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Reduction of Carbon Dioxide Emission by Using Microbial Fuel Cells during Wastewater Treatment

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ABSTRACT

To explore the feasibility of simultaneous carbon reduction and energy saving/recycling during wastewater decolorization, this study used naturally-occurring microbes (e.g., *Aeromonas* and *Klebsiella* sp.) for dye decolorization as well as energy and materials recycling. These microbes were tested for capabilities of bioelectricity generation in parallel with dye treatment for simultaneous energy recovery. The supplementation of electron-shuttling mediators (e.g., aminophenols) significantly increased the electron-transfer efficiency of electrochemically-active microorganisms. Moreover, the presence of decolorized intermediate(s) might repress intracellular accumulation of the biodegradable polyhydroxyalkanoates (PHAs), likely due to toxicity of aromatic amines. Microbial fuel cells (MFCs) appear to be feasible for use in the reduction of CO_2 emitted (ca. 40–60 Faraday efficiency) during the generation of bioenergy (e.g., bioelectricity) and biomaterials (e.g., PHAs) in wastewater treatment, thus aiding the development of cradle-to-cradle sustainable designs.

Keywords: Wastewater decolorization; Microbial fuel cells; Polyhydroxyalkanoate production; Carbon reduction.

INTRODUCTION

Since the meltdown at Japan's crippled Fukushima Daii-chi nuclear power plant after the tsunami disaster, seeking safe and renewable energy for global needs apparently becomes top-priority issue worldwide for sustainable development. In fact, the approach of simultaneous energy saving and carbon reduction becomes the appropriate strategy to face problems of Today's gradually-depleted energy resources over the globe. Among alternative resources of renewable energy, microbial fuel cells (MFCs) can use naturally-occurring microorganisms as biocatalysts to extract sustainable energy from oxidation of organic matter for bioelectricity production and wastewater treatment (Du *et al.*, 2007). As a matter of fact, our prior study (Chen *et al.*, 2010a, 2011a, 2012a) used indigenous pollutant-degrading bacteria to recover bioelectricity via dye decolorization in MFCs. The findings also suggested that decolorized intermediates (e.g., phenyl methadiamine) might play a crucial role of electron-shuttling mediator to enhance simultaneous bioelectricity generation and color removal (SBG & CR). This feasibility study unveiled the effects of model decolorized intermediates upon the performance of azo dye decolorization and bioelectricity generation not only unveiled the effects of model decolorized intermediates upon the performance of azo dye decolorization and bioelectricity generation, but also tended to reduce carbon dioxide emission during wastewater treatment. As known, reducing the emitted CO_2 (i.e., one of greenhouse gases) could attenuate the risk of global warming. Moreover, to consider simultaneous materials recycling during carbon reduction and bioelectricity generation facultatively anaerobic Gram-negative rod *A. hydrophila* was also used to be an excellent strain for polyhydroxyalkanoate (PHA) production during treatment. Thus, the model polymer of PHA, poly-3-hydrobutyrate (PHB) was selected as a form of energy storage molecule to be metabolized via carbon assimilation under nutrient-limited conditions. As known, PHB is capable to be used as myriads of biomaterials with specific physical and chemical characteristics (Sevastianov *et al.*, 2003). Here, to consider the perspective of energy saving and carbon reduction, this first-attempt study disclosed whether dye-decolorizing or pollutant-degrading bacteria could own not only bioelectricity-generating, PHA-accumulating potential but also capability to decrease accumulation of emitted CO_2 for energy and materials recycling and CO_2 reduction during wastewater treatment. Comparative assessment upon PHB generation in the presence of model amine intermediates also suggested the feasibility of bioelectricity generation and PHA production in parallel with CO_2 reduction for simultaneous biofuel and biomaterials recycling and reuses.

In fact, according to National Energy Conservation and

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Carbon Reduction Master Program (NEC & CRMP; 2010), the quantity of greenhouse gas (GHG) emissions in 2020 and 2025 should be reduced to that in 2005 and 2000, respectively. Moreover, to achieve Taiwan's goal of "nuclear-free homeland" and NEC & CRMP, Taiwan's power-generation sector has set up GHG control protocols (e.g., renewable biomass energy development and process control analysis, Lin *et al.*; 2012) to reduce GHG emissions (Taiwan Power Company, 2010). For example, CO_2 and O_2 emission in the flue gases of the power plant were approx. 12.0–12.8% and 4.90–5.80%, respectively (Wang *et al.*, 2010). These contents were fairly applicable to MFC applications for recycling and reusing the emitted CO_2 in wastewater for the generation of bioenergy- CH_4 (Villano *et al.*, 2010) and biodiesel (Powell and Hill, 2010) or the production of materials- formic acid (Zhao *et al.*, 2012) and PHA (Yagi *et al.*, 1996). In fact, several CO_2-utilizing bacteria were considered to produce bioenergy and biomaterials (Saini *et al.*, 2011); for example, *Chloroflexus aurentiacus*, *Escherichia coli*, *Metallosphaera sedula*, *Acidianus brierleyi*, autotrophic *Sulfolobales* and *Ignicocccus hospitalis*. This study was linked into scheduled grids for economic development of Lanyang Plain according to Yi-Lan County. In particular, on the way from Taipei City to Lanyang Plain in Yi-Lan, the fifth longest tunnel in the world, 12.9 km Hsueh-Shan Tunnel provided convenient traffic for northeast Taiwan. However, such a closed long tunnel caused severe air pollution due to poor diffusive conditions (or so-termed the piston effect; Ma *et al.*, 2011). To solve such a problem of air quality as aforementioned, some innovative MFC system (e.g., microbial electrolysis cell or MEC) as a viable carbon recycling method was proposed to take electricity and directly convert CO_2 and water to formic acid for CO_2 reduction (Zhao *et al.*, 2012a, b). Note that the theoretical potential for electrochemically reducing CO_2 to formic acid at standard conditions is –0.199 V (vs. NHE): $CO_2 + 2H^+ + 2e^- \rightarrow HCOOH$ and the lowest reduction potential of CO_2 is –1.5 V (vs. Ag/AgCl on a Pb electrode). Therefore, emphasis of this first-attempt study MFC/MEC for simultaneous CO_2 reduction and materials reuse afterwards would give on-site professionals a promising chance to examine feasibility of renewable bioenergy and materials before they launch globally.

