The use of sludge as micronutrient for improvement of biogas production from seaweed: A case of integration of two sources of environmental concerns to bring new opportunities

Mostafa Rezaei[1], Mohammadali Ebrahimi-Nik[1*], Silvia Tedesco[2], Reyhaneh Zeynali[3]

[1] Department of Biosystems Engineering, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran,

[2] Department of Mechanical Engineering, School of Mechanical Engineering, Manchester Metropolitan University, Dalton Building, Chester Street, Manchester M1 5GD, UK,

[3] Faculty of architecture and engineering, University of Bologna, Via Zamboni, 33 - 40126 Bologna, Italy

* Corresponding author: E-mail: ebrahimi-nik@um.ac.ir (M. Ebrahimi-Nik)

Abstract

High presence of seaweed in marine environments and coastal areas can cause serious hygienic and environmental problems. Anaerobic digestion could provide a solution being beneficial at producing bioenergy and fertilizer. However, AD of algae biomass has some limitations and consolidation of the process is required. To so increase the process efficiency, batches of 350 mL of feedstocks contained seaweed biomass (*Sargassum sp.*), inoculum and different dosages of sludge from drinking water treatment (DWTS as a micronutrient source to improve biogas production) were digested in 500 mL glass reactor and at mesophilic conditions, leading to significantly enhanced methane production. The highest methane yield (199 Nml g^{-1} VS) was observed when 6 mg L^{-1} DWTS was added, which showed a 30% improvement compared to control digester and accounted for 249.4 kWh increase in net energy per ton. Furthermore, the biodegradability index following DWTS addition also increased by 10% compared with the control.

Keywords: anaerobic digestion, biodegradability index, drinking water treatment sludge, algae, trace element

1. Introduction

Seaweed is a type of multicellular algae, which is highly spread in marine environments [(1)]. On average, photosynthesis efficiency of terrestrial plants is around 0.5%, which is considerably lower compared to most species of seaweeds. Photosynthetic efficiencies of marine plants vary between 3% to 8% [(2, 3)]. Furthermore, excess wealth of nutrients in coastal areas usually causes a dense growth of algae (phenomena called algal blooms). If these algae are not collected, serious hygienic and environmental issues might arise [(1)]. For instance, the recreational use of the beaches will be very hard or even impossible. On the other hand, disposal of the collected algae is environmentally and economically challenging [(4, 5)] which requires deliberate measures.

Seaweed (or macroalgae) biomass mainly contains of easily biodegradable carbohydrates and a low portion of lignin [(5)]. Additionally, for the most of countries seaweed does not compete with food production and land use. These features make seaweed a suitable feedstock for anaerobic digestion (AD) [(6)]. AD is a capable technology to help address issues related to the environment and energy associated with waste disposal, whilst simultaneously producing a valuable energy carrier (biomethane) [(7, 8)]. Several studies on lab scale have examined the AD of macro-algae. Considering the variable composition of algae, how much biomethane is producible has been the main question of the most of them. Mhatre at al. [(9)] explored that the biomethane potential of green macroalgae *Ulva lactuca* is 211 mL CH_4 g^{-1} VS and after treatment and removal of 'sap' (a mineral-rich extract) and ulvan, it reaches 408 mL CH_4 g^{-1} VS (70.9% of maximum theoretical). Tedesco and Daniels [(10)] also examined AD of five different species of brown seaweed and concluded that seaweed residues have a great potential to produce methane. They found the highest biomethane potential for L. *saccharina sp.* peaking at 535 mL CH_4 g^{-1} VS and after solvent extraction.

Digestion of macroalgae however, might exhibits some limitations with the AD process requiring consolidation [(15)]. In this respect, the challenges involve the presence of recalcitrant compounds with very low biodegradability. These organics cannot be degraded through conventional AD due to the low conversion rate and, consequently, high retention times are required. Also, the low C:N ratio typically found in algal biomass would likely lead to ammonia inhibition (NH_4^+). Another common issue is its high sensitivity to fluctuations in operational parameters such as digesting temperature, which extremely reduces the methane formation efficiency [16]. Finally, AD of algal biomass is ~~also~~ highly affected by microorganism adaptation to salinity levels [17] and any nutrients imbalance in the reactor could result in inhibition and AD failure [18].

