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**In-situ power generation and nutrients recovery from waste activated sludge -
long-term performance and system optimization**

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ABSTRACT

This study reports an integrated sludge treatment and resource recovery system that consisted of a sludge pre-treatment reactor and a microbial fuel cell (MFC) aiming for simultaneous energy and nutrients recovery. Nearly one year performance of the system in terms of power generation, nutrients removal efficiency, key dissolved organic matters (DOMs) transformation and microbial community change were investigated and reported. Volatile fatty acids (VFAs) and other soluble organic matters was produced from fermentation of waste activated sludge under thermophilic alkaline condition. Fermented liquor (FL) was used as feed for MFC, which produced maximum voltage of 0.477 V and power density of 8.07 W m⁻³. Humic-like substances (HSs) were removed by 48.27% within a 5 days cycle. The analysis on DOMs transformation revealed both high and low molecular weight protein and polysaccharides, and HSs were converted and contributed to power generation. Some unknown compounds in FL could enhance the power generation by 5.5 times more compared to pure VFA as feed. 90.59% of ammonium was removed with majority concentrated in the cathode. Phosphorus was removed and stored in the biomass in the form of polyphosphate (poly-P). Microbial community analysis indicated that *Exoelectrogenic* was the dominant community while *Aquamicrobium*, *Nitrosomonas*, *Achromobacter* and *Rhodocyclaceae* were also enriched in the anode. This work demonstrates that the integrated system can successfully remove and recover nutrients and generate power simultaneously with long-term stable performance.

Keywords: Sewage sludge; Fermented liquor; Microbial fuel cell; Nutrients; Long-term operation.

1. Introduction

Biological wastewater treatment plants discharge large amount of waste activated sludge (WAS). Its increasing quantity and complicate characteristics make it difficult and costly to deal with before releasing into the environment [1]. Conventional sludge treatment employs anaerobic digester to convert organic matter in the sludge to biogas and further to electricity or heat. However, the efficiency of such energy conversion is relatively low ($47.01 \text{ J g}^{-1} \text{ COD d}^{-1}$) [1, 2]. Thus, it would make more sense to recover higher value products and directly convert the energy source to reduce the efficiency loss.

Prior to resource recovery, sludge solubilization is a crucial step to transfer the compounds of interests into the soluble form. Thermophilic alkaline treatment has been reported to be a very efficient method to solubilize the sludge [3]. Short-chain fatty acid is a common and dominant product from WAS treatment. In addition, other organic matters, such as proteins (PNs), polysaccharides (PSs) and humic-like substances (HSs), are also commonly present in the fermented liquid (FL) [3, 4]. The concentration of phosphorus and ammonium is relatively high in the FL with efficient sludge treatment. Thus, organic matters and nutrients co-exist in the FL. In order to reduce the recovery efficiency loss, one-step or simultaneous resource recovery would be preferred compared to sequential recovery.

Microbial fuel cell (MFC) or microbial electrolysis cell (MEC) can be a useful process to directly convert the compounds in the FL to other resources, e.g. electrons and hydrogen [5-7]. Chen et al. [8] used the FL of WAS and kitchen waste as the feed for a MFC which generated 0.556 V of maximum voltage power. Xu et al. [9] reported the highest energy recovery efficiency of $169 \pm 0.1\%$ and the maximum soluble chemical oxygen demands (sCOD) removal efficiency of $51.4 \pm 0.6\%$ were

obtained with 0.8 V external applied voltage in a MEC system. Hence, the complex organics from the FL could be used as the carbon source for a MFC and converted to electrical energy directly [10-12].

Bioelectrochemical systems (BES) have also been used for nitrogen and phosphorus removal and/or recovery. For example, nitrification [13, 14] and denitrification [15] could occur in the anode or single chamber air cathode of MFC. The form of $\text{NH}_4^+/\text{NH}_3$ and the chemical status of phosphorus can be affected by the pH of electrolyte [16]. Therefore, they can be recovered at high pH conditions [17]. Although previous studies showed BES was a promising application in nitrogen and phosphate recovery, there still are several challenges to overcome. For example, phosphate precipitates are normally formed on the cathode electrode. The collection of those precipitates and replacement/regeneration of the cathode electrode will be a great challenge [18]. To date, most of the organics, nitrogen and phosphorus removal/recovery processes are carried out in separated systems due to the various requirements on pH values, redox potential, reaction locations, and external energy. This requires specific design of the system to target each individual compound, which likely increases the overall capital and operating costs.

