Recovery of biohydrogen in a single-chamber microbial electrohydrogenesis cell using liquid fraction of pressed municipal solid waste (LPW) as substrate

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Abstract

The use of liquid fraction of pressed municipal solid waste (LPW) for hydrogen production was evaluated via electrohydrogenesis in a single-chamber microbial electrolysis cell (MEC). The highest hydrogen production (0.38 ± 0.09 m^3 m^{-3} d^{-1} and 30.94 ± 7.03 mmol g^{-1} COD_{added}) was achieved at an applied voltage of 3.0 V and pH 5.5, increasing by 2.17-fold than those done at the same voltage without pH adjustment (pH 7.0). Electrohydrogenesis was accomplished by anodic oxidation of fermentative end-products (i.e. acetate, as well as propionate and butyrate after their acetification), with overall hydrogen recovery of 49.5 ± 11.3% of COD_{added}. These results affirm for the first time that electrohydrogenesis can be a noteworthy alternative for hydrogen recovery from LPW and simultaneous organics removal. Electrohydrogenesis efficiency of this system has potential to improve provided that electron recycling, electromethanogenesis and deposition of non-conductive aggregates on cathode surface, etc. are effectively controlled.

Keywords: Hydrogen; Microbial electrolysis cell (MEC); Electrohydrogenesis; Electromethanogenesis; Electron recycling

1. Introduction

Continuing growth in global energy demand in the most recent years has aggravated the depletion of non-renewable resources like fossil fuels. It, as per IEA's latest data, increased to 13579 million tons of oil equivalent (Mtoe) in 2013 worldwide, up from 8790 Mtoe in 1990 [1]. The world is at a critical stage in its efforts to combat energy crisis. Exploring sustainable energy alternatives to fossil fuels is becoming of great importance to alleviate the energy shortage. One of the potential alternative renewable energy resources that are currently under-utilized is dissolved organic matters present in wastewater streams [2, 3]. Bioenergy produced during wastewater treatment can help offset the costs of operation and increase self-sufficiency. During the past years, various methods thus have been developed to exploit wastewaters for energy recovery and simultaneously realize their environmental-friendly treatment: upflow anaerobic sludge blanket (UASB) [4], expanded granular sludge bed (EGSB) [5], anaerobic submerged membrane bioreactor (AnSMBR) [6], anaerobic baffled reactor [7], etc.

Microbial electrolysis cell (MEC) is a lately designed biotechnological device that can reduce carbon dioxide for the generation of multi-carbon biofuels such as methane, acetate, etc. (called electromethangenesis) [8, 9], or utilize organic matters in wastewater to form hydrogen (referred to as electrohydrogenesis) [10]. In an electrohydrogenesis MEC process, anode respiring bacteria (ARB, or exoelectrogens) populated at the anodic biofilms decompose organic substances into bicarbonate, protons and electrons [11]; the electrons by means of a small energy input, in addition to that (–0.3 V) produced by ARB, are transferred to the cathode and react with protons, generating H_2 via the hydrogen evolution reaction (HER): $2H^+ + 2e^- \rightarrow H_2$ (g) (–0.42 V at pH 7) [3, 12]. The MEC has multifold advantages over the conventional hydrogen-producing applications, such as lower energy consumption

(0.2–1.0 V) [13] compared to water electrolysis (1.23 V) [14], and nearly stoichiometric conversion of a substrate to hydrogen [11] versus only 33% by dark fermentation [15]. A wide range of substrates with different biodegradability have been tested so far in MEC, from single carbon sources such as volatile acids [13, 16], methanol, glucose, glycerol, and starch [11, 12, 17], to complex feedstocks including domestic [18], swine [3], human urine [19], fermentation [20], saline [21] and winery wastewaters [22].

The liquid fraction of pressed municipal solid waste (LPW) obtained from the pressing of the biofraction of municipal solid garbage, is a potential alternative starting material [23]. LPW, as a special high-strength wastewater, is rich in organic matters, nitrogen sources, heavy metals and salinity; especially, its properties may vary with the seasons of refuse collected. In view of the high complexity and variability, LPW represents a bottleneck in waste management to be broken through. The treatment of LPW thus has attracted considerable attention, taking into consideration its severe threats to ecologic environment and human health. Very recently, several technical attempts have been carried out by Koók et al. [23] and to adopt LPW in microbial fuel cell (MFC) for bioelectricity valorization. To date, no work with LPW as the substrate of MEC has been referred to in literature, except that Mahmoud et al. [24] used landfill leachate in MEC for electron recovery, and Kargi and Catalkaya [25] for hydrogen production. It is likely that LPW with unique physiochemical characteristics and more complex components has higher difficulties in hydrogen production, in comparison with other common wastewaters. Whether or not MEC system can catalyze LPW to form hydrogen gas still keeps unknown. This will not only provide a cleaner, more energy-efficient technology for LPW valorization but also advance the widespread applications of MEC technologies in water industry provided that LPW can be converted via electrohydrogenesis.

Therefore, the objective of this work was to investigate the potential opportunities of LPW for net hydrogen production in a single-chamber MEC equipped with graphite felt, and Ti/RuO_2 mesh covered with a layer of carbon cloth as anode and cathode, respectively. Series of batch-scale bioelectrochemical experiments were conducted under different operational conditions to optimize the electrohydrogenesis process for the maximum hydrogen output. At the end of tests, both anode and cathode were removed and the development of the biofilms was characterized by scanning electron microscope (SEM) and fluorescence *in-situ* hybridization (FISH), with the purpose of revealing the principal mechanisms for hydrogen evolution. To the best of the authors' knowledge, this is the first report within the MEC-related scientific community to assess bioenergy recovery from LPW.

2. Materials and methods

2.1 LPW samples

LPW samples from a regional solid waste treatment plant located in Királyszentistván, Hungary, were collected and air-transported in cooled containers within 2 days to National Institute of Environmental Studies (NIES), Japan. The main stages of waste pressing and LPW collection were detailed in previous publications [23]. The basic characteristics of LPW material were as follows: pH 4.5 ± 0.0, total solids (TS) 85.7 ± 0.9 g/L, volatile solids (VS) 51.3 ± 0.3g/L, VS/TS ratio 0.60 ± 0.0, total chemical oxygen demand (TCOD) 108.6 ± 0.6 g/L, soluble chemical oxygen demand (SCOD) 90.0 ± 0.2 g/L, particulate COD (PCOD) 18.5 ± 0.5g/L, total proteins (PN) 16.5 ± 0.4 g/L, and total polysaccharides (PS) 10.6 ± 0.1g/L. The samples were stored at 4 °C prior to use in the tests.

2.2 Single-chamber MEC construction and operation

The single-chamber membrane-less MEC reactor consisted of a wide mouth glass bottle (Pyrex® Laboratory Glassware, Japan) sealed with butyl rubber stopper and screw top with phenolic open-top cap (SIBATA, Japan) (Fig. 1S in supplementary information). The effective working volume of the reactor was 100 mL with 20 mL of headspace. Anode was a three dimensional graphite felt (projected area 12 cm^2, thickness 0.5 cm; Xuesheng Technology Co. Ltd., China); the cathode was made of a Ti/RuO_2 mesh (3.0 × 4.0 cm) physically fixed with the same size of carbon cloth. The electrodes were fixed in parallel at a distance of 2 cm, and connected through a titanium wire that extended through the rubber stopper to a regulated digital DC power supply (AD-8735, A&D Co., Japan) for controlling the electrical voltage. A digital multimeter (Model: PC720M, Sanwa Electric Instrument Co., Ltd., TOKYO, Japan) was used to measure and register the voltage as a function of time across a 10 Ω resistor (Artec, Japan) incorporated in the electrical circuit as time, and the current was then calculated using Ohm's law.