Stable methane production from seaweed can be enabled by micro- and macro-nutrients [19], particularly metal nutrients since they act as major elements of enzymes' active sites [19-21]. Many researchers examined the biomethane production in relation to the presence of one or two metals. Synergism of multiple trace elements supplementation can be better interpreted through comparison of impact levels of single trace element addition showing better AD performances. [20, 22]. Interrelated effects of trace elements occur simultaneously in all stages of AD, and collaborative supplementation is needed to prevent negative effects on performance [23]. Therefore, the correct combinations of trace elements could lead to a more effective digestion. Identifying such interactive effects between and among the different trace elements is one of the research gaps that must be bridged for a more effective process performance [24]. At the same time, the extensive addition of micro-nutrients is limited by their high cost. Hence, adding these elements to the digester will be economically feasible if inexpensive sources are available [25]. The source should contain micro-nutrients in a suitable range of concentration, which will otherwise result in inhibition [26]. Interplays of these elements should be fully understood because of the probability of existence of antagonistic or synergistic effects between them [27-29]. The authors have previously found that drinking water treatment sludge (DWTS) could play such a role in anaerobic digestion {Ebrahimi-Nik, 2018 #25}. DWTS is characterized by the presence of different trace elements, which remain in the residual effluent after treatment of drinking water, where a portion of sludge is generated [30]. This comprises mostly alkaline compounds, trace elements, heavy metals and clay [31, 32]. Current DWTS management methods (e.g., disposal in sanitary landfills or burring in deserted soil) causes serious environmental and health problems. Land and groundwater pollutions are the main environmental issues derived from such disposal routes [33, 34]. Diverting DWTS from landfill to use in AD digesters would provide an inexpensive solution for nutrients recovery and trace elements addition to supplement AD microbial consortia, while avoiding pollution of land and water. Many researchers have utilized this waste to improve the AD process. For instance, Wu et al., [35] declared that DWTS can boost total dissolved nitrogen and dissolved phosphorus removal efficiencies in AD of excess activated sludge. The release of dissolved organic matter by DWTS may raise the concentration of dissolved organic carbon, resulting in the accumulation of non-biodegradable acid-like substance in aerobic and anaerobic digestion liquid. Therefore, the knowledge on the effect of DWTS on AD of algae is not yet clear. The aim of the present work was to examine whether this additive can improve the AD performance from algae (*Sargassum sp.*). To estimate the conversion performance a number of indicators such as methane yield, net energy, biodegradability index and volatile solids (VS) destruction rates were investigated.

2. Materials and Methods

2.1 Seaweed, inoculum, and micronutrients

Sargassum sp. of seaweed (SSW) was collected from Southeast shores of Iran (Chabahar port) in winter 2017. The algae were sun dried and then transferred to the biogas laboratory of Ferdowsi University of Mashhad for further process. The samples were then milled to 0.15-0.63 mm, using a grinder model Rqueen, Iran, and stored at 4 °C in a refrigerator. Volatile solids of the substrate in the digester was 49.6 g L^{-1}, which is within the recommended range of 20-60 g L^{-1} [(36)]. In addition, digestate from a laboratory scale digester fed with cow manure was used as inoculum. Prior to the experiments, it was kept in a water bath for 2 weeks at 37 °C [(36)]. Also, DWTS from a water treatment plant [water treatment plant (number 1) in Mashhad, Iran] was air dried and then grinded to 0.63 mm followed by 2h of calcining at 550 ºC [(31)]. Tables 1 and 2 summarize the substrates characterization.

2.2 AD and experimental design

Batch AD experiments were done in glass jars (volume of 500 ml and effective volume of 350 ml) as bioreactors and in mesophilic condition (37°C). In order to evaluate the biomethane potential from SSW, digestion of 350 mL of feedstocks was carried out. Five levels of DWTS addition were examined as 0, 2, 6, 12, and 18 mg L^{-1} and were labelled as B (control), D2, D6, D12, and D18, respectively. The ratio of inoculum to substrate was fixed to 2:1 as is suggested by Holliger et al., (2016). Therefore, each reactor contained 26 g seaweed residual, 324 g inoculum and a defined proportion of DWTS. Also, digestion of microcrystalline cellulose (Sigma-Aldrich) in the same experimental condition was performed (the positive control test), which it helps to confirm the ‘’viability of methane potential test’’ of the AD experiments [(36)].