METHODS

Microbial Fuel Cell (MFC) Operation

Membrane-free air-cathode single-chamber MFCs (refer to Chen *et al.* (2010a) for schematic setup of MFC) were constructed and operated as described elsewhere (Chen *et al.*, 2011; Chen *et al.*, 2012a). Chemical oxygen demand (COD) was colorimetrically determined via US EPA method 410.4.

SBG & CR Using Model Aromatic Amines (MAAs)

The MAAs (Chen, 2006) used for assessment of decolorization and electron-shuttling mediator to MFCs included 2-, 3-, 4-aminophenol (2AP, 3AP, 4AP), 3-aminobenzoic acid (3ABA) and aniline 2-sulfonic acid (A2SA). In addition, 2AP was also supplemented to mixed culture MFCs to compare performance of power generation.

Cultures for PHA Production

To probe PHA-producing microbes in the population of pollutant-degrading bacteria, batch cultures in lauric acid-bearing MR medium (Chen *et al.*, 2011b, 2012b) were conducted using seeding cells precultured in Bacto LB medium for 12–16 h. The nutrient medium and chemical reagents used for cell cultures, the method of Sudan black B (SB) staining for probing possible PHA-accumulating microbes and GC analysis to determine PHA content were discussed elsewhere (Chen *et al.*, 2011b).

Standard Normal Deviate (Z Scores)

To reveal whether the augmentation of carbon source could stimulate or inhibit PHA production and microbial growth, statistical standard normal deviate (Z scores) was used for significant testing as described in Chen *et al.* (2012b).

MEC Reactor Construction

The MEC system was composed of one MEC for CO_2 reduction and a dry cell (ca. 1.5V) to simulate MFC voltage in series for extra power supply. The MEC was transformed from a two-chambered MFC (ca. 500 mL for each compartment). Carbon fiber was used as the anode and the cathode for the two-chambered MFC. All electrodes used in the MFCs had a projected surface area of ca. 18 cm^2. The electrode spacing was ca. 10 cm in the two-chamber. The two compartments for the two-chambered MFC were separated by a proton exchange membrane (PEM, Nafion117, 5 cm diameter, Dupont, U.S.A.). The PEM was sequentially boiled in H_2O_2 (30%), deionized water, H_2SO_4 solution (0.5 M), and again in deionized water (each for 1 h) and then immersed in deionized water for use (Liu *et al.*, 2004). Air was aerated continuously into the phosphate buffer solution (0.1 M) to supply O_2 as the electron acceptor for the cathodic chamber of the two-chambered MFC.

MEC was modified from the two-chambered MFC as aforementioned by changing the cathode and the catholyte. A plate of Pb (2×8 cm^2, 99.9%, Jiehan Technology Corporation) was used as the cathode of the MEC, and it was pretreated in H_2SO_4 solution (1 M) at room temperature for 10 min and then successively rinsed with acetone and ultra-pure water. A $KHCO_3$ solution (0.1 M) was used as the catholyte for CO_2 reduction. The MEC were connected in series with a 10 Ω resistor (to allow the circuit current measurement) when the electrolysis of CO_2 was conducted.

Regarding CO_2 Electrolysis, CO_2 was electro-reduced in the cathodic chamber of the MEC. An electrolysis time of 300 min was applied for each batch. The catholyte of $KHCO_3$ solution (0.1 M) was saturated with CO_2 (99.5%) before each electrolysis process, and CO_2 gas was continuously aerated at a rate of 30 mL/min during the electrolysis process. The products in the cathodic solution after electrolysis, including formic acid, were analyzed by ion chromatography (METROHM 861ADVANCED COMPACT IC, column using METROSEP a supp4). The

sampling was set at 100, 200, and 300 min from the start of electrolysis. The Faraday efficiency for the formation of formic acid (FE_{HCOOH}) was calculated as follows:

$$FE_{HCOOH} = \frac{n_{HCOOH} \times n \times F}{\int_0^t Idt} \times 100\%, \quad (1)$$

where n_{HCOOH} is the moles of formic acid harvested; n represents the number of electrons required for the formation of one molecule of formic acid from CO_2 (n = 2 here); F is Faraday's constant (96,485 C/mol of electrons); and I is the circuit current.

