Bulgarian Chemical Communications, Volume 54, Issue 4 (pp. 343-348) 2022 *DOI: 10.34049/bcc.54.4.NC03*

Influence of the applied external voltage on anaerobic digestion with integrated microbial electrolysis cell

P. G. Velichkova*, S. G. Bratkova, A. T. Angelov

Department of Engineering Geoecology, University of Mining and Geology "St. Ivan Rilski", Sofia 1700, Bulgaria

Received: July 26, 2022; Revised: November 16, 2022

An external microbial electrolysis cell (MEC) was integrated into the ethanol stillage anaerobic digestion reactor (AD). The AD-MEC system is a promising technology for enhancing biogas production. It accelerated methane production and stabilized the process. A change in applied external voltage could change microbial metabolism and affect substrate degradation rate, volatile fatty acid (VFA) generation and biogas production. The effect of applied external voltage on biodegradability, VFA and methane content in produced biogas was studied. Four values of external voltage were selected - 0.6, 0.8, 1.0 and 1.2 V. The kinetics of biogas production for 15 days was monitored. Methane yields increased to 84, 88 and 82 % ("vol.) at 0.6, 0.8 and 1.0 V voltages of the microbial electrolysis cell, respectively. Higher voltage (1.2 V) did not increase the methane content *vs* AD-only process (75 %, vol.). The best biodegradability was achieved at low voltages (0.6 and 0.8 V) - reduction of chemical oxygen demand by 88-89 % and purification of ethanol stillage from sulfates. Acetic acid decreased by 96 % at 0.6 V, 95 % at 0.8 V, 74 % at 1.0 V and 65 % at 1.2 V.

Keywords: Anaerobic digestion, microbial electrolysis cell, ethanol stillage, VFA, COD

INTRODUCTION

The microbial electrolysis cell (MEC) is the commonest bioelectrochemical system with anaerobic digestion (AD) being studied. The integration of MEC into an AD reactor (AD-MEC) improves the process by alleviating volatile fatty acids (VFA) accumulation, shortening solid retention time, enhancing hydrolysis/acidogenesis rates, and enriching exoelectrogen/methanogen activities, so improving the AD performance [1]. Moreno *et al.* found that MEC could enhance biomethane production rate, resulting from the alleviation effects of reduced VFA accumulation [2]. In recent research was reported that methane yield even exceeds 90 % from an AD-MEC system [3-5]. The advantages of AD-MEC include less energy (voltage < 1.0 V) which was required for hydrogen production compared to water electrolysis (voltage > 1.2 V) process and higher hydrogen yield compared to conventional fermentation-based processes [1].

The biomethane productivity responds to voltage application are highly substrate-dependent. In addition, the change in VFAs content or decrease in chemical oxygen demand (COD) also varies for different substrates. For example, in terms of methane productivity, there existed an optimum applied voltage for palm oil mill effluent, while voltage application shows insignificant impact on swine manure [1]. The applied voltage affects both the methane enhancement and productivity. Results showed that AD-MEC operated at 0.4–0.8 V have better biomethane productivity, suggesting the existence of an optimal applied voltage for microbial growth and associated electrochemical bioreaction rates. High voltage (>1.0 V) can be harmful to microorganisms, while the applied voltage should exceed a threshold value to overcome the thermodynamic barrier for the desired electrochemical bioreactions [1, 6]. The VFAs are generated by AD but could not be converted mainly to methane. An extra energy input is needed to convert VFAs to biogas [7]. They can be converted into hydrogen by applying voltage.

In the present study, the influence of the applied external voltage on the reduction of VFAs (lactic, acetic, propionic and butyric acids) and organic matter was studied. Analyzes were performed in four operating modes of AD-MEC - with 0.6, 0.8, 1.0 and 1.2 V external voltage. The process was monitored for 15 days by sampling the inlet and outlet of the reactor at different voltages. The samples were analyzed for COD and VFAs.

EXPERIMENTAL

Microbial electrolysis cell configuration

The scheme of the laboratory installation is shown on Fig. 1a and the picture – on Fig. 1b. The anaerobic reactor has a working volume of 5 dm^3. Two graphite plates measuring 100 × 100 × 6 mm were used as electrodes.