Biogas produced from each reactor was bubbled through a bottle containing 3M NaOH solution to absorb carbon dioxide content. The bubbled-through gas (which should contain mostly of methane) was collected in a Tedlar Bag. Measurement of daily methane production were conducted until the cumulative total volume of gas was lower than 1% [36]. The measured gas volumes were converted to their equivalent methane volume at standard conditions using the method described by [31]. The ambient temperature and pressure, which are needed to calculate the biomethane yield based upon standard temperature and pressure (STP), were recorded daily. The data reported correspond to the averaged amount of three identical tests for each set of experimental conditions. Completely randomized design were used to statistically analyze the data.

2.3 Analytical method

Compositional characteristics such as total solids (TS) and volatile solids (VS) of the raw materials were determined through proximate analysis in agreement with the standard of the American Public Health Association [37]. Other feedstocks properties such as carbon, nitrogen, sulfur, and hydrogen content were measured through ultimate analysis and using CHNS Elemental Analyzer (Flash EA 1112). Presence of different oxides and elements in DWTS was identified by X-ray fluorescence (XRF) analysis.

2.4 Theoretical methane potential and biodegradability index

Theoretical methane potential (TMP) at the standard condition for temperature and pressure was calculated [38, 39]. Equation 1 is based on the elemental composition ($C_aH_bO_cN_d$).

$$\text{TMP} = \frac{\left(\frac{a}{2}+\frac{b}{8}-\frac{c}{4}-\frac{3d}{8}\right)\times 22400}{12a+b+16c+14d}\ \text{mL g}^{-1}\ \text{VS} \qquad \textbf{(1)}$$

The biodegradability index, as defined in equation 2, was used to evaluate the efficiency of digestion using biochemical methane potential (BMP). The BMP was calculated as percentage of TMP yield of the extracted feedstock achieved at the end of the digestion period.

$$\text{BI\%} = \frac{\text{BMP}}{\text{TMP}} \times 100 \qquad \textbf{(2)}$$

Besides, VS per liter content of the reactor were measured before and after digestion to get to the measure of VS destruction in each experiment.

2.5 Energy balance

A theoretical energy balance was carried out to establish the technical viability of digestion of algae with DWTS. The AD efficiency after addition of DWTS was evaluated considering speculative energy equilibrium for a pilot-scale analysis using experimental data achieved in the present study. For pilot-scale analysis, the macroalgal biomass was assumed to be one ton, based on the work of [40].

Energy required to prepare the AD feedstock and energy spend during the digestion were considered as input energy (E_i). As E_i was equal in all the treatments, its calculation was not accounted. Output energy was calculated as per Equation (3):

$$E_o = M_E \times HV_m \qquad \textbf{(3)}$$

where E_o is the output energy (kWh), M_E is the methane yield based on the laboratory tests (m^3), HV_m is the heating value of methane (9.3 kWh m^{-3} [41]).

The difference between the input and the output energies is the net energy (E_N) defined by Eq. (4).

$$E_N = E_i - E_o \qquad \textbf{(4)}$$

3. Results and discussion

3.1 DWTS and Substrates characterization

The main constituents of DWTS were Fe_2O_3, SiO_2, CaO, and Al_2O_3, respectively (Table 1). It is worth mentioning that despite sampling occurred in different year periods, the values of the aforementioned compounds were found to be approximately the same as those reported by the authors [(42)]. The water treatment plant in Mashhad, Iran, adds ferric chloride for water cleanup and calcium carbonate for pH adjustment. High quantity of Fe_2O_3 in DWTS is therefore mainly due to the mentioned chemical addition for the plant operations. Another widely present component in the water is SiO_2 which is used to enhance the suspended solids removal. Small quantities of other oxides and some trace elements were also found in DWTS (Table 1).

It can be seen that the C:N ratio is in lower optimum range as proposed by [(43)] for the digestion of seaweed. Composition of macroalgae are varied based on several environmental factors such as season, growth condition, geographical location [(44)], and water pollution [(45)]. Therefore, environmental variations could extremely affect the elemental composition of Sargassum. As reported by [(46)], C:N ratio for SSW is between 12 and 22, where the ranges for Carbon and Nitrogen are 12-40% and 0.6-2.0%, respectivelly.

