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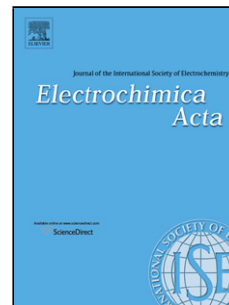
In situ acetate separation in microbial electrosynthesis from CO₂ using ion-exchange resin

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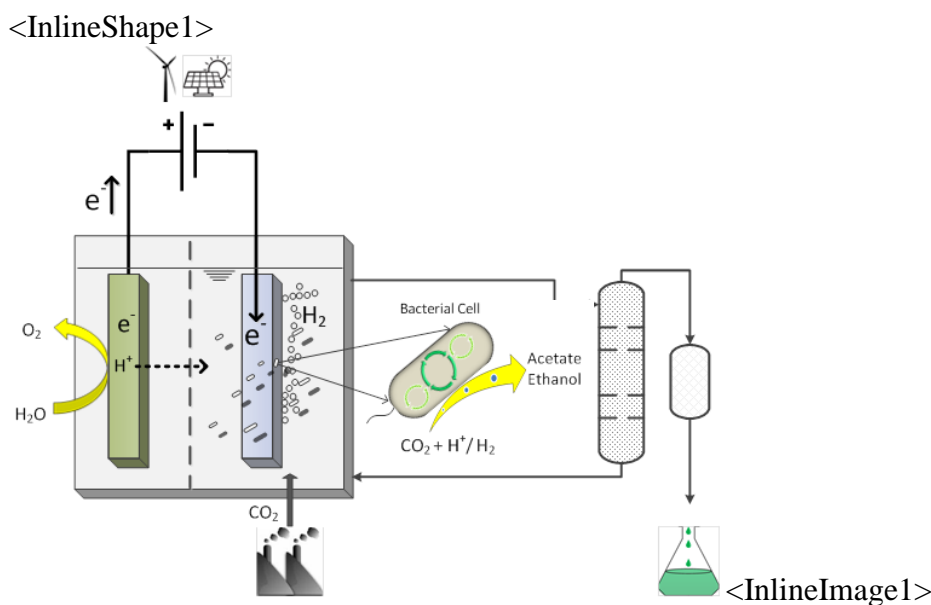
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GRAPHICAL ABSTRACT

ABSTRACT

Bioelectrochemical reduction of carbon dioxide (CO_2) to multi-carbon organic compounds particularly acetate has been achieved in microbial electrosynthesis (MES) using the reducing equivalents produced at the electrically polarized cathode. MES based on CO_2 reduction produced $7-10$ g L^{-1} acetate at the cathode while operating the CO_2 fed reactor in batch mode using the homoacetogenic activity enriched mixed culture. An integration of acetate extraction from the catholyte is interesting, firstly to recover the product and secondly to reduce the probable product inhibition due to the accumulation of fatty acids. We investigated acetate production from CO_2 in

MES in combination with a batch-wise removal of acetate from the broth using a commercially available anion-exchange resin (Amberlite^{TM} FPA53). Acetate sorptions of $10-20$ mg g^{-1} resin were observed from the catholyte broth. The production of acetate from CO_2 continued at 0.5 g L^{-1} d^{-1}after the acetate removal by sorption. Overall, an MES system for the production and separation of acetate from CO_2 was technically feasible through the integration of MES with an anion exchange resin.

Keywords: Ion-exchange resin; ; ; ; , In situ separation, Adsorption, MES, CO_2 reduction,

Acetate

1. INTRODUCTION

World economy still relies greatly on the non-renewable fossil resources for the production of bulk chemicals and liquid fuels. Because these fossil resources are available in a finite stock and the emissions from the combustion of them cause environmental pollution and global warming [1,2], alternatives are desired to secure our long-term need for energy, fuels and chemicals and to reduce our carbon footprint. In recent decades, advances were made in the field of production of value-added compounds, especially via electricity-driven bioprocesses [3–6]. It has been demonstrated that microorganisms are able to use electricity as the source of energy to reduce oxidized molecules, such as CO_2 into suitable building blocks chemicals such as volatile fatty acids (VFAs) [3,7], in a process referred to as microbial electrosynthesis (MES). MES is a promising technology to produce bio-commodities from CO_2 with the input of electricity from renewable sources. In fact, MES can be presented as an excess energy-storing system for an intermittently produced renewable electricity [6]. MES of biochemicals from CO_2 reduction can lower our dependency on fossil fuel and also utilize CO_2 to mitigate the climate change issues [6,8].

Several studies have shown the use of a mixed culture as biocatalyst in MES to form a robust biocathode for CO_2 reduction with high product yield (i.e. electron recovery) and acetate accumulating up to 10 g L^{-1} [9] and 11 g L^{-1} [10] at cathode potential ≤ -0.59 V versus standard hydrogen electrode (V vs SHE). Here acetate complies the sum-up of dissociated form-acetate as well as the undissociated form-acetic acid; and as such, we use the term 'acetate' as the collective name for the sum of both forms hereafter, otherwise the dissociated acetate and undissociated (acetic acid) form of acetate are specifically stated. It has been repeatedly observed that long-term operation of CO_2 reduction in MES (>300 days) using an homoacetogenic activity enriched mixed biocatalyst produced up to 7−10 g L^{-1} of total acetate during fed-batch operation [11]. The concentrations of acetate produced in MES are likely not yet sufficient for economically sound product extraction as compared to the industrial fermentation processes; for instance, 20−200 g L^{-1} of organic acids were produced in industrial fermentations [12]. To achieve these concentrations in MES, a further intensification of the process of CO_2 conversion to acetic acid is needed. Under such conditions, (potential) product inhibition should also be circumvented by *in situ* product removal. It is indeed known that the undissociated form of acetic acid can pass-through the cytoplasmic membrane of the microorganisms and disrupt the proton-motive force [13]. Hence it could hamper further acetate production from CO_2 reduction.

