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Short Communication

Bioelectrosynthesis technology for enhancing methane production using a thermophilic methanogenic consortium

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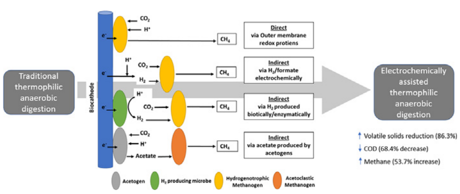
GRAPHICAL ABSTRACT



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ABSTRACT

This work investigated the electrocatalytic activity of a thermophilic methanogenic consortia (TMC) for developing a bioelectrosynthesis process to convert food and paper wastes to methane. Electroanalytical techniques were used to analyze the electrocatalytic activity of the TMC biofilm formed onto the electrodes. The developed electromethanogenesis process enhanced the yield of methane by 54.7% than control experiments. Scanning electron micrographs of the TMC bioelectrodes showed that the electrosynthesis process accelerates biofilm formation onto the electrodes leading to enhanced direct electron transfer reactions at electrode–electrolyte interface. This study will help in developing a novel approach for valorization of food and paper waste to biofuels.

1. Introduction

Global warming and related environmental issues have instigated development of environmental benign and facile technologies to significantly lessen the conventional fossil fuels utilization rates. Anaerobic digestion (AD) is a widely used technology for reduction of solid organic waste (SOW) that recovers energy in the form methane. AD is a multi-step biological process which utilizes a biocatalytic activity of a complex microbial consortium for mediating the different steps of the process. It has numerous advantages over aerobic waste treatment processes including low energy consumption, less sludge production, and being more economical. However, low substrate hydrolysis, low methane yield, VFA accumulation, and limitations with respect to reaction kinetics (Tripathi et al., 2015) are some of the bottlenecks. Different engineering strategies have been employed to improve AD process by optimizing the process factors such as temperature (Wang et al., 2014), pH and alkalinity (Chen et al., 2015), C:N ratio of substrate (David et al., 2018) ammonia (Chen et al., 2016) and VFA concentrations (Park et al., 2018), organic loading rates (Jansson et al., 2019), hydraulic retention times, reactor configuration (Rabii et al., 2019), and physical and chemical pretreatments (Porselvam et al., 2017; Singh & Kumar, 2019).

Thermophilic anaerobic digestion (TAD, 50–60 °C) helps to overcome some of the drawbacks of conventional mesophilic digestion such as utilize better substrate hydrolysis leading to higher methane yield and lower hydraulic retention time than its mesophilic counterpart due

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to increased solubility of the organic substrate as well as higher growth rate and metabolic reactions of thermophilic microbes. In addition, TAD process also aids in pathogen removal from the digested sludge (Labatut et al., 2014). The thermophilic methanogenic consortium (TMC) is generally composed of both facultative and obligate anaerobic microbial groups and plays a crucial role in hydrolysis, acidogenesis, acetogenesis, and methanogenesis. Furthermore, the use of bioelectrocatalysis approach is an attractive option to accelerate the rates of waste utilization by the TMC and increase methane yield. Applying the specific oxidation potential, will help in accelerating the rate of oxidation of electron donor (increasing the rate of substrate utilization) leading to enhanced methane production. This helps to avoid any expensive pretreatment measures for improving substrate hydrolysis but accelerates microbial methane production providing electrons at the biocathode (Venkata Mohan et al., 2014). Thus, aids in overcoming limitations with respect to reaction kinetics. Microbial bioelectrosynthesis technology is gaining widespread significance as it helps in harnessing the electrocatalytic activity of microbes to convert wastes to storable and/or transportable chemical energy. This led to different electrochemical approaches being attempted to increase methane production in the anaerobic digestion process. For instance, Song et al. (2016), reported that applying the potential of 0.3 V offered better volatile solids reduction (70.5%), specific methane production rate (407 mL L^{-1} d^{-1}), and the methane content (76.9%) with shorter HRTs when compared with the conventional anaerobic digestion technologies. Park et al., 2019 reported that the bacterial communities change in bio-electrochemical anaerobic digestion reactors which in turn influenced COD removal and methane production rates. The bio-electrochemical methane production can be mediated either by direct or indirect (hydrogen mediated) electron transfer (Fu et al., 2015). In direct electron transfer, the methanogens can accept electrons directly from cathode for electrocatalytic synthesis of methane. In indirect electron transfer, an intermediate product i.e. hydrogen is formed by transfer of electrons to H^+ ions (abiotic hydrogen production) in solution or transfer of electrons to hydrogen producing microbes present in the methanogenic consortium (Blasco-Gomez et al., 2017; Mateos et al., 2020). Then, hydrogenotrophic methanogens produce methane using hydrogen and carbon dioxide.