3.2 Biomethane potential from SSW and the effect of DWTS addition

Positive control set up generated a biomethane volume of 360 Nml g^{-1} VS. This result aligns with the normal range mentioned in a guideline by [(36)], which demonstrates the sustainability of the utilized inoculum and the accuracy of employed biomethane measurement method.

As can be seen in Figure 1 and Figure 2, the methane yield of D6, was significantly higher than the other digesters. Table 3 illustrates the variance analysis to evaluate the effect of addition of 6 mg L^{-1} DWTS to the SSW feedstock (D6) on the cumulative methane production. According to the results, methane production had a significant difference (at the level of 5%) and led to a 1.31-fold increase in methane volume compared to the control. Among the various levels of DWTS, D2, D12, and D18 did not have any significant improvement in comparison to the control (B). The highest methane yield was recorded in D6 and was around 200 mL g^{-1} VS. It resulted a total production yield of around 1.31 times than the control reactor (B). In this reactor, daily methane was much greater relative to the control, which exhibits two perceptible peaks at around days 3-4 and 5 - 6 (Figure 1). The second highest peak occurred at day 5 and was also significantly higher than the control.

We envisage the first peak could be due to digestion of low-carbon organic compounds, whilst degradation of poorly biodegradable matters would be responsible for the following peak, which shows that an enhancement in digestion of SSW with poor biodegradability can occur by adding small amount of DWTS.

However, the higher concentration of DWTS introduced on the other hand has caused a reduction in methane yield, which could be an indication of proximity to toxic levels of DWTS concentration for the microbial consortium. Identifying the optimal range of the DWTS additive is vital for optimization of the AD and preventing the inhibitory effects. A previous study by the authors on AD of food waste also reported 6 mg L^{-1} of DWTS as a suitable concentration (31). Further research is needed to confirm if this concentration is the maximum possible regardless of the substrate.

A prevalent trace element of the DWTS is ferric oxide (Fe_2O_3), with almost 40 wt. % (Table 1). Fe_2O_3 is one of several semi-conductive iron oxides, which are reported to improve the methanogenesis step in AD. Ferric iron is a key element in the iron cycle and can oxidize various organic substances when acting as an electron acceptor [(47)]. From the literature, three main mechanisms are identified for the improvement of biogas production in the presence of iron oxide, these are dissimilatory iron reduction (DIR), direct interspecies electron transfer (DIET) and extracellular polymers substances (EPS).

DIET is an intrinsically faster mechanism of electron transfer than interspecies H_2 transfer [(48)], and is promoted by semi-conductive oxides, which might be the reason of higher methane yield [(49-51)]. DIR affects iron-reducing bacteria (IRBs) [(52)]. Despite not having direct involvement in anaerobic transformation pathway and not being essential microorganisms in AD, IRBs can improve AD performance remarkably by using surplus H_2 and acetate [(53)]. On the other hand, IRBs can compete with methanogenic microorganisms in term of nutrients (acetate and H_2) consumption [(54)]. Finally, iron oxide can also positively impact anaerobic process via a mechanism that alters the properties of extracellular polymers substances (EPS) [(55)]. It has been proved that EPS had more noticeable stimulatory effects on DIET processes after addition of Fe_2O_3 [(56)]. The degradation of organic matter increases because of enhancement in electron transfer rate from secondary fermenting bacteria to methanogens [(50)]. However, if Fe accumulation in the cells increases and exceeds tolerated levels, it may bring the cell to death [(57)].

Baek, Kim (53) showed that co-digestion of waste activated sludge and iron oxyhydroxides increases methane production by accelerating the decomposition of complex substances, mentioning a boost to DIET could be given by semi-conductive iron oxides. Wang, Zhang (51) evaluated the positive effects of Fe_2O_3 Nano particles (NPs) in term of methane production of waste activated and granular sludge. At the end, addition of Fe_2O_3 NPs resulted in more methane production in compare to the control test, reporting a negligible ions diffusion from Fe_2O_3 NPs. Also, another study by Lu, Zhang (49) showed increased accumulation of methane from AD of swine manure in presence of Fe_2O_3.