The operating pH of MES remains at near neutral pH, which means that the acetate (charged) form predominates over the acetic acid form. Under such conditions, electrodialysis and ion exchange can be commonly considered for *in situ* product removal following the decision tree given in Van Hecke et al. [14] for the choice of *in-situ* product removal. *In situ* acetate separation using ion-exchange membranes electrolysis has already been integrated in the CO_2 reducing MES [15]. The membrane electrolysis technique is attractive, but more electric power has to be applied to maintain the same cathode potential due to the additional membrane and transport resistance offered by the additional compartment in the MES reactor. Unlike using ion-exchange membranes, sorption processes in ion-exchange resins do not require high power input. Sorption processes associated with ion-exchange resins are the common methods of separation in industry. Sorption technology collectively include absorption, adsorption and ion-exchange mechanisms for the separation processed and has already been used in separating organic acids *in situ* from aqueous fermentation medium with minimum energy input [16,17]. Typically, sorption is more appropriate for the low pH bioprocesses however ion-exchange resins can support removal of charged product

by ion-exchange process at an operating pH above the dissociation constant of the organic acids [18]. Ion-exchange resins have charged groups which adsorbs counter ions from a solution based on ion-exchange phenomenon. The operating pH of MES are at near neutral pH, which means that the dissociated acetate form predominate over acetic acid form and can have a charge interaction with the charged groups in the resin. A weakly basic ion exchange resin could be used to extract acetic and related acids [16,19]. In addition to the charge interactions in ion-exchange resins, surface adsorption of non-ionic portion of organic acid to the internal surface of the resin also occurs simultaneously as another mechanism of organic acids adsorption in ion-exchange resins [18].

The objective of this study is to check the feasibility of the application of an ion-exchange resin to recover total acetate produced from the CO_2 reduction in MES process. Separation techniques using ion-exchange resins are often applied in industrial water treatment, i.e. heavy metal removal [20,21], nutrient removal [22] or drinking-water softener [23]. In the present work, we investigated acetate production from CO_2 in MES, in combination with a batch-wise extraction, by using Amberlite[TM] FPA 53 anion-exchange resin. An integration of acetate extraction in MES is intended to achieve product recovery and enrichment, as well as to reduce the product inhibition effects if exist at higher concentration levels.

2. EXPERIMENTAL

2.1. Microbial electrosynthesis reactor set-up & operation

CO_2 reduction to acetate experiments were performed in a double chamber H-type reactor as described earlier [24]. The MES reactor consists of two compartments, each 250 ml, separated by a cation exchange membrane. A dimensionally stable anode (DSA), i.e. a ruthenium/iridium oxide coated titanium mesh (Magneto Anodes, Netherlands), serves as anode. The cathode was a graphite stick with two graphite felts (Mast Carbon, UK) wrapped around it with 30 cm^2 of exposed surface area. An Ag/AgCl in 3 M KCl reference electrode (Radiometer Analytical) was positioned close to the cathode. This MES cell was operated with the same anolyte and catholyte buffer solution as described earlier [24]. The reactor was closed with airtight stoppers, and continuously stirred maintaining a temperature of 35-37 °C with an electric heater. The headspace was intermittently flushed with N_2:CO_2 (20:80) to render anaerobic condition as well as to provide CO_2 for reduction. The cathode potential was controlled by applying chronoamperometry using a potentiostat (Biologic VMP3).

The complete cathode (biocathode) for CO_2 reduction in this MES experiment was taken from the previously operated MES reactor described in [11]. The biocathode was transferred in this new MES reactor in anaerobic condition. The biocathode had already been developed over a long-term (367 days) operation [11] with a selectively enriched mixed culture inoculum from biological sludge which was additionally supplemented with an acetogenic species, *Clostridium ljungdahlii*. The biocathode had already produced 7−10 g L^{-1} of acetate from CO_2 reduction at -1 V vs. Ag/AgCl in previous experiment described earlier [11]. A new batch was started with 200 mL of fresh new buffer medium. The days counting of this MES operation continued from day 367 as the biocathode was the same from the previous MES. The MES reactor was operated in fed-batch mode with intermittent N_2:CO_2 (20:80) bubbling. Samples were taken at least twice a week for the analyses of VFAs (C1-C4) plus ethanol in Agilent 1200 series HPLC with a Agilent Hi-Plex H column and a Agilent 1260 infinity refractive index detector (Agilent Technologies) as described previously [25]. Each time the samples were taken, the reactor was sparged with N_2:CO_2 (20:80)

for at least 20−30 minutes and 2–4 mL of mineral medium was added to replace the sample volume. Whenever the pH of catholyte rose, the flushing of N_2:CO_2 (20:80) gas in the cathode compartment also lowered the catholyte pH. The pH of catholyte was maintained between 7 and 8 by CO_2 gas mixture sparging and addition 1 M $NaHCO_3$ solution.

2.2. Anion exchange resins and Pre-treatments

A commercially available Amberlite™ FPA53 anion exchange resin was used to investigate the removal of acetate from the catholyte broth. This resin was selected (from around 10 types of resins) based on pre-screening tests of adsorption capacity for carboxylic acids. The selected resin Amberlite™ FPA53 (Dow Chemicals) has a cross-linked acrylic gel structure with tertiary amine functional groups and was obtained in its free base form of weight 700 g L^{-1}. According to Dow product data sheet [26], it is a weak base resin with bead size 0.5−0.75 mm and a total exchange capacity of $\geq$ 1.6 equivalents (eq) L^{-1}. As a pre-treatment, the ion-exchange resins were washed several times with demineralized water. The washed resins were kept soaked in demineralized water for at least 12 h, then the suspension with the resins was filtered using a Whatman$^{\circledR}$ 589/1 blackband filter to collect the resins prior to use.

2.3. *Ex situ* acetate sorption tests by anion-exchange resin

2.3.1. Effect of pH on acetate removal
For the investigation of acetate removal from the spent MES catholyte at different pH values, the MES reactor's catholyte from previous batch operation in Bajracharya et al. [11] was used. The catholyte (i.e. medium broth) contained VFAs mainly acetate (upto ~ 10 g L^{-1} [11]). A number of 15 mL centrifuge tubes each containing a mixture of 8 mL of filtered catholyte sample (via a 0.45 μm syringe filter) and 0.8 g of pretreated anion exchange resins (10% w/v) were taken. The pH values of the media in different tubes were adjusted in duplicates to 2, 3, 4, 5, 7 and 8 by using 3 M HCl. The tubes were shaken using a tube rotator for 24 h. Samples of catholyte were taken at the start and after 24 h of shaking and pH was also measured at the same time. The samples were analyzed for acetate, butyrate and ethanol in Agilent 1200 series HPLC (Agilent Technologies) as described earlier [25].