This study aims to investigate the feasibility of simultaneous electricity generation and in-situ nutrients recovery. The long-term performance of the system under different operation phases was investigated. The specific objectives of study are to (i) evaluate the efficiency of energy and nutrients recovery, (ii) understand the conversion and transformation of the key dissolved organic matters (DOMs) during the operation, (iii) analyze the microbial communities and reveal the relationship between the performance and microbial activities.

2. Materials and methods

2.1 Sludge pretreatment

The feed sludge was mixed primary and secondary sludge (w/w: 1:1) from a local water reclamation plant. The fermentation of sludge was conducted in a 7 L fermenter with 3 L working volume at 55 °C and pH 9.9 for volatile fatty acids (VFAs) production. The detailed operation condition can be found in Chen et al. [3]. Prior to entering the anode of MFC, the FL was separated with the remaining solids and the pH was adjusted to 7.0 ± 0.2 with 1 M HCl. The characteristics of the FL are displayed in Table 1. Total organic carbon (TOC) was around $1247.75 \text{ mg L}^{-1}$, with 47.6% in the form of VFAs. High content of HSs was also found. Phosphorus concentration was much higher than that of ammonium due to the involvement of primary sludge and the high phosphorus content of the secondary sludge. The performance of the fermenter and characteristics of the FL were relatively stable during the operation period.

2.2 MFC reactor setup and operation

MFC reactors were constructed using two cubic chambers ($5.0 \times 5.0 \times 5.0 \text{ cm}^3$) separated by a cation exchange membrane (Ultrex CMI-7000, Membranes International, Ringwood, NJ, USA). Each chamber had a working volume of 28 mL. Carbon felt and carbon rod ($1.5 \times 1.5 \times 1.0 \text{ cm}^3$) were used as the anode and cathode electrodes respectively and connected with a titanium wire. All reactors were connected with an external resistant of 1000Ω . The potential and output voltage was collected by an automatic data collection system (PISO-813, Hongge Co., Taiwan).

The anode chambers of MFCs were seeded with the culture from a parent MFC reactor with acetate as feed [19-21]. The anolyte contained acetate was sparged with

N₂ for 15 min before feeding at the beginning of the cycle. The cathode was filled with potassium ferricyanide solution (100 mM K₃[Fe(CN)₆] and 20 mM PBS). After one month acclimation, power output from all the reactors was relatively stable. Two randomly selected reactors were operated under open circuit (OC) mode as control. The feed of another three reactors was changed to FL. It should be noted that there are many other complex substances in the FL besides VFA, e.g. HSs, which may enhance electrons transfer [22]. To understand if the complex organics can enhance power production, synthetic FL that only contained VFA but with the same COD concentration as real FL was used to act as a control in another reactor. Composition of VFAs (acetic, propionic, iso-Butyric and n-Butyric) followed the proportion distribution reported in Table 1, amounting to a total of 7852 mg COD L⁻¹.

The systems fed with FL were operated for almost 300 days in a cyclic feed-draw manner. Each cycle lasted for around 5 days and the new feed was fed into the anodes at the beginning of the new cycles. There were two operation stages during the whole operation period (stage I and II). In stage I, there were three phases (A, B, C), while stage II had multiple phases A and B. The acclimation phases were named phase A, during which the systems may encounter feed changes or biomass withdrawal. Phase B in all the stages represented the stable performed cycles. The phase C in stage I showed deteriorated performance of the system due to the overgrowth of biomass. Detailed performance and stage/phase categorization can be found in Fig. 5.

2.3 Analytical methods

Samples were collected in random cycles every week and centrifuged at 12000 g for 10 min. The supernatant was filtered through a 0.45 µm membrane filter for further analysis. Total chemical oxygen demand (tCOD), ammonium and phosphorus were measured according with the standard methods [23]. TOC was measured by a

TOC analyser (TOC-5000A, Shimadzu) using the combustion-infrared method. PNs and HSs were determined by the Lowry-Folin method [24].

