Microbial Electrolysis of Synthetic Acids for Biohydrogen Production: Influence of Biocatalyst Pretreatment and pH with the Function of Applied Potential

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Abstract

Bioelectrolysis of synthetic acids (acetate, butyrate and propionate) was evaluated in a single chamber Microbial Electrolysis Cell (MEC) to produce biohydrogen (H₂). The influence of culture pretreatment (untreated and acid pretreated) and pH (6 and 7) conditions on electrolytic process were studied. MEC was operated at three optimized potentials viz., 0.2, 0.6 and 1.0 V along with control operated without any applied potential. Maximum Hydrogen Production Rate (HPR), Cumulative Hydrogen Production (CHP) and Specific Hydrogen Yield (SHY) were registered at 0.6 V followed by 1.0 and 0.2 V operations under all the experimental conditions studied. Culture pretreatment and pH variation showed influence on the MEC process. Pretreatment (PTr) operation at pH 7 showed good process performance than pH 6. MEC with untreated (UTr) at pH 6 and 7 showed lower performance compared to PTr operations. About 53% removal of synthetic acids was registered during the process which is a good sign for MEC as a wastewater treatment unit. Electrokinetic evaluation through Tafel slope showed that MEC operations with PTr and UTr at pH 6 recorded lower redox slopes and lower polarization resistance (R_p) at 0.2 V and 0.6 V whereas pH 7 recorded lower redox slopes and R_p at 0.6 V and 1.0 V.

Keywords: Biohydrogen; Microbial electrolysis cell; Poised potential; Pretreatment; Wastewater treatment

Introduction

In recent times, microbial electrolysis is emerging as a promising technology for biohydrogen (H₂) production when compared to the conventional dark fermentation process [1-3]. Dark-fermentation is a potential process for the production of H₂ using wastewater as substrate with simultaneous wastewater treatment [4,5]. But dark fermentation process has the disadvantage of low substrate conversion efficiency and low yield (4 mol H₂/mol of glucose, compared to a stoichiometric potential of 12 mol H₂/mol). Most of the organics (60-70%) remain as soluble fermentation products mainly in the form Volatile Fatty Acids (VFA) such as acetate and butyrate. The treatment of VFA is indispensable prior to discharge [4-7]. Further energetic gains can be made by using wastewater effluent as substrate for the production of additional H₂ which results in the reduction of Chemical Oxygen Demand (COD) and solid content of the effluent [6-9]. MEC process is achieved with input of a small electric current and has the potential of converting a wide variety of dissolved organic matter present in the wastewater and secondary effluents to H₂ [1,3,6,9,10]. Production of H₂ by applying external voltage to the Microbial Fuel Cells (MFCs) is investigated and many reports are available in this aspect [11-15]. Microbial electrolysis cell (MEC) facilitated a new approach for H₂ production. Lower energy investment compared to the conventional water electrolysis makes MEC as an attractive system for H₂ production. Process efficiency of MEC is influenced by several physical, operational and biological factors. Among them, biological and operational factors like biocatalyst selection and operating pH are significant. Employing mixed culture is a better option for treatment of complex and non-sterile wastewater. In this context, biocatalyst plays a significant role in influencing the overall process efficiency especially with wastewater-mixed culture microenvironment. But the feasibility of H₂ production with typical anaerobic consortia is limited as it gets rapidly consumed by methanogens [16,17]. Pretreatment is one of the strategies which can be applied to parent inoculum to facilitate the selective enrichment of Acidogenic Bacteria (AB) which are capable of producing H₂ as the end-product with simultaneous prevention of hydrogenotrophic methanogens [17]. pH of the system can bring about alterations in several primary physiological parameters of the biocatalyst like change in membrane potential and proton motive force. pH of the system can also influence the substrate metabolism and release of metabolic byproducts [5]. pH range of 5.5 to 6 is ideal for effective H₂ production due to the suppression of methanogens [16] and H₂ evolution. Moreover, neutral pH is the optimum condition for many bacterial growth and metabolic activities [18]. At pH 7, electrochemically active bacteria can typically oxidize organic compounds and also effective for H₂ production in single chamber MEC [6,19,20]. In a previous study [6], we employed an integration strategy where effluents from dark fermentation rich in VFA (acetate, butyrate and propionate) are used as substrate in MEC process. Production of more H₂ with better wastewater treatment was achieved under optimized poised potentials [6]. In the present study, we tried to use acetate, butyrate and propionate of commercial grade to evaluate the conversion efficiency to H₂ under applied potential conditions. Further, in the present study, an attempt was made to evaluate the influence of culture pretreatment and pH of the system on MEC process for H₂ production using synthetic acids (acetate, butyrate and propionate) at a loading rate of 3000 mg/l concentration.
Material and Methods