The MEC reactor was started up by inoculation with 20 mL of mixed microorganisms obtained form an existing long-term anaerobic MEC system fed with grounded submerged aquatic plants (*Egeria densa*) in our lab [26]. The synthetic buffer solution was supplemented to get final working volume. The electrolyte medium used here contained (per liter, pH around 7.0): $MgCl_2 \cdot 6H_2O$ 1.0 g; $CaCl_2 \cdot 2H_2O$ 0.375; NH_4Cl 1.25 g; K_2HPO_4 2.18; KH_2PO_4 1.7 g; $NaHCO_3$ 2.5 g; CysteinHCl·H_2O 0.5 g; extract yeast dried 0.5 g; and trace minerals 1 mL as previously reported [8]. The mineral solution contained following (per 100 mL): $FeCl_2 \cdot 4H_2O$ 0.5 g; $CoCl_2 \cdot 6H_2O$ 0.0425 g; $ZnCl_2$ 0.0175 g; H_3BO_3 0.015 g; $MnCl_2 \cdot 2H_2O$ 0.125 g; $NiCl_2 \cdot 6H_2O$ 0.01 g; $CuCl_2 \cdot 2H_2O$ 0.0068 g; and $NaMoO_4 \cdot 2H_2O$ 0.0063 g. 2-bromoethanesulfonate (BES) was added as methanogenic activity

inhibitor at a concentration of 50 mM according to Montpart et al. [17]. LPW was diluted using buffer solution (1: 100 dilution) (i.e. initial COD of 1080 mg/L, pH = 7.0) and used as carbon source for all electrochemical experiments. After around two weeks of acclimation, the reactor was drained, filled with synthetic diluted LPW, and sparged with ultra-high purity nitrogen gas (99.999%) for 5 min to remove oxygen before regulating a potential from 0 to 3.0 V. The reactor was operated under fed-batch mode with each cycle lasting 48 h. At the end of each cycle, biogas volume was determined using the known headspace volume (20 mL) and a gas bag (100 mL) method. The 0.5 mL of biogas was taken using an airtight syringe for analysis of the compositions. Hydrogen and methane production rate (Q) (m^3 m^{-3} d^{-1}) and yield (Y) (mmol g^{-1} COD_{added}) were calculated mainly according to Selembo et al. [11]. Liquid samples were collected and then subjected to further measurements. The reactor was run at 35 °C in an EW-100RD water bath (ASKUL, Japan) under magnetic stirring (slow stirrer SW-500S, Nissin Scientific Corp., Japan). All batch MEC tests were performed in duplicates and results are presented as the arithmetic mean with standard deviation from the duplicate analysis.

In addition, control experiments in identical reactor configuration lacking the electrochemically-active bioanode were performed to reveal (i) the existence of indigenous H_2 production activity of LPW (at 0 V applied voltage) and the (ii) H_2 formation that might occur from water splitting at higher external voltage supplementations (>1 V).

Besides, the pH is a critical factor influencing the activities of hydrogen-producing bacteria because of its effect on the hydrogenase activity and the metabolism pathway. It is commonly reported that the optimal pH value for hydrogen production is 5.5 [27]. For the purpose of comparison, the effect of pH 5.5 on hydrogen production efficiency of MEC system was evaluated as well. The pH was adjusted using 1 mol/L HCl solution.

2.3 Analytical methods

TS and VS were analyzed according to Standard Methods [28]. The pH was determined using a HORIBA Compact pH meter (B-212, Japan). TCOD and SCOD were measured using COD Digest Vials (HACH, Loveland, CO, USA) in accordance with the manufacturer's instructions. Particulate COD (PCOD) was calculated as follows: PCOD = TCOD – SCOD. Proteins (PN) and polysaccharides (PS) were estimated by the corrected Lowry procedure and the phenol-sulfuric acid method, respectively. Percentages of H_2, CH_4, CO_2 and N_2 of the produced biogas were analyzed using a gas chromatograph (GC-8A, Shimadzu, Japan) equipped with a thermal conductivity detector (TCD, 80 mA) and a 2 m stainless steel column packed with Shicarbon ST (Shimadzu GLC). The temperatures of the column and the detector were maintained at 100 °C and 120 °C, respectively. The concentrations of individual volatile fatty acids (VFAs) including acetic, propionic, iso-butyric, n-butyric, iso-valeric and n-valeric acids were determined by a gas chromatograph (GC14B, Shimadzu, Japan) equipped with a flame ionization detector (FID) using helium as carrier gas (50 kPa) and a StabiliwaxR-DA capillary column (Restek, Bellefonte, PA, USA). The oven temperature was increased from 100 °C to 250 °C. The temperature of injector and detector both was 250 °C.

Small pieces of the biofilm materials well-developed on the electrodes (including graphite felt and carbon cloth) were cut and collected for scanning electron microscopy (SEM) combined with energy dispersive X-ray spectra (EDS) analysis. The detailed procedures for SEM analysis were explained in our previous publications [8].

Cells suspensions were sampled from the anodic surface (ultrasonication at 60 W for 5 min) for fluorescence *in-situ* hybridization (FISH) analysis. The samples were pretreated according to Zhen et al. [8]. Hybridizations were performed at 46 °C for 3 h with

hybridization buffer (900 mM NaCl, 20 mM Tris-HCl [pH 7.2], 35% formamide, 0.01% sodium dodecyl sulfate and 1% blocking reagent (w/v, RocheDiagnostics, Mannheim, Germany)) containing 0.5 μM of specific fluorescent probe and then washed at 48 ˚C for 15 min with washing buffer (900 mM NaCl, 20 mM Tris-HCl [pH 7.2], 35% formamide and 0.01% sodium dodecyl sulfate). The oligonucleotide probe EUB338 (*Bacteria*, GCTGCCTCCCGTAGGAGT) [29] was used to target the 16S rRNA of the domain *Bacteria*. After hybridization, the cells on slides were stained with 4, 6-diamidino-2-phenylindole (DAPI). The samples were then observed via an Eclipse E1000 research-level microscope (Nikon, Japan) equipped with a HAMAMATSU ORCA-ER digital camera and a computer-based image analysis system (AQUA-Lite software).

2.4 Calculations

Coulombic efficiency (γ_{CE}) was calculated according to Eq. (1) [16, 17].

$$\gamma_{CE}(\%) = \frac{\int_0^t I(t)dt}{S \cdot b \cdot F \cdot V_R} \quad (1)$$

where t is time (s), S is the substrate added in terms of COD (mol O_2/L), b is the stoichiometric number of electrons produced per mol of oxygen (4 mol-e^-), F is Faradaic constant (96485 C/mol-e^-) and V_R the working volume (L).

Cathodic hydrogen recovery (γ_{CAT}) was calculated from the number of electrons consumed for the formations of reduced products during electrohydrogenesis process via Eq. (2):

$$\gamma_{CAT}(\%) = \frac{m_{H2} \cdot n_{H2} \cdot F}{\int_0^t I(t)dt} \quad (2)$$

where *m* is the number of moles of products harvested, *n* is the number of electrons required for the formation of hydrogen (2 eq/mol) [9].

Overall hydrogen recovery (γ_{H2}) was estimated on the basis of the total recovered moles of hydrogen versus that theoretically possible via Eq. (3).

$$\gamma_{H2}(\%) = \frac{m_{H2} \cdot n_{H2}}{S \cdot b \cdot V_R} \quad (3)$$

Energy recovery (η_W) based on electricity input was estimated as presented in Eq. (4).

$$\eta_W(\%) = \frac{m_{CH4} \cdot \Delta H_{CH4} + m_{H2} \cdot \Delta H_{H2}}{W_{in}} \quad (4)$$

where $\triangle H_{H2}$ is the heat of combustion of hydrogen (the upper heating value, 285.8 kJ/mol), $\triangle H_{CH4}$ is the heat of combustion of methane (894.4 kJ/mol), and W_{in} is the amount of energy added by the power source and $W_{in} = \int_0^t (I \cdot E_{ps} - I^2 \cdot R_{ex})dt$. E_{ps} (V) is the voltage poised and R_{ex} is the external resistance (10 Ω).

Overall energy recovery (η_{W+S}) based on electricity and substrate input was calculated by Eq. (5).

$$\eta_{W+S}(\%) = \frac{m_{CH4} \cdot \Delta H_{CH4} + m_{H2} \cdot \Delta H_{H2}}{W_{in} + W_S} \quad (5)$$

where W_S is the energy contributed by organic matters present in influent and $W_S = S \cdot V_R \cdot 13.9$ kJ/g-COD.