* To whom all correspondence should be sent:
E-mail: polina.velichkova@mgu.bg

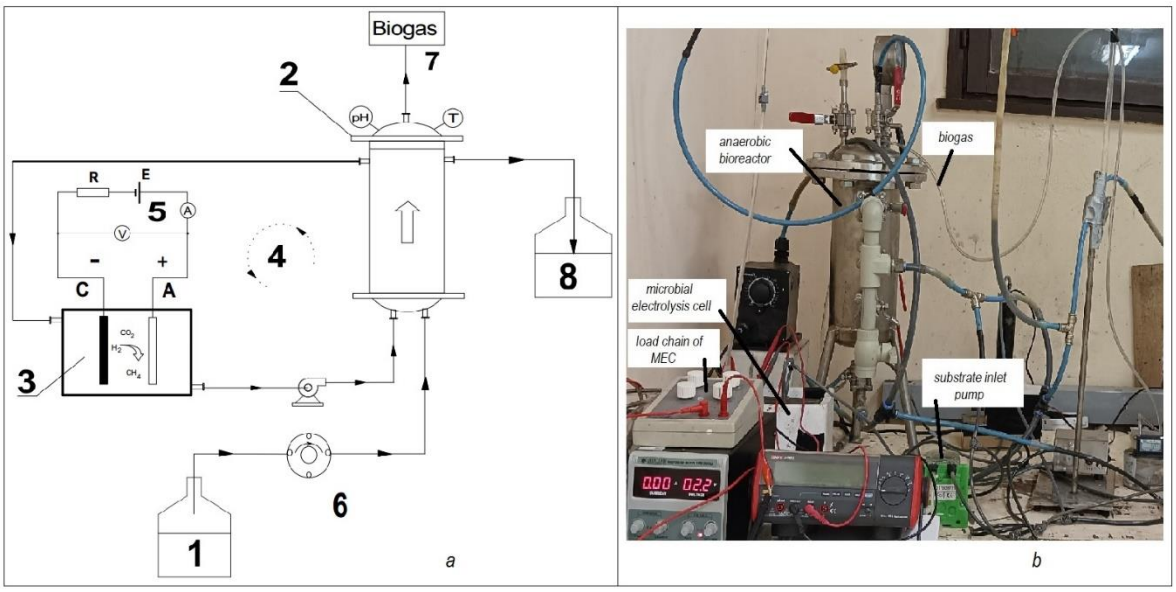

Fig. 1. Scheme (a) and picture (b) of an integrated AD-MEC system. Legend: 1- substrate input, 2- anaerobic bioreactor (UASB), 3- microbial electrolysis cell (MEC), 4- recirculation flow, 5- load chain of MEC, 6- substrate inlet pump, 7-biogas, 8- reactor outlet.

Spacing between the anode and cathode placed on opposite sides measured 20 mm. Recirculation pumps moved the flow.

The microbial electrolysis cell is integrated outside the volume of the anaerobic reactor. The cathode and anode electrodes were connected to the power supply, with an external resistance of 10 Ω. The reactor system was filled with ethanol stillage and 10 % of volume of substrate inoculum from a mixed methanogenic consortium (activated sludge). 273 ml/day of stillage is pumped to the reactor.

Stillage and activated sludge

Stillage was obtained after the separation of ethanol obtained by acid hydrolysis of starch-containing plants from "Kehlibar" Winery Ltd, Svetovrachene, Bulgaria. The stillage was stored in a refrigerator. Before use, it was neutralized to pH 7.5 with NaOH. Activated sludge was taken from "Almagest" AD, Verinsko, Bulgaria.

Analytical methods

Chemical oxygen demand was measured with COD Ultra-High Range Reagent Vials, EPA method (Hanna Instruments). The sulfate concentration was determined using a spectrophotometric method at λ 420 nm using $BaCl_2$ as a reagent. The contents of CO_2, CH_4, H_2S and H_2 in the produced biogas were measured using a portable gas analyzer "Draeger X-am 7000". The acid composition in the ethanol stillage at the inlet and outlet of the installation was determined by HPLC at the Institute of Chemical Engineering - BAS. Scanning electron microscopy (SEM) was used for scanning the biofilm on electrodes. Prior to SEM, the samples were fixed with 2 % glutaraldehyde in 0.1 M phosphate buffer overnight in the fridge (4 ºC) and then were dried with ethanol. After that, the samples were kept in a desiccator for 48 hours and then were coated with a thin layer of graphite.

RESULTS AND DISCUSSION

Change in the composition of volatile fatty acids

Samples were taken on the 14th day from the beginning of the decomposition of the ethanol stillage in AD-MEC for each of the applied external voltages at the inlet and outlet of the reactor- 0.6, 0.8, 1.0, 1.2 V. The obtained results for lactic, acetic, butyric and propionic acids are given in Table 1.

