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Bio-electrocatalytic reduction of CO_2: Enrichment of homoacetogens and pH optimization towards enhancement of carboxylic acids biosynthesis

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ABSTRACT

The microbial catalyzed electrochemical reduction of CO_2 is gaining significant attention in the field of energy and environment as it provides dual benefits of product recovery with simultaneous CO_2 neutrality. Specific reduction of single carbon unit (C1-CO_2) to two-carbon (C2-CH_3COOH) carboxylic acids was studied in a bio-electrochemical system (BES) via a three stage experimental design using BESA treated and untreated (control) anaerobic consortia. During stage-I, enrichment of homoacetogenic culture was carried out by supplementing H_2 and CO_2 in the reactors containing BESA treated and control cultures respectively. Optimization of pH was carried out in stage-II to enhance the carboxylic acids production at diverse pH range (acidic to alkaline viz., pH 5, 6.5, 8.5 and 10), where pH 10 was found to be optimum for maximum carboxylic acids (VFA: volatile fatty acids) generation in BESA treated (3500 mg/l) and control cultures (1200 mg/l) respectively followed by pH 8.5 utilizing bicarbonate. Interestingly, reduction in VFA concentration was observed after 24 h which can be attributed to its consumption by other groups of bacteria that co-exist along with the enriched culture. During stage-III, bioelectrocatalytic production of acetate was evaluated by considering the optimized pH 10 and under applied potential of -0.8 V vs Ag/AgCl (S) in two BES viz., BES_B (BES with BESA treated consortia) and BES_C (BES with parent consortia as control) using CO_2 and bicarbonate respectively. Maximum acetate production of 1.7 g/l (2.88 mmol/d)/2.1 g/l (3.55 mmol/d) was recovered in BES_B through the bio-electrochemical reduction of CO_2/bicarbonate, which correlated well with the observed higher reduction currents and columbic efficiency.

1. Introduction

Carbon dioxide (CO_2) is a greenhouse gas with rapidly increasing concentrations in the atmosphere due to the vehicle, industrial activities, etc. [1]. On the other hand, CO_2 is a potential carbon resource available on the earth. There is an alarming effect of global warming during the last two centuries due to the CO_2 emissions [2]. Hence, there exists a need to sequester or reduce the CO_2 concentration towards zero emissions or carbon neutrality. Many methods/processes are being implemented to sequester CO_2 viz., physical, chemical fixation, geological sequestration, terrestrial utilization, membrane technology, MEA scrubbing process, etc. [3]. On the other hand, biological processes could potentially make a significant contribution to carbon capture, as they can be deployed in a sustainable and renewable manner [4]. One of the economically viable biological routes to sequester CO_2 with simultaneous value addition is the microbial catalyzed electrochemical reduction in bio-electrochemical systems (BES) [5,6]. BES is an emerging technology in the current field of bioenergy and environmental research that utilizes waste towards value addition (Ex: CO_2 to C2, C3, C4 units) by using the enriched microorganisms as biocatalysts in cathode chamber specifically for reduction reactions [7–9]. A key advantage of BESs is that renewable energy and value added products can be generated at low costs with high efficiency [10,11]. Various factors viz., biocatalyst in biocathode, pH, applied potentials, reactor design, etc., play a key role in BES performance.

Biocatalysts play a major role in oxidizing/reducing the substrate towards value added short-chain carboxylic acids (volatile fatty acids (VFA)) viz., acetic acid (AA), butyric acid (BA), propionic acid (PA), etc. [12–15]. Many studies were carried out using pure culture as biocatalyst in BES systems for product recovery [16,17]. However, use of mixed consortia as biocatalyst in biocathodes is an emerging interest as it can suffice the economics

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of the process with simultaneous product yield [18,19]. Specific enrichment of biocatalyst is of prime focus in BES where the substrate (e.g., CO_2) is reduced to a specific product (e.g., acetate) [20]. Pre-treatment of biocatalyst is an attractive option to produce the targeted product by selectively enriching the biocatalyst of desired characteristics [11]. Examples of pretreatment methods for the specific enrichment of bacteria are chemical, heat, alkaline treatments, etc. [21]. Enrichment of homoacetogens for acetate recovery was reported previously [22]. In addition, pH also plays a major role in the product synthesis by regulating the system redox conditions [12,23]. More specifically, the solubility of CO_2 is greatly dependent on pH of the solution [24]. An optimum pH range is necessary for the effective functioning of BES, as the redox microenvironment decides the fate of carboxylic acids synthesis [25]. Optimal pH conditions also enhance the rate and yield of carboxylic acids synthesis which are of high economical value.

In BES, the applied potentials are considered to be the major factor for carboxylic acids synthesis, as they drive the redox equivalents for substrate reduction [26,27]. Studies were carried out using various ranges of applied potentials for the reduction of CO_2 towards carboxylic acids, using mixed consortia [28,29]. There is a great variation in product formation (C1–C6 units) from CO_2 by varying the applied potentials [18]. In order to emphasize the impact of various factors, an attempt is made in the present study to evaluate the specific reduction of single carbon (CO_2) to two-carbon carboxylic acids (acetate) by integrating the optimization of process parameters viz., enrichment of biocathode biocatalyst and optimization of pH via a three stage strategy. Acetate formed as an end product during the process has wide range of applications and can be used as a building block for a variety of value added chemicals. Experiments were designed and carried out in a two chambered BES system using the anaerobic mixed consortia as biocatalyst at anode along with the pretreated and untreated biocatalyst at cathode in two BES respectively. The first stage emphasizes the enrichment of homoacetogenic culture as cathode biocatalyst through selective pretreatment followed by the optimization of pH towards maximization of carboxylic acids in stage-II and the evaluation of the optimized factors in BES during stage-III operation for the specific reduction of CO_2 towards carboxylic acid (acetate) synthesis.