RESULTS AND DISCUSSION

Power Generation Performance

As shown in time courses of COD degradation and cell voltage (Fig. 1), a sharp rise of output was observed when a new energy source (i.e., 0.2x LB medium) was provided. Moreover, the decrease of COD was almost in parallel with a decline of output voltage in MFC. This portion of decrease in COD was due to biodegradable organic carbon (e.g., primary and secondary alcohols; Chen *et al.*, 2012a) provided for bioelectricity generation in MFCs. However, the cell voltage was steadily stabilized at ca. 80 mV, indicating that residual COD was very likely not effectively bioavailable for bioelectricity generation. These parallel decreasing profiles of COD degradation and cell voltage confirmed that energy-recycling (or saving) and carbon reduction was feasible for MFCs.

Effects of Exogenous Mediator on Bioelectricity Generation and Color Removal

Regarding exogenous electron-shuttling mediator, prior study (Chen *et al.*, 2010a) suggested that phenyl methadiamine produced via reductive decolorization could mediate electron transfer in anodic biofilm of *P. hauseri* for power generation in MFC (Chen *et al.*, 2010b). As shown in Fig. 2 (MFC-A), appropriate amount of 2AP supplemented could significantly enhance the performance of bioelectricity generation compared to 2AP-free MFC (i.e., 0 mg/L), revealing its crucial role to skyrocket electron-transfer efficiency in anodic biofilm of MFC. The output voltages were gradually increased with respect to an increased concentration of 2AP, suggesting that current production of *P. hauseri* in MFC could be significantly stimulated by the supplementation of 2AP as an exogenous electron-transfer mediator. However, not all isomeric aminophenols could effectively work as redox mediators or electron shuttles for extracellular electron transfer from *P. hauseri* cells to the anode. For example, the supplementation of 3AP (i.e., a model decolorized intermediate) repressed the performance of power production in MFC (MFC-B; Fig. 2) likely due to an increase in electron transfer resistance after supplementation. Recently, Chen *et al.* (2012a) suggested that the absence of reduction and oxidation peak potentials for 3AP in cyclic voltammograms (CVs) led to low feasibility for 3AP to be an electron-shuttling mediator. Moreover, although 4AP could show the presence of reduction and oxidation peak potentials in CV (data not shown), zero output voltage was still observed in 4AP-supplemented MFC due to significant toxicity of 4AP on *P. hauseri*. As a matter of fact, the toxicity potency of 4AP might be functionally similar to that of 4-aminopyridine, 4AP might penetrate the cell membrane, act on the cytoplasmic side of potassium ion channel (Kv), block the Kv channels, and become trapped in the channel once it is closed or inactivated (Zhan *et al.*, 2008). This point also confirmed that a reduced dye (i.e., aromatic

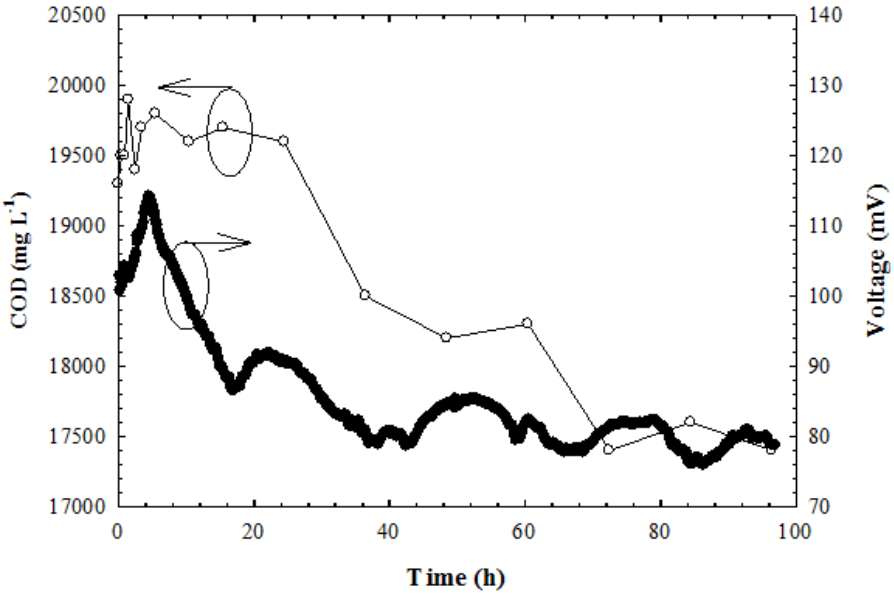

Fig. 1. Time courses of COD degradation and cell voltage in *Acinetobacter* sp. x72-dominated MFC using 0.2x LB medium as energy source (external resistance 1 KΩ). Initial decay rate constant for bioelectricity generation and initial decay rate constant of COD degradation were ca. 0.45 and 0.0015 h^{-1}, respectively.