Theoretical methane production of *Sargassum sp.* was estimated to be 448 mL g^{-1} VS and by using theoretical methane potential (TMP) procedure. Biodegradability index (BI) is the ratio between the experimental methane production and the theoretical methane production. The BI value for control sample was 34% while for D2, D6, D12 and D18 were 36, 44, 38 and 33%, respectively. As indicated in Figure 3, the differences in the final volatile solids (the weight of DWTS was subtracted when calculating the removal efficiencies) between Control (B) and D6 was not significant ($p > 0.05$). Nevertheless, adding 12 and 18 mg L^{-1} DWTS reduced the final VS. However, no significant difference in methane volume produced between the control and D12 and D18 reactors was observed. Therefore, D6 constituents greatly stimulated the methanogenic activity and contributed to better methane production efficiency.

3.3 Effect of DWTS on energy considerations

Various pretreatments have been proposed to improve the methane efficiency from macroalgal biomass [58, 59]. However, often the high energy consumption of these methods makes them economically difficult to apply. Large industrial applications of these techniques is limited due to the issue of cost [60]. A mixture of cheap or even free of charge micronutrient like DWTS, which can improve methane production without extra energy input could be a promising solution. It was found that DWTS can significantly enhance methane yield and net energy (E_N). As mentioned earlier, the highest methane yield (199 Nml g^{-1} VS) was observed when 6 mg L^{-1} DWTS was added. While the methane production of control (B in Figure 3) was 152 Nml g^{-1} VS. This shows an increase of 31% in comparison with the control digester. The net energy of D6 is greater than control, as the input energy is the same in all treatments. The output energy calculated for D6 was 1057.5 kWh per ton of SSW, and it was 807.8 kWh for the control (Table 1). This shows a net energy increase of 249.7 kWh per ton of seaweed biomass. Its result is reduction of environmental issues related to water treatment sludge without any increase in input energy and cost. Mechanical pretreatment mostly result in a negative net energy obtained like the report from Tamilarasan, Kavitha (61) where a net energy production of -2531 kWh per ton was achieved. Overall, using DWTS as an additive to AD of algal biomass is economically as well as environmentally more beneficial than using algal biomass alone.

4. Conclusion

DWTS was applied as a cheap additive for biogas production from algal biomass (*Sargassum sp.*). DWTS which contained essential nutrients for the anaerobic digestion showed a significant improvement on biomethane production by stimulating the methanogenic activities. A maximum methane yield of 199 Nml g^{-1} VS was observed by adding 6 mg L^{-1} DWTS. This was 44% of theoretical methane potential and showed an increase of 249.7 kWh in net energy per ton of macroalgae biomass. In conclusion, adding DWTS to algal biomass leads to reduction of environmental impacts of sludge from water treatment without any increase in input energy and cost.

Acknowledgment

The authors appreciate the financial support from Ferdowsi University of Mashhad (grant number 46167).