2. Materials and methods

2.1. Bio-electrochemical system configuration

Two bio-electrochemical systems (BES) were fabricated using Schott-Duran glass bottles (Fig. 1). BES used in the study is a double chambered set up consisting of anode and cathode chambers each with a total/working volume of 2.5/2.0 l separated by a proton exchange membrane (PEM; Nafion117, Sigma–Aldrich). Prior to use, NAFION 117 sheet was arch punched to 50 mm size and pretreated by boiling sequentially in 30% H_2O_2, deionized water (pH 7.0), 0.5 M H_2SO_4 and deionized water each for 1 h to increase the porosity. Subsequently, PEM was fixed between washers and clamped in the hollow tube connecting both the chambers. Non-catalyzed graphite plates (4 × 4 cm; 1 mm thickness) were used as electrodes in both the chambers for microbial catalyzed electrochemical redox reactions. Bioanode and biocathode were used in the operation of both BES reactors, each inoculated with specific and different biocatalysts (15% of the total volume of the reactor) at anode and cathode respectively. The two double chambered BES systems were operated with the same anaerobic culture (untreated) as biocatalyst in anode chamber. The cathode chamber was inoculated with the enriched homoacetogenic culture (BESA treated) in BES_B and untreated culture but with enriched homoacetogenic culture (control) in BES_C respectively. Anode and cathode were completely submerged in anolyte and catholyte respectively and proper recirculation and mixing was ensured during operation with the help of magnetic stirrers. Provisions were made in the design for CO_2 sparging, sampling, wire input, inlet and outlet ports. Copper wires sealed with an epoxy sealant were used to maintain contact with the electrodes. Leak proof sealing was employed to maintain anaerobic microenvironment in the system.

2.2. Biocatalyst

2.2.1. Anodic biocatalyst

Anaerobic and untreated culture was used as anodic biocatalyst and pretreated cum enriched homoacetogenic culture was used as cathodic biocatalyst in both BES_B and BES_C respectively. An indigenous mixed anaerobic consortium collected from a full scale anaerobic bioreactor treating sewage wastewater was used as parent inoculum. The anode chamber (bio-anode) was inoculated with anaerobic consortia as biocatalyst without any pretreatment in both the BES viz., BES_B and BES_C. Prior to inoculation, the biocatalyst was enriched in the synthetic wastewater (SW: glucose: 1.5 g/l; NH_4Cl: 0.5 g/l, KH_2PO_4: 0.25 g/l, K_2HPO_4: 0.25 g/l, $MgCl_2$: 0.3 g/l, $CoCl_2$: 25 mg/l, $ZnCl_2$: 11.5 mg/l, $CuCl_2$: 10.5 mg/l, $CaCl_2$: 5 mg/l, $MnCl_2$: 15 mg/l, $NiSO_4$: 0.16 g/l, $FeCl_3$: 0.03 g/l).

2.2.2. Cathodic biocatalyst

Prior to inoculation, the parent inoculum was subjected to chemical pretreatment with 2-bromoethanesulfonic acid (BESA (B); 0.2 g/l) to suppress/inhibit specific methanogenic activity of bacteria [11]. BESA, a structural analog of co-enzyme-M, specifically found in methanogens, facilitates the selective inhibition of methanogenic activity [21]. Subsequently, the enriched acidogenic bacteria were subjected to selective enrichment of homoacetogens, by supplementing H_2 and CO_2 (80:20) in the reactor [30]. The resulting enriched culture was used as a biocatalyst in the cathode chamber during the operation of BES_B. In addition, anaerobic consortia (without BESA pretreatment and with CO_2 and H_2 supplementation) was used as cathodic biocatalyst (control (C)) in BES_C for the comparative evaluation. Prior to inoculation, the biocatalysts were enriched in the synthetic wastewater (SW: NH_4Cl: 0.5 g/l, KH_2PO_4: 0.25 g/l, K_2HPO_4: 0.25 g/l, $MgCl_2$: 0.3 g/l, $CoCl_2$: 25 mg/l, $ZnCl_2$: 11.5 mg/l, $CuCl_2$: 10.5 mg/l, $CaCl_2$: 5 mg/l, $MnCl_2$: 15 mg/l, $NiSO_4$: 0.16 g/l, $FeCl_3$: 0.03 g/l).

2.3. Experimental methodology

The experimental operation for the reduction of CO_2 towards acetate synthesis was carried out in three stages (Fig. 1). In the stage-I, the biocatalyst (mixed consortia) to be used during the BES operation was pre-treated using BESA and was subjected to a headspace of CO_2 and H_2 for the selective enrichment of homoacetogens at pH 6 using 1 N HCl or 1 N NaOH. In the stage-II, in order to increase the process efficiency in terms of volatile fatty acid (VFA)/carboxylic acid production, optimization of pH was carried out at four different set pH (using 1 N HCl or 1 N NaOH) viz., 5, 6.5, 8.5 and 10 using sodium bicarbonate as the sole carbon source supplemented in SW. This was evaluated with two different consortia viz., BESA treated and control culture as biocatalysts. In the stage-III, the optimized pH condition was evaluated for the formation of acetate through CO_2 (CO_2 sparged through the provision made in cathode chamber) and bicarbonate reduction in two BES viz., BES_B and BES_C under an applied voltage of −0.8 V vs Ag/AgCl (S) applied via Chronoamperometry (CA) by potentiostat–galvanostat system (Autolab-PGSTAT12, Ecochemie). BES was operated in fed batch mode at room temperature (29 ± 2 °C). Prior to feeding, pH of the anolyte was adjusted to 7 ± 0.1 using 1 N HCL/1 N NaOH. Feeding and sample collection was