2.3.2. Concentration effect on acetate removal
Acetate uptake by anion-exchange resins was examined by suspending 10% w/v resin to a series of acetate solutions with increasing concentrations. The acetate solutions in duplicates contained 4, 6, 8, 10, 15 and 20 g L^{-1} of acetic acid for the concentration effect test. To ensure mixing and contact over a large surface area of the resin, the tubes were rotated head-over-head for 24 h. The pH measurement and HPLC analysis for acetate, butyrate and ethanol was performed before and after the experiment.

2.4. *In situ* acetate removal from the running MES catholyte applying anion exchange resins column

A glass column (diameter 3 cm; height 12 cm) was filled with 35 g of Amberlite™ FPA53 resin. The MES catholyte was recirculated through the column using a Watson-Marlow 323 peristaltic pump (Watson-Marlow Fluid Technology Group) at the rate of 50 mL min^{-1}. A spacer (100 μm mesh) was placed in the column to prevent flow of resin into the MES reactor. The schematic representation of the set-up used for the application of resins to the operating MES reactor are shown in **Fig. 1** (Photographs are as shown in Fig. SM-1 in supplementary material).

The reactor medium was recirculated through the resin column for 2 days to remove the acetate. The sorbed acetate in the resin column was eluted by recirculating 50 ml of regenerant (eluent) (1 M NaOH) for two days. This regeneration cycle was repeated at least two times to remove any remaining acetate from the column. Then the column was washed with demineralized water to remove eluent from the column so that column was ready for the next reuse. A sample for HPLC analysis was taken each day to check any uptake or desorption of acetic acid from the reactor medium and resin respectively.

2.5. Calculations

2.5.1. Acetate production rate in CO_2 reduction
In the batch mode operation of MES, acetate production rate in g L^{-1} d^{-1} was calculated according to the following equation.

$$P_{acetate} = \frac{\left(C_{acetate,t} - C_{acetate,t_0}\right)}{t - t_0} \tag{1}$$

Here, t_0 and t refer to two subsequent samples, P is the production rate in g L^{-1} d^{-1} and $C_{acetate}$ is concentration of acetate in catholyte (g L^{-1}). Here, it should be noted that Eq. (1) is not valid for the production rate calculation during the integration of resin column for the acetate removal.

The number of moles of acetate produced at any time t was calculated according to following equation.

$$N_{acetate,t} = \frac{V_{cat} \times \left(C_{acetate,t} - C_{acetate,t_0}\right)}{M_{acetate}} \tag{2}$$

Here, t_0 and t refer to two subsequent samples, N is the number of moles acetate produced, V_{cat} is total volume of catholyte, $C_{acetate}$ is concentration of acetate (g L^{-1}) and $M_{acetate}$ refers to molar conversion of acetate (g mol^{-1}).

2.5.2. Coulombic efficiency (CE) of production
Coulombic efficiency (CE) was calculated by using the Eq. (3)

$$CE \text{ in } \% = \frac{n_{e,acetate} \times F \times N_{acetate,t}}{\int_{t0}^{t} I \, dt} \times 100 \% \tag{3}$$

Here $N_{acetate,t}$ is the moles of acetate produced between time t_0 and t, $n_{e,acetate}$ represents the molar electron equivalent conversion factor (8 electron equivalent per mole for acetate), F is Faraday constant (96,485 C mol^{-1} of electron equivalent) and I is electric current (A).

Acetate equivalent from electric current input in the MES is calculated as

$$\text{Acetate equivalents from current in moles} = \frac{\int_{t0}^{t} I \, dt}{n_{e,acetate} \times F} \tag{4}$$

2.5.3. Acetate sorption capacity
Specific acetate sorption by the resin, expressed as uptake per gram of resin, was calculated using the following equation:

$$\text{Specific acetate sorption (mg } g^{-1}) = \frac{V \times (C_{acetate,start} - C_{acetate,end})}{m_{resin}} \tag{5}$$

Here $C_{acetate}$ is concentration of acetate (mg L^{-1}) in the catholyte sample at the start and end of experiment, V is volume of catholyte liquid (L) and m_{resin} is amount of resin used (g).

2.5.4. Acetate recovery from the resin

The acetate sorbed on the anion exchange resin is extracted from the column by washing the resin with a basic eluent solution. The amount of acetate extracted from the column is calculated by multiplying the acetate concentration in the regenerant analyzed in the HPLC analysis with the volume of regenerant solution. Next, the efficiency of the removal was calculated by dividing the amount of eluted acetate during regeneration with the acetate sorbed by the resin. These calculations are according to the following equation.

$$\eta_{acetate\ recovery}\ (\%) = \frac{m_{acetate\ in\ regenerant(s)}}{m_{acetate\ in\ resin}} \times 100 \tag{6}$$

$$m_{acetate\ in\ regenerant(s)} = V_{regenerant} \times \left(C_{acetate\ in\ regenerant,t} - C_{acetate\ in\ regenerant,t_0} \right) \tag{7}$$

$$m_{acetate\ in\ resin} = m_{resin} \times \text{specific acetate sorption} \tag{8}$$

Here η denotes recovery percentage, initial acetate concentration new regeneration $C_{acetate\ in\ regenerant,\ t0}$ is null, $m_{acetate\ in\ regenerant(s)}$ is the amount of acetate present in the regenerant (mg) and $m_{acetate\ in\ resin}$ is the amount of acetate adsorbed in the resin (mg).

3. RESULTS AND DISCUSSION

3.1 Amberlite^{TM} FPA 53 resin sorbs acetate from the spent MES catholyte

Accumulation of acetate to high concentrations in reactor broths is needed to be considered for separation. So far acetate concentrations in MES from CO_2 reached typical concentrations of 10-11 g L^{-1} [9–11]. Acetate uptake in FPA 53 anion-exchange resin from the series of acetic acid solutions of various concentrations are as depicted in **Fig. 2A**. The sorption of acetate in FPA 53 resin increased when the concentration of acetic acid solutions increased from 4 to 20 g L^{-1}. High acetate sorption was observed with FPA 53 from the concentrated acetic acid solutions. According to the kinetic models of sorption, the higher concentration/availability of ions gives higher sorption [27]. Thus, the sorption of acetate was high from the higher concentrations solution. For 20 g L^{-1} acetic acid solution, the achieved sorption in the FPA 53 resin was 100 mg g^{-1} **[Fig. 2(A)]**.