Biocatalyst

Anaerobic mixed consortium acquired from a full-scale Upflow Anaerobic Sludge Blanket (UASB) bioreactor treating composite wastewater was used as biocatalyst. Prior to inoculation, the culture was washed twice with saline buffer (5000 rpm, 20°C). The culture was then enriched with designed synthetic wastewater [DSW; glucose-3 g/l, NH₄Cl-0.5 g/l, KH₂PO₄-0.25 g/l, K₂HPO₄-0.25 g/l, MgCl₂-0.3 g/l, CaCl₂-25 mg/l, ZnCl₂-11.5 mg/l, CuCl₂-10.5 mg/l, CaCl₂-5 mg/l, MnCl₂-15 mg/l, NiSO₄-16 mg/l, FeCl₂-25 mg/l] under anaerobic microenvironment at pH 7.0 (100 rpm; 48 h).

Acid pretreated biocatalyst: Native untreated anaerobic mixed consortium was treated with concentrated orthophosphoric acid under anaerobic environment for 24 h at pH 3. This method of pretreatment was employed to inhibit the methanogenic bacteria present in the mixed consortia.

MEC configuration

Single chamber membrane less MEC [(working/total volume, 1.2/1.6 l with 0.4 l head space)] made of perspex material with non-catalyzed graphite plates (5×5 cm, surface area 70 cm²) as electrode materials was used in the present study. The electrodes were connected with copper wires by an epoxy sealant. They were placed in MEC such that both are held opposite to each other at a distance of 1 cm. The copper wires were passed through the small provisions made in MEC such that both the electrodes were connected to the potentiostat (Span control DC regulator power supply) for applying external potential. Provisions were made at appropriate positions for substrate feeding and sample collection. H₂ sensing port was arranged on the top of the MEC to measure the H₂ gas produced.

Operation

Prior to the start up of the experimental study, six cycles were operated in order to acclimatize the biocatalyst to the new environment until stabilized performance was attained with respect to H₂ production. Applied potentials were given to all the MECs with the help of a potentiostat by poising both the electrodes. Optimum potentials were chosen from the previous reported work and used in the present study [3]. MEC was then operated under batch mode operation at different poised potentials viz, 0.2, 0.6 and 1.0 V using cyclic Voltammetry (CV) using potentiostat-glavanostat system (PGSTAT12, Ecochemie). All electrochemical assays were performed in situ by considering anode and cathode as working and counter electrodes against Ag/AgCl (S) reference electrode in the electrolyte. CV was performed by applying a potential ramp to the working electrode (anode) at a scan rate of 30 mV/s over the range from +0.5 to -0.5 V. Tafel analysis was carried out with voltammetric profiles using GPES (version 4.0) software and conclusions were drawn in terms of Tafel slope and polarization resistance.

Bio-electrochemical analysis

Bio-electrochemical behavior of micoflora under applied potential conditions with respect to electron discharge was studied by employing Cyclic Voltammeter (CV) using potentiotstat-galvanostat system (PGSTAT12, Ecochemie). All electrochemical assays were performed in situ by considering anode and cathode as working and counter electrodes against Ag/AgCl (S) reference electrode in the electrolyte. CV was performed by applying a potential ramp to the working electrode (anode) at a scan rate of 30 mV/s over the range from +0.5 to -0.5 V. Tafel analysis was carried out with voltammetric profiles using GPES (version 4.0) software and conclusions were drawn in terms of Tafel slope and polarization resistance.

Results and Discussion

Bioelectrolytic H₂ production

Biocatalyst pretreatment, system pH and applied potential showed significant influence on MEC performance for H₂ production. H₂ production trend was observed in the following order with respect to culture pretreatment and pH studied.