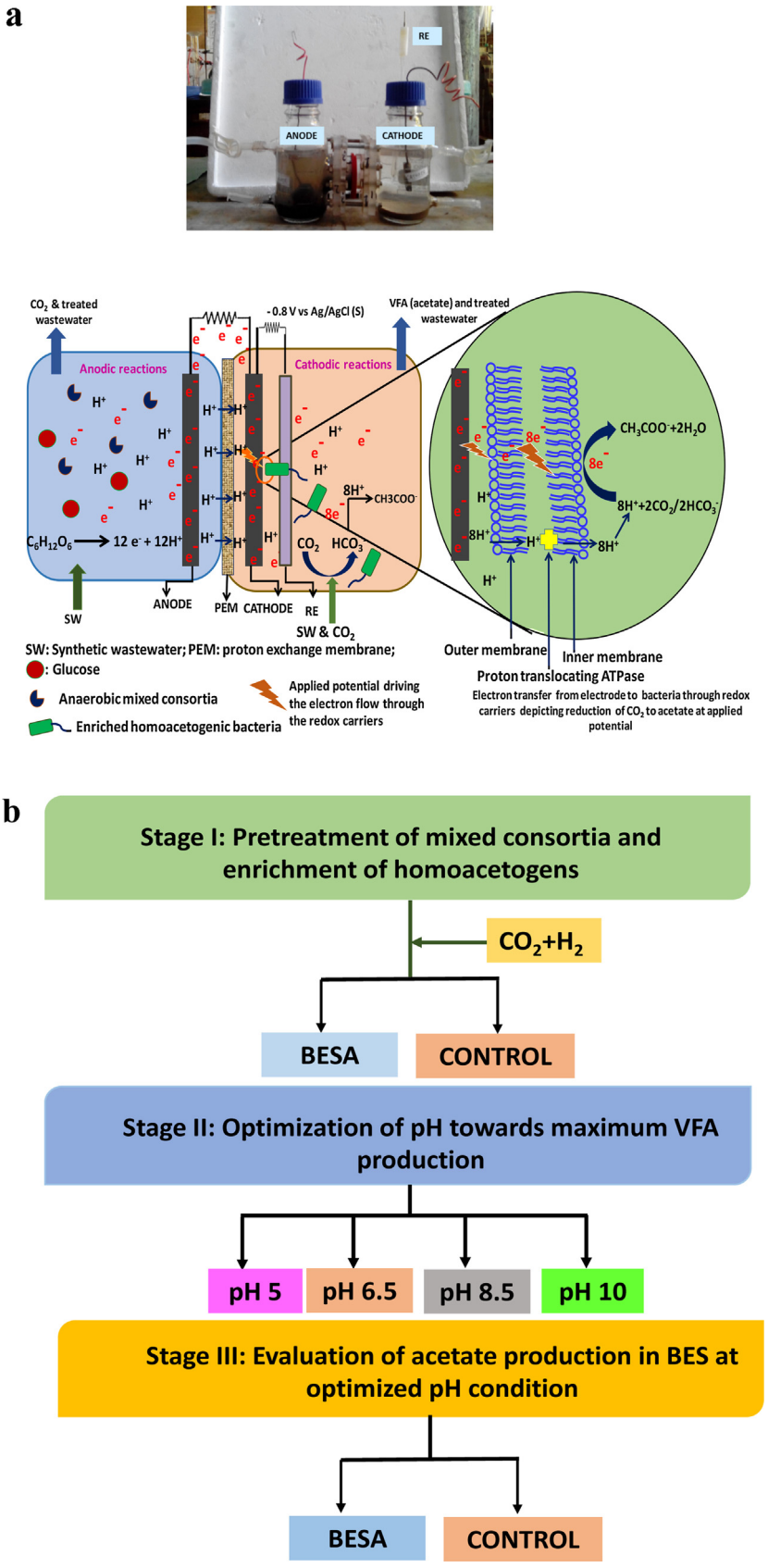

Fig. 1. (a) Photographic and schematic representation of bio-electrochemical system (BES) depicting the electron transfer mechanism from electrode to bacteria and (b) flow chart depicting the experimental methodology in three stages.

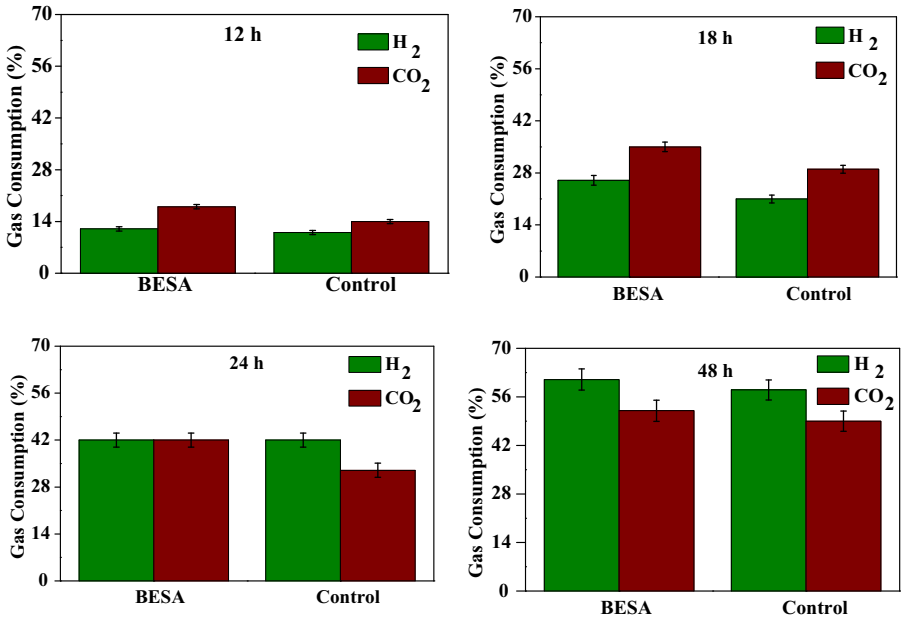

Fig. 2. H_2 and CO_2 consumption profiles during the homoacetogenic bacteria enrichment.

done through the appropriate provisions provided in BES. Nitrogen gas was sparged into the reactor for 5 min after every feeding and sampling event to maintain anaerobic conditions. BES was operated for a cycle period of 48 h based on the reduction in carbon source. Both the BES were operated for 6 cycles and the experiment was performed in triplicates for each reactor setup.

2.4. Process monitoring

The performance of BES was evaluated at every stage in regular time intervals. The enrichment of homoacetogens in Stage-I was evaluated by monitoring the consumption profiles of CO_2 and H_2 gas through gas chromatography (GC; NUCON 5765) using thermal conductivity detector (TCD) with 1/8 × 2 m Heysep Q column employing (argon as carrier gas). The injector and detector were maintained at 60 °C each and the oven was operated at 40 °C isothermally. Quantitative estimation of VFA was analyzed using high performance liquid chromatography (HPLC; Shimadzu LC10A) employing UV–Vis detector (210 nm) and C18 reverse phase column (250 mm × 4.6 mm diameter; 5 μm particle size, flow rate: 0.6 ml/h; wave length: 210 nm). Mobile phase of (40% acetonitrile in 1 mN H_2SO_4; pH, 2.5–3.0) and 20 μl sample injection was used. Bio-electrochemical behavior of biocatalysts along with the redox catalytic currents generation during the operation was studied by Cyclic voltammetry technique using a potentiostat–galvanostat system by applying a potential ramp (+1.0 to −1.0 V), at a scan rate of 30 mV/s (Autolab-PGSTAT12, Ecochemie). The potential of −0.8 V vs Ag/Agcl (S) was applied through chronoamperometry (CA) analysis by potentiostat–galvanostat system (Autolab-PGSTAT12, Ecochemie). Biochemical parameters viz., pH, VFA and bicarbonate reduction were estimated according to the standard methods [31]. All the experiments were carried out in triplicates for validation of the results.