As per the specification given by the supplier for the FPA 53 resin [26], the total exchange capacity is $\geq$ 1.6 eq L^{-1} which corresponds to the acetate exchange capacity of $\geq$ 137 mg g^{-1} tentatively. Total exchange capacity of the resin refers to the sorption capacity based on the exchange sites in resin including voids. In the experiments, the sorption obtained for the acetic acid solutions comes closer to the expected exchange capacity of the resin regardless to the mechanism of sorption.

According to adsorption isotherms, there would be an optimum anion concentration in the medium resulting the highest sorption in the resin depending on the ion-exchange capacity of the resin [19]. However in the current experimental setting, the optimum acetic acid concentration was not encountered indicating that the maximum sorption might be beyond 20 g L^{-1}. Since the concentration of acetate in the catholytes of MES reactors reached only up to 10−15 g L^{-1}, the optimum acetate concentration corresponding to the highest acetate sorption by FPA 53 we did not further investigate this.

Another test with the filtered spent reactor medium was performed to investigate effect of pH on acetate sorption. pH of solution affects the sorption, because it determines the fraction of charge species of organic acids and also the charge density of exchangeable ions adsorbed on the surface of the adsorbent [28]. The pH of solution governs the distribution of the fraction of undissociated

acetic acid or dissociated acetate. At a pH lower than the pKa (4.75), the majority of acetate remains as undissociated acetic acid. Fig. 1B shows the acetate sorption to FPA 53 from the spent MES catholyte at initial pH ranging from 2 to 8. Fig. 1B shows that at a lower pH, more acetic acid was sorbed in the resin, reaching a maximum of 22 mg.g^{-1} at pH 2 whereas only 4 mg g^{-1} sorption in the resin at pH 7. The pH of the solution rose after the sorption of acetate/acetic acid; the pH 2 solution reached pH 7.5 and pH 7 solution reached pH 9.5. Because the anion-exchange resins have previously adsorbed alkaline groups (like OH$^-$) which are first neutralized with acetic acid and later exchanged with the dissociated acetate during the sorption, it makes the medium less acidic after the acetate exchange. An increase in pH lowers the availability of protons and therefore decreases the possibility of ion pairing between the protonated amine group and the carboxylate [12]. Base neutralizing effect of acetic acid provides more free sites for the adsorption of acetate on the resin which increases the sorption capacity. During the sorption, the resin exchanges previously adsorbed OH$^-$ ions for acetate and the OH$^-$ ions released in the solution, turned it alkaline. Indeed it was shown in the experiments that pH increased during sorption at all the initial pH values. The highest pH rise from pH 2 to pH 7.5 was observed with more acidic mixture **[Fig. 2(B)]**.The pH rise after acetate sorption was high when the sorptions were higher. This suggests that low initial pH increased the adsorption of acetic acid and thus the sorption was higher for acetic acid than that for dissociated acetate.

It is apparent from **Fig. 2** that the acetate sorption from the spent MES catholyte (~6 g L^{-1} acetate) was lower than the sorptions from the pure acetic acid solutions of same concentration. Acetate sorption from the spent MES catholyte at initial pH 3 was 20 mg g^{-1} whereas for acetic acid solution of 6 g L^{-1}, acetate sorption was 61 mg g^{-1}. Lower pH has positive influence on the sorption by the resin. The difference was most probably due to the competition of acetate with other volatile fatty acids and other interfering species in the MES catholyte (e.g. propionate, butyrate and other molecules) which were also sorbed on the resin. When pH is low, more acetate was sorbed on the resin. As mentioned before, at a pH lower than the pKa of acetate (4.75), the majority of acetate should be undissociated acetic acid and doesn't have charge with which the functional group of resin can interact. In this situation, the adsorption of non-ionic acetate on to the internal surface of the resin would be the dominant mechanism of sorption. This suggests that the actual functional group on the ion-exchange is not needed for the removal of acetic acid from watery fermentation broths. According to Magalhaes et al. [19] and Shi et al. [29], the uptake of carboxylic acids depends on the pH of the matrix wherein the resins reside. The uptake of acetate by the resin was higher at lower pH which is the indication of absorption on the resin-matrix in addition to the ion-exchange on the functional group. Yang et al. [30] also described that tertiary amine groups have a tendency for acetic acid uptake because an acetic acid molecule can dimerize with the acetate molecule attached to the functional group. Accordingly, acetate molecules that were present in the catholyte solution bind to the resin and dimerize with another acetic acid molecule via hydrogen bonds. This phenomenon can also contribute to a higher uptake at low pH. However, when the solution is acidic, the acetate is predominantly in its undissociated form and as such no acetate available to dimerize with another acetic acid molecule.

Furthermore, at higher pH, ion-exclusion mechanisms can also take place and acetate anions are repulsed (excluded) due to high surface charge density (crowding) of previously occupying alkaline anions (mainly by OH$^-$), thereby the sorption of acetate remained low at higher pH. On the contrary, at acidic condition, the non-ionic acetic acid molecules can easily enter the resin network and the surface charge density of resin decrease due to the neutralization of OH$^-$ groups.

Further research on the discussed mechanisms can reveal which actual sorption mechanism is most important and therefore to be used to further optimize the extraction process.

So far, the acetate sorption in the FPA 53 resin from the spent catholyte containing ~6 g L^{-1} acetate was between 4-8 mg g^{-1} at near neutral pH which was fairly low. However, the acetate sorption in the resin was shown as being feasible. For the application within MES, anion-exchange resin for acetate separation could be attractive while MES are operated at slightly acidic pH. Moreover, if MES biocathodes can be developed at more acidic pH [31], the efficiency of the used resins could be further enhanced.