2.5 Statistical analysis

Analysis of variance (ANOVA) was performed using Microsoft Excel 2013 to determine statistical differences in the results obtained from different conditions.

3. Results and discussion

3.1 Biogas composition and hydrogen production

Fig. 1 shows the cumulative biogas formation and composition for the MEC operation with LPW as substrate at different applied voltages and pHs. There was no hydrogen evolution detected in the absence of external power (0 V), except a small amount of methane observed (5.6 ± 4.7%). The biogas exclusively contained methane (53.7 ± 3.7%) when a voltage of 1.0 V was supplied to the LPW, with the corresponding methane production rate (Q_{CH4}) and yield (Y_{CH4}) rising to 0.06 ± 0.01 $m^3\ m^{-3}\ d^{-1}$ and 4.72 ± 1.16 mmol g^{-1} COD_{added} respectively, revealing that methanogenic inhibitor, 50 mM 2-bromoethanesulfonate used herein, was not able to completely suppress methanogenesis. Yet, the electrohydrogenesis did not seem occur while the applied voltage was continuously increased to 2.0 V, where there was still a positive electromethanogenesis although it has been suppressed to a certain degree. Possibly ascribed to the complex compositions of substrate used in this study, a considerable hydrogen was produced only when a voltage of at least 3.0 V was invested with H_2

concentration of around 36.7 ± 16.5%. Q_{H2} and Y_{H2} in this condition reached up to 0.12 ± 0.06 m^3 m^{-3} d^{-1} and 9.76 ± 4.65 mmol g^{-1} COD_{added}, respectively, within 48 h; comparatively, the change in electromethanogenesis performance was only marginal (Q_{CH4} 0.04 ± 0.03 m^3 m^{-3} d^{-1} and Y_{CH4} 3.33 ± 2.34 mmol g^{-1} COD_{added}).

According to the description in Section 2.2., control tests were conducted to determine the significance of non-bioelectrochemical H_2 generation coming either (i) from the activity of indigenous microflora contained in the LPW substrate or (ii) from water electrolysis in the higher voltage regions. In the former case, the results indicated clearly that H_2 evolution from LPW self-fermentation (at 0 V applied voltage) was lower than the experimental error, thus it had a negligible contribution to the measured H_2 production capacity of the MEC. As for the H_2 formation from waster splitting reaction at applied voltages above 1 V, some not so remarkable amounts of hydrogen could be detected at 3 V, meaning that its extent relative to the total (bioelectrochemical plus non-bielectrochemical) H_2 production taking place in the MEC did not exceed 4 %. This outcome coincides well with the report of Kargi and Catalkaya [25], presenting H_2 production in a single chamber electrohydrolysis process using landfill leachate (similar to LPW) as substrate between 0.5-5 V applied voltages. In fact, the authors revealed that H_2 from water electrolysis at 3 V played only a less than 5 % role in the overall H_2 generation. More lately, similar data were published by Kargi and Uzuncar [37] on cheese whey wastewater.

These observations provide a fair conviction that the bioelectrohydrogenesis process of LPW organics – rather than indigenous fermentation and water splitting – was the dominant participant of the realized hydrogen evolution in this study. Moreover, it is evident that for a stable and efficient electrohydrogenesis from LPW, higher voltages (3.0 V) seem quite necessary (Fig. 1) and compared with other wastewaters LPW will face notable challenges for biohydrogen recovery in MEC. This value (3 V) is to a certain degree higher

than the commonly reported ones (0.6–0.9 V) using sole carbon sources e.g. acetate [16], methanol [30], or glycerol [11], and even also higher than those attained with complex substrates for instance domestic wastewater (1.0 V) [31], cellulose fermentation wastewater (1.0–1.2 V) [20], etc., presumably attributed to the large portion of refractory biodegradable organics present in LPW. Nonetheless, some studies, such as those done by Kargi and co-workers [25,37,38] extracted hydrogen by the electrochemical treatment of various substrates at voltage requirements of 3 V or even above.

It is commonly reported that the methanogenesis is a pivotal hamper for a successful electrohydrogenesis process due to (i) competing with anode respiration bacteria (ARB) for substrate acetate ($acetate^- + H_2O \rightarrow CH_4 + HCO_3^-$, $\triangle G^{0\prime} = -31.0$ kJ $reaction^{-1}$) or (ii) scavenging H_2 ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$, $\triangle G^{0\prime} = -135.6$ kJ $reaction^{-1}$) from the electrohydrogenesis to form methane. The pH 5.5 was thus used to inhibit methanogenesis activity according to the findings of Fang and Liu [27], where it was concluded that pH 5.5 was able effectively inactivate *Archaea* while simultaneously upgrading hydrogenase activity. To dissect the influence of pH on MEC performance, experiments were conducted using pH 5.5 medium solution at voltage 3.0 V. As it was previously expected, adjusting pH played a beneficial effect on hydrogen production. At an applied voltage of 3.0 V and pH 5.5, Q_{H2} and Y_{H2} amounted to 0.38 ± 0.09 $m^3\ m^{-3}\ d^{-1}$ and 30.94 ± 7.03 mmol g^{-1} COD_{added}, respectively, increasing by approximately 2.17-fold compared with those achieved without pH adjustment (*p*-value = 0.0250 < 0.05, ANOVA); the hydrogen content at this very moment was about 40.4 ± 5.4%. No significant improvement in methane production was noted with the estimated Q_{CH4} and Y_{CH4} of 0.08 ± 0.02 $m^3\ m^{-3}\ d^{-1}$ and 6.18 ± 1.67 mmol g^{-1} COD_{added}, respectively. Our results suggested that adjusting pH to 5.5 could be a potential strategy to avoid the methanogenesis and enhance hydrogen recovery. These findings in part contradict those of Hu et al. [15], who argued that lowering the pH did not suppress methanogens on the

basis of comparable methane concentrations at pH 5.8 (1.4%) and 7.0 (1.5%) with 0.6 V voltage application. Such disagreements might be associated with the differences in the components of substrates, the history of inoculum, the configuration of MEC and the operating circumstances.

Fig. 1.

3.2 Changes in liquid phase

Particulate LPW organics are not accessible to the biocatalysis unless they are solubilized and liberated into the liquid phase. Soluble organics in terms of SCOD, soluble proteins (PN), soluble polysaccharides (PS) and VFAs were measured by the end of the experiments to quantify the effect of poised potentials on the hydrolysis and mineralization of LPW organics (Fig. 2). Up to 48 h of running (Fig. 2a), SCOD left in the test without the external power was still as high as 802.4 ± 44.6 mg L^{-1}, highly compared to the initial value of 900.3 ± 1.6 mg L^{-1} (p-value = 0.0899 > 0.05, ANOVA), which hinted that the single anaerobic fermentation is not cost-effective in the metabolism of organics and bioenergy formation. In contrast, the investment of voltages remarkably magnified the biodegradation and removal of SCOD significantly. SCOD was decreased to 284.7 ± 108.2 mg L^{-1} at poised voltage of 1.0 V (p-value = 0.0249 < 0.05, ANOVA), and it was further decreased to 142.8 ± 22.3 mg L^{-1} when the voltage was set to 2.0 V (p-value = 0.0028 < 0.05, ANOVA). It means that the application of lower power input (≤ 2.0 V) is still relatively advantages to the methanogenesis of LPW although it could not enable electrohydrogenesis. Interestingly, at an applied voltage of 3.0 V where successful electrohydrogenesis had taken place, the SCOD retained in the effluent, reversely, increased (i.e. 232.8 ± 32.6 mg L^{-1}), 63.0% higher than

that observed at 2.0 V. Guo et al. [32] communicated similar results when studying the effect of bioelectrochemical process on methane production from waste activated sludge that the SCOD could be enhanced along with increasing the applied voltage. These findings demonstrate that the application of suitable voltages (≥ 3.0 V) could not only greatly accelerate the decomposition and conversion of the readily available soluble organics such as acetate into hydrogen but also facilitate the solubilization of insoluble organics into the liquid phase, which accordingly favored subsequent acidification (especially acetification) and electrohydrogenesis. Moreover, pH adjustment (5.5) could further promote the enhancement effect. When pH was set as 5.5, the residual SCOD after electrohydrogenesis approached about 290.3 ± 4.8 mg L^{-1}, 24.7% higher than with only power input. Analogously to the SCOD variation, soluble PN and soluble PS experienced a similar tend (Fig. 2b and c). Thanks to the synergistic effect of electric input and pH adjustment, high solubilization/hydrolysis was therefore achieved.