PT r pH 7>UT r pH 7>PT r pH 6>UT r pH 6

Application of poised potentials showed higher H₂ production compared to the control (without any applied potential) (Figures 1 and 2). Maximum H₂ production was observed at 0.6 V irrespective of pH and nature of the biocatalyst studied. Electron discharge pattern of the biocatalyst get enhanced under applied potential conditions [9,25,26]. Application of external potential increases the overall potential difference between anode and microbial cell mobilizing the electron flow to the external environment. Increase in applied potential increases H₂ production [9]. Along with applied potential, system pH and nature of the biocatalyst studied also showed influence on the MEC performance (Figures 1 and 2). Pretreated biocatalyst (PT r) showed higher H₂ production compared to untreated biocatalyst (UT r) at pH 7. 0.6 V with PT r at pH 7 documented higher H₂ production rate (HPR, 2.42 mmol/h) and cumulative H₂ production (CHP, 8.66 mmol), followed by 1.0 V (2.185 mmol/h; 7.804 mmol) and 0.2 V (1.482 mmol/h; 5.073 mmol). The same trend was noticed with UT r biocatalyst at pH 7 but comparatively less H₂ production was observed at 0.6 V (HPR, 2.210 mmol; CHP, 7.024 mmol) followed by 1.0 V (HPR, 2.029 mmol/h; CHP, 6.09 mmol) and 0.2 V (HPR, 1.795
At operating pH and biocatalyst used. At pH 6, MEC operated with UTr biocatalyst registered 0.546 mmol/h of HPR and CHP of 1.560 mmol whereas PTr biocatalyst registered HPR of 0.858 mmol/h and CHP of 3.356 mmol. MEC operated at pH 7 with UTr biocatalyst showed HPR of 1.092 mmol/h and CHP of 3.590 mmol/h and with PTr biocatalyst showed HPR of 1.170 mmol/h and CHP of 4.604 mmol of H\textsubscript{2}. Acid pretreatment of mixed culture suppressed the methanogens so that the produced H\textsubscript{2} is not consumed by the methanogens and H\textsubscript{2} yield is increased simultaneously \cite{17,18}. This might be the reason for more H\textsubscript{2} in the PTr operation compared to UTr. During the process, irrespective of the experimental conditions studied, maximum H\textsubscript{2} production was noticed between 12\textsuperscript{h} and 16\textsuperscript{h} of operation. H\textsubscript{2} production was found from 4\textsuperscript{h} of operation and attained maximum between 12\textsuperscript{h} and 16\textsuperscript{h} of operations and decreased gradually by the end of 24\textsuperscript{h} of operation. Thus, H\textsubscript{2} production was significantly influenced by the culture pretreatment employed and the pH of the system.

**Utilization of acid intermediates**

**VFA removal:** Synthetic VFA present in the feed served as carbon source for H\textsubscript{2} production during MEC operation at different experimental conditions studied. Initially, the organic loading of the feed at 3000 mg VFA/l was given in all the experiments. A gradual reduction in VFA was observed during initial phase of the operation under all the experimental conditions studied (Figure 3). MEC operation with UTr biocatalyst showed maximum VFA removal efficiencies at pH 6 and 7. Maximum VFA removal efficiency was observed at 0.6 V (pH 6 (48%), pH 7 (53%)) than PTr biocatalyst (pH 6 (46%), pH 7 (50%)). Control system comparatively showed less VFA removal efficiencies [UTr, pH 6 (30%), pH 7 (37%); PTr, pH 6 (35%), pH 7 (34%)] than MEC operations (Figure 3). At applied external potential conditions, more H\textsubscript{2} are produced by the electro hydrolysis of VFAs where maximum H\textsubscript{2} yields can be obtained by the reduction of produced H\textsuperscript{+} with the electrons given externally by applying potential \cite{9].