VFAs were measured through a gas chromatograph (GC7890A, Agilent, USA) equipped with flame ionization detector. The composition of VFAs was analyzed with a DB-FFAP fused-silica capillary column. DOMs were characterized through the LC-OCD-OND system (DOC-LABOR, Karlsruhe, Germany). Detailed methods can be found in Chen et al. [3]. The quantification of biopolymers (high molecular weight (HMW) compounds of PNs and PSs), HSs, building blocks, low molecular weight (LMW) acid, LMW neutrals and hydrophilic organic carbon (HI DOC), was carried out through the software program (ChromCALC, DOC-LABOR, Karlsruhe, Germany) [25].

2.4 Visualization of poly-P granules and polyhydroxyalkanoates (PHAs)

An improved DAPI and Nile red staining procedure was used in this study to stain samples for poly-P and PHAs respectively [26, 27]. Detail methods could be found in supplementary information.

2.5 Electrochemical tests

The electrochemical tests of the MFCs were performed with a potentiostat (SP-150, BioLogic, Singapore) using the anode as a counter electrode and cathode as the working electrode. Before the linear sweep voltammetry measurement, the cells were in open circuital mode until the potential was stabilized and then tested from 0.0 V to the open circuital potential at a scan rate of 0.1 mV s^{-1} [19]. A three-electrode system, using cathode electrode as working electrode, an Ag/AgCl as the reference (195 mV vs. SHE) near the working electrode and a Pt filament as a counter electrode,

was set-up for CV test. A Pt filament was used to replace the cathode to avoid any possible chemical reaction in cathode.

2.6 DNA extraction and high-throughput sequencing

The biofilm from anode was collected in cycle 1 (day 5), 2 (day 10), 14 (day 70), 30 (day 150) and 45 (day 225) respectively for microbial community analysis. The DNA of the samples was extracted using the fast DNA extraction Kit for soil (MP Biomedicals, Singapore) and then stored at -20 °C before sequencing. Primer of 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGG-TATCTAAT-30') were used for high-throughput sequencing targeting V3 and V4 regions of rRNA genes [28]. The low-quality sequences and artificial replicate sequences were removed by quality control (QC) assessment (NovogeneAIT Genomics Singapore Pte Ltd). The QC passed sequences were clustered into operational taxonomic units (OTUs) at 97% similarity.

3. Results and discussion

3.1 Cyclic performance

The FL contained large amount of organic matter from the solubilisation of sludge. PNs, PSs, HSs, and VFAs were the dominant organic compounds. In one typical cycle, TOC concentration could be reduced from $1247.25 \pm 42.07 \text{ mg L}^{-1}$ to $252.75 \pm 5.3 \text{ mg L}^{-1}$ in 120 h with a removal efficiency of 79.74% (Fig. 1A). Correspondingly, soluble PNs and PSs degradation followed first-order kinetic reduction (Fig. S2). The degradation rate of PSs was higher than that of PNs, indicating that PSs was probably a preferred carbon source compared to PNs for power generation, and the final removal efficiency was higher than that of PNs (68.34%

v.s 48.26%) [29, 30]. Notwithstanding, the degradation rate of protein in the MFC reactors was still higher than that in a conventional anaerobic process ($41.27 \text{ mg L}^{-1} \text{ d}^{-1}$ v.s. $36.92 \text{ mg L}^{-1} \text{ d}^{-1}$) [31, 32].

It is believed that HSs are recalcitrant in a normal biological system and their degradation can only be carried out with chemical oxidation. For example, Katsumata et al. [33] reported the 10 mg L^{-1} HSs can be completely degraded by H_2O_2 and Fe(II) after 8 h reaction. In this study, nearly half of the HSs (800 mg L^{-1}) was reduced within a 120 h cycle (Fig. 1A). Degradation rate of HSs was calculated as $6.77 \text{ mg L}^{-1} \text{ h}^{-1}$ which was 4.41 times faster than the Photo-Fenton process. The dark brown color of FL was decolorized steeply during the operation time and became clear at the end of the cycle (Fig. S1).

VFAs were rapidly utilized in the anode of MFC (Fig. 1B). The degradability and conversion of different VFAs were clearly dependent on chain length of the VFAs [6]. The higher removal rates were found with the simpler VFAs structures. The degradability of the VFAs was in the following order: Acetic acid > Propionic acid > n-Butyric acid > iso-Butyric acid. Within a cycle, 92.5% of acetic acid, 79.3% of propionic acid, 72.6% of n-butyric acid and 36.1% of iso-butyric acid could be converted.