Electrohydrogenesis works on the premise that the soluble organic content of the substrate is hydrolyzed and acidified. Fig. 2d presents the levels of the three major VFAs components (i.e. acetate, propionate and butyrate) under different operating conditions. As it can be seen, the batch without external power produced the highest VFAs build-up, especially acetate (187.9 ± 17.1 mg L^{-1}). Acetate sharply declined once the external power source was supplied, and even no signs of acetate were detected at the applied voltages greater than 2.0 V, in accordance to the production of bioenergy (hydrogen and methane) (Fig. 1). This indicates the efficient conversion driven by microbial electrocatalysis (e.g. acetoclastic methanogenesis, electrohydrogenesis, etc.). More importantly, in addition to acetate, propionate and butyrate were also characterized by a gradual decrease in conjunction with the ascending voltage, in particular at 3.0 V and pH 5.5 where the VFAs have been depleted completely. This is in high levels as compared with other MEC researches done by

Guo et al. [32], Dhar et al. [33], and Cheng and Logan [13]. There is a consensus in literature that ARB primarily grow on acetate for electron generation in MEC but cannot directly utilize propionate and butyrate. The most rational explanation for the removals of propionate and butyrate thus can be the fermentation of the simple organic acids to acetate and H_2 first, as suggested by Dhar et al. [33]. The conversions are unfavorable thermodynamically (propionate$^-$ + $3H_2O$ → acetate$^-$ + HCO_3^- + H^+ + $3H_2$, $\triangle G^{0\prime}$ = +71.67 kJ reaction^{-1}; butyrate$^-$ + $2H_2O$ → 2acetate$^-$ + H^+ + $2H_2$, $\triangle G^{0\prime}$ = +48.3 kJ reaction^{-1}) due to the strict requirements for extremely low concentrations of the reaction products (acetate and H_2) (i.e. acetate concentration: 10^{-4}–10^{-1} mol/L; H_2 partial pressure required for propionate: 10^{-6}–10^{-4} atm; and for butyrate: (1.0–7.0) ×10^{-3} atm) [33, 34]. The energy barrier must have been overcome through faster ARB's oxidization which is capable of reducing acetate and H_2 level sufficiently, ultimately inducing a favorable Gibbs free energy change for *β*-oxidation of propionate and butyrate. This might be the most critical driving force for the elimination of the simple carboxylic acids and the concomitantly enhanced hydrogen output at the higher applied voltages.

Fig. 2.

3.3 Fate of COD added

In order to describe the fate of COD added during the bioelectrochemical process, COD balance was calculated (Fig. 3). It is apparent that there was a large variation in the recovery of electrons from the COD added, closely dependent upon the operating conditions. The test in the absence of the power source, as said before, was the least efficient with respect to energy recovery (around 1.8 ± 1.6%), with up to 98.1% of COD discharged. Energy

conversion rate of COD increased considerably at applied voltages of 1.0 and 2.0 V (*p*-values < 0.05, ANOVA), giving 30.2 ± 7.4% and 29.3 ± 1.4% as averages, respectively, in spite of being recovered as methane. The organic matters in the LPW were successfully transformed into molecular hydrogen gas at a poised voltage of 3.0 V. At voltage 3.0 V and pH 5.5, hydrogen conversion rate accounted for up to 49.5 ± 11.3% of total COD; the ratio of residual COD in this case fell to only around 26.7 ± 0.4%. The pH adjustment might have suppressed the menthanogenesis to a certain extent and driven more efficient conversion of COD into hydrogen (Fig. 4). These results confirm the superiority of MEC process plus pH adjustment over single fermentation for renewable energy recovery from wastewater streams.

Fig. 3.

Fig. 4.

3.4 Current generation

Current response under each condition was monitored over time to assess (i) the enrichment of bioelectrochemical active biofilms on electrodes, and (ii) the overall performance of the MEC configuration. Fig. 5 depicts the variations of current intensity determined by means of box plots. As can be seen, the average current density was 1.94 ± 1.14 mA at 1.0 V, and it only exhibited a slight rise into 3.81 ± 2.76 mA when increasing the voltage to 2.0 V, presumably associated with the complex characteristics of the substrate, causing the relatively great Ohmic losses and thus low electric current output; moreover, the measured current in those two scenarios was highly variable with time, as exhibited by large standard deviations relative to the mean, indicating the severe process upset and instability. Nonetheless, the current when applying the voltage 3.0 V was magnified and reached an

increase up to 2-fold (11.59 ± 0.72 mA), in line with the results shown in Figs. 1 and 3. Meanwhile, the current in this stage kept relatively stable. Hydrogen production rate relies heavily upon current density and faster degradation of substrate needs higher current values, which explained the cause of elevated hydrogen yield at an applied voltage of 3.0 V. Furthermore, it is obvious from the results of Fig. 5 that despite the fact that the pH 5.5 had increased electrohydrogenesis efficiency, it, however, lowered current production. The current in this case stabilized at 9.22 ± 1.15 mA, slightly lower than that without pH control. Electric power consumption is the product of electric current and applied voltage [19]. Considering this, it can be noted that pH adjustment not only enhances the rate of electrohydrogenesis but it also reduce electric power investment.

Fig. 5.

3.5 Hydrogen and energy efficiency

For the purpose of evaluating the electrohydrogenesis performance of the MEC-based system, several well-accepted process parameters, namely coulombic efficiency (γ_{CE}), cathodic hydrogen recovery (γ_{CAT}), overall hydrogen recovery (γ_{H2}), as well as energy recoveries (η_W, η_{W+S}), were computed, and the corresponding results are plotted in Fig. 6. The electrohydrogenesis efficiency of the MEC showed a close dependency on the poised voltage. The γ_{CE} was 23.3 ± 19.5% and 47.5 ± 19.4% (over 48 h) at 1.0 and 2.0 V, respectively (Fig. 6a); the values of both γ_{CAT} and γ_{H2} were nearly zero. Afterwards, the MEC had the γ_{CE} value that exceeded 100% at 3.0 V, being as high as 152.0 ± 4.9%, but γ_{CAT} and γ_{H2} were still not indeed satisfactory, averaging 10.3 ± 4.9% and 15.6 ± 7.4%, respectively. The low hydrogen recoveries in the LPW-fed MEC system was primarily due to low

conversion rate of electrons to hydrogen (i.e. cathodic hydrogen recoveries), namely although the electrons captured from the substrate were transferred into current, however, most of them were not successfully utilized for proton reduction. The other reason for this might be linked to hydrogen recycling effect occurring between the anode and cathode, as exhibited by γ_{CE} greater than 100%. Part of hydrogen formed through HER at cathode was re-converted into acetate through homoacetogenesis, and after that ARB oxidize further acetate which led to the electron/current production ($H_2 + CO_2 \rightarrow$ acetate $\rightarrow e^- \rightarrow$ current [33]) and thus hydrogen recycling. Some researchers even note that ARB is capable to directly oxidize cathodic H_2 for electron production ($H_2 \rightarrow 2H^+ + 2e^- \rightarrow$ current) [30]. The hydrogen recycling phenomenon was also confirmed by other researchers in literature, such as Ullery and Logan [2], who, in comprising complex effluent treatability in mini and larger-scale cube MECs, documented that the cells both gave γ_{CE} values greater than 100%, and Montpart et al. [30], who, in a methanol-driven MEC, reported a γ_{CAT} even reaching up to 296%. As a result of the occurrence of hydrogen recycling effect, net hydrogen output was reduced. Interestingly, the electron recycling was likely restricted to a certain degree as indicated by lower current production (Fig. 5) provided that the MEC was operated with pH 5.5 medium solution, possibly due to the inhibitory effect of pH 5.5 on the proliferation of homoacetogenic bacteria. The γ_{CE} deteriorated sharply to around 117.7 ± 13.8%, which contributed to high cathodic hydrogen recoveries, i.e. γ_{CAT} 41.8 ± 4.6% and γ_{H2} 49.5 ± 11.3%. From the perspective of maximizing hydrogen recovery, electron recycling had better be avoided.