SHY was also calculated in the present study with respect to the amount of VFA degraded and H\textsubscript{2} produced (Figure 4). At pH 7 with PTr biocatalyst, 0.6 V (5.99 mol/kg VFA\textsubscript{a}) showed maximum SHY followed by 1.0 V (5.87 mol/kg VFA\textsubscript{a}) and 0.2 V (3.81 mol/kg VFA\textsubscript{a}) which was more than that observed at pH 6 (0.6 V (6.07/4.8 mol/kg VFA\textsubscript{a}); 1.0 V (3.54 4.5 mol/kg VFA\textsubscript{a}); 0.2 V (3.453.4 mol/kg VFA\textsubscript{a})). The same trend was observed with UTr biocatalyst but with low SHY (pH 7, 0.6 V (4.89 mol/kg VFA\textsubscript{a}); 1.0 V (4.52 mol/kg VFA\textsubscript{a}); 0.2 V (3.40 mol/kg VFA\textsubscript{a}); pH 6, 0.6 V (3.9 mol/kg VFA\textsubscript{a}); 1.0 V (3.67 mol/kg VFA\textsubscript{a}); 0.2 V (3.68 mol/kg VFA\textsubscript{a})). Control operation showed less SHY than MEC operations [Untreated, pH 6 (1.44 mg/kg VFA\textsubscript{a}); pH 7 (3.03 mg/kg VFA\textsubscript{a}); pretreated, pH 6 (3.25 mg/kg VFA\textsubscript{a}); pH 7 (4.14 mg/kg VFA\textsubscript{a})]. SHY corroborated well with the substrate degradation in terms of VFA\textsubscript{a}. The pattern of VFA, varied with the poised potential. Substrate degradation favored H\textsubscript{2} yield during the experimental study.

Quantitative estimation was also performed during the study by HPLC analysis to elucidate the variation in composition of the VFAs and their utilization with time (Figure 5). Variation in the composition of VFAs with respect to time was also evaluated. The inlet composition of feed consists of a mixture of synthetic acids viz, acetate, butyrate and propionate (HAc+HBu+HPr) contributing to a total of 3 mg/ml concentration. At 0\textsuperscript{h}, the concentrations of each acid were 1 mg/ml (HAc, 1 mg/ml, HBu, 1 mg/ml, HPr, 1 mg/ml). During the course of experiments, concentration of synthetic acids was found to decrease indicating their contribution in the metabolic process which ultimately converted to H\textsubscript{2}. Applied potential increases the H\textsubscript{2} production using VFA \cite{6,8}. In the present study also, applied potential employed mmol/h; CHP, 4.682 mmol). At pH 6, using PTr, maximum HPR and CHP of 1.560 mmol/h; 7.258 mmol was observed respectively at 0.6 V followed by 1.248 mmol/h; 5.151 mmol at 1.0 V and 1.170 mmol/h; 4.604 mmol at 0.2 V, respectively. Comparatively UTr showed maximum HPR of 1.404 mmol/h and 5.463 mmol and 4.526 mmol at 0.6 V and 1.0 V followed by 0.936 mmol/h; 3.980 mmol of H\textsubscript{2} at 0.2 V. At operating pH 6, H\textsubscript{2} production was observed during the experimental process but comparatively less than pH 7 for both PTr and UTr. Overall control registered less H\textsubscript{2} production with respect to
showed positive influence on the bioelectrolytic conversion of synthetic acids to $H_2$. 0.6 V registered maximum utilization of VFA [HAc (0.46 mg/ml), HBu (0.65 mg/ml) and HPr (0.80 mg/ml)] by the end of 24th h of operation. Almost equal or high consumption of VFA was registered at 0.2 V than 1.0 V particularly at PTr operations than UTr operations. Even though consumption was observed more at 0.2 V but due to the applied potential used, more electrons might have released in the case of 1.0 V to make $H_2$ [9,25,26] and this might be the reason for the observed high $H_2$ production at 1.0 V than 0.2 V. Maximum utilization of acetate was visualized during the study indicating that biocatalyst was able to utilize acetate but bytrate and propionate due to its simpler form. At applied potential of 0.6 V, 50% utilization of acetate was noticed which indicated that the system favored electro-hydrolysis. About 1 $\mu$W/h, 5 $\mu$W/h and 27.7 $\mu$W/h of energy were given continuously during the operation at 0.2, 0.6 and 1.0 V, respectively. Electrohydrolysis of synthetic VFA was visualized in the study from the observations in the VFA removal pattern which determines their utilization for $H_2$ production. Normally water electrolysis plants operate at electrolyte temperature between 70-90°C with applied potential of 1.85-2.05 V [27]. In the present study, as the MEC was operated at room temperature (28 ± 2°C) and applied potentials below 1.0 V, the observed $H_2$ production can be assumed is by microbial electrolysis process [3,7,9]. Tuna et al. [9] reported 85.5 ml of CHP with 56% of energy efficiency using effluents from dark fermentation whereas our previous study using effluents from dark fermentation produced 78 ml of CHP at 0.6 V [6]. Therefore from the results obtained, maximum performance was visualized at lower applied potentials indicating the potential of the system for $H_2$ production and wastewater treatment.