The lactic, acetic, butyric and propionic acids were consumed as a food source for the microbial communities. The best degradability was achieved at 0.8 V, followed by 0.6, 1.2 and 1.0 V, respectively. The acetic acid is considered as the key intermediate product for methane production. The decrease in acetic acid concentration indicated that exoelectrogens on the anodes might actively consume acetic acid for the current generation, which was used for the reduction at the cathodes for the methane production. Such results are reached by Arvin *et al.* [7], where the acetic acid content decreases from 317 mg/L in the first phase to 278.3 mg/L in the second, 79.7 mg/L in the third, and 70.6 mg/L in the fourth phase in AD-MEC with external voltage 1.0 V.

Table 1. Content of VFAs in the ethanol stillage before and after methanation in AD-MEC at different external voltage

VFA	Sample							
	AD-MEC 0.6V		AD-MEC 0.8V		AD-MEC 1.0V		AD-MEC 1.2V	
	input	output	input	output	input	output	input	output
Lactic acid, g/L	0.67	<0.01	2.5	<0.01	1.98	0.15	<0.01	<0.01
Acetic acid, g/L	5.20	0.19	2.8	0.15	4.39	1.14	4.03	1.42
Propionic acid, g/L	5.93	0.12	3.54	<0.01	5.44	0.75	5.01	0.67
Butyric acid, g/L	1.15	<0.01	0.15	<0.01	0.52	0.08	1.20	<0.01

Table 2. COD and sulfates value input and output of reactor at different external voltage

Parameter	AD, control experiment	AD-MEC with applied voltage			
		0.6V	0.8V	1.0V	1.2V
COD_{input}, g O_2/L	99.44	58.48	87.36	104.12	69.44
COD_{output}, g O_2/L	34.08	5.68	9.76	15.4	47.48
$SO_4^{2-}{}_{input}$, mg/L	847.45	860.23	562.16	978.38	875.67
$SO_4^{2-}{}_{output}$, mg/L	450	<1	<1	153.75	<1

The results in Table 1 show that acetic acid decreased from 5.2 to 0.19 g/L (by 96 %) at 0.6 V, from 2.8 to 0.15 g/L (by 95 %) at 0.8 V, from 4.39 to 1.14 g/L (by 74 %) at 1.0 V and from 4.03 to 1.42 g/L (by 65 %) at 1.2 V. At the end of biogas production, 0.12, 0.75 and 0.67 g/L propionic acid were detected at 0.6, 1.0 and 1.2 V, respectively, while at 0.8 V it was consumed by the methanogens. The lactic acid was also completely degraded at 0.6 and 0.8 V, while at 1.0 V 0.15 g/L were detected. At 1.2V, it was not detected either at the inlet or outlet of the installation, which may be due to a chemical change that occurred during storage of the substrate. The butyric acid was consumed in 3 of the cases - at 0.6, 0.8 and 1.2 V. At 1.0 V a minimum quantity was detected - 0.08 g/L butyric acid. This improves the microbial activities and does not interfere with the process of methanogenesis.

Venkata and Lenin proved that a change in applied voltage could change microbial metabolism and consequently affect substrate degradation rate, volatile fatty acid generation and biohydrogen production. The activity of dehydrogenase enzyme and biohydrogen production had a maximum value at applied voltage of 0.6 and 1.0 V, respectively [8].

Change in chemical oxygen demand and sulfates

Table 2 shows the values of the two most important parameters related to the results of wastewater treatment - COD and sulfates. As can be seen from the data in Table 2, the degradability of organic matter at 0.6, 0.8, 1.0 and 1.2 V external voltage is 89.72 %, 88.83 %, 85.21 % and 31.62 %, respectively. Sulfates from the ethanol stillage are reduced to hydrogen sulfide and sulfides by anaerobic bacteria. In the presence of an electric field, sulfides are oxidized to elemental sulfur on the surface of the anode. Sulfates are not detected in the output stream of the AD-MEC system at 0.6, 0.8 and 1.2 V (<1 mg/L), while at 1.0 V minimal amounts are observed (153.75 mg/L), which points to 84 % degradability. The organic removal rate was successfully increased by AD-MEC with 0.6, 0.8 and 1.0 V external voltage, which was 66 % with the cell off. At 1.2 V, no improvement was observed - even the biodegradability decreased to 31.62 %. Sulfate removal was also improved with integrated MEC compared to AD-only process, where it was only 46.90 %. Therefore, it can be concluded that in terms of biodegradability there is not significant difference between the applied low voltages- 0.6 and 0.8 V, while at higher - 1.0 and 1.2 V, there is either a residue of sulfates and insufficient purification of stillage (at 1.0 V) or incomplete removal of organic matter (at 1.2 V). These results again prove the conclusions of Venkata and Lenin that the metabolism of bacteria is strongly influenced by the applied external voltage - at high voltages (in the case of 1.2 V), the microbial metabolism is inhibited and the degree of organic decomposition decreases [8].