3.2. Microbial electrosynthesis of acetate from CO_2 reduction accumulated high acetate

The MES operation reported here was a continuation of the next batch operation from the biocathode of "MES 2" of previous study reported in Bajracharya et al. [11]. Microbial electrosynthesis was carried out using chronoamperometry with the constantly polarized biocathode at -1 V vs Ag/AgCl. Bioelectrochemical reduction of CO_2 at the cathodes of the MES resulted to the production of mainly acetate and a minor amount of butyrate and ethanol (< 0.2 g L^{-1}). Intermittent bubbling of 20:80 mixture of N_2:CO_2 in the reactor resulted to acetate accumulation which reached up to 10 g L^{-1} after 100 days of operation. The average production rate of 100 mg L^{-1} d^{-1} was obtained in the batch operation which was similar to acetate production from CO_2 as in the previous MES reactor as mentioned earlier in Bajracharya et al. [11] with the same biocathode. The products profiles in MES are shown in **Fig. 3(A)**. The current demand of the polarized biocathode polarized is shown in **Fig. 3(B)**. The maximum acetate production rate calculated from the acetate accumulation in this MES was 248.6 mg L^{-1} d^{-1} between day 436 and 439. Acetate production rate was higher after day 422 than at the initial days of operation and after reaching ~10 g L^{-1}, the acetate production almost stopped and then the concentration declined due to other possible conversions of acetate. The increase in production after day 422 was associated with the manual addition of $NaHCO_3$ for controlling the catholyte pH between 7-8. $NaHCO_3$ was an additional CO_2 source in the MES reactor. At the initial phase of MES operation before day 422, pH was not manually controlled and the pH decreased to 5-6 due to CO_2 sparging (See pH profile in supplementary information Fig. SM-2).

The current densities in the MES experiment were recorded between 5 to 20 A m^{-2} [**Fig. 3(B)**]. High current densities imply higher current demand by the cathodic reactions. High current densities of 10-20 A m^{-2} were observed up to day 417 whereas after day 427 the current densities remained 5-10 A m^{-2}. The change in current densities were due to manual pH controlling; before day 417 the pH was maintained below 6 whereas after day 427 the pH was manually raised to 7-8 by addition $NaHCO_3$ solution. Multiple electrochemical and bioelectrochemical phenomena were simultaneously occurring in the reactor which resulted in increase/decrease in the current density. Hydrogen evolution was clearly visible from the MES biocathode polarized at -1 V vs Ag/AgCl. Indeed, the catholyte mixing irregularities and hydrogen evolution were two of the prominent phenomena that interfer the electrical current. Due to the hydrogen evolution at cathode, the protons were constantly consumed. Noticeably, the pH of the solution was also fluctuating due to the intermittent CO_2 sparging which changes the CO_2 availability for the reduction reaction. In addition, the pH of the catholyte changes due to the irregularities in ion transfer through the proton exchange membrane between the anode and cathode chamber.

The current density profile of the MES operation also showed several spikes which were due to the electrochemical disturbances created when the CO_2 gas was sparged. The operation of MES reactor was

done with the intermittent CO_2 sparging instead of continuous sparging. The availability of CO_2 on gas sparging and the lowering of pH associated with the CO_2 dissolution accompanied the spikes in electric current. Additionally, a number of spikes in current densities were also due to the operation of cyclic voltammetry (CV) on the cathode as programmed periodically in potentiostat (for example on days 377, 387, 397, 407, 496 and 508 etc.). A representative CVs performed on day 377, 407 and 508 are provided in Fig. SM-3 and the most prominent feature in the CVs was hydrogen evolution from the biocathode at -1 V vs Ag/AgCl.

In **Fig. 3(B)**, acetate equivalents profile of the operation of MES is shown as a potentially obtainable acetate concentration from the electric current demand. The acetate equivalents from current demand attained 60-70 g L^{-1} on day 477 whereas the acetate accumulated in catholyte on day 477 was 10 g L^{-1}. This means almost 6-7 times higher acetate equivalents were available from the recorded current densities. Thus, the electric power input in this MES reactor can support even higher acetate production than currently achieved. Highest coulombic efficiency (CE) of acetate production in the MES was calculated at 40-50% for the batch operation. CE of 40-50% means almost the half of the electron equivalents provided by the electric current was assimilated as acetate via CO_2 reduction and other half was lost or assimilated into unmeasured products. Hydrogen evolution at the cathode was the main cathode reaction other than CO_2 reduction though the amount of hydrogen produced was not measured (CVs in Fig. SM-3). But due to the escaping of hydrogen gas from the cathode chamber, CE of the MES was calculated only up to 50%. Therefore improvement in electrode design and operations is required which could result up to a 100% electron recovery as in Jourdin et al. [10].

After reaching 10 g L^{-1}, the acetate concentration in the catholyte did not increase which could be due to the possible conversion to other compounds such as ethanol, butyrate or other undetected products. In this regards, it can be speculated that the high accumulation of acetate in the catholyte might become inhibitory to the biocathode for further CO_2 reduction to acetate. Thus, the separation of acetate from the MES catholyte was tested to regain the acetate production. In any case, the accumulated acetate to 10 g L^{-1} in the MES catholyte can still favor the acetate recovery since the acetate sorption by the resin from the spent medium of lower concentration was shown feasible in the *ex situ* acetate removal tests.

3.3 *In situ* acetate separation from MES catholyte was feasible

A glass column filled with FPA 53 was integrated in the experimental setting when the amount of acetate in the reactor started to fall after reaching ~10 g L^{-1}. The acetate profile in the MES reactor showing the days of ion-exchange resin column integration for acetate separation and the current densities are illustrated in **Fig. 3**. After the first extraction run with FPA 53 resin column, the acetate concentration in the MES catholyte decreased from 8.2 g L^{-1} to 6 g L^{-1} and remained stable for about two weeks after which it started to rise again reaching 6.8 g L^{-1}. The production of acetate resumed after the application of ion exchange resin. During the acetate removal period, 80% CO_2 containing gas mixture was continuously bubbled instead of intermittent supply in the MES reactor so as to neutralize the pH rise induced due to ion-exchange in FPA 53 resin. Possible pH rise due to the ion-exchange process as observed in *ex situ* acetate sorption test might be harmful to the MES biocathode. In **Fig. 4**, the variation of catholyte pH after day 470 is shown. The catholyte pH dropped instead of usual increase during the integration of resin column due to the acidifying effect of continuous bubbling of 80% CO_2 containing gas mixture.