Among SOW, food and paper waste (FPW) constitutes a significant fraction (~42%) of municipal solid wastes (MSWs) and there is an urging need to ensure safe disposal of FPW (David et al., 2020). According to Environmental protection agency, nearly 6.3 percent of the food waste generated in the Unites states is wasted in composting process (EPA, 2017). Reports also showed that only 64.7% of the paper and paperboard waste is recycled. The remaining 28.4% of it is landfilled and 6.9% is incinerated (EPA, 2017). This clearly indicates that FPW can be used as an inexpensive feedstock in TAD process to produce methane. Therefore, this study aims to investigate the electrocatalytic activity of a thermophilic methanogenic consortium developed in our lab which is capable of digesting FPW. The goal of this investigation is to improve the TAD performance in terms of methane production and substrate utilization.

2. Materials and methods

2.1. Consortium

The inoculum was obtained from the anaerobic digester of the Wastewater Reclamation plant, Rapid city, SD. It was purged with nitrogen gas after collection to maintain anaerobic conditions and stored at −20 °C until use. The thermophilic methanogenic consortium (TMC) was enriched through sub-culturing technique described in our previous work (David et al., 2020). An organic loading of 1% volatile solids (VS) was used for the sub-culturing experiment as well as for the electrochemically assisted AD process investigated in this study. The TMC was revived by growing it at 60 °C on food waste (1% w/v VS) in 250-mL serum bottles containing 100 mL anaerobic medium described previously (David et al., 2018). The revived consortium was used for the electrochemical investigations and electrosynthesis studies. Microbial diversity analysis of the enriched TMC revealed the dominance of syntrophic acetate oxidation coupled with hydrogenotrophic methanogenesis (our unpublished data).

2.2. Fabrication of electrodes

TMC biofilms for electrochemical investigations were formed onto fabricated carbon felt electrodes (dimension: 1 cm × 1 cm). The TMC bioelectrodes were used to investigate the electrocatalytic activity of the microbial consortium (Rathinam et al., 2015). Sodium phosphate buffer solutions (0.1 M, pH 7) was used as electrolyte for the experiments.

2.3. Electrochemical analysis

Cyclic voltammetry was used to elucidate the electrocatalytic activity of the TMC bioelectrodes. Cyclic voltammograms (CV) of bioelectrodes was analysed in phosphate buffer (0.1 M, pH 7) at a scan rate of 10 mV/s with glucose as the electron donor. Silver wire and platinum (Pt) were used as a pseudo-reference and counter electrodes, respectively. The electrochemical investigations were conducted at aseptic conditions, and the temperature was maintained at 60 °C. Cyclic voltammograms of bioelectrodes were recorded at scan rate of 10 mV/s with glucose as the electron donor (Rathinam et al., 2013).

2.4. Bioelectrosynthesis experiment

Bioelectrosynthesis of methane was carried out by applying a specific oxidation potential using amperometry (Rathinam et al., 2018, 2014). Experiments were conducted with 1% VS (w/v) food and paper waste mixture as feedstocks in 100 mL of anaerobic medium as described in our previous study (David et al., 2018). The effects of applied electrochemical potential on the substrate utilization, COD removal rate, methane production, and biofilm formation were investigated. Control experiments were conducted with bioelectrodes without applied oxidation potential. Electrolyte samples collected before and after the experiment were used for quantification of COD levels. Changes in the quantity of volatile solids in both bioelectrocatalysis and control experiments with food and paper waste as substrates were analysed. The effect of applied electrochemical potential on the biofilm formation were analysed using a Zeiss Supra40 variable-pressure field-emission SEM.