3. Results and discussion

3.1. Enrichment of homoacetogenic bacteria (Stage-I)

Selective enrichment of homoacetogenic bacteria to be used as biocatalyst in bio-cathode for acetate production was carried out in two steps. Most of the homoacetogenic bacteria can grow chemolithoautotrophically on H_2 plus CO_2 as energy and cell carbon source [20]. In the absence of electron acceptors other than CO_2, consumption of H_2 is only possible by methanogenic archea and homoacetogenic bacteria. Conversion of $4H_2 + CO_2$ to $CH_4 + 2H_2O$ ($\Delta G = -130$ kJ) is thermodynamically more favorable than conversion of $4H_2 + 2CO_2$ to $CH_3COOH + 2H_2O$ ($\Delta G = -55$ kJ) [32]. This thermodynamic advantage seems to be the reason that methanogens are able to utilize H_2 at higher concentrations than homoacetogenic bacteria. Therefore, in order to suppress the methanogenic activity, pretreatment of the anaerobic consortia was carried out using BESA. Application of BESA pretreatment will specifically eliminate the methanogenic activity facilitating the growth of other bacteria such as acidogens [11]. Co-enzyme M reductase complex, a chief component for methanogenic bacteria facilitates the function of methanogenic activity. BESA, a structural analog of co-enzyme-M, when added to the bacterial culture dissociates the bound enzyme complex and gets attached in the place of enzyme complex thereby facilitating the selective inhibition of methanogenic activity [33]. Once, methanogenic activity was suppressed, the resulting culture was subsequently subjected to a gas mixture of H_2 and CO_2 (4:1) in the headspace of reactor for the specific enrichment of homoacetogenic bacteria. The enrichment of homoacetogenic bacteria was elucidated by the gas consumption profiles of CO_2 and H_2 with time (Fig. 2). The reduction in CO_2 and H_2 with time can be presumed to be the enrichment of homoacetogenic bacteria that consume the gas mixture for its growth and specific product synthesis. The gas consumption profiles were observed to increase with respect to time, studied at a cycle HRT of 48 h for both pretreated (BESA) and untreated culture (control). Cumulative H_2 and CO_2 consumption of 61% and 52% were observed with BESA treated culture, while it was 58% and 49% with untreated (control) culture. In the BESA-pretreated culture, during 12 h, a maximum reduction in H_2 and CO_2 of 12% and 18% was observed followed by an increment in consumption up to 26% and 35% during 18 h, respectively. During 24 h, an equal percentage (42%) of H_2 and CO_2 consumption was observed followed by a maximum increment in consumption up to 61% and 52% by the end of operation. Whereas, in the case of control, H_2 and CO_2 consumption during 12 h was observed to be 14% and 13% followed by an increment up to 21% and 29% respectively during 18 h. A maximum increment in consumption

of H_2 and CO_2 until 42% and 33% was observed during 24 h followed by an increment up to 58% and 49% respectively during 48 h. Comparatively, slightly higher consumption of H_2 than CO_2 by untreated culture (control) is observed which might be attributed to the activity of methanogens co-existing in the mixed culture that rapidly utilizes H_2 due to its thermodynamically flexibility towards methane formation [32].

3.2. Enhancement of carboxylic acids synthesis (Stage-II)

3.2.1. Optimization of bio-cathode pH

pH plays a major role in governing the overall performance of a system as the redox processes are coupled with the function of pH. Any change or variation in system pH contributes for a marked change in the overall process efficiency. More specifically, the carboxylic acids (C1–C4) or fatty acid synthesis of any anaerobic system is fundamentally regulated by the system pH, because most of the bacteria cannot survive in extremely acidic or alkaline environments [34]. An optimal pH range based on the system conditions viz., biocatalyst and substrate is desirable to achieve enhanced process efficiency. Therefore, in order to enhance the process efficiency in terms of carboxylic acids/VFA production, optimization of pH was carried out in stage-II, after the enrichment of homoacetogenic bacteria. The experiment was designed and carried out at four different set pH viz., 5, 6.5, 8.5 and 10 using 1 N HCl/NaOH to elucidate the optimum pH for maximum VFA production. The study was carried out in single chambered Schott Duran glass bottles at diverse range of pH viz., acidic to alkaline conditions using sodium bicarbonate as carbon source. The optimal pH values for the production of carboxylic acids are mainly in the range of 5.25–11, but the specific ranges are dependent on the type of waste and bacteria used [35].

The pH optimization studies were intended to employ the optimized pH in bio-cathode chamber of BES to induce maximum VFA generation that can be coupled to electrochemical reduction. Simultaneous experiments were carried out in eight individual Schott Duran glass bottles (total/working volume: 250/200 ml) operated at four different set pH using the BESA treated and untreated biocatalysts enriched in SW medium at all the experimental conditions. A total of eight experiments ($4 \times 2 = 8$; four different pH and two different biocatalysts) were carried out to monitor VFA production with the variations in pH. In stage-II, the initial pH depicted significant influence on carboxylic acids synthesis (Fig. 3). Initially, the system was optimized using sodium bicarbonate as carbon source (9.5 g/l based on our previous studies with respect to the glucose concentration used) to monitor carboxylic acids production with respect to the operational pH [36,37]. Variation in pH associated with carboxylic acids generation was monitored at regular time intervals of 0, 24 and 48 h during the stage-II operation studied with a HRT of 48 h.

3.2.2. Variation in pH with time

The employed pH was observed to vary with respect to time. In the acidic range, pH 5 was observed to increase at 24 h (BESA (B): 6.13; Control (C): 7.35) followed by a marginal decrement at 48 h (B: 5.92; C: 6.64) in both BESA and control cultures, respectively. Relatively higher acidogenic conditions prevailed in BESA in comparison to control at pH 5, might be attributed to the functional effect of BESA pretreatment in enriching the acidogens. However, operation at near neutral pH, i.e., 6.5 showed a continuous increment in pH till 48 h in both BESA (24 h: 6.95; 48 h: 7.4) and control (24 h: 6.71; 48 h: 6.75) cultures respectively. This variation in pH is ascribed to the buffering action of bicarbonate and carbonate in the system in maintaining the pH with slight variations. In addition, operation at alkaline (pH 8.5) and highly alkaline (pH 10) ranges did not show significant variation in system pH operated with BESA [pH 8.5: 24 h (8.17); 48 h (8.29); pH 10: 24 h (10.11); 48 h (10.03)] culture. However, operation with control showed a slight decrement in pH at pH 8.5: 24 h (7.27); 48 h (7.3) and at pH 10: 24 h (9.62); 48 h (9.31) respectively. The near constant/marginal variation in pH at alkaline conditions (pH, 8.5-10) is attributed to the buffering action of bicarbonate in the system due to the favorable alkaline microenvironment for bicarbonate dissolution and dissociation.