References

1. Xia A, Jacob A, Tabassum MR, Herrmann C, Murphy JD. Production of hydrogen, ethanol and volatile fatty acids through co-fermentation of macro-and micro-algae. Bioresource technology. 2016;205:118-25.
2. Demirbas A, Demirbas MF. Algae energy: algae as a new source of biodiesel: Springer Science & Business Media; 2010.
3. Lardon L, Hélias A, Sialve B, Steyer J-P, Bernard O. Life-cycle assessment of biodiesel production from microalgae. ACS Publications; 2009.
4. McKennedy J, Sherlock O. Anaerobic digestion of marine macroalgae: A review. Renewable and Sustainable Energy Reviews. 2015;52:1781-90.
5. Nkemka VN, Murto M. Evaluation of biogas production from seaweed in batch tests and in UASB reactors combined with the removal of heavy metals. Journal of Environmental Management. 2010;91(7):1573-9.
6. Jung KA, Lim S-R, Kim Y, Park JM. Potentials of macroalgae as feedstocks for biorefinery. Bioresource technology. 2013;135:182-90.
7. Cecchi F, Pavan P, Mata-Alvarez J. Anaerobic co-digestion of sewage sludge: application to the macroalgae from the Venice lagoon. Resources, Conservation and recycling. 1996;17(1):57-66.
8. Jung H, Kim J, Lee C. Effect of enhanced biomass retention by sequencing batch operation on biomethanation of sulfur-rich macroalgal biomass: Process performance and microbial ecology. Algal research. 2017;28:128-38.
9. Mhatre A, Gore S, Mhatre A, Trivedi N, Sharma M, Pandit R, et al. Effect of multiple product extractions on bio-methane potential of marine macrophytic green alga Ulva lactuca. Renewable Energy. 2019;132:742-51.
10. Tedesco S, Daniels S. Optimisation of biogas generation from brown seaweed residues: Compositional and geographical parameters affecting the viability of a biorefinery concept. Applied energy. 2018;228:712-23.
11. Cudjoe D, Han MS, Nandiwardhana AP. Electricity generation using biogas from organic fraction of municipal solid waste generated in provinces of China: Techno-economic and environmental impact analysis. Fuel Processing Technology. 2020;203:106381.
12. Piñas JAV, Venturini OJ, Lora EES, del Olmo OA, Roalcaba ODC. An economic holistic feasibility assessment of centralized and decentralized biogas plants with mono-digestion and co-digestion systems. Renewable energy. 2019;139:40-51.
13. Rouhollahi Z, Ebrahimi-Nik M, Ebrahimi SH, Abbaspour-Fard MH, Zeynali R, Bayati MR. Farm biogas plants, a sustainable waste to energy and bio-fertilizer opportunity for Iran. Journal of Cleaner Production. 2020;253:119876.
14. Zeynali R, Khojastehpour M, Ebrahimi-Nik M. Effect of ultrasonic pre-treatment on biogas yield and specific energy in anaerobic digestion of fruit and vegetable wholesale market wastes. Sustainable Environment Research. 2017;27(6):259-64.
15. Assemany P, de Paula Marques I, Calijuri ML, da Silva TL, Reis A. Energetic valorization of algal biomass in a hybrid anaerobic reactor. Journal of environmental management. 2018;209:308-15.
16. Saratale RG, Kumar G, Banu R, Xia A, Periyasamy S, Saratale GD. A critical review on anaerobic digestion of microalgae and macroalgae and co-digestion of biomass for enhanced methane generation. Bioresource technology. 2018;262:319-32.
17. Pragya N, Pandey KK, Sahoo P. A review on harvesting, oil extraction and biofuels production technologies from microalgae. Renewable and Sustainable Energy Reviews. 2013;24:159-71.

18. Demirel B, Yenigün O. Two-phase anaerobic digestion processes: a review. Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology. 2002;77(7):743-55.
19. Demirel B, Scherer P. Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. Biomass and bioenergy. 2011;35(3):992-8.
20. Facchin V, Cavinato C, Fatone F, Pavan P, Cecchi F, Bolzonella D. Effect of trace element supplementation on the mesophilic anaerobic digestion of foodwaste in batch trials: the influence of inoculum origin. Biochemical Engineering Journal. 2013;70:71-7.
21. Gustavsson J, Yekta SS, Sundberg C, Karlsson A, Ejlertsson J, Skyllberg U, et al. Bioavailability of cobalt and nickel during anaerobic digestion of sulfur-rich stillage for biogas formation. Applied energy. 2013;112:473-7.
22. Zhang W, Zhang L, Li A. Enhanced anaerobic digestion of food waste by trace metal elements supplementation and reduced metals dosage by green chelating agent [S, S]-EDDS via improving metals bioavailability. Water research. 2015;84:266-77.
23. Moestedt J, Nordell E, Yekta SS, Lundgren J, Martí M, Sundberg C, et al. Effects of trace element addition on process stability during anaerobic co-digestion of OFMSW and slaughterhouse waste. Waste management. 2016;47:11-20.
24. Choong YY, Norli I, Abdullah AZ, Yhaya MF. Impacts of trace element supplementation on the performance of anaerobic digestion process: A critical review. Bioresource technology. 2016;209:369-79.
25. Huiliñir C, Montalvo S, Guerrero L. Biodegradability and methane production from secondary paper and pulp sludge: effect of fly ash and modeling. Water Science and Technology. 2015;72(2):230-7.
26. Lo H, Chiu H, Lo S, Lo F. Effects of micro-nano and non micro-nano MSWI ashes addition on MSW anaerobic digestion. Bioresource technology. 2012;114:90-4.
27. Menon A, Wang J-Y, Giannis A. Optimization of micronutrient supplement for enhancing biogas production from food waste in two-phase thermophilic anaerobic digestion. Waste management. 2017;59:465-75.
28. Romero-Güiza M, Vila J, Mata-Alvarez J, Chimenos J, Astals S. The role of additives on anaerobic digestion: a review. Renewable and Sustainable Energy Reviews. 2016;58:1486-99.
29. Voelklein MA, O'Shea R, Jacob A, Murphy JD. Role of trace elements in single and two-stage digestion of food waste at high organic loading rates. Energy. 2017;121:185-92.
30. Osalo TP, Merufinia E, Saatlo ME. Phosphorus Removal from Aqueous Solution by Bentonite: Effect of Al2o3 Addition. Journal of Civil Engineering and Urbanism. 2013;3(5):317-22.
31. Ebrahimi-Nik M, Heidari A, Azghandi SR, Mohammadi FA, Younesi H. Drinking water treatment sludge as an effective additive for biogas production from food waste; kinetic evaluation and biomethane potential test. Bioresource technology. 2018;260:421-6.
32. Wang C, Yuan N, Pei Y, Jiang H-L. Aging of aluminum/iron-based drinking water treatment residuals in lake water and their association with phosphorus immobilization capability. Journal of environmental management. 2015;159:178-85.
33. Babatunde A, Zhao Y. Constructive approaches toward water treatment works sludge management: an international review of beneficial reuses. Critical Reviews in Environmental Science and Technology. 2007;37(2):129-64.
34. Muisa N, Hoko Z, Chifamba P. Impacts of alum residues from Morton Jaffray Water Works on water quality and fish, Harare, Zimbabwe. Physics and Chemistry of the Earth, Parts A/B/C. 2011;36(14-15):853-64.
35. Wu Y, Du H, Li F, Su H, Bhat SA, Hudori H, et al. Effect of Adding Drinking Water Treatment Sludge on Excess Activated Sludge Digestion Process. Sustainability. 2020;12(17):6953.
36. Holliger C, Alves M, Andrade D, Angelidaki I, Astals S, Baier U, et al. Towards a standardization of biomethane potential tests. Water Science and Technology. 2016;74(11):2515-22.