Biogas production from ethanol stillage

Figure 2 shows a comparative graph of the kinetics of a process of biogas production in AD-MEC at different external voltages- 0.6, 0.8, 1.0 and 1.2 V. The graph shows that 27, 31, 29 and 35 liters of biogas were produced in 15 days in the AD-MEC system at 0.6, 0.8, 1.0 and 1.2 V, respectively. Although the highest yield is at 1.2 V, the fastest process is at 0.8 V- until the 10th day. Then 24 liters of biogas are produced in both and the predominance is for 1.2 V. The produced biogas for 0.6 and 1.0 V is 16 and 18 liters, respectively, on the 10th day.

Table 3. Gas composition in produced biogas by different external voltage

Gas composition	CH_4, vol. %	CO_2, vol. %	H_2S, ppm	H_2, vol. %
AD-MEC with 0.6 V	84	14	0	1
AD-MEC with 0.8 V	88	8	120	3
AD-MEC with 1.0 V	82	15	287.5	3
AD-MEC with 1.2 V	75	16	2000	7

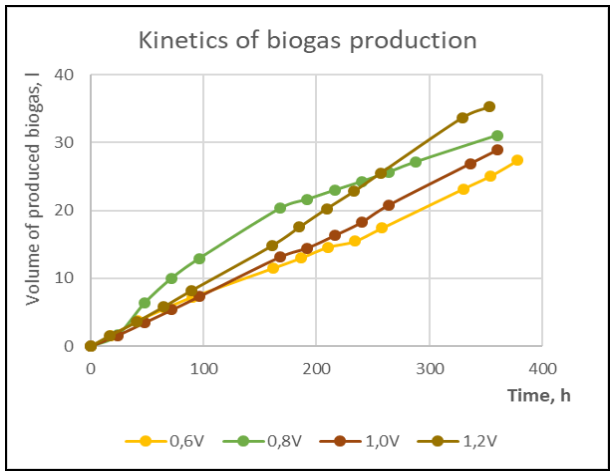

Fig. 2. Comparative graph of the kinetics of a process of biogas production at different external voltages in the AD-MEC system.

Table 3 shows the gas composition in the produced biogas at different external voltages. As can be seen, methane yields are highest at 0.8 V (88 vol. %), followed by 0.6 (84 vol. %), 1.0 (82 vol. %) and 1.2 V (75 vol. %). Generation of hydrogen (7 vol. %) and methane (75 vol. %) was observed at 1.2 V, with carbon dioxide and hydrogen not being converted into additional amounts of methane. Most likely, this is due to ongoing electrolysis of water or inhibition of the metabolism of microorganisms. Choi *et al*. found that methane production increased when an external voltage of 0.5-1.0 V was applied and decreased at 1.5 V [6]. Linji *et al*. found too that 0.8 V is the optimal external voltage for energy recovery from waste activated sludge [9]. From the obtained results, which correspond to literature data from other authors, it can be concluded that the optimal external voltage applied to AD-MEC is 0.6-0.8 V.

Stillage (substrate) was not treated for H_2S by 1.2 V - there is 2000 ppm in the produced biogas. In other cases, the concentration of hydrogen sulfide in the biogas was below 300 ppm or even 0 (at 0.6 V). The contents of carbon dioxide and hydrogen decrease and the methane content increases at 0.8 V. In the cases of 0.6 and 1.0 V the carbon dioxide content is 14-15 vol. %.

Current density

A relatively constant voltage was recorded over the duration of the assay. From MEC surface area and current, the current density for 0.6 and 1.2 V external voltage was calculated. The graph with the data is shown on Fig. 3 for 15 days of operation of AD-MEC at the respective external voltage. At 0.6 V the change in voltage and current is relatively small during the process (15 days). The maximum current was 0.043 A (achieved in the first 24 h) and the minimum was 0.030 A (on the 15th day). The current density varies from 2.15 to 1.5 A/m^2. At 1.2 V, a drastic change in current density is observed - from 4.45 to 2.95 A/m^2 (current from 0.890 A on the first day to 0.590 A on the 15th day). These results confirmed the conclusions made so far about the advantages of applying lower over higher external voltage.