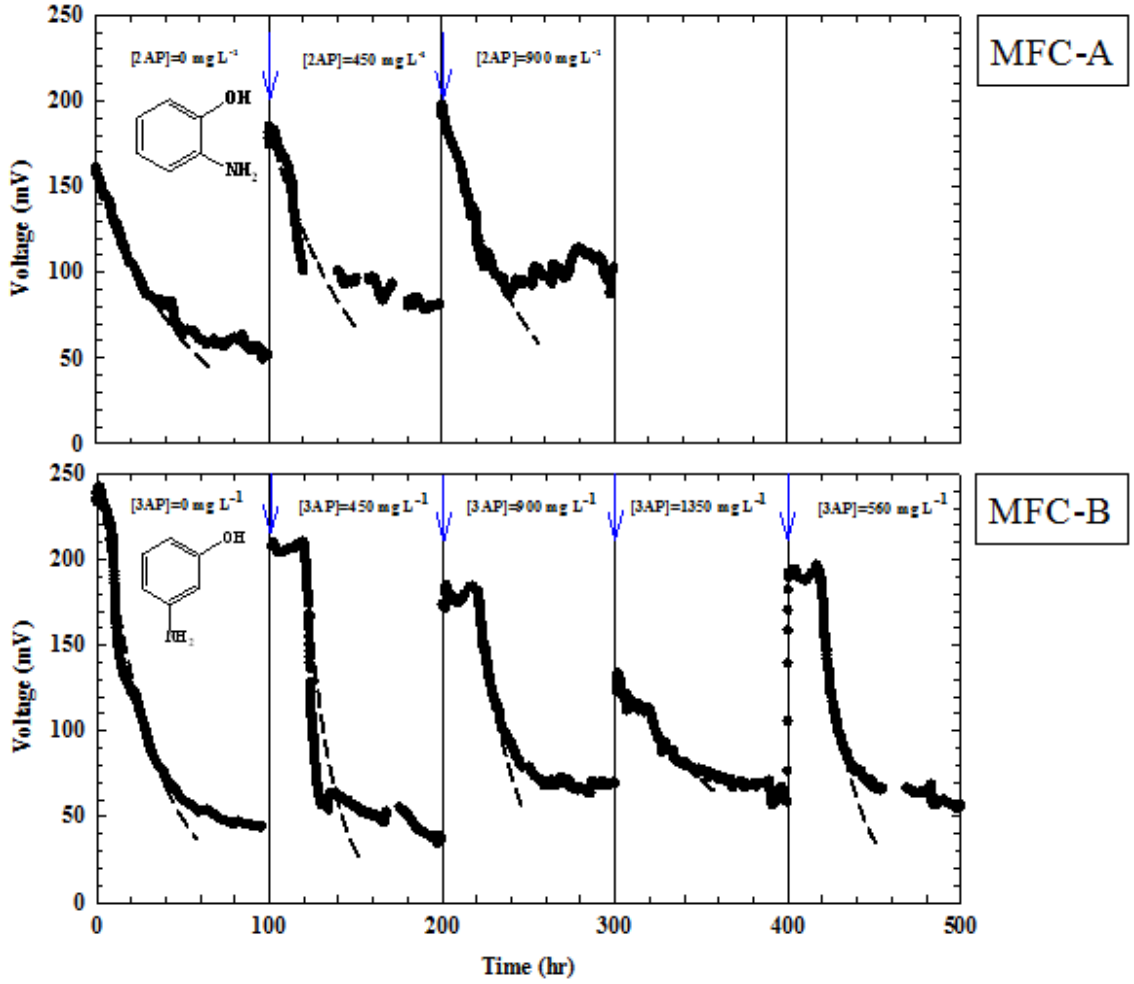

Fig. 2. Time course of bioelectricity generation of *Proteus hauseri* ZMd44 in MFC-A and MFC-B supplemented with various concentrations of 2-AP, 3-AP (external resistance = 1 KΩ). Dashed curves denoted model prediction of output voltage profiles (raw data after Chen *et al.* (2011a)).

amine) with relatively less biotoxicity might be feasible as an exogenous mediator to stimulate electron transfer to anodic biofilm in MFC (e.g., 2AP). In fact, 2AP was found to be a possible electron-shuttling mediator to Gram-negative aerobic rod *Acinetobacter* sp. x72 and facultatively anaerobic Gram-negative rod *Enterobacter* sp. m30 dominant mixed culture MFC at 120, 170 mg/L, appreciably increasing the power-generating efficiency ca. 79 and 151%, respectively (Fig. 3). It was thus concluded that 2AP seemed to be the most electrochemically promising isomer for mediating electron transfer in MFC. This study also confirmed that decolorized amine intermediate(s) could play a role for electron-shuttling mediator in MFCs.