37. APHA A. WPCF, Standard Methods for the Examination of Water and Wastewater, 20th. Ed, APHA, NW, Washington DC. 1998.
38. Buswell AM, Neave SL. Laboratory studies of sludge digestion. Bulletin (Illinois State Water Survey) no 30. 1930.
39. Hidalgo D, Martín-Marroquín JM. Effects of inoculum source and co-digestion strategies on anaerobic digestion of residues generated in the treatment of waste vegetable oils. Journal of environmental management. 2014;142:17-22.
40. Gao L, Li D, Gao F, Liu Z, Hou Y, Chen S, et al. Hydroxyl radical-aided thermal pretreatment of algal biomass for enhanced biodegradability. Biotechnology for biofuels. 2015;8(1):194.
41. del Real Olvera J, Lopez-Lopez A. Biogas production from anaerobic treatment of agro-industrial wastewater. Biogas. 2012.
42. Yazdani M, Ebrahimi-Nik M, Heidari A, Abbaspour-Fard MH. Improvement of biogas production from slaughterhouse wastewater using biosynthesized iron nanoparticles from water treatment sludge. Renewable Energy. 2019;135:496-501.
43. Wang X, Yang G, Feng Y, Ren G, Han X. Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresource technology. 2012;120:78-83.
44. Tedesco S, Hurst G, Imtiaz A, Ratova M, Tosheva L, Kelly P. TiO2 supported natural zeolites as biogas enhancers through photocatalytic pre-treatment of Miscanthus x giganteous crops. Energy. 2020;205:117954.
45. Lamare MD, Wing SR. Calorific content of New Zealand marine macrophytes. New Zealand Journal of Marine and Freshwater Research. 2001;35(2):335-41.
46. Marquez GPB, Santiañez WJE, Trono Jr GC, Montaño MNE, Araki H, Takeuchi H, et al. Seaweed biomass of the Philippines: sustainable feedstock for biogas production. Renewable and Sustainable Energy Reviews. 2014;38:1056-68.
47. Kim SJ, Park SJ, Cha IT, Min D, Kim JS, Chung WH, et al. Metabolic versatility of toluene-degrading, iron-reducing bacteria in tidal flat sediment, characterized by stable isotope probing-based metagenomic analysis. Environmental microbiology. 2014;16(1):189-204.
48. Zhuang L, Tang J, Wang Y, Hu M, Zhou S. Conductive iron oxide minerals accelerate syntrophic cooperation in methanogenic benzoate degradation. Journal of hazardous materials. 2015;293:37-45.
49. Lu T, Zhang J, Wei Y, Shen P. Effects of ferric oxide on the microbial community and functioning during anaerobic digestion of swine manure. Bioresource technology. 2019;287:121393.
50. Wang M, Zhao Z, Niu J, Zhang Y. Potential of crystalline and amorphous ferric oxides for biostimulation of anaerobic digestion. ACS Sustainable Chemistry & Engineering. 2018;7(1):697-708.
51. Wang T, Zhang D, Dai L, Chen Y, Dai X. Effects of metal nanoparticles on methane production from waste-activated sludge and microorganism community shift in anaerobic granular sludge. Scientific reports. 2016;6:25857.
52. Lovley DR, Anderson RT. Influence of dissimilatory metal reduction on fate of organic and metal contaminants in the subsurface. Hydrogeology Journal. 2000;8(1):77-88.
53. Baek G, Kim J, Cho K, Bae H, Lee C. The biostimulation of anaerobic digestion with (semi) conductive ferric oxides: their potential for enhanced biomethanation. Applied microbiology and biotechnology. 2015;99(23):10355-66.
54. Lovley DR, Phillips EJ. Rapid assay for microbially reducible ferric iron in aquatic sediments. Appl Environ Microbiol. 1987;53(7):1536-40.
55. Ye J, Hu A, Ren G, Chen M, Tang J, Zhang P, et al. Enhancing sludge methanogenesis with improved redox activity of extracellular polymeric substances by hematite in red mud. Water research. 2018;134:54-62.
56. Xiao Y, Zhang E, Zhang J, Dai Y, Yang Z, Christensen HE, et al. Extracellular polymeric substances are transient media for microbial extracellular electron transfer. Science advances. 2017;3(7):e1700623.