The second test of acetate sorption from the MES catholyte was performed with the same column after the resin regeneration by washing with 1 M NaOH. However, the treatment during the second run was slightly different from the first run. For the second run, the resin in the column was pre-treated with 0.5 M

HCl solution to decrease the alkalinity of the resin gained due to washing by NaOH solution so that it could lower the pH rise effect in following acetate sorption. An overview of the results of the acetate removal by FPA 53 in MES reactor is shown in Table 1. After the integration of ion-exchange column for the acetate removal in the MES, the production of acetate from CO_2 reduction re-established after a short interval; specifically for the first acetate removal, the production of acetate regained after 16 days and in case of second test, the production regained after 2 days. However, after the second acetate removal test, 155 mL of MES catholyte was replaced with fresh catholyte. Therefore, the concentration of acetate was much lower after day 508 in the **Fig. 3(A)**. But production of acetate restarted after day 509. Overall, the bioproduction process from bioelectrochemical CO_2 reduction was not stopped by the temporary integration of *in situ* acetate removal resin column.

The *in situ* acetate sorption in the first run was higher than the sorption from the spent MES catholyte in the *ex situ* acetate separation tests at same pH (shown in Fig. 1B). At a pH of around 7, the acetate sorption according to the *ex situ* separation tests was expected to be around 4–5 mg g^{-1} (see Fig. 1(B)) instead of which 18 mg g^{-1} was obtained in the *in situ* separation process. When compared with the values obtained from the sole acetic acid solutions, this acetate sorption was 22−23% of the observed sorption from the acetic acid solution of same concentration as in the *ex situ* test (see Fig. 1(A)).

In the second *in situ* acetate separation test (after the regeneration of FPA 53 resins, as would be discussed later), a lower acetate sorption (only 4 mg g^{-1}) on the resin was observed than in the first test but this acetate sorption was still more or less similar with the acetate sorbed from the spent MES catholyte in the *ex situ* test. The lower acetate sorption in the second *in situ* separation could be due to the low acetate concentration of the catholyte. High acetate concentration and low pH of the catholyte were the main controlling factor for the higher sorption of acetate in the resin. To enhance the acetate concentration in the catholyte the CO_2 reduction process should be improved. In another case, higher concentration of acetate in MES could be achieved with a concentrating step such as *in situ* electrodialysis as performed by Gildemyn et al. [15], after which the acetate could be removed with the resin more effectively.

In general, sorption techniques using commercial weak anion exchanger are more effective for the separation of undissociated carboxylic acids [12]. Lowering pH of the solution, the fraction of undissociated carboxylic acids increases which then get sorbed on the anion-resin. Evidently, the acetate sorption in the *ex situ* test was 80 mg g^{-1} for 8−10 g L^{-1} acetic acid solution (Figure 1A), which was stimulated by the lower pH (<3). In case of MES with *in situ* acetate removal, in order to increase the acetate sorption capacity of the resins, pH control at much lower values could be done provided that the MES biocathodes can withstand more acidic pH (with acidophilic and electrophilic microorganisms). At very low pH, the sorption capacities of resin for acetic acid can reach as high as 27.6 g g^{-1} from 24.6 g L^{-1} initial acetic acid solution using Amberlite IRA-67 anion exchange resin [27] and 250-260 mg g^{-1} from the acetic acid solutions of 4.5-5 g L^{-1} equilibrium concentration using Purolite A133S anion exchange resin [32]. In this study, the highest sorption capacity Amberlite FPA 53 anion resin was obtained ~100 mg g^{-1} from 20 g L^{-1} initial acetic acid solution during the *ex situ test*. The sorption capacity for Amberlite FPA53 appeared lower than the capacities of resins in the literature but they are not comparable directly since the operational methods and calculations were different in each case. Nevertheless, high sorption capacity resins are required in case of acetate sorption from MES catholyte so as to make the *in situ* acetate separation attractive.

Moreover, during the *in situ* acetate sorption on resin, the microorganisms in the catholyte could attach on the resin, which could be deactivated and denature by the action of alkaline eluent. This live and denatured microbial biomass could also contribute to the resin clogging, thereby decreasing the acetate

sorption, especially in the second run. In this situation, the clogging should be avoided by settling the biomass or filtering the catholyte before passing in the resin column.

3.4 Acetate recovery up to ~70% from the resin by washing with water and NaOH

After the sorption of acetate in the resin, the acetate was desorbed from the resin by recirculating 50 ml of 1 M NaOH each time through the column in two consecutive washing step. For the first test, the first washing was performed with 30 ml demineralized water for two hours. However, significant amounts of acetate (almost 23% of sorbed acetate) were washed out during this washing. The amounts of acetate recovered from the ion-exchange column in a number of resin regeneration steps are given in the Table 2. Washing with water was not performed in the second test of *in situ* acetate extraction. The concentration of acetate recovered in the eluents was up to 5.0 g L^{-1} which is not yet significant to be applied in real application. In the literature, recovery of carboxylates at higher concentration up to 100 gL^{-1}were reported using alcohols like ethanol and n-propanol as eluent for the recovery of acetic acid, propionic acids and butyric acids from purelite A133S anion exchange resin in number of washing steps and the recovery was reached 90-99% [32].

Desorption of acetate in the first run by NaOH was more effective than in the second run. Approximately 72% of acetate sorbed in the ion-exchange column was recovered during the two cycles of elution. For the second test of acetate sorption and recovery, the first elution cycle with NaOH was less effective compared to the previous test, as the amount of acetate desorbed was only 21 mg. The second elution cycle gave a better acetate desorption of ca. 60%. Overall, the acetate recovery percentage from the resin column in both tests remained ~70%. Moreover, the selectivity of recovered acetate was not 100%, since other trace amounts of VFAs (butyrate) and ethanol were also sorbed on the resin. Beside this, the eluate could comprise a part of microorganisms and other elements from the electrolyte. Due to the high pH of the eluent, microorganisms attached on the column are likely to be deactivated and lysed. Hence, a proper technology is desirable to remove these impurities for high end application of the separated acetate.

4. CONCLUSIONS

Amberlite^{TM} FPA 53 resin can separate acetate and other VFAs from the reactor medium *in situ* without hindering the bioelectrochemical CO_2 reduction and other biochemical production. Both *ex situ* as well as *in situ* resin application were shown to be technically feasible. Acetate sorption of 10−20 mg g^{-1} resin was observed for the MES catholyte. The production of acetate in MES retained after the removal of acetate. Acetate desorption from the FPA 53 resin resulted in ~ 70% recovery in two washing and up to a final concentration of 5.0 g L^{-1} of acetate in the eluent. In this study, *in situ* separation of acetate from the MES catholyte was performed which can be beneficial for the products like acetate that remain unstable and undergo further conversions when accumulate to some extent in the catholyte. However, the effects of product removal on CO_2 reduction and bioproduction were not observed. Higher sorption capacity of resin for acetate can be achieved by increasing the concentration of the acetate in the catholyte and by lowering the pH. Long-term operation of *in situ* acetate removal may lead to the lowering of sorption capacity of resin due to the fouling and cell lysis during elution of the column with NaOH. To prevent microbial fouling, a suitable filter or membrane can be included in the system. A MES system for the production and separation of acetate from CO_2 was technically feasible through the integration of MES

with anion exchange resin sorption. However, the feasibility in terms of economics and applicability in scaled-up MES is still to study.