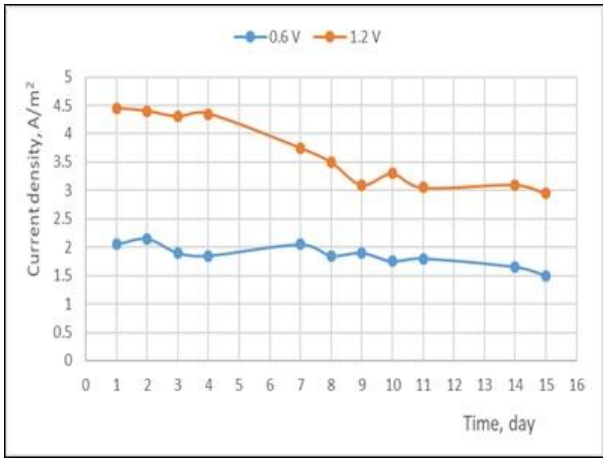

Fig. 3. Comparative graph between current density by 0.6 and 1.2V applied voltage in AD-MEC

Bacterial attachment on MEC electrodes

The SEM images on the anode surface showed more bacterial cells colonization vs cathode (Fig. 4). Electrodes were covered with attached biofilm after 15 days of biomethanation in the AD-MEC system. The formed biofilm of the electrodes was analyzed at a supply of 0.8 V external voltage, as it showed the best decomposition of organic matter and the highest yield of biomethane. Hassanein *et al*. (2020) found that the adhesion of cells to the graphite electrode was better than that on stainless steel [10]. They also proved the presence of

exoelectrogenes, electricigens and anodophilic bacteria at the anode after 11 days of the MEC inclusion to AD. Hydrogenotrophic methanogens such as *Methanobacterium, Methanospirillum, Methanobrevibacter, Methanosarcina, Methanoculleus and Methanocorpusculum* have been reported to grow on the anode [11]. Similar to their results, SEM analysis showed a very well formed biofilm on the anode. Rod-shaped and coccoid bacteria dominated on the anode surface. These cells were likely exoelectrogens and hydrogenotrophic methanogens, such as *Methanobacterium, Methanosarcina, Methanobrevibacter* and *Methanosphaera*.