2.5. Analytical methods

The total solids and volatile solids in the electrolyte were quantified using APHA standard methods. The COD levels in the electrolyte at different time were calculated using the COD digester (DRB200, Hach). The methane content of the biogas produced by TMC was quantified using gas chromatography (Agilent Technologies 7890A) equipped with Thermal conductivity detector and Supelco Porapak Q column.

3. Results and discussion

3.1. Electrogenic activity of TMC

The cyclic voltammograms of the TMC bioelectrodes was recorded from a potential range of −0.8 V to + 0.8 V (vs PRE) at a scan rate of 5 mV/s (shown in Fig. 1). A redox peak at 0.55 mV (vs PRE) in the cyclic voltammogram increased with addition of glucose as the electron donor. With addition of 100 µL of glucose (0.1 mM) oxidation current increased from 0.9913 mA at 0.563 V to 1.059 mA at 0.565 V. Subsequent additions of glucose (100 µL) increased the oxidation current to

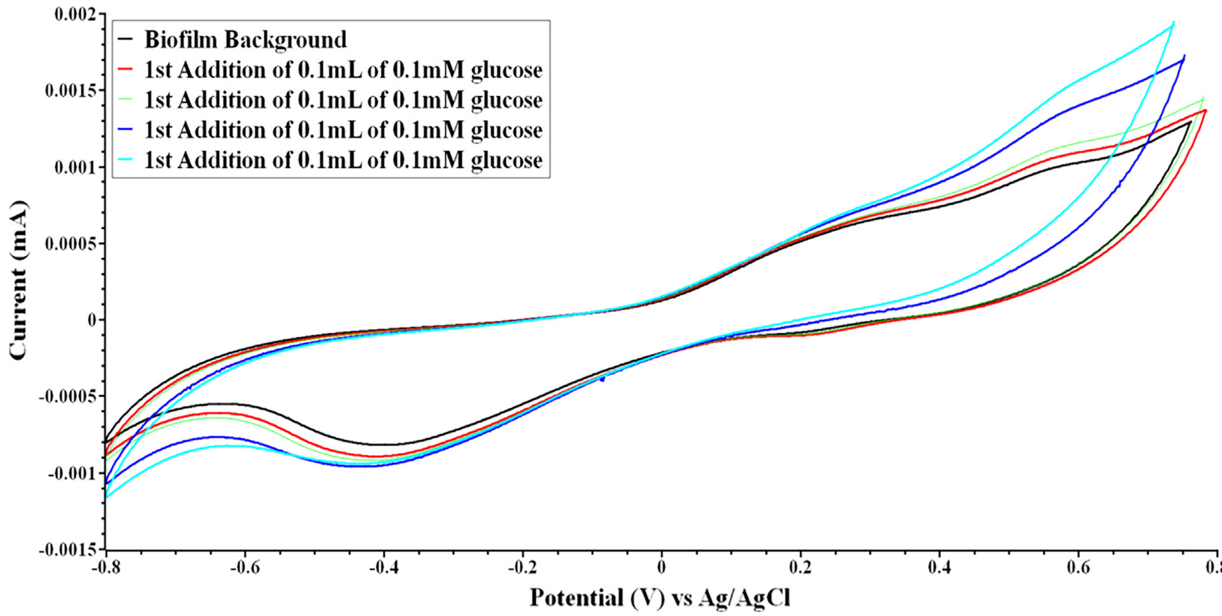

Fig. 1. Cyclic voltammograms of TMC bioelectrodes on bioelectrocatalysis of glucose.

1.13 mA, 1.29 mA and 1.463 mA respectively. The increase in oxidation current with increasing glucose concentration confirm the electrocatalytic activity of TMC bioelectrode. The peak at 0.55 mV (vs PRE) in the cyclic voltammogram is related to key enzymes involved in glucose oxidations viz. glucose oxidase or glucose dehydrogenase and corroborates well with the previous reports (Rathinam et al., 2020).