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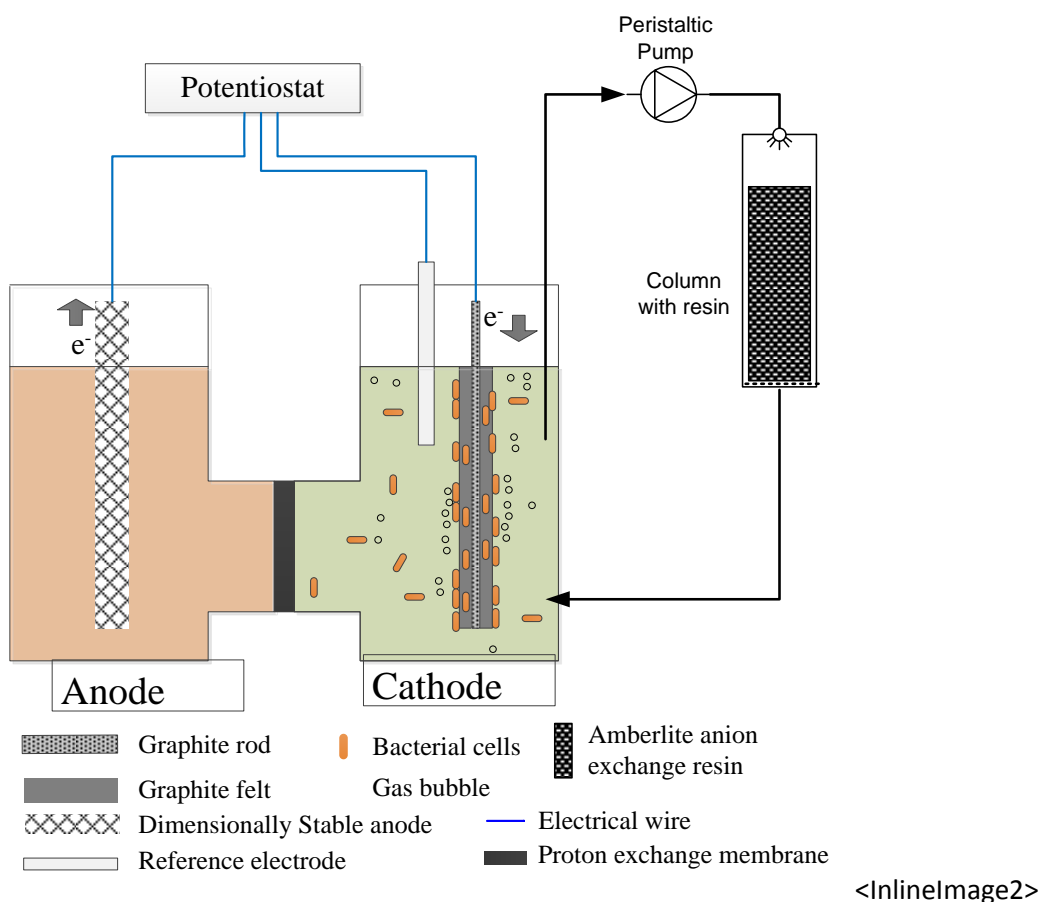
Fig. 1: Schematic representation of MES reactor for CO_2 reduction integrated with a column with Amberlite anion exchange resin
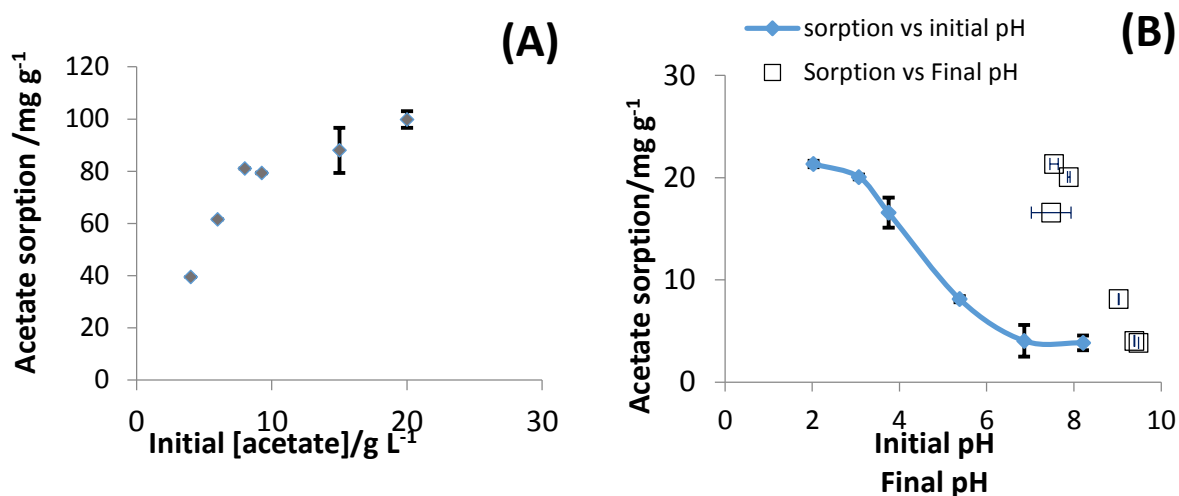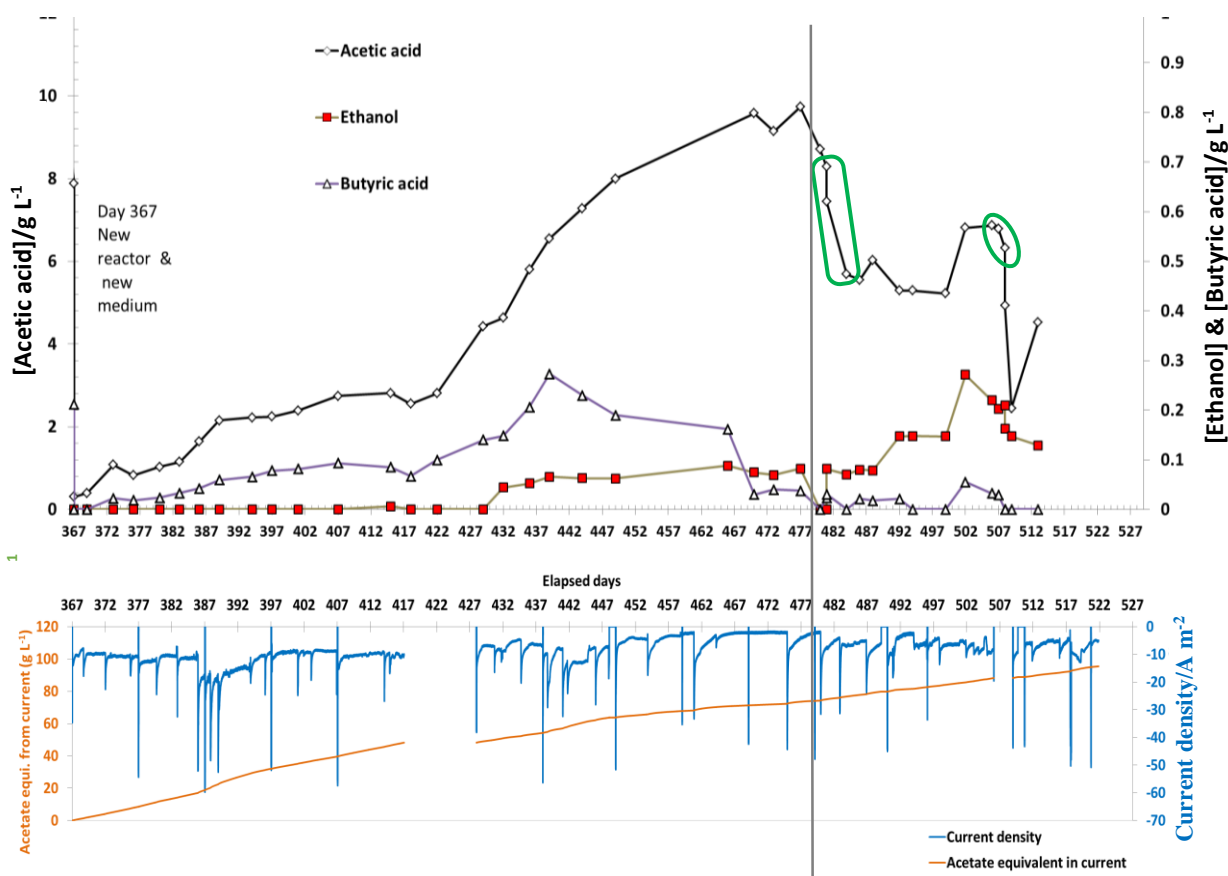
Fig. 2 (A) Acetate sorption in FPA 53 resin from the acetic acid solutions (initial pH 2.6 to 2.9) as a function of concentration (4 to 20 g L^{-1}). **(B)** Acetate sorption by FPA 53 resin and final pH of mixture when suspended for 24 h in the spent MES catholyte (~6 g L^{-1} acetate) at different starting pH. Error bars are showing the standard deviations calculated from the duplicates.