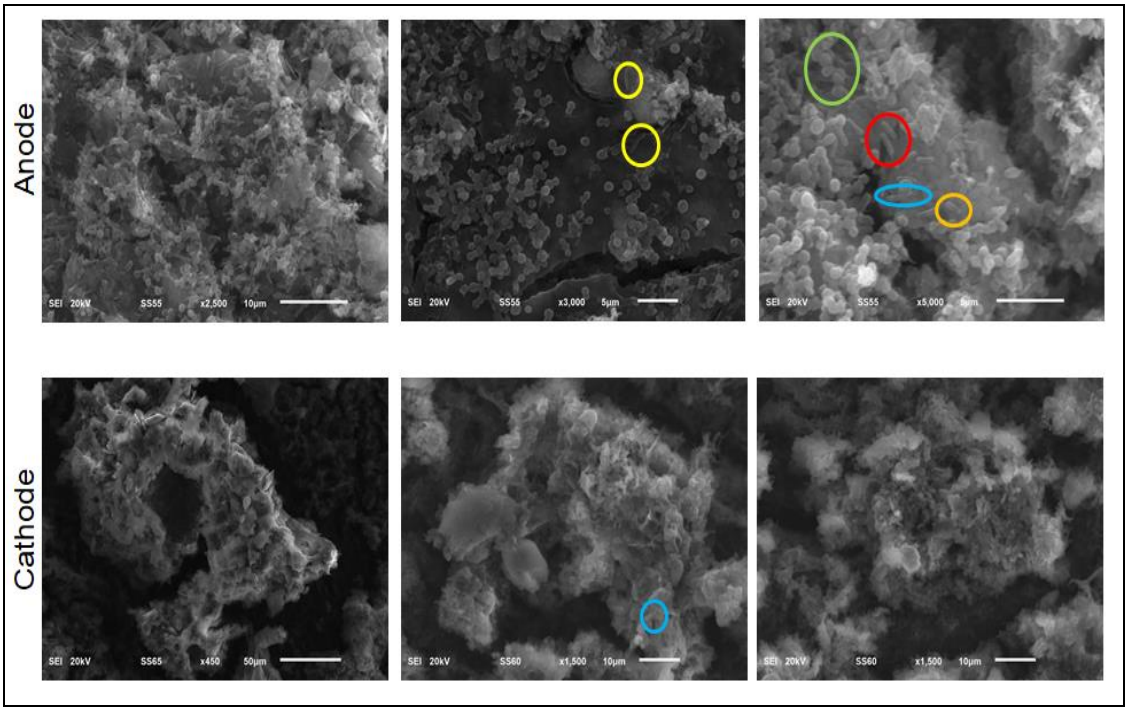

Fig. 4. Morphological characteristics of anode and cathode by SEM - coccoid bacteria with green circle, 1-1.5 μm rod-shaped bacteria with orange circle, 1.5-2.0 μm rod-shaped bacteria with blue and red circle, curved rod-shaped bacteria around 1.5 μm with yellow circle.

Less rod-shaped bacteria and no coccoid bacteria were present on the cathode, maybe because the biofilm was wrinkled a lot.

CONCLUSION

From the obtained results it could be concluded that when purifying an ethanol stillage in an AD-MEC system it is best to apply 0.8 V external voltage. Under these conditions, wastewater is purified from sulfates and organic matter (89 % biodegradability), VFAs are degraded and do not inhibit the process of methanogenesis. Also, the produced biogas has a high methane content of 88 vol. % and a low CO_2 content of 8 vol. %, which is an advantage and a prospect for eliminating the next step in the purification of biogas, and direct application. Good results for biodegradability and reduction of VFAs, as well as high methane yield were obtained at 0.6 and 1.0 V external voltage. At higher voltages (such as 1.2 V) there was poor biodegradability (32 %), partial reduction of VFAs, which are inhibitors of methanogenesis at high values in the bioreactor, and low methane yield (75 vol. %). The current density was 2.15-1.5 A/m^2 at 0.6 V and 4.45-2.95 A/m^2 at 1.2 V. Rod-shaped and coccoid bacteria dominated in the biofilm on the anode but not on the cathode. In addition, SEM analysis distinguished bacteria that may be methanogens - rod-shaped bacteria such as *Methanobacterium, Methanobrevibacter* and coccoid bacteria such as *Methanosarcina, Methanosphaera*.

Acknowledgement: *This research was supported by the Bulgarian National Science Fund, Grant № KP-06-M47/3 from 26.11.2020.*

REFERENCES

1. W. Wang, J.-S. Chang, D.-J. Lee, *Bioresource Technology*, **345**, 126519 (2022).
2. R. Moreno, E. Martínez, A. Escapa, O. Martínez, R. Díez-Antolínez, X. Gomez, *Fermentation*, **4**(1), 2 (2018).
3. T. Y. Gao, H. M. Zhang, X. T. Xu, J. H. Teng, *Water Res.*, **190**, 116679 (2021).
4. C. Q. Liu, D. Z. Sun, Z. Q. Zhao, Y. Dang, D. E. Holmes, *Bioresour. Technol.*, **291**, 121877 (2019).
5. H. H. Zhou. D. F. Xing, M. Y. Xu, Y. Y. Su, Y. F. Zhang, *Appl. Energy*, **269**, 115101 (2020). DOI: 10.1016/j.apenergy.2020.115101.
6. K.-S. Choi, S. Kondaveeti, B. Min, *Bioresour. Technol.* **245**, 826 (2017).
7. A. Arvin, M. Hosseini, M. M. Amin, G. N. Darzi, Y. Ghasemi, *Biochemical Engineering Journal*, **144**, 157 (2019).
8. M. S. Venkata, B. M. Lenin, *Bioresour. Technol*, **102**, 8457 (2011).
9. X. Linji, L. Wenzong, W. Yining, W. Aijie, L. Shuai, J. Wei, *Int. J. Hydrogen Energy*, **38** (35), 15801 (2013).
10. A. Hassanein, F. Witarsa, S. Lansing, L. Qiu, Y. Liang, *Sustainability*, **12**, 8491 (2020).
11. A. A. Pawar, A. Karthic, S. Lee, S. Pandit, S. P. Jung, *Environ. Eng. Res*., **27**(1), 200484 (2022). https://doi.org/10.4491/eer.2020.484.