Fig. 6b and c display energy recoveries based on (i) electricity input (η_W) and (ii) electricity and substrate input (η_{W+S}). For single electrohydrogenesis, the lowest efficiencies were obtained at 1.0 V (η_W-H_2 = 0.2 ± 0.3%; η_{W+S}-H_2 = 0.1 ± 0.1%) and the highest were at 3.0 V and pH 5.5 with a η_W-H_2 of 21.2 ± 2.4% and η_{W+S}-H_2 of 15.9 ± 2.3%, as expected. In

sharp contrast, the overall energy efficiencies for both recovered hydrogen and methane showed a reverse trend. The MEC described here produced the maximal η_W-H_2-CH_4 and η_{W+S}-H_2-CH_4 (209.5 ± 134.4% and 25.2 ± 2.9%, respectively) at the lower applied voltage of 1.0 V, but the lowest values of on average 11.0 ± 3.5% and 8.7 ± 2.7% respectively, at the higher applied voltage of 3.0 V due to low cathodic hydrogen recoveries, large ohmic losses as well as long hydraulic retention time (48 h). Decreasing the medium pH substantially improved energy and substrate efficiencies for reasons described above, with η_W-H_2-CH_4 of 34.9 ± 2.7% and η_{W+S}-H_2-CH_4 of 26.1 ± 1.3% (*p*-values < 0.01, ANOVA), notwithstanding still being far from considering the system energetically attractive.

Several representative MEC results obtained with different targets as the feedstock are summarized in Table 1 for comparison. Because of the scarcity of reports featuring electrohydrogenesis with LPW (with very few exceptions of works using landfill leachate [25]), the results reported on other substrates also have been included in the list. It can be found that the electrohydrogenesis efficiencies varied significantly with the types of substrates. In general, the configurations with easily biodegradable carbon sources would have high H_2 production and energy efficiencies. The performance becomes much worse once the real wastewater, in particular LPW studied in this study, has been fed, resulting from the complex/recalcitrant components, toxic substances and inorganic salts present, which means that the practical applications of MEC technology in simultaneous LPW treatment and renewable energy recovery represent numerous technical challenges to be addressed. It is important bearing in mind that even though the successful hydrogen production from LPW has been evidenced in here, more efforts in eliminating hydrogen recycling and maximizing electrohydrogenesis efficiencies still should be paid in future work. Except hydrogen recycling evidenced in here and the commonly cited causes, e.g. architectural design of MEC, the history of microbial inoculum, types of substrates etc. in literature, another factor

governing electrohydrogenesis process could be the electrochemical properties of electrode materials adopted. Electron transport inside the cathode biofilm decides its combination with proton and thus hydrogen formation. This is an important factor for future studies, which should focus on developing new electrode materials to provoke electron transfer efficiency and cathodic hydrogen liberation.

Fig. 6.

Table 1

3.6 Characterizations of microbial biofilm growth on the electrodes

In order to visualize the enrichment and deposition of electroactive microbial biofilm on the surface of electrodes during the electrohydrogenesis process, scanning electron microscopy (SEM) combined with energy dispersive X-ray spectra (EDS) analysis was conducted. Fig. 7 displays the typical SEM images of the electroactive biofilm developed by the end of tests. Anodic graphite felt, consisting of a framework of micro- and nano-scale fibers, possesses open pore structure and thus may afford substantial habitats for the adherence and proliferation of ARB. As illustrated in Fig. 7a, it plotted the surface of the electrode material with multiple small "bumps". Further observation at high magnification clearly noticed that the minute “bumps” were rob- and cocci-shaped bacterial cells likely affiliated with the ARB, in line with the results of FISH analysis (Fig. 2Sa in supplementary information). The observation that the microbes did not colonize as densely as those previously identified within the anodic biofilm of acetate-fed bioelectrochemical systems could be expected owing to the harsh surroundings and the complex components of LPW. Nonetheless, the microbial biofilm attached to the graphite felt in a very close manner, revealing the successful formation of anodic biofilm community. According to the results

from 16S rDNA sequencing conducted by Carmona-Martínez et al. [21] and Nam et al. [20], the predominant bacteria resemble the genus *Geobacter* spp., etc., the well-known exoelectrogens capable of using acetate as the primary electron donor to liberate electrons and produce current. Besides, some of the detected bacteria might be assigned to fermentative bacteria, or homoacetogens. The presence of the microbes as the syntrophic partners drove hydrolysis of particulate matters and subsequent acetification and provided the preferred acetate for the growth and enrichment of ARB. In this regard, a stable hydrogen from LPW through electrohydrogenesis process can be maintained. The syntrophic interactions were also highlighted in many previous bioelectrochemical studies using complex feedstocks, such as glucose digestate [35], ground corn silage [36], and fermentation wastewater [20], etc. as substrates.

In addition, taking account of an appreciable methane output being detected in the presence of external power source, which reflected the occurrence of electromethanogenesis, the cathodic carbon cloth was further characterized (Fig. 7b). Thick amorphous aggregates were observed to accumulate on the surface of carbon cloth. A further close-up of the surface elucidated several rod-shaped microbes likely related to electrotrophs (e.g. *Methanobacterium* spp.) (FISH results in Fig. 2Sb in supplementary information)), which are able to uptake electrons transferred from anodic biofilm (direct extracellular electron transfer, i.e. direct EET) ($CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + H_2O$, E = –0.244 V vs. SHE) or consume cathodic hydrogen gas (indirect EET, or hydrogenotrophic methanogenesis) ($4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) to catalyze CO_2 for methane production (i.e. electromethangenesis) [8, 9]. This finding coincides with many previous studies with hydrogen as the target that also noticed the cathodic enrichment of electrotrophs [15]. The presence of electromethanogenesis could compete e^- and H^+/H_2 with electrohydrogenesis, thereby weakening cathodic hydrogen efficiencies to a certain degree, which in part explained the suboptimal energy efficiency

achieved even at high applied voltage. These observations point to a need for more efficient strategies over pH adjustment to prevent methanogenesis from the perspective of maximizing biohydrogen production from the LPW. So as to suppress methanogenesis, Catal et al. [10] have employed various inhibitors such as neomycin (NS), bromoethanesulfonate (BES), chloroethanesulfonate (CES) and hypoxanthine (AHX). It also should be stressed that besides the cathodic electroactive cells, another responsible factor limiting cathodic hydrogen efficiencies might be the deposition of various inorganic salts (e.g. Na, Mg, Ca, etc.) as indicated by EDS analysis, due to high salinity of LPW used. Such amorphous aggregates with non-conductive properties near to the cathode could have occupied some of the reactive sites and decreased the reaction probability of electron and H^+. The adverse impact exerted by deposits was also stated by Carmona-Martínez et al. [21] in electrohydrogenesis fed with saline wastewater, and by Zhen et al. [8] in electromethanogenesis with CO_2 as electron donor.

Based on the above mentioned results, to map the main bioelectrochemical pathways involved within the LPW-fed MEC system as well as their separate contribution to hydrogen evolution, a schematic diagram was drafted and is depicted in Fig. 8. It is worth to remark that this is the first scientific report to extract biohydrogen from LPW by means of MEC process to our knowledge, and thus more works in deciphering the mutual-aid relations between phylogenetically diverse microbial populations (e.g. fermentative bacteria, homoacetogens, ARB, etc.) during electrohydrogenesis treatment of LPW with microbiological techniques have to be done.