3.2.3. Variation in carboxylic acids production with time

Carboxylic acids production was observed to vary based on the pH and the utilization of carbon source (Fig. 4). Though the pH in the system did not show significant variation, the carboxylic acids production monitored during 24 h and 48 h depicted significant disparity. Maximum carboxylic acids generation was observed at pH 10 (3500 mg/l; 1200 mg/l) followed by pH 8.5 (3150 mg/l; 1102 mg/l), 6.5 (2300 mg/l; 898 mg/l) and 5 (1980 mg/l; 787 mg/l) in both BESA treated as well as control cultures, respectively during 24 h. Since, the carbon source used in the experiment is CO_2/$NaHCO_3$, solubility in water will be greatly influenced by the system pH. A marginal variation in carboxylic acids generation was observed between pH 8.5 and 10 as the alkaline pH range is suitable for CO_2 solubility into bicarbonate form (bicarbonate dissociation is also favored). The availability of carbon source in the form of bicarbonate is relatively higher than in the form of CO_2during alkaline conditions. This might have contributed for higher carboxylic acids production than the corresponding acidic pH. In addition, sodium bicarbonate enhances the rate of carboxylic acids production in the system maintaining the optimal alkalinity [38]. Besides, alkaline redox conditions also suppress the growth of methanogens. The enriched homoacetogenic bacteria seemed to have grown chemolithotrophically utilizing CO_2 towards maximum VFA generation. The carboxylic acids concentration showed a decrement pattern from 24 h to 48 h in both BESA and control cultures irrespective of the operational pH. During 48 h, a decrement in carboxylic acids concentration is noticed up to 1533 and 645 mg/l at pH 5, 1865 and 756 mg/l at pH 6.5, 2675 and 976 mg/l at pH 8.5 and 2677 and 923 mg/l at pH 10 for BESA and control respectively. The initial production of carboxylic acids during 24 h might be ascribed to the utilization of carbon source (C1– $NaHCO_3$) by the enriched bacterial consortia towards the carboxylic acids synthesis (C1–C4 compounds). However, the decrement in carboxylic acids concentration might be attributed to the consumption of C1–C4 compounds by the bacteria. Since maximum carboxylic acids synthesis was observed at pH 10, the composition was quantitatively analyzed by HPLC to elucidate the C1–C4 fractionation of fatty acids in BESA (B) and control (C) systems. Maximum fraction of C2 organic acids (acetic acid (AA): B: 1600 mg/l; C: 800 mg/l) followed by C3 (propionic acid (PA): B: 44 mg/l; C: 10 mg/l) and C4 (iso-butyric acid (BA): B: 15 mg/l; C: 8 mg/l) organic acids were observed. Alkaline pH (8.5-10) improves the acetic acid accumulation/production and its percentage in the total VFA than the corresponding carboxylic acids [39]. The organic acids formation observed through HPLC not only depicts the activity of the enriched homoacetogens, but also the activity of mixed culture which would have resulted in multi-carboxylic acids synthesis utilizing the carbon source [16]. Also, alkaline condition enhances the hydrolysis through ionization of the charged groups (e.g., carboxylic groups) of the extracellular polymeric substances in the sludge [35]. Consequently, more soluble substrates are available for the production of carboxylic acids under alkaline conditions. The formed intermediates/carboxylic acids viz., acetate, butyrate and propionate serve as good carbon source to the mixed bacterial population to be consumed easily for its cell metabolic activities in both BESA and control cultures. Consumption/accumulation of fatty acids is

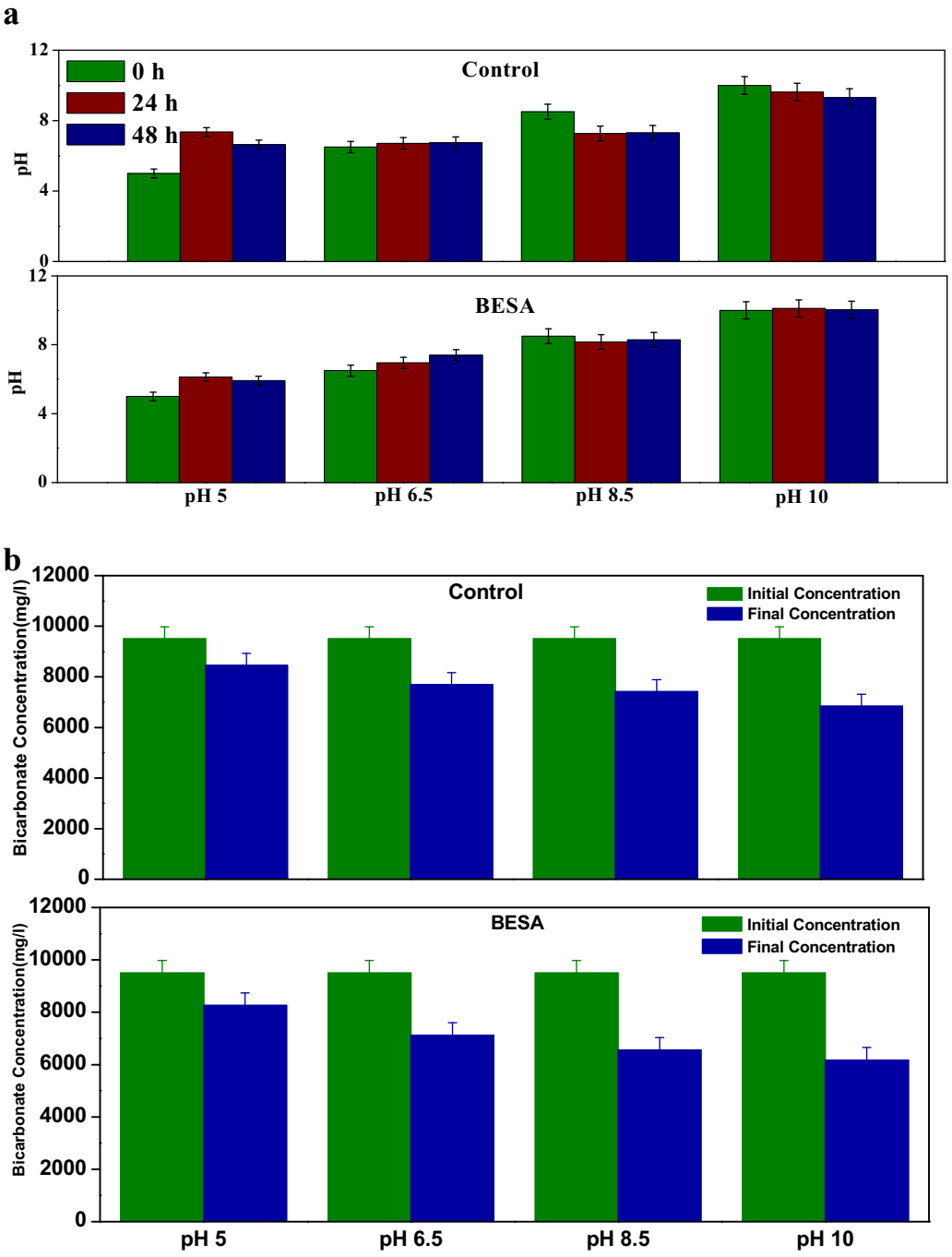

Fig. 3. (a) pH variation in BESA and control cultures with respect to time and (b) reduction in bicarbonate concentration (mg/l) in BESA and control cultures.