**pH variation:** pH of the system plays a crucial role in carrying out the metabolic reactions in all the living systems. pH influences the efficiency of substrate metabolism, protein synthesis, synthesis of storage material and metabolic by-product release [16]. Depending on the organism and growth conditions, changes in external pH can bring about subsequent alterations in several primary physiological parameters, including internal pH, concentration of other ions, membrane potential and proton-motive force. In the present study, MEC was operated at acidic pH 6 and at neutral pH 7 in order to evaluate the behavior of the biocatalyst under these two pH environments. Variation in the pH profiles with respect to substrate degradation corroborated well during the process (Figure 6). Marginal variation was noticed in the system performance with respect to the substrate degradation and $H_2$ production at these two pH values. At initial stage of operation, a sudden drop in the pH was noticed but subsequently the pH got stabilized without further drop. This might be due to the utilization of synthetic acids in the metabolic process which further got converted to $H_2$. Acidogenic environment prevailed under both the pH conditions studied indicating the favorable conditions for $H_2$ production with PTr at pH 6. During experimentation at pH 6, the system pH was found in the range of 5.7 ± 0.5 and at pH 7, the system pH was found to be in the range of 6.2 ± 0.1. The observed pH also influenced substrate metabolism and the release of metabolic by-products.

**Dehydrogenase activity**

DH enzyme function is important for shuttling of the generated proton (generated during anaerobic metabolism) between the metabolic intermediates with the help of redox mediators/electron donors.
Variation in pH profiles at different applied potentials, pH and biocatalyst used with function of time.

carriers viz., NAD/NADH, FAD, FMN, etc. [22]. DH mainly involves in the inter conversion reactions and provides higher availability of proton in the cell rather than degradation, which is especially important for H₂ production. DH activity will be higher under anaerobic operation [22]. In our previous study, we tried to optimize the best potentials below water electrolysis potential (1.2 V) and tried to evaluate the H₂ production efficiency at each applied potential using designed synthetic wastewater and at the same time we also reported the biocatalyst behavior under each applied potential with respect to dehydrogenase activity [3]. Whereas, in our present study, using those optimized applied potentials we evaluated the efficiency of synthetic acids of commercial grade as substrate to produce H₂. DH activity was estimated at regular time intervals in all the modes of experimental conditions studied. DH activity was found to increase gradually from initial stage and attained maximum at 12th h of operation and gradually decreased till 24th h of operation (Figure 7). The same trend was followed in all the experimental conditions studied but the activity varied based on the applied potential, nature of the biocatalyst and pH of the system. Overall, maximum DH activity was noticed at 12th h of operation in all the experimental conditions studied except at pH 7 UTr and PTr pH 6 (8th h). Maximum DH activity was observed at 0.6 V of 3.36 µg/ml and 3.32 µg/ml at pH 6 and 7 of PTr operations. At pH 6, UTr operation also showed maximum DH activity at 0.6 V (3.36 µg/ml) and at pH 7, showed DH activity of 2.89 µg/ml. Control operation registered less DH activity (UTr pH 6, 2.02 µg/ml; UTr pH 7, 2.43 µg/ml; PTr pH 7, 1.86 µg/ml) than MEC operations except at pH 6 PTr operation where high DH activity (2.75 µg/ml) was observed than 0.2 V (2.50 µg/ml). Applied potential conditions registered maximum DH activity than control might be due to enhanced activity of the biocatalyst at applied potential conditions [3,25]. Maximum DH activity observed during the study inferred the generation of more protons and electrons by substrate degradation which ultimately got reduced to H₂ at cathode.