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<InlineShape2>

Fig. 3: Products of CO_2 reduction at the cathode of MES reactor at -1 V vs. Ag/AgCl. **(A)** Concentration profiles of acetate, ethanol and butyrate in the catholyte over time. **(B)** Current densities and equivalent acetate concentration (g L^{-1}) derived from electric current. *In situ* extractions were performed on day 481-484 and day 506-508 with FPA 53 resin in a glass column. The duration of *in situ* acetate separations are indicated with the green shapes.
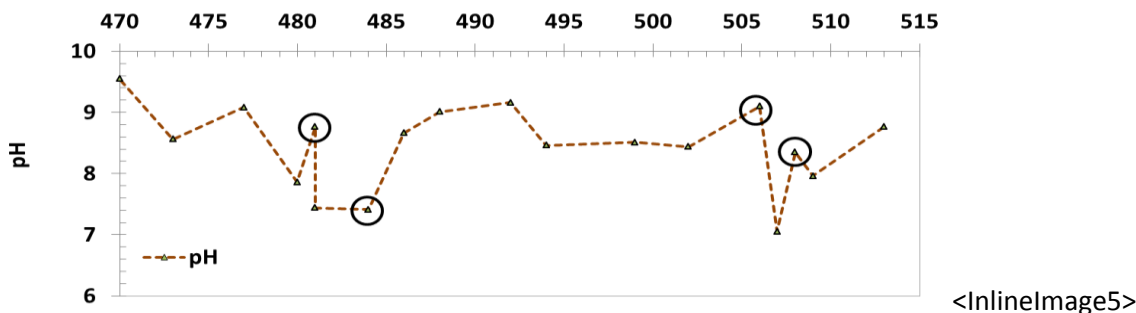
<InlineImage5>

Fig. 4: Changes in catholyte pH over the elapsed days during the integration of *in situ* acetate separation with FPA 53 resin in a glass column. The circles indicate the time of *In situ* acetate sorptions performed from day 481 to day 484 and from day 506 to day 508.

Table 1: Acetate sorption by FPA 53 resin in column from the MES catholyte

Parameters	First run	Second run (HCl pre-treated)
pH of catholyte after acetate sorption	7.44	7.05
Acetate concentration in catholyte at start of column integration (g L^{-1})	8.28	6.78
Acetate sorbed by resin $m_{acetate\ in\ resin}$ (mg)	649	137
Specific acetate sorbed/uptake by resin (mg g^{-1})	18.3	3.9

Table 2: Amount of acetate desorbed from the resin using different regenerants and final acetate recovered

	Acetate recovered during desorption steps (mg) $m_{acetate\ in\ regenerant(s)}$ [calculated using Eq. (7)]			Total acetate recovery from resin (%) $\eta_{acetate\ recovery}$ [calculated using Eq. (6)]
# run	Washing demi water	NaOH cycle 1	NaOH cycle 2	
1	150	245	59	72%

| 2 | - | 21 | 81 | 74% |