generally associated with the methanogenesis either by acetoclastic (pH, 6–8) or hydrogenoclastic (pH, 9–10) archea and syntrophic acetogenesis by syntrophic acetogens to produce H_2, CO_2 and acetate by utilizing the carboxylic acids (as carbon source) [39,40]. The aforementioned bacterial population will be existing in control (methanogens, homoacetogens and syntrophic acetogens) and BESA (homoacetogens and syntrophic acetogens) respectively which would have contributed towards carboxylic acids consumption. Fermentation microenvironment particularly alkaline redox microenvironment favored higher carboxylic acid production compared to corresponding neutral and acidic conditions.

The carbon source (sodium bicarbonate) was observed to decrease gradually at a HRT of 48 h in both BESA and control cultures, respectively. The reduction in sodium bicarbonate concentration depicts the consumption of carbon source chemolithotrophically by the enriched bacteria. By the end of cycle period, maximum bicarbonate reduction to 6175 mg/l (35%) and 6840 mg/l (28%) was observed at pH 10 with BESA and control cultures respectively, followed by pH 8.5 (B: 6555 mg/l (31%); C: 7410 mg/l (22%)), 6.5 (B: 7125 mg/l (25%); C: 7695 mg/l (19%)) and 5 (B: 8265 mg/l (13%); C: 8455 mg/l (11%)). The bicarbonate (C1) reduction is simultaneously associated with carboxylic acids (C1–C4) production. Maximum bicarbonate reduction observed at pH

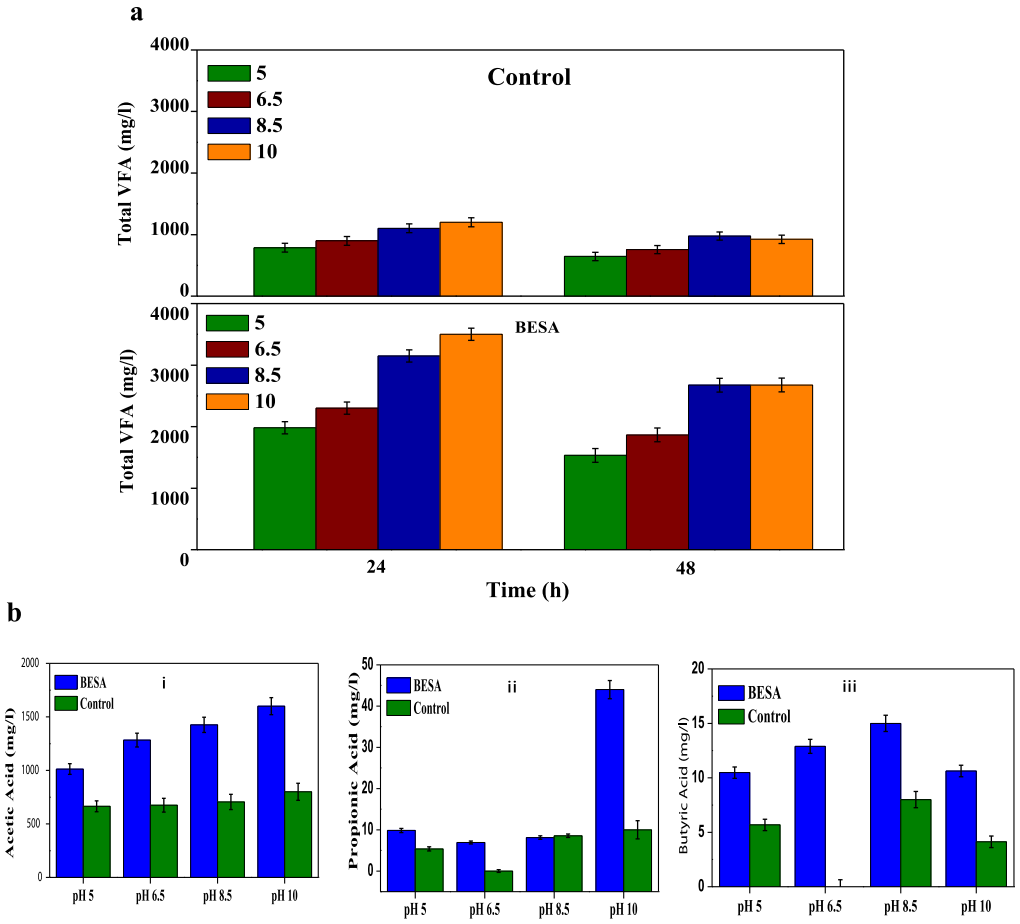

Fig. 4. (a) Total VFA concentration (mg/l) at varied pH in BESA and control cultures and (b) individual fatty acids concentration ((i) acetic acid; (ii): propionic acid and (iii): butyric acid) in BESA and control at varied pH conditions.

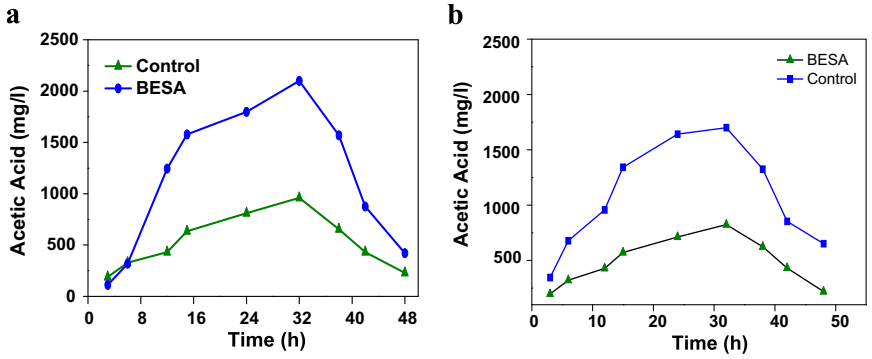

Fig. 5. (a) Acetic acid synthesis via bicarbonate reduction and (b) CO_2 reduction at pH 10.