It should be noted that the systems also achieved good performance in terms of in-situ nutrients separation and recovery (Fig. 1A). 80-90% of ammonium in the FL was recovered in the cathode chamber, which was due to the function of cationic exchange membrane [34]. This enabled the concentration and recovery of ammonium in the cathode. It is interesting to note that around 42% of phosphorus was also removed in every cycle. It was proved that there was no precipitation in the anode chamber ($\text{pH } 6.7 \pm 0.2$). Instead, poly-P granules and PHAs were found in the

biomass discharged in the effluent (Fig. 2A). The presence of poly-P granules indicated the existence of polyphosphate-accumulating organisms in the reactors, which will be discussed further later. Along with the accumulation of phosphate, microbial accumulation of PHAs was also observed in the biomass (Fig. 2B). Early studies indicated that ATP from poly-P degradation was the primary source of energy used by polyphosphate-accumulating organisms for substrate uptake, PHAs synthesis and cell maintenance under anaerobic conditions [35]. The observation of PHAs and poly-P evidenced the active metabolism of polyphosphate-accumulating organisms in the system. It is suggested the polyphosphate-accumulating organisms removed phosphate and stored it as poly-P in the cell and possibly used it as the energy source for the synthesis of PHAs. Yet, further research is needed to assess the accurate pathway of biological phosphate removal in the MFC.

In a typical cycle, the reactor generated highest voltage of 0.477 V and average of 0.388 V (Fig. 3A). The anode achieved average anode potential of -0.185 V which was close to that from a reactor with acetate as feed (-0.23 V, 1 g L⁻¹ of sodium acetate). The result demonstrates acetate enriched exoelectrogenic culture have the capability to utilize complex organics to generate electrons. Increase of anode potential after 30 h was observed, which was corresponding to the rapid degradation of VFAs (Fig. 1B) and other organic matters, e.g. PSs, during the first 24 h (Fig. 1A).

As shown in Fig. 3B, the detected DOMs were mainly divided into HI DOC, biopolymers (>20 kDa, including HMW PSs, HMW PNs and amino-sugars), HSs, building blocks (300-500 Da), LMW acid (<350 Da) and LMW neutrals (<350 Da, including alcohols, aldehydes, ketones and mono-oligosaccharides) in terms of respective properties and molecular weights. The results present the detailed organic matter transformation within a cycle. The HI DOC (the sum of building blocks, LMW

neutrals, LMW acids, HMW protein and HMW polysaccharide) decreased by 67.63% from 1139.87 mg L⁻¹ in 120 h (Table 2). The removal of hydrophilic organic compounds was consistent with TOC results (Fig. 1A). However, biopolymers which include HMW PNs and HMW PSs exhibited a decreasing trend at the beginning of the cycle and then increased at the later stage (Fig. 3B). The degradation of HMW PNs was also only observed during the first 48 h of power generation (Fig. 3B). As shown in Table 2, the HMW PNs was removed by 46.7% in the first 48 h and then gradually increased to original level at the end of the cycle. Meanwhile, the HMW PSs followed the similar trend (Table 2). 94.9% removal of LMW acids was observed, however, a slight increase of LMW acid was also detected at the end of the cycle. It suggests that most organics were consumed in the first 30 h for power generation and biomass growth, while the hydrolysis and acidogenesis continued in the anode that further released organic matters which may be less biodegradable (Fig. 3A). Thus, the power generation was slowed down although the organic matter was still available.

Previous studies reported that VFAs used as the organic substrate and electron donor could produce stable power in MFC [5, 6]. To investigate the influence of other compounds in FL on power generation, the synthetic FL with the same COD concentration was prepared using VFAs. Interestingly, the maximum power density obtained with the FL as the feed was 5.5 times than that with VFAs feed although VFAs should be the preferred carbon source (Fig. 4A). Concomitant with power density, the open circuit potential with FL feed was also higher. The anode with FL fed exhibited obvious oxidation peak at 0.75 V (Fig. 4B), whereas anode with VFAs fed did not show electrochemical signal. The results clearly suggest that certain organic compounds in the FL significantly contributed to the power generation. It has been reported that the HSs in the FL can alter the propensity of reactor performance

and play an important role in electron transfer efficiency as electron mediator [22, 36]. Given the high concentration of HSs in the FL fed reactors, it is likely that HSs may enhance the electron transfer and the overall power generation performance.