Tafel slope analysis

Tafel plots provide a visual understanding of the losses present in the system, which helps to interpret the biocatalytic activity based on the derived kinetic parameters viz., oxidative Tafel slope (βₒ), reductive Tafel slope (βₐ) and polarization resistance (Rₑ) in Ω [28,29]. Low potentials (+0.5 to -0.5 V) were chosen for the linear regression analysis to minimize mass transfer limitation, and the best fit of multiple linear regressions within the chosen potential range which was used to calculate kinetic parameters. Based on the Tafel equation, the Y-axis intercept is the logarithm of the exchange current densities (ln iₒ).

Higher slope indicates the requirement of higher activation energy that makes the redox reactions less favorable [28,29]. PTr and UTr operations under various poised potentials and two pH ranges (pH 6 and 7) are evaluated in terms of redox slopes as well as Rₑ (Figure 8 and 9). At pH 6, PTr operation recorded less redox slopes than the UTr operation. Oxidative slope (βₒ) varied in a similar manner and was found to be less in both the UTr as well as the PTr operations. Comparatively, 0.2 V operation recorded lower βₒ (PTr, 0.049; UTr, 0.043 V/dec) followed by 0.6 V (PTr, 0.053; UTr, 0.05 V/dec), 1.0V (PTr, 0.049; UTr, 0.046 V/dec) followed by 1.0V (PTr, 0.049; UTr, 0.046 V/dec).
The number of protons and electrons from the biocatalyst to make it to the redox slopes. Even though low redox slopes were observed at 0.2 V, H₂ production was comparatively less than other applied potentials with 0.6 V (0.073 V/dec) and control (0.115 V/dec). Unlike in PTr, UTr recorded lower βc with 0.1 V (0.06 V/dec) followed by 0.6 V (0.078 V/dec), 0.2 V (0.089 V/dec) and control operation (0.116 V/dec), βc varied significantly in both the PTr and UTr operations under different poised potentials. In PTr, control operation (0.64 V/dec) showed a lower slope followed by 0.2 V (0.67 V/dec), 0.6 V (0.72 V/dec) and 1.0V (0.84 V/dec) whereas in UTr condition, 0.6 V recorded lower βc (0.42 V/dec) followed by control (0.46 V/dec), 0.2 V (0.49 V/dec) and 1.0 V (0.53 V/dec). The observed lower slopes with 0.6 V and 1.0 V reveal high oxidation and reduction processes during the metabolism. DH activity also supported the above observation that supports the good shuttling activity of free protons inside the system. Rp was observed to follow a similar pattern in all the poised potential operations with both the PTr and UTr biocatalyst. Comparatively, lower Rp was observed with PTr than the UTr biocatalyst. 1.0 V recorded lower Rp (PTr, 37.94; UTr, 41.59 Ω) followed by 0.6 V (PTr, 39.38; UTr, 52.88 Ω), 0.2 V (PTr, 44.85; UTr, 67.78 Ω) and control (PTr, 50.81; UTr, 72.22 Ω). The observed lower Rp with 0.6 V and 1.0 V showed good electron discharge properties of the biocatalyst. Electrokinetic evaluation enumerated that pH 6
operation with PTR and UTr biocatalyst recorded lower redox slopes and $R_e$ with 0.2 V and 0.6 V whereas pH 7 operation recorded lower redox slopes and $R_e$ with 1.0 V and 0.6 V operation. This trend might be attributed to the activation of the energy levels of the biocatalyst at varied conditions and potential ranges that could be sufficient enough to meet their reduction potential towards $H_2$ production.

Conclusions

The present study inferred the potential of MEC operation for converting synthetic pollutants containing wastewater to biohydrogen with simultaneous treatment. Applied potential showed influence on $H_2$ production and on bioprocess. Along with applied potential, nature of the biocatalyst and pH of the system also influenced the MEC operation with respect to $H_2$ and also treatment. Pretreated operation at pH 7 showed better performance than pretreated operation at pH 6. Maximum substrate utilization was registered during the study indicating that MEC could be used as wastewater treatment unit along with $H_2$ production. Overall process performance was enhanced at 0.6 V compared to 1.0 V and 0.2 V and hence depicts the potential of 0.6 V for MEC operation. Tafel slopes at 0.6 V showed lower redox slopes and polarization resistance in the both UTr and PTR experimental conditions studied corroborated well with the $H_2$ production and treatment.

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