Fig. 7

Fig. 8

4. Conclusions

Hydrogen was successfully produced from LPW via electrohydrogenesis in a single-chamber MEC system with the optimal performance (Q_{H2} 0.38 ± 0.09 m^3 m^{-3} d^{-1}, Y_{H2} 30.94 ± 7.03 mmol g^{-1} COD_{added}, γ_{CE} 117.7 ± 13.8%, γ_{CAT} 41.8 ± 4.6%, γ_{H2} 49.5 ± 11.3%, η_W-H_2-CH_4 34.9 ± 2.7% and η_{W+S}-H_2-CH_4 26.1 ± 1.3%) observed at 3.0 V and pH 5.5. Electrohydrogenesis was accomplished by anodically oxidizing the fermentative products (i.e. acetate, and propionate and butyrate after their acetification). These results for the first time demonstrate that electrohydrogenesis is an alternative strategy for LPW treatment and simultaneous hydrogen recovery.

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Figure Captions:

Fig. 1. Biogas compositions (a), and cumulative biogas production rate (Q) and yield (Y) (b) at different used voltages and pHs after 48 h of operation.

Fig. 2. Variations of SCOD (a), soluble PN (b), soluble PS (c) and VFAs (d) retained in the effluent at different used voltages and pHs after 48 h of operation.

Fig. 3. Fate of the COD added in MEC experiments under different applied voltages and pHs.

Fig. 4. Possible effects of power source investment and pH adjustment on H_2 and CH_4 evolution pathways.

Fig. 5. Current generation under different applied voltages and pHs. Description of box plots: bottom and top of box = 25^{th} and 75^{th} population percentiles; top and bottom whisker end = 10^{th} and 90^{th} population percentiles; solid line in the box = median value; dotes in the box = mean value; dotes that protrude out of the box = outliers.

Fig. 6. Coulombic efficiency (γ_{CE}), cathodic hydrogen recovery (γ_{CAT}) and overall hydrogen recovery (γ_{H2}) (a); and energy recoveries based on electricity input (η_W) (b) and based on electricity and substrate input (η_{W+S}) (c) under different applied voltages and pHs.

Fig. 7. Typical scanning electron microscopy images of the electroactive biofilm enriched on the surface of anodic graphite felt (a) and cathodic carbon cloth (b).

Fig. 8. Basic bioelectrochemical processes involved within the LPW-fed MEC system.

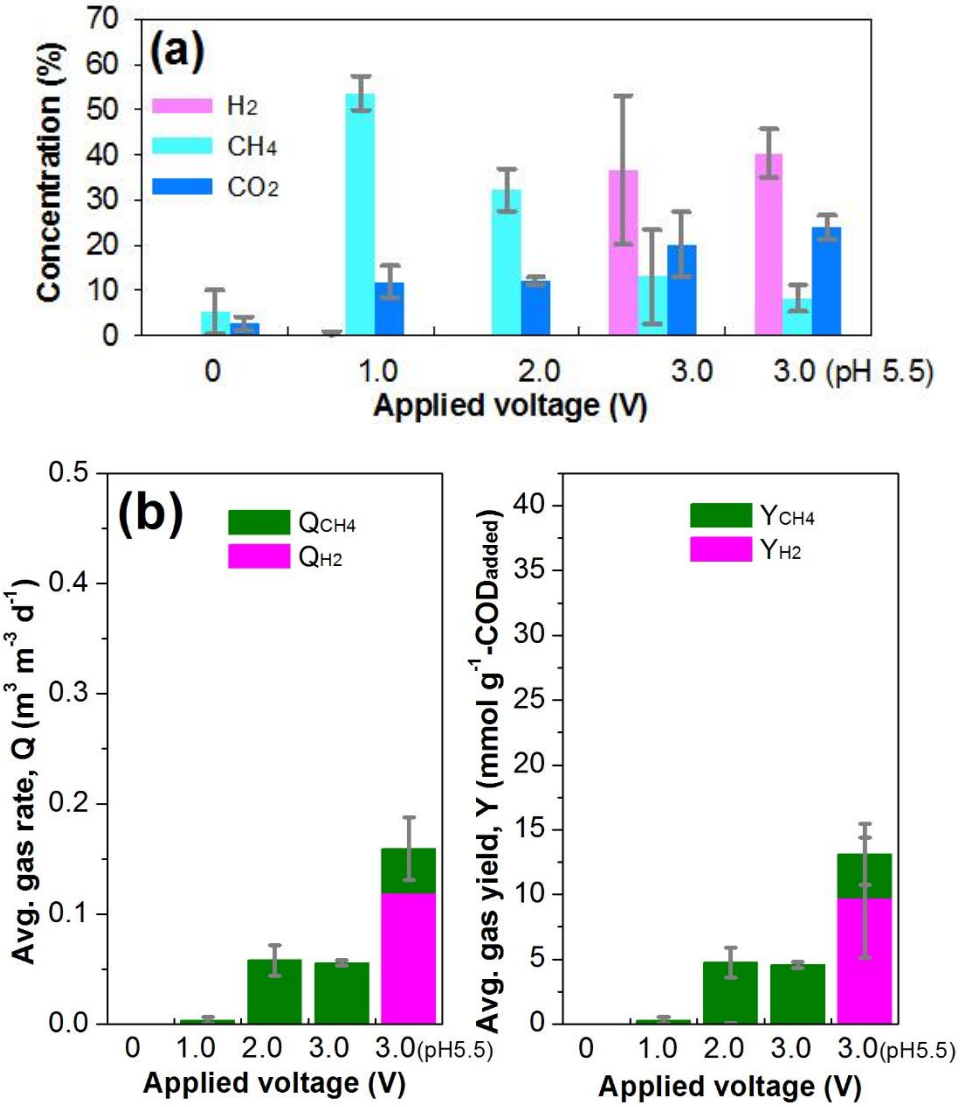

(a)
Concentration (%)
H2
CH4
CO2
Applied voltage (V)
(b)
QCH4
QH2
Avg. gas rate, Q (m3 m-3 d-1)
Applied voltage (V)
YCH4
YH2
Avg. gas yield, Y (mmol g-1-CODadded)
Applied voltage (V)

Fig. 1.

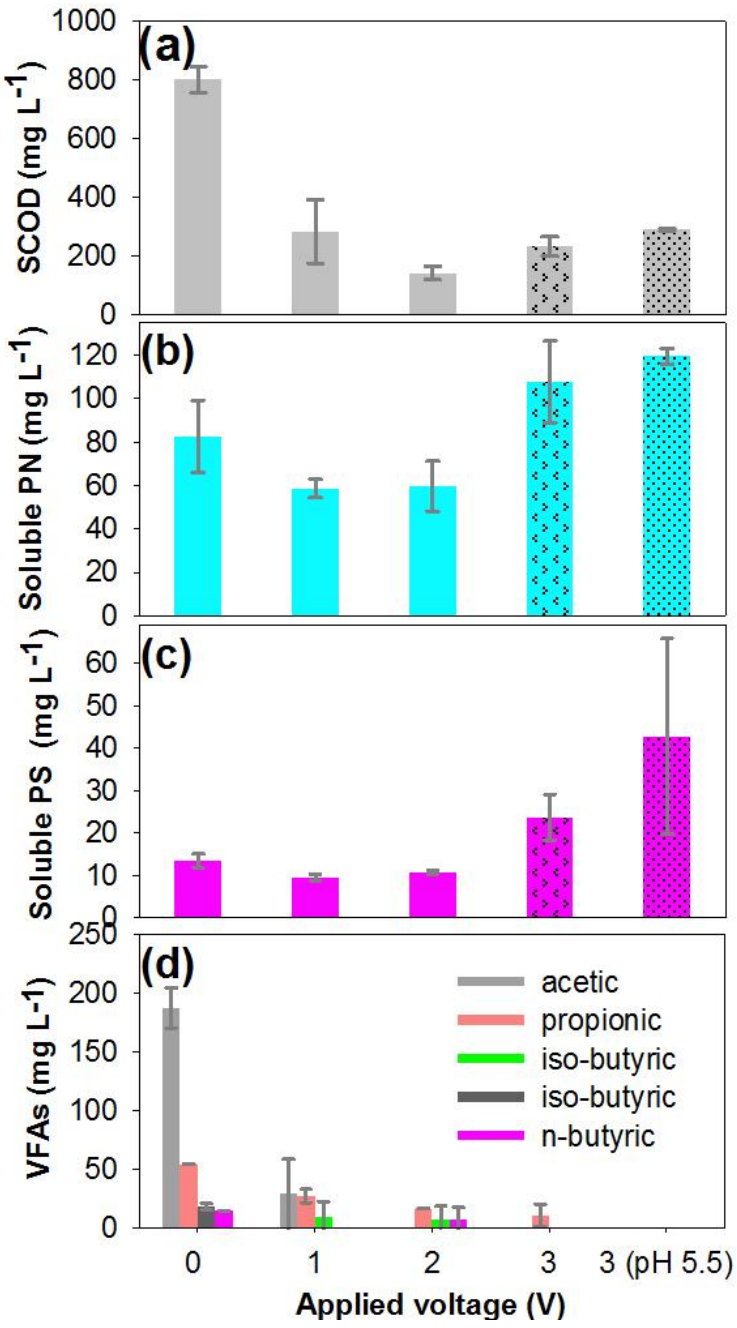
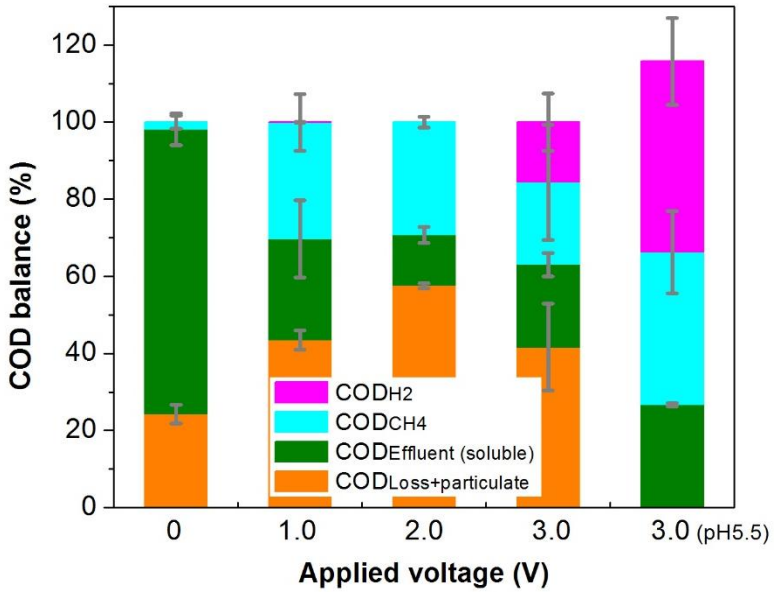