3.2. Substrate utilization and methane production

Electrosynthesis experiments were conducted with 1% (w/v) VS of mixed paper and food waste as the feedstock by applying a potential of −1 mV (vs PRE) for 5 days. The effect of the applied voltage on by volatile solids reduction and COD removal rate was investigated. Volatile solids (VS) includes the organic fraction of total solids of a substrate that are biodegradable (David et al., 2018). Therefore, tracking their consumption gives a better measure of utilization of the substrate i.e. food and paper waste. Samples were taken before and after the electrosynthesis experiment. As depicted by the VS reduction of the test and control reactors, electromethanogenesis increased the substrate utilization by 11.2% when compared to control (without applied voltage). Applying the voltage reduced the volatile solids by 86.3%. Fig. 2A depicts the effect of applied voltage on methane production. The electrosynthesis approach also increased the methane production from 165.4 to 254.2 mLg^{-1} VS which marked a 53.7% increase when compared with the systems without applied voltage. The increase in substrate utilization and subsequently methane production as a result of applied potential can be attributed to the following – Firstly, the electrons provided by applied external potential can directly reduce CO_2 to CH_4 with the help of outer membrane redox proteins of microbes; Secondly, the applied potential of 1 V can increase hydrogen production by either supplying electrons to abiotic H^+ ions in solution or by providing electrons to hydrogen producing microbes, both resulting in increased hydrogenotrophic methane production. Lastly, acetogenic methanogens can also take up electrons and increase the acetogenic methane production. Thus, application of external potential accelerates the different steps of TAD process and the cumulative effect increases substrate degradation from hydrolysis step to methanogenesis step. Our future work will focus in deciphering which of the above-mentioned mechanism is the major cause of increased methane yield. for In a similar study conducted with glucose as substrate, Choi et al., 2017 reported that on applying the potential of 1 V (vs. Ag/AgCl) to the wastewater inoculum, the methane yield was increased by 30.3% when compared to control (without applied voltage).

Further, COD levels were monitored to consider the soluble metabolites produced by the TMC (Fig. 2B). COD values includes the amount of substrate as well as the metabolites produced by the microbial electrocatalysis process in the electrolyte. Thus, COD values could correlate the rates of metabolism as well as microbial interspecies interaction in the TMC. As shown in Fig. 2B, COD levels increased in the first 3 days in both cases with and without applied voltage. Subsequently, the COD levels started decreasing as methane levels started increasing. The initial increase in the COD (during first 3 days) is caused by the hydrolysis and acidification of the complex solid wastes which increases the concentration of soluble metabolites in solution. The initial COD increase was higher when the external voltage was applied indicating faster microbial metabolism in the bottles with applied voltage compared to bottles without applied voltage. The decrease in COD after day 3 is due to utilization of the soluble metabolites for methanogenesis. The results of COD utilization rates with applied oxidation potential corroborate well with the substrate utilization (in terms of VS reduction) and methane production rates. On applying the oxidation potential, the COD removal rate from day 3 to day 5 was increased by 68.4% indicating that the electromethanogenesis process (by applying external potential) was more efficient in COD removal as compared to conventional methanogenesis. These results clearly indicate that this approach is promising for treatment of solid and liquid wastes with high COD levels including domestic and industrial effluents.

3.3. Biofilm formation

SEM analysis revealed that the electrodes with applied external voltage had a denser biofilm formation when compared with the control. SEM images showed that the applied voltage drives biofilm formation by promoting the formation of TMC biofilms on the electrode surface leading to improved microbial electrocatalysis (Rathinam et al., 2018). The external voltage serves as a driving force for biofilm formation which offers advantage to the TAD system. The denser biofilm also helps in biogas upgradation (i.e. increasing methane fraction of biogas) due to the physical separation of the organic matter oxidation from microbial methane production. Bioelectrosynthesis process enhances the rate of oxidation of electron donor leading to enhanced