10 depicts the optimum pH range (alkaline range of pH 8.5–10) for enhanced bicarbonate dissociation (pK_a above 8.2) towards carboxylic acids synthesis. In addition, the presence of bicarbonate in the system will aid in the enhancement of carboxylic acids production [38].

3.3. BES for carboxylic acid production with optimized pH (Stage-III)

Stage-III operation was carried out in two BES systems to evaluate the C2-carboxylic acid (acetic acid) production at the optimized pH condition, i.e., 10 (obtained from stage-II) under applied potential using BESA and control cultures. Fig. 5a and b depicts the acetic acid formation using sodium bicarbonate and CO_2, respectively as carbon source. Initially, bio-anode chamber was inoculated with anaerobic culture without any pretreatment and operated at an organic load of 1.5 g/l glucose in both BES_B and BES_C, respectively. The initial pH of the anodic chamber of both the BES is set at 7 using 1 N HCL/1 N NaOH [11]. Operation at pH 7 enables maximum substrate degradation thereby liberating more number of reducing equivalents (e^- and H^+) to be transferred to cathode chamber for reduction, which can also suffice in minimizing the applied potential. The cathode chamber of both BES_B and BES_C was inoculated with the enriched homoacetogenic culture set at pH 10 from stage-II. Initially, BES was operated using sodium bicarbonate as carbon source in the cathode chamber of BES_B and BES_C, respectively under an applied potential of −0.8 V vs Ag/Agcl (S) [41,42] to evaluate the acetic acid production. Acetic acid (C2) production was observed to start during 4 h and a gradual

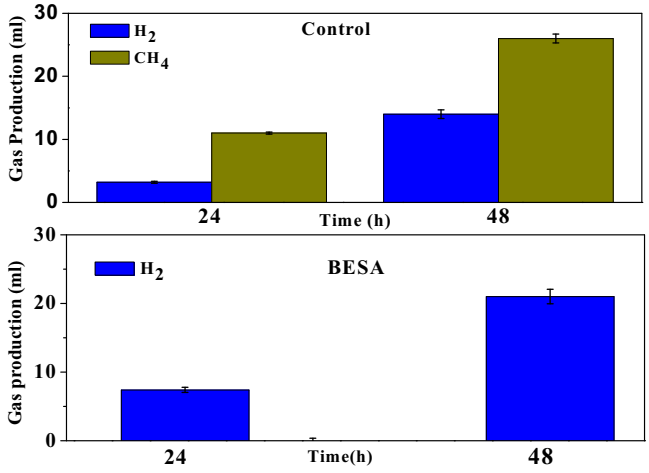

Fig. 6. Gas production along with gas composition analyzed during stage-III in concurrence to the decrement in acetic acid concentration with respect to time in control and BESA cultures.

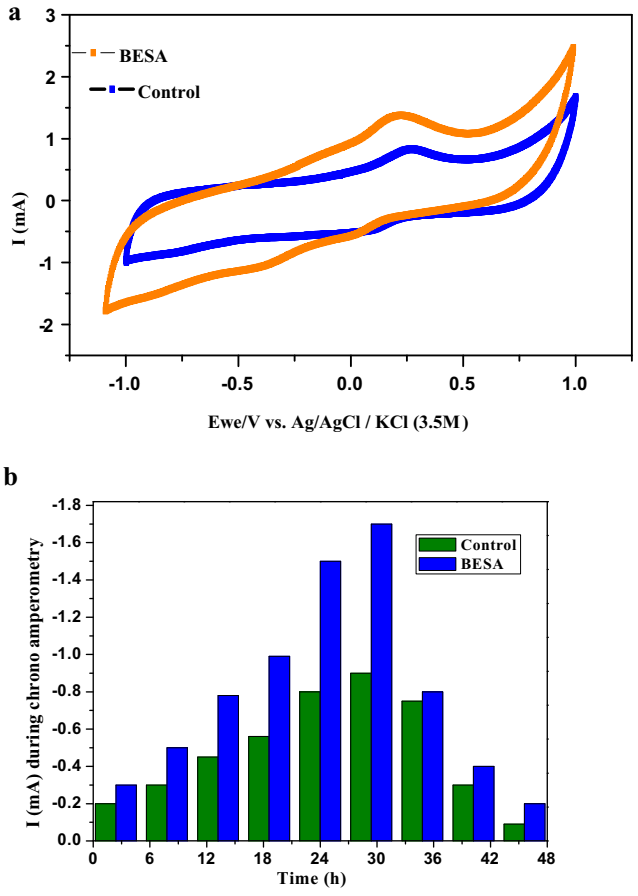

Fig. 7. (a) Cyclic voltammograms depicting the redox catalytic currents and (b) current generation during chronoamperometry analysis with BESA and control BES systems.

increment in acetic acid concentration was noticed up to 24 h and 32 h followed by a decrement till the end of cycle in BES_B and BES_C respectively. Maximum acetic acid concentration of 2100 mg/l (3.55 mmol/d; 32 h) and 960 mg/l (1.62 mmol/d) were observed with BES_B and BES_C respectively, when operated using sodium bicarbonate. However, operation with CO_2 as carbon source depicted a gradual increment in concentration up to 32 h and 36 h followed by a decrement till the end of cycle in both the BES. Maximum acetic acid concentration of 1700 mg/l (2.88 mmol/d) and 823 mg/l (1.39 mmol/d) was observed with BES_B and BES_C respectively, when operated using CO_2. The reducing equivalents generated through the applied potential would have enabled in the synthesis of acetic acid at a higher rate. On the contrary, the decrement in acetic acid concentration can be attributed to the consumption of acetic acid for the growth and metabolic activities of bacteria. The consumption of carboxylic acids (more specifically acetate), by the co-existing bacteria can be attributed to the readily available simpler form of carbon source rather than the bicarbonate/CO_2. In addition, the bacterial population would be diverse in both BESA and control cultures, being the major proportion of homoacetogens followed by the presence of syntrophic acetogens. However, acetotrophic methanogens along with the aforementioned two groups of organisms would co-exist in control as no pretreatment was applied. These bacterial groups would have resulted in carboxylic acids consumption as carbon source. The consumption of VFA also resulted in the production of bio-H_2 (21 ml) in BESA and CH_4 (26 ml), H_2 (14 ml) in control cultures respectively, which depicts the utilization of redox equivalents towards gas production (Fig. 6). The methanogens existing in BES_C (control) would utilize the redox equivalents towards CH_4 formation rather than towards acetate due to its thermodynamic flexibilty, which was elucidated during stage-I as well. In general, the syntrophic acetogens produce H_2, CO_2 and acetate utilizing the substrate which would have favored the bio-H_2 production. Also, the acetotrophic methanogens utilizes VFA in the form of acetate which undergoes a dismutation reaction to produce CH_4 and CO_2 [29,40]. On the whole, the C2-carboxylic acids synthesis was appeared to be more specific and higher during stage-III (electrotrophic phase-electron driven externally) which is ascribed to the combined action of enrichment, pH optimization as well as application of external potential. The consolidated performance of BES in all the three stages is depicted in Table 1.