57. Wu J, Zhu G, Yu R. Fates and impacts of nanomaterial contaminants in biological wastewater treatment system: A review. Water, Air, & Soil Pollution. 2018;229(1):9.
58. Carrere H, Antonopoulou G, Affes R, Passos F, Battimelli A, Lyberatos G, et al. Review of feedstock pretreatment strategies for improved anaerobic digestion: from lab-scale research to full-scale application. Bioresource technology. 2016;199:386-97.
59. Nkemka V, Murto M. Exploring strategies for seaweed hydrolysis: effect on methane potential and heavy metal mobilisation. Process biochemistry. 2012;47(12):2523-6.
60. Montingelli M, Tedesco S, Olabi A. Biogas production from algal biomass: A review. Renewable and Sustainable Energy Reviews. 2015;43:961-72.
61. Tamilarasan K, Kavitha S, Banu JR, Arulazhagan P, Yeom IT. Energy-efficient methane production from macroalgal biomass through chemo disperser liquefaction. Bioresource technology. 2017;228:156-63.

Table 1. *The components of sludge from drinking water treatment*

Components	Quantity [wt. %]	Elements	Quantity [ppm]
Fe_2O_3	39.96	Ba	749
SiO_2	24.51	Co	93
Al_2O_3	7.01	Cr	127
CaO	9.61	Cu	93
LOI	11.17	Ni	89
MgO	2.22	Zr	107
K_2O	2.47	Cl	990
TiO_2	0.45	Zn	1803
MnO	1.03	Rb	75
Na_2O	0.4	Sr	282
P_2O_5	0.38	V	123
		S	1283
		Ce	85

Table 2. *The composition of Sargassum sp.*

Parameter	*Sargassum sp.*
TS [%]	88.5
VS [%]	64.5
Nitrogen [%]	2.23
Carbon [%]	34.39
Hydrogen [%]	4.66
Sulphur [%]	0.98
C:N ratio	15.42

Table 3. *The one-way ANOVA table for the effect of DWTS addition on AD*

	df	Sum of Squares (SS)	Mean Sum of Squares (MSS)	F-value	P-value
Effect of DWTS addition	4	4844	1210.9	5.87	0.011*
Error	10	2063	206.3		
Total	14	6907			

* a significance level of 0.05