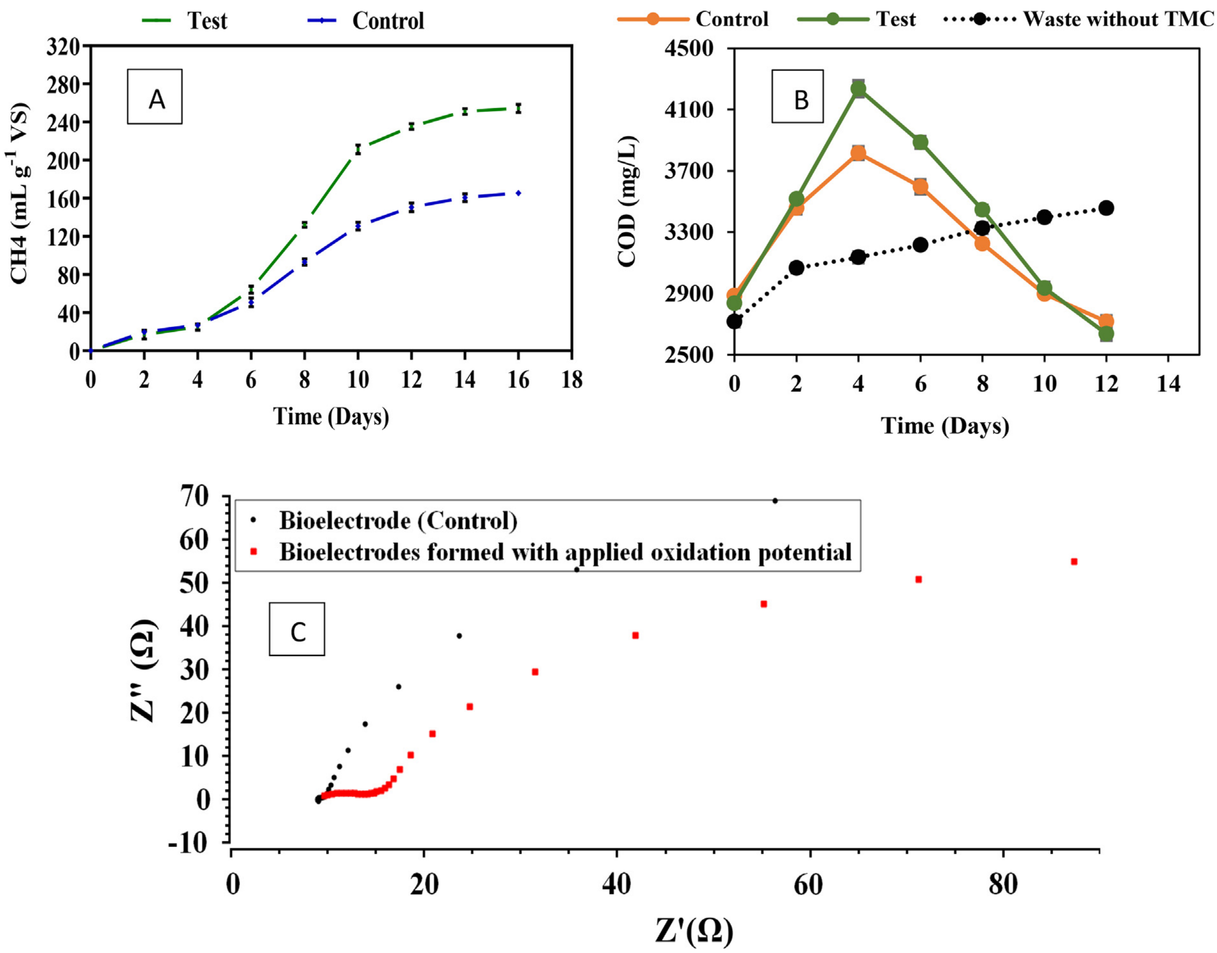

Fig. 2. (A) Effect of applied voltage on biomethane production. (There was no methane production in food and paper waste without TMC – data not shown) (B) COD changes during TAD of food and paper waste in the presence and absence of an applied external potential. (C) Nyquist Plots for bioelectrodes formed with and without applied external voltage.

production of electrons (Dou et al., 2018). In addition, the denser biofilm also lessens the exposure of the methanogenic consortium to inhibitory compounds that may be present in the waste substrate (Villano et al., 2011).