Probing PHA-accumulating Strains

In fact, carbon reduction of wastewater decolorization could be used not only for bioelectricity generation, but also for materials recycling (e.g., PHA and formic acid production). To qualitatively reveal the feasible bacteria for PHA production, optical microscopic observations upon pollutant-degrading microorganisms (e.g., *Aeromonas hydrophila* NIU01, YTl1, KB23, *A. salmonicida* 741, *Acinetobacter johnsonii* NIU-x72, *Proteus hauseri* ZMd44, *Enterobacter cancerogenus* BYm30, *Pseudomonas plecoglossicida* NIU-Y3, *Klebsiella pneumoniae* ZMd31 and *Chromobacterium violaceum* P1; (Chen *et al.*, 2011b)) after SB staining indicated that more significant black blue granules accumulated in strains of *Aeromonas* and *Klebsiella* genera (Fig. 4), revealing that those were likely promising microbes for PHA production. Plus, relatively lower level accumulations of PHA were found for *Pseudomonas* sp. and *Chromobacterium* sp. In contrast, significant accumulation of PHA granules were not observed in intracellular compartments of *Acinetobacter* sp., *Enterobacter* sp. and *Proteus* sp., simply suggesting that these strains were likely not feasible for PHA-producing microbes (Fig. 4). After identifying PHA-producing bacteria via SB staining, quantitative assessment upon PHA productivities of 24 pollutant-degrading microbes was also carried out by using 5 g/L lauric acid as the sole carbon source. The findings indicated that feasible strains of PHA-accumulating microbes were *Aeromonas* sp. NIU01, KB23, YTl1 and 741, *Pseudomonas* sp. NIU-Y3, *Klebsiella* sp. ZMd31 and *Chromobacterium* sp. P1. In particular, the high PHB

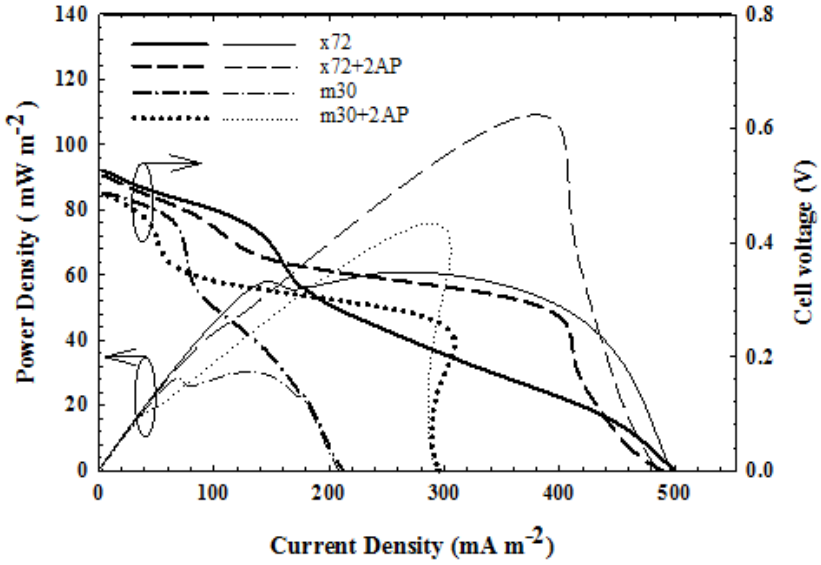

Fig. 3. Power density and polarization curves for *Acinetobacter* sp. x72 (x72) and *Enterobacter* sp. m30 (m30) dominant mixed culture MFCs using 2-aminophenol (2AP) at ca. 120 and 170 mg/L as electron shuttle mediator, respectively.