3.4. Bio-electrocatalytic behavior-electron transfer

Bio-electrochemical behavior of BES_B and BES_C was studied using cyclic voltammetry (CV) and Chronoamperometry (CA), the electrochemical techniques to characterize the electron transfer interactions between microorganisms or microbial metabolites by applying an external potential [37,43]. CV analysis was carried out for both BES_B and BES_C considering bio-cathode containing enriched culture as working electrode and bio-anode containing anaerobic consortia as counter electrode against Ag/AgCl (S) as reference electrode at an applied potential ramp of −1 to +1 V at a

Table 1
Consolidated performance of BES operated with BESA and control cultures.

Biocatalyst	Stage-I	Stage-II at pH 10			Stage-III	
	CO_2 and H_2 consumption (%)	Maximum VFA (mg/l)	Maximum bicarbonate reduction (%)	VFA composition	Maximum acetic acid via bicarbonate/ CO_2 reduction (mg/l)	Gas composition
BESA treated	52 and 61	3500	35	AA > PA > BA	2100/1700	H_2 (21 ml)
CONTROL	49 and 58	1200	28	AA > PA > BA	960/823	H_2 and CH_4 (14, 26 ml)

scan rate of 30 mV/s. Voltammograms (vs. Ag/AgCl (S)) measured in situ through CV visualized marked variation in the redox catalytic currents with the function of nature of biocatalyst (Fig. 7a). Higher redox catalytic currents (reduction currents (RC) and oxidation currents (OC)) were observed with BES_B (OC: 2.2 mA; RC: −2 mA) in comparison to BES_C (OC: 1.3 mA; RC: −1 mA). OC and RC were observed to be nearly simultaneous (slight variation during oxidation and reduction) in both BES_B and BES_C, which is attributed to the effective redox reactions carried out by the biocatalysts present at anode and cathode, respectively. Though the biocatalyst used at anode chamber in BES_B and BES_C is same, the enriched homoacetogenic culture with the aid of BESA pretreatment would have contributed for effective substrate utilization towards the liberation of more number of redox equivalents towards the generation of higher redox catalytic currents in BES_B in comparison to BES_C. During the voltammetric analysis, a quasi reversible peak was identified on voltammetric signature. The identification of peak depicts the involvement of an electron shuttle/redox species (RS) in the electron transfer process. The obtained peak potential (E_{peak}) against Ag/AgCl(S) reference electrode was tailored with respect to standard hydrogen electrode (SHE) to contrast with the biological tower of electrons donors and acceptors chart [44]. The peak was identified at −0.042 V potential, which corresponds to the involvement of cytochromes, the membrane bound proteins that act as electron carrier during the electron transfer process in both BES_B and BES_C. In addition, a slight catalytic wave portion appeared on the reduction side of the voltammogram in BES_B, corresponding to the involvement of NAD/NADH redox couple (-0.32 V) in the electron transfer process aiding towards the reduction reactions. Besides the voltammetry analysis, CA analysis was performed to evaluate the reduction catalytic current generation in response to CO_2 reduction towards the synthesis of carboxylic acids, specifically acetate. A continuous monitoring of current generation during the BES operation was ensured by CA analysis at an applied potential of −0.8 V vs Ag/Agcl (S) by recording the reduction currents for every 300 s. The reduction currents were observed to be -1.7 ± 0.3 mA and -0.9 ± 0.2 mA for BES_B and BES_C respectively during CA analysis (Fig. 7b). In addition, the columbic efficiency (CE) was calculated for both BES_B and BES_C systems during the acetic acid synthesis by utilizing CO_2 [45]. The CE was found to be 24% and 15% for BES_B and BES_C respectively for acetate synthesis by utilizing CO_2. However, the conversion efficiency was observed to be 41% and 32% for BESA and control operations respectively, when compared with the theoretical production values.

4. Conclusion

The present study illustrates the carboxylic acids synthesis through CO_2 sequestration in BES using BESA treated and control cultures by employing a three stage strategy. Stage-I was carried out for the specific enrichment of homoacetogenic culture which was depicted by the gas consumption profiles of H_2 and CO_2 presuming the enrichment of homoacetogenic culture for specific reduction of single carbon atom (CO_2) to two-carbon atoms (CH_3COOH). Stage-II was carried to evaluate the optimum pH for maximum carboxylic acids generation at a diverse range of acidic to alkaline pH. Alkaline pH 10 followed by pH 8.5 was found to be optimum for enhanced VFA/carboxylic acids generation, which was also in support with the CO_2 solubility at the alkaline range. Reduction in VFA concentration was observed after 24 h of operation, which can be attributed to its consumption as substrate by the mixed bacterial population. During stage-III, specific reduction of single carbon unit to two carbon units (CO_2 and bicarbonate to acetate) was observed by employing the optimized pH (10) from stage-II under applied potential of −0.8 V vs Ag/AgCl (S) in BES. Maximum acetic acid concentration of 1.7 g/l and 0.82 g/l was recovered in BES_B and BES_C respectively under optimized conditions in this study. A gradual reduction in acetate concentration was observed after certain time which is attributed to the partial utilization of redox equivalents towards gas production and partially towards the utilization of acetate as substrate. Higher acetate recovery was noticed with BES_B than BES_C, which indicates the significant influence of the pretreated and enriched biocatalyst in BES_B along with the combined effect of pH. The synthesized acetic acid is an industrially important chemical which has wide scope for various applications. In addition, reduction catalytic currents and CE were observed to be higher for BES_B in comparison to BES_C depicting the influence of enriched biocatalyst in reducing the CO_2 concentration significantly towards carboxylic acids (acetate) synthesis.

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