(a)
SCOD (mg L-1)
1000
800
600
400
200
0
(b)
Soluble PN (mg L-1)
120
100
80
60
40
20
0
(c)
Soluble PS (mg L-1)
60
50
40
30
20
10
0
(d)
VFAs (mg L-1)
250
200
150
100
50
0
acetic
propionic
iso-butyric
iso-butyric
n-butyric
0
1
2
3
3 (pH 5.5)
Applied voltage (V)

Fig. 2.


COD balance (%)
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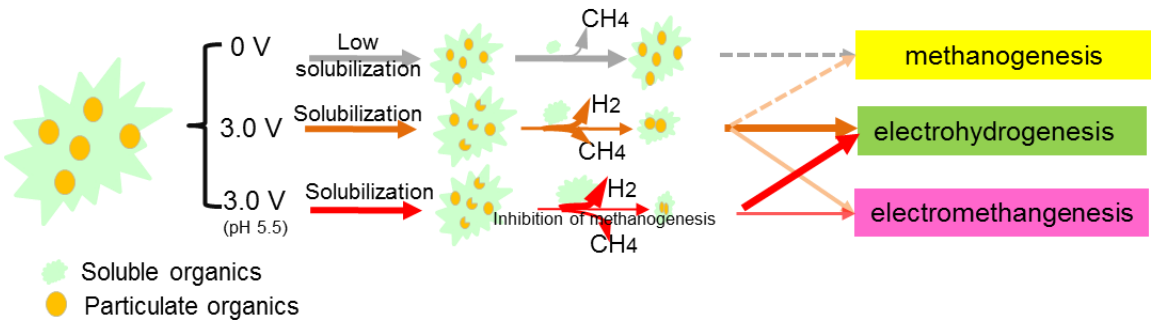
100
80
60
40
20
0
COD$_{H2}$
COD$_{CH4}$
COD$_{Effluent\ (soluble)}$
COD$_{Loss+particulate}$
0
1.0
2.0
3.0
3.0 (pH5.5)
Applied voltage (V)

Fig. 3.


0 V
Low solubilization
3.0 V
Solubilization
3.0 V
(pH 5.5)
Solubilization
CH4
H2
CH4
H2
CH4
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electrohydrogenesis
electromethangenesis
Soluble organics
Particulate organics

Fig. 4.

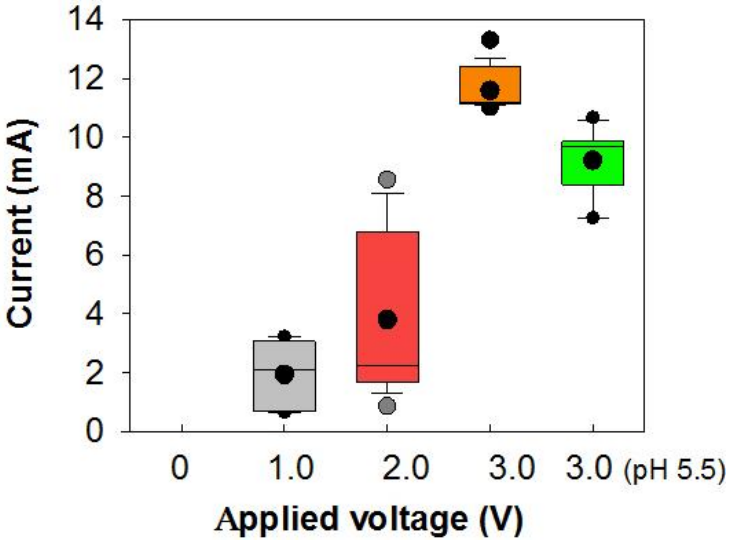

Fig. 5.

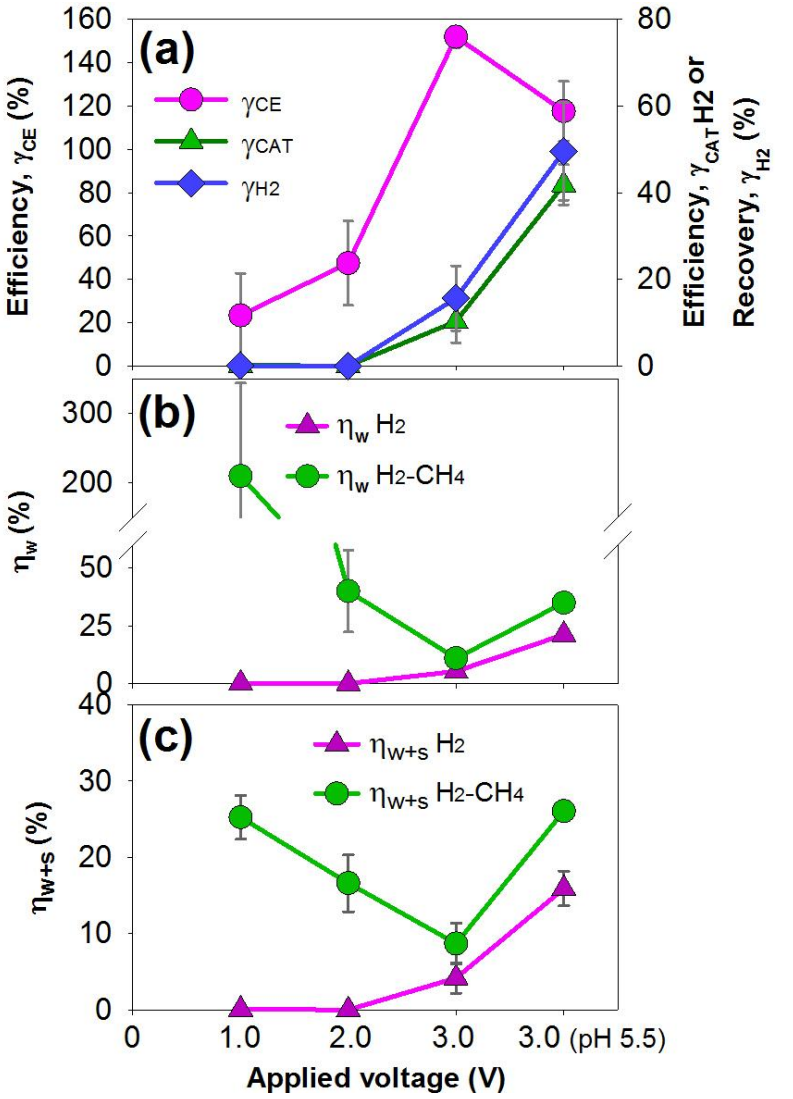
(a)
γCE
γCAT
γH2
Efficiency, γCE (%)
Efficiency, γCAT H2 or
Recovery, γH2 (%)
(b)
ηw H2
ηw H2-CH4
ηw (%)
(c)
ηw+s H2
ηw+s H2-CH4
ηw+s (%)
Applied voltage (V)
3.0 (pH 5.5)

Fig. 6.