3.4. Electrochemical Impedance Spectroscopy

Electrochemical Impedance Spectroscopy is a reliable technique to decipher the kinetics of biofilm formation as well as biofilm characteristics. Nyquist plots can provide insights on the solution resistance of the electrolyte (depends on microbial cell density, composition, and metabolites) and charge transfer resistance at the electrode–electrolyte interface (depends of the adherence of cells to the electrode/electrolyte interface). Fig. 2C shows Nyquist plots of the electrodes with TMC in phosphate buffered anaerobic culture medium. The results showed that the solution resistance of the electrolyte increased from 2 Ω to 8 Ω on applying the external voltage when compared with the control. Increased solution resistance indicates higher solubilization of substrate, higher microbial density resulting in higher metabolite production in the presence of external potential. Further, charge transfer resistance also increased from 60 Ω to 90 Ω on applying the external voltage indicating enhanced biofilm formation. The improved biofilm formation as indicated by increase in charge transfer resistance allows maintenance of high concentration of active microbes near or onto the electrode, especially the ones having lower growth rates (such as the methanogenic archaea), which in turn can improve synergism between different microbial groups in TMC resulting in increased methane production. This result on the role of applied voltage on improved cell density in the electrolyte and improved biofilm formation corroborate well with our previous report on the bioelectrosynthesis for enhanced lignocellulosic degradation in *Geobacillus* strain WSUCF1 (Rathinam et al., 2020).

4. Conclusion

This study reported a novel and facile strategy for accelerating the anerobic digestion process for enhanced substrate utilization as well as product yield. The use of applied external voltage positively promoted the adherence of biofilm onto the electrodes. This will be a promising strategy for incommodity engineering applications such as biovalorisation of food and paper wastes to biofuels and value-added compounds. Further attempts will be made to improve the process yield and cutdown the operational costs using bioelectrochemical reactor configurations with new electrode materials and electrode functionalizing strategies.

