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 Sustain Energy Rev 16(1):861–877
11. Oladejo J, Shi K, Luo X, Yang G, Wu T (2019) A review of sludge-to-energy recovery methods. Energies. https://doi.org/10.3390/en12010060
12. Wang D, Zhao J, Zeng G, Chen Y, Bond PL, Li X (2015) How does poly(hydroxyalkanoate) affect methane production from the anaerobic digestion of waste-activated sludge? Environ Sci Technol 49(20):12253–12262. https://doi.org/10.1021/acs.est.5b03112
13. Shanmugam P, Horan NJ (2009) Simple and rapid methods to evaluate methane potential and biomass yield for a range of mixed solid wastes. Bioresour Technol 100(1):471–474. https://doi.org/10.1016/j.biortech.2008.06.027
14. Hansen TL, Schmidt JE, Angelidaki I, Marca E, JlC J, Mosbæk H, Christensen TH (2004) Method for determination of methane potentials of solid organic waste. Waste Manag 24(4):393–400. https://doi.org/10.1016/j.wasman.2003.09.009
15. Raposo F, Fernández-Cegrí V, De la Rubia MA, Borja R, Béline F, Cavinato C, Demirer G, Fernández B, Fernández-Polanco M, Frigon JC, Ganesh R, Kaparaju P, Koubova J, Méndez R, Menin G, Peene A, Scherer P, Torrijos M, Uellendahl H, Wierinck I, de Wilde V (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. https://doi.org/10.1002/jctb.2622
16. Filer J, Ding HH, Chang S (2019) Biochemical methane potential (BMP) assay method for anaerobic digestion research. Water. https://doi.org/10.3390/w11050921
17. APHA; WWA & WEF APHAWWAaWEF (1999) Standard methods for the examination of water and wastewater. Solids. Amer Public Health Assn, New York
18. Usepa Usepa (1993) The determination of chemical oxygen demand by semi-automated colorimetry. Cincinnati, Ohio 45268
19. Gebreeyessus GD (2015) Effect of anaerobic digestion temperature on sludge quality. Gent, Gent University. https://lib.ugent.be/fulltxt/RUG01/002/217/086/RUG01-002217086_2015_0001_AC.pdf
20. Bitton G (2005) Anaerobic digestion of wastewater and biosolids. Wastewater microbiology. Wiley, New York, pp 345–369
21. Zanetti L, Frison N, Nota E, Tomizioli M, Bolzonella D, Fatone F (2012) Progress in real-time control applied to biological nitrogen removal from wastewater. A short-review. Desalination 286:1–7. https://doi.org/10.1016/j.desal.2011.11.056
22. Meyer SS, Wilderer PA (2004) Reject water: treating of process water in large wastewater treatment plants in germany—a case study. J Environ Sci Health Part A 39(7):1645–1654. https://doi.org/10.1081/ESE-120037866
23. Parker WJ, Jones RM, Murthy S (2008) Characterization of the COD/VSS ratio during anaerobic digestion of waste activated sludge: experimental and modeling studies. Proc Water Environ Fed 17:524–533. https://doi.org/10.2175/193864708788735592
24. Kabouris JC, Tezel U, Pavlostathis SG, Engelmann M, Dulaney J, Gillette RA, Todd AC (2008) The mesophilic and thermophilic anaerobic digestion of municipal sludge and FOG. Proc Water Environ Fed 9:6756–6775. https://doi.org/10.2175/193864708790893756
25. 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(6):755–781
26. Sung S, Liu T (2003) Ammonia inhibition on thermophilic anaerobic digestion. Chemosphere 53(1):43–52. https://doi.org/10.1016/S0045-6535(03)00434-X
27. Carrere H, Dumas C, Battimelli A, Batstone DJ, Delgenes JP, Steyer JP, Ferrer I (2010) Pretreatment methods to improve sludge anaerobicdegradability: a review. J Hazard Mat 183(1–3):1–15
28. Pontoni L, D'Alessandrob G, d'Antoniob G, Espositoa G, Fabbricinob M, Frunzoc L, Pirozzib F (2015) Effect of anaerobic digestion on rheological parameters and dewaterability of aerobic sludges from MBR and conventional activated sludge plants. Chem Eng Trans 43:6. https://doi.org/10.3303/CET1543386

29. Carrere H, Antonopoulou G, Affes R, Passos F, Battimelli A, Lyberatos G, Ferrer I (2016) Review of feedstock pretreatment strategies for improved anaerobic digestion: from lab-scale research to full-scale application. Bioresour Technol 199:386–397. https://doi.org/10.1016/j.biortech.2015.09.007
30. Girault R, Bridoux G, Nauleau F, Poullain C, Buffet J, Peu P, Sadowski AG, Béline F (2012) Anaerobic co-digestion of waste activated sludge and greasy sludge from flotation process: batch versus CSTR experiments to investigate optimal design. Bioresour Technol 105:1–8. https://doi.org/10.1016/j.biortech.2011.11.024
31. Ge H, Jensen PD, Batstone DJ (2011) Increased temperature in the thermophilic stage in temperature phased anaerobic digestion (TPAD) improves degradability of waste activated sludge. J Hazard Mat 187(1):355–361
32. Wang D, He D, Liu X, Xu Q, Yang Q, Li X, Liu Y, Wang Q, Ni B-J, Li H (2019) The underlying mechanism of calcium peroxide pretreatment enhancing methane production from anaerobic digestion of waste activated sludge. Water Res 164:114934. https://doi.org/10.1016/j.watres.2019.114934