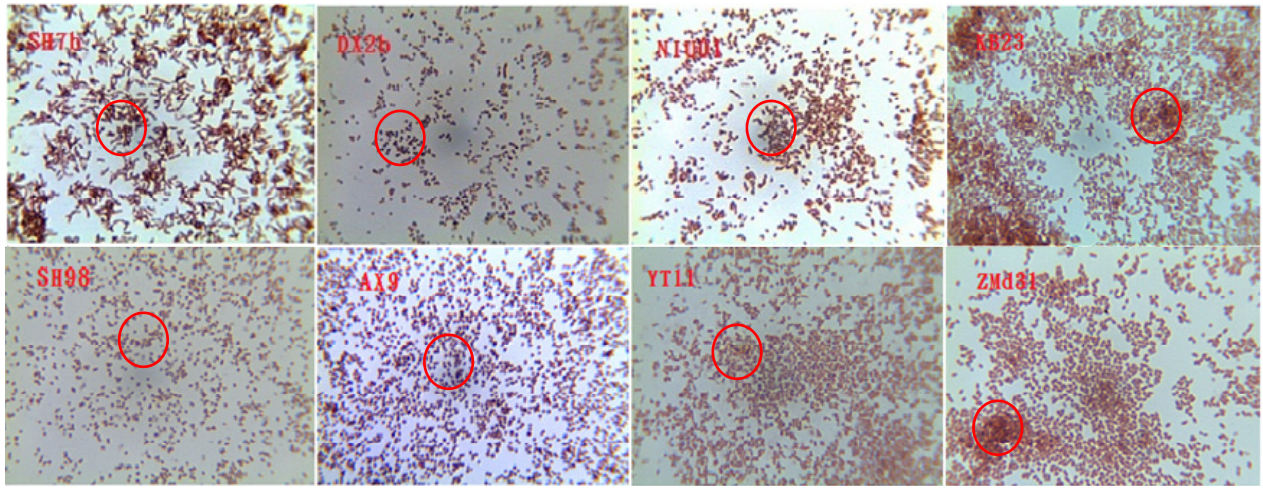

Fig. 4. Optical microscopic observation upon possible PHA accumulation of *Microvirgula aerodenitrificans* SH7b, *Rahnella aquatilis* DX2b, *Pseudomonas* sp. SH98, *Acinetobacter guillouiae* AX9, *Aeromonas hydrophila* NIU01, YTl1, KB23 and *Klebsiella pneumoniae* ZMd31 (black colors (i.e., red-circled portions) and purple colors denoted (+) and (–) response of possible strains, respectively).

contents of dye-decolorizing bacterial strains NIU01, KB23, YTl1, ZMd31 and 741 were found at ca. 19.35, 22.52, 24.48, 18.25 and 13.28 wt%, respectively (Chen *et al.*, 2011b).

Effects of Decolorized Intermediates- Aromatic Amines

To uncover the feasibility of PHA production during wastewater decolorization, this study also disclosed whether different model amines at various concentrations would stimulate or inhibit PHA-generating capabilities. Apparently, augmentation of amines seemed to provoke biotoxicity to impede gene expression for microbial growth and PHA generation (e.g., 2AP, 3ABA; Fig. 5). In contrast, PHA-generating capabilities were not affected by the presence of 3AP and 4AP in cultures. For *A. hydrophila* YTl1, no matter which amines were used (e.g., 3AP and 4AP) PHA contents evidently declined due to the provoked toxicity of amines (Chen, 2006, 2009). In particular, at 1000 mg/L 4AP inhibitory effects on microbial growth would be inevitable. In summary, PHA-generating capabilities of dye-decolorizing microbes would not be affected in 3AP, 4AP-containing cultures. In contrast, *A. hydrophila* NIU01 owned a higher capability to tolerate biotoxicity of amines (e.g., 3AP and 4AP) for cell growth as well as PHA production. In addition, Chen (2006, 2009) indicated that dye-decolorizer NIU01 could express higher resistance to toxicities of azo dye(s) and/or derived amine intermediates. As NIU01 was originally isolated from a selection pressure of highly toxic dye- reactive red 141 (Chen *et al.*, 2008), such a comparably higher tolerance to dyes and dye-derived intermediates was reasonably resulted. That was why at

higher concentrations of dye and amine (e.g., at 3800 mg/L RR141 and 1000 mg/L amines) *A. hydrophila* NIU01 still expressed higher bioactivity than *Pseudomonas luteola* did (Chen *et al.*, 2009). As a matter of fact, recent findings also revealed that strain NIU01 was a promising microorganism for simultaneous bioelectricity-generation and dye-decolorization (data not shown). These all suggested that a high resistance of strain NIU01 in dye-bearing environment seemed to be prerequisite to efficiently transcribe associated metabolically-functioning capabilities (e.g., PHA-production or power generation). That is, strain NIU01 seemed to be more appropriate to be used for materials and energy recycling and reuses in wastewater decolorization. Beyond dispute, this study offered a systematic framework for implementing appropriate response measures upon the feasibility of simultaneous energy and materials recycling using indigenous pollutant-degrading microbes.

CO_2 Reduction via Electrolysis

To inspect the feasibility of CO_2 via MEC, electrolysis was taking place in the cathode of two-chambered MFC with CO_2 purge. The amount of CO_2 collected was sufficient for the test in which only 500 mL of electrolyte (0.1 M $KHCO_3$) was used. The maximum formic acid production rate and the corresponding Faraday efficiency reached 63.1 mg/L/h and 62.2% (Fig. 6). That is, it is feasible to electro-