CRediT authorship contribution statement

Aditi David: Investigation, Data curation, Writing - original draft. **Navanietha Krishnaraj Rathinam:** Conceptualization, Project administration, Supervision. **Rajesh K. Sani:** Formal analysis, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Blasco-Gomez, R., Batlle-Vilanova, P., Villano, M., Balaguer, M.D., Colprim, J., Puig, S., 2017. On the edge of research and technological application: a critical review of electromethanogenesis. Int. J. Mol. Sci. 18 (4).
Chen, H., Wang, W., Xue, L., Chen, C., Liu, G., Zhang, R., 2016. Effects of ammonia on anaerobic digestion of food waste: process performance and microbial community. Energy Fuels.
Chen, S., Zhang, J., Wang, X., 2015. Effects of alkalinity sources on the stability of anaerobic digestion from food waste. Waste Manag Res 33 (11), 1033–1040.
Choi, K.-S., Kondaveeti, S., Min, B., 2017. Bioelectrochemical methane (CH4) production in anaerobic digestion at different supplemental voltages. Bioresour. Technol. 245, 826–832.
David, A., Govil, T., Tripathi, A., McGeary, J., Farrar, K., Sani, R., 2018. Thermophilic anaerobic digestion: enhanced and sustainable methane production from co-digestion of food and lignocellulosic wastes. Energies 11 (8).
David, A., Tripathi, A.K., Sani, R.K., 2020. Acetate production from cafeteria wastes and corn stover using a thermophilic anaerobic consortium: a prelude study for the use of acetate for the production of value-added products. Microorganisms 8 (3).
Dou, Z., Dykstra, C.M., Pavlostathis, S.G., 2018. Bioelectrochemically assisted anaerobic digestion system for biogas upgrading and enhanced methane production. Sci. Tot. Environ. 633, 1012–1021.
EPA Sustainable Management of Food, United States Environmental Protection Agency 2017.
Fu, Q., Kuramochi, Y., Fukushima, N., Maeda, H., Sato, K., Kobayashi, H., 2015. Bioelectrochemical analyses of the development of a thermophilic biocathode catalyzing electromethanogenesis. Environ. Sci. Technol. 49 (2), 1225–1232.
Jansson, A.T., Patinvoh, R.J., Sárvári Horváth, I., Taherzadeh, M.J., 2019. Dry anaerobic digestion of food and paper industry wastes at different solid contents. Fermentation 5 (2), 40.
Labatut, R.A., Angenent, L.T., Scott, N.R., 2014. Conventional mesophilic vs. thermophilic anaerobic digestion: A trade-off between performance and stability? Water Res. 53, 249–258.
Mateos, R., Escapa, A., San-Martín, M.I., De Wever, H., Sotres, A., Pant, D., 2020. Long-term open circuit microbial electrosynthesis system promotes methanogenesis. J. Energy Chem. 41, 3–6.
Park, J.-G., Lee, B., Jo, S.-Y., Lee, J.-S., Jun, H.-B., 2018. Control of accumulated volatile fatty acids by recycling nitrified effluent. J. Environ. Health Sci. Eng. 16 (1), 19–25.
Park, J.-G., Shin, W.-B., Jun, H.b., 2019. Changes of bacterial communities in an anaerobic digestion and a bio-electrochemical anaerobic digestion reactors according to organic load. Energies 12.
Porselvam, S., Soundara Vishal, N., Srinivasan, S.V., 2017. Enhanced biogas yield by thermo-alkali solubilization followed by co-digestion of intestine waste from slaughterhouse with food waste. 3 Biotech 7 (5), 304.
Rabii, A., Aldin, S., Dahman, Y., Elbeshbishy, E., 2019. A Review on anaerobic co-digestion with a focus on the microbial populations and the effect of multi-stage digester configuration. Energies 12 (6), 1106.
Rathinam, N.K., Rengasamy, K., Berchmans, S., Chandran, S., Pal, P., 2013. Functionalization of electrochemically deposited chitosan films with alginate and Prussian blue for enhanced performance of microbial fuel cells. Electrochimica Acta 112, 465–472.
Rathinam, N.K., Berchmans, S., Pal, P., 2015. The three-compartment microbial fuel cell: a new sustainable approach to bioelectricity generation from lignocellulosic biomass. Cellulose 22 (1), 655–662.
Rathinam, N.K., Berchmans, S., Pal, P., 2014. Symbiosis of photosynthetic microorganisms with non-photosynthetic ones for the conversion of cellulosic mass into electrical energy and pigments. Cellulose 21, 2349–2355.
Rathinam, N.K., Tripathi, A.K., Smirnova, A., Beyenal, H., Sani, R.K., 2018. Engineering rheology of electrolytes using agar for improving the performance of bioelectrochemical systems. Bioresour. Technol. 263, 242–249.
Rathinam, N.K., Gorky, Bibra, M., Salem, D.R., Sani, R.K., 2020. Bioelectrochemical approach for enhancing lignocellulose degradation and biofilm formation in Geobacillus strain WSUCF1. Bioresour. Technol. 295, 122271.
Singh, R., Kumar, S., 2019. A review on biomethane potential of paddy straw and diverse prospects to enhance its biodigestibility. J. Clean Prod. 217, 295–307.
Tripathi, A., Kumari, M., Kumar, A., Kumar, S., 2015. Generation of biogas using pine needles as substrate in domestic biogas plant. Int. J. Renew Energy Res. 5, 716–721.
Venkata Mohan, S., Velvizhi, G., Vamshi Krishna, K., Lenin Babu, M., 2014. Microbial catalyzed electrochemical systems: A bio-factory with multi-facet applications. Bioresour. Technol. 165, 355–364.
Villano, M., Monaco, G., Aulenta, F., Majone, M., 2011. Electrochemically assisted methane production in a biofilm reactor. J Power Sour 196, 9467.
Wang, X., Lu, X., Li, F., Yang, G., 2014. Effects of temperature and carbon-nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. PLoS ONE 9 (5), e97265.