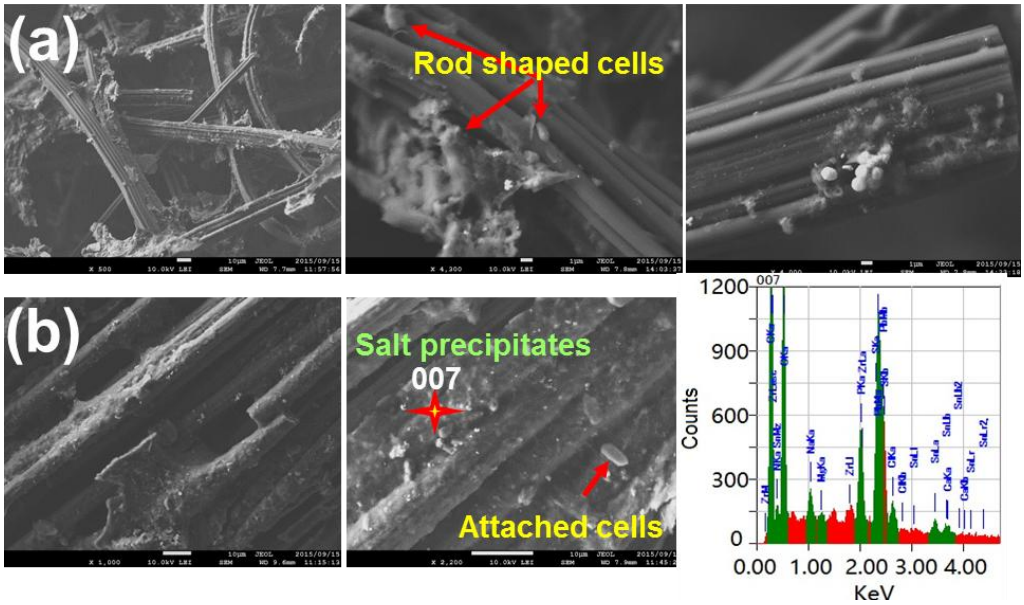
(a)
Rod shaped cells
(b)
Salt precipitates
007
Attached cells
007
1200
900
600
300
0
Counts
0.00 1.00 2.00 3.00 4.00
KeV

Fig. 7.

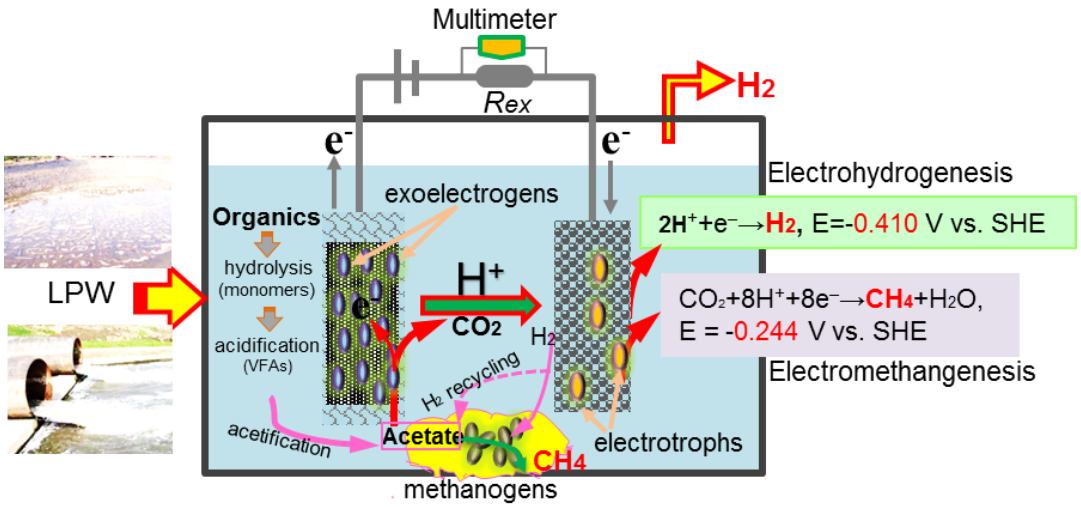
Multimeter
Rex
H2
e-
e-
Electrohydrogenesis
exoelectrogens
Organics
2H+ +e− → H2, E=-0.410 V vs. SHE
hydrolysis
(monomers)
LPW
H+
CO2
CO2+8H+ +8e− → CH4+H2O,
E = -0.244 V vs. SHE
H2
acidification
(VFAs)
H2 recycling
Electromethangenesis
acetification
Acetate
CH4
electrotrophs
methanogens

Fig. 8.

Table 1 Summary of MEC results obtained with different wastewater as the feedstock.

Substrate	Chamber	Voltage (V)	γ_{CE} (%)	$\gamma_{CAT\ H2}$ (%)	γ_{H2} (%)	Q_{H2} ($m^3\ m^{-3}\ d^{-1}$)	Q_{CH4} ($m_3\ m^{-3}\ d^{-1}$)	Y_{H2} (mmol g^{-1} COD)	Y_{CH4} (mmol g^{-1} COD)	$\eta_{W\ H2}$ (%)	$\eta_{W\ H2\text{-}CH4}$ (%)	$\eta_{w+S\ H2}$ (%)	$\eta_{w+s\ H2\text{-}CH4}$ (%)	References
Acetate	Single	0.8	98±0[a]	96±1		3.12				194		75		[16]
Acetate	Single	0.6(5.8)	73	87	64	0.69				215		60		[15]
Acetate	Single	0.6			91	1.10		57.0[a,b]		261		82		[13]
Lactate					91	1.04		56.7[a,b]		283		82		
Glucose	Single	0.9	105±10[a]	88±5		0.80±0.08		37.5[a,b]		152±8		62±4		[11]
B-Glycerol			91±10[a]	65±14		0.41±0.13		16.1[a,b]		107±25		37±7		
Domestic wastewater	Two	0.2-0.6	10-26[a]	2-43	0.2-10[a]			6.25[a,b]						[18]
Milk	Single	0.8	52	13		0.09								[17]
Winery wastewater	Single	0.9				0.07±0.04	0.02							[22]
Swine wastewater	Single	0.5	43±2	53±6	23±4	0.8±0.2		17.5[b]		179±4				[3]
Landfill leachate	Single	4.0						107.1		35				[25]
Diluted LPW	Single	3.0 (7.0)	152±5	10±5	16±7	0.12±0.06	0.04±0.03	9.8±4.7	3.3±2.3	5±2	11±3	4±2	9±3	This study[c]
		3.0 (5.5)	118±14	42±5	50±11	0.38±0.09	0.08±0.02	30.9±7.0	6.2±1.7	21±2	35±3	16±2	26±1	

a The γ_{CE}, γ_{H2} and Y_{H2} were calculated based on the unit gram of COD removal.
b Parameter calculated from the data presented in the specific publication.
c The γ_{CE}, γ_{H2} and Y_{H2} in this study were calculated based on the unit gram of COD added (these values could be higher when calculated based on the unit gram of COD removal).