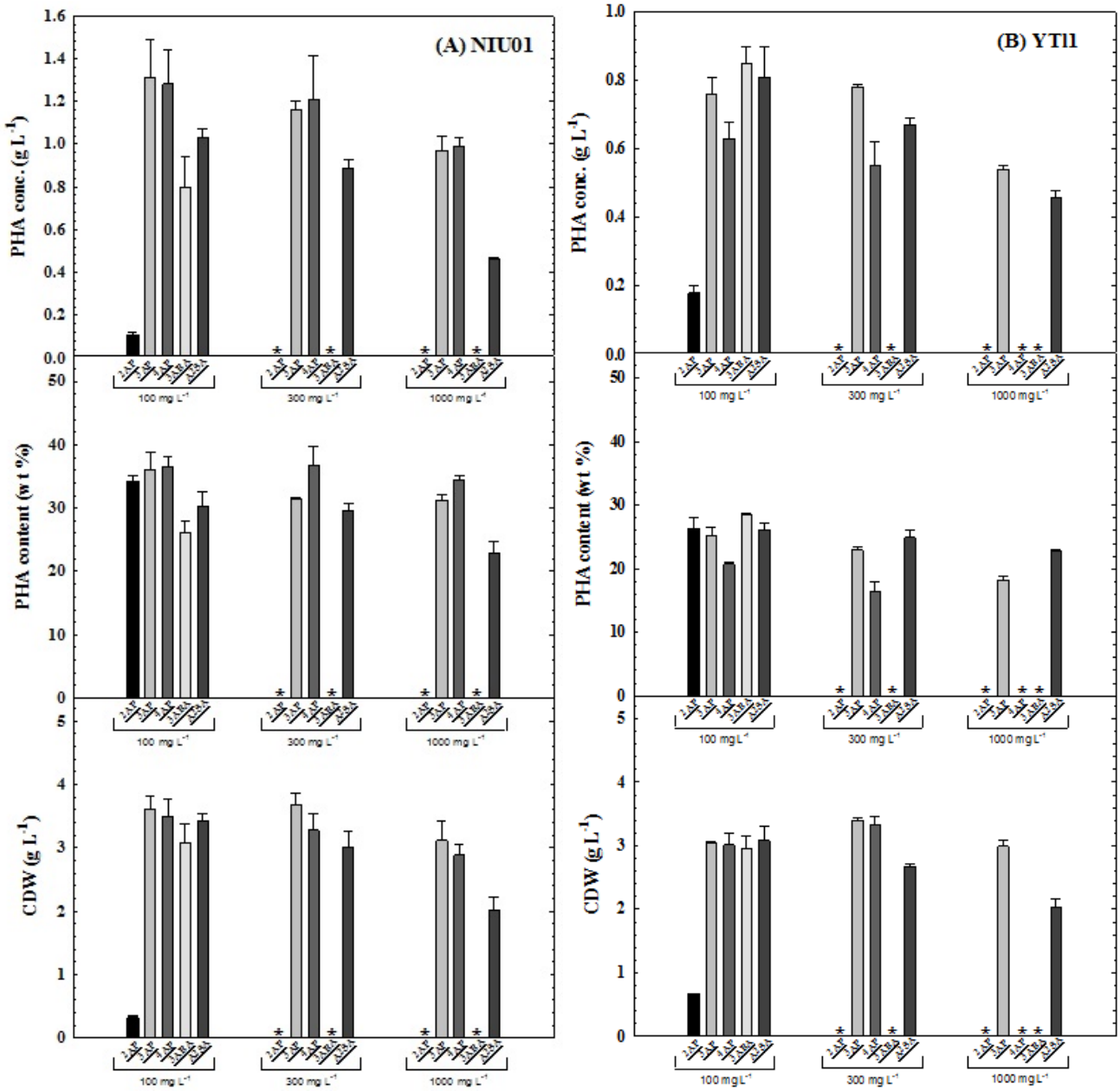

Fig. 5. Comparison on performance of cell growth and PHA production for dye-decolorizing strains (A) *Aeromonas hydrophila* NIU01, (B) *Aeromonas hydrophila* YTl1, (C) *Aeromonas hydrophila* KB23 and (D) *Aeromonas hydrophila* 741 at various concentrations of model amines (lauric acid = 10 g/L) (star symbol (*) denoted "negligible and not detectable").

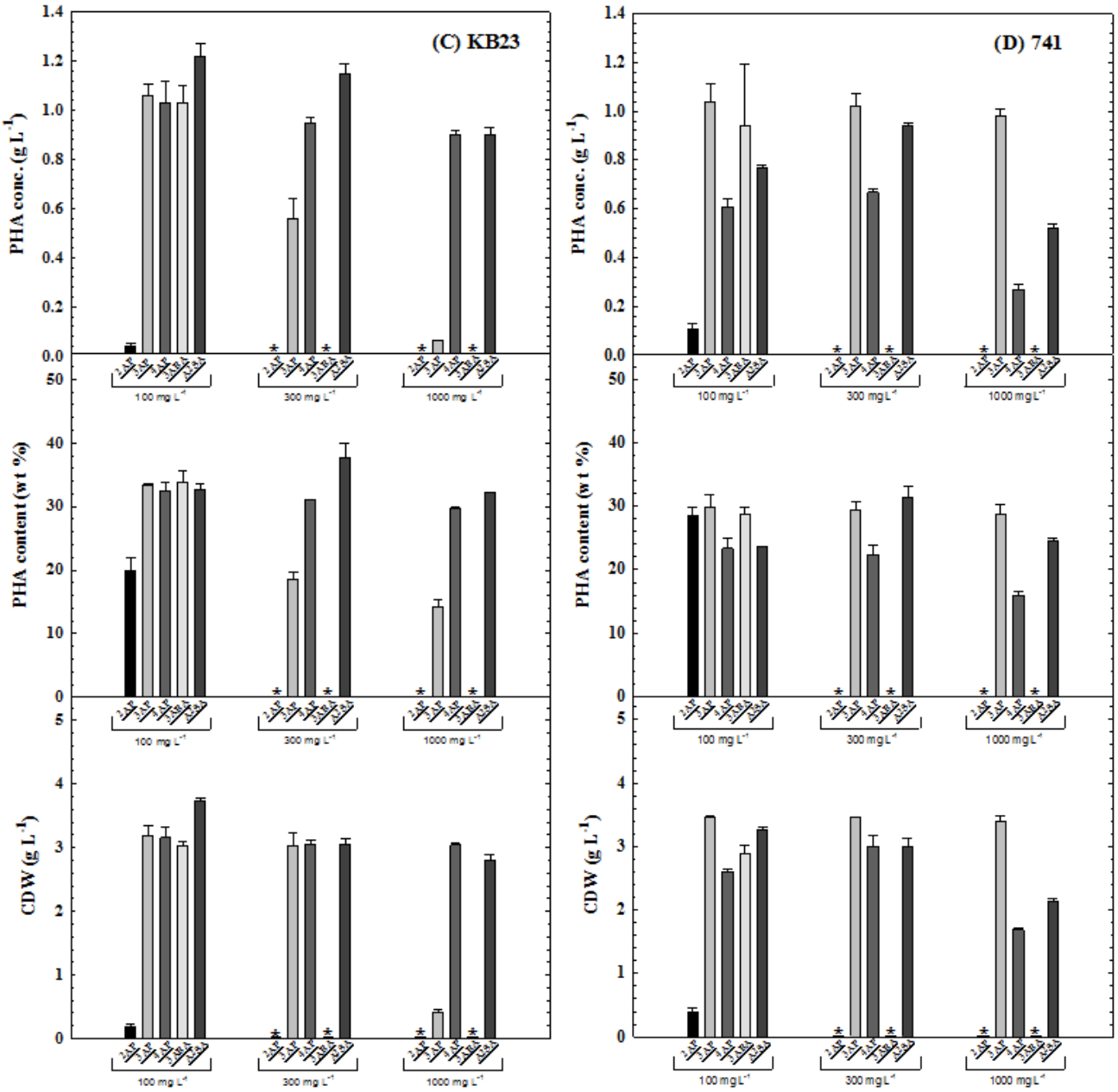

Fig. 5. *(continued).*

reduce the emitted CO_2 from MFC for CO_2 reduction. In the organic wastewater or waste-treatment processes based on MFCs, biological electricity would be generated while the organic matter was degraded to produce CO_2. If the device combined with electrolysis cell were optimized on a large scale and the MFCs could be fed with organic wastewater or wastes, the CO_2 generated during wastewater processing and waste treatment could be converted into valuable chemicals (e.g., formic acid) for CO_2 reduction.

CONCLUSIONS

As SBG & CR was technically viable for promising biodecolorizers (e.g., *Proteus hauseri*, *A. hydrophila*), capabilities of bioelectricity-generation and reductive decolorization were apparently non-growth associated. Gene expression of PHA production for NIU01 was very likely repressed in the presence of decolorized amine intermediate(s). In addition, PHA production should be separated from SBG & CR for considering industrial optimal operation of materials and energy recycling afterwards. That is, MFC applications for recycling and reusing the emitted CO_2 in wastewater (ca. 40–60% Faraday efficiency to formic acid) for the generation of bioenergy and materials were technically feasible.

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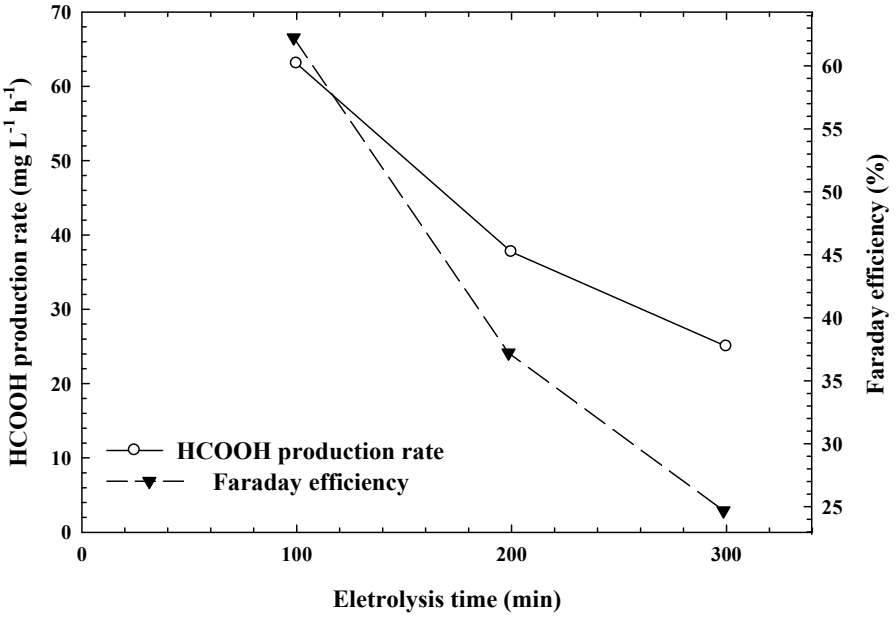

Fig. 6. Variations in the formic acid production rate and the Faraday efficiency with electrolysis time in CO_2 electrolysis process.

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DISCLAIMER

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