3.2 Long term performance of the system

The potential profile of anode and cathode during the 300 days operation can be found in Fig. 5. Phases A represents the acclimation period of the system when the system for the first time received the fermented liquid or the period after the biomass withdrawal. Phases B represents the system performance at the stable stage. Phase C shows system deterioration by the overgrowth of biomass. From the potential profile, the whole operation period can be separated into 2 stages, where stage 1 (first phases A, B, C) experienced acclimation, stabilization and deterioration, stage 2 (second, third and fourth phases A, B) had acclimation and stabilization phases.

During all phases A, anode potential was relatively low (-0.27 V) while high overall potential of 0.537 V was observed due to the change of feed or biomass amount. Generally, phases A lasted for two cycles, and then the reactor entered the stable power generation phases B, which produced $0.424 - 0.477$ V voltage. It is noteworthy that the performance in phases B was not as good as phases A in terms of organic matters removal as well as power generation. This is likely due to the microbial community shifts which will be discussed later. The phases B generally sustained for 10 to 12 cycles before the voltage decreased (Fig. 5).

In the stage I, it was observed that large amount of biomass was accumulated in the anode as well as in the effluent. Although the biomass would be discharged together with the effluent, the discharge rate seemed to be slower than the growth rate. During entire Phase C, the system was unable to resume its performance as Phase A or B. At the end of Phase C, 20% of the biomass was withdrawn manually from anode,

and the performance was immediately resumed in the stage 2 Phase A (Fig. 5). After 2 cycles operation, the system was stabilized in Phase B. In the following stage II, biomass was withdrawn regularly at the end of each Phase B as soon as the power generation was reduced. This confirms that amount of biomass should be controlled and regularly removed from the system to ensure the system stable performance.

The removal efficiencies of organic compounds coincided with the power generation performance. Table 3 displays the major organic matter removal efficiencies during the operation. The average TOC removal efficiency was the highest in Phases A (70%), followed by Phases B and then C. PNs, PSs, HSs, VFAs had similar degradation trends as TOC. Phosphate removal, on the other hand, showed better performance at the latter phase. As chemical phosphate removal was not observed, it is hypothesized biological P removal occurred in the system. Hence, the system would need to take some time to build up the P-removal microbial community. Ammonium removal was relatively consistent other than Phase C where the potential was low.

Conventional anaerobic sludge digestion produces biogas from the organic matters in WAS. The produced biogas cannot be in-situ utilized and required to convert to other forms of energy source, e.g. heat and electricity. The conversion process may have substantial energy loss and it has been estimated that only 30–35% of the energy in methane can be converted to electricity [2]. Marti et al. [1] reported a digestion system with specific biogas production rate of $0.94 \pm 0.27 \text{ L g}^{-1} \text{ TVS}_{\text{rem}}$ that contained $64 \pm 1\%$ of methane. The system had COD removal efficiency of $60 \pm 9\%$ from $40650 \text{ mg COD L}^{-1}$. The 135 L digester was operated with solids retention time of 20 days. Based on the heat value of methane (35.8 MJ m^{-3}), the totally energy conversion from methane to electricity was estimated to be $55.72 \text{ J g}^{-1} \text{ COD d}^{-1}$.

Based on the performance of the MFC in this study, the system fed with FL was able to produce 167.4 C electrons and average of 0.388 V voltage for 120 hours (Fig. 3A) which was 224.4 J g⁻¹ COD d⁻¹ equivalent electricity. Hence, compared with the conventional anaerobic digestion, the energy efficiency of MFC was 19.82 times higher.

The thermophilic anaerobic digestion can improve the methane production. With 55 °C thermal alkaline condition, Chen et al. [3] reported the methane yield reached 165.80 mL g⁻¹ VSS. The energy conversion from methane to electricity could be 1403 J g⁻¹ COD d⁻¹, which was much higher than normal anaerobic process (based on 35% conversion rate). However, thermophilic anaerobic digestion requires high energy input to achieve sufficient turnover. The thermal condition almost consumed 126 kJ L⁻¹ energy to maintain the high temperature (from 25 °C to 55 °C). Within a 5-day cycle (phase B), MFC converted almost 45.02% carbon source to electricity (based on anodic columbic efficiency) while a 6-day anaerobic digestion process with in-situ pretreatment only convert 20.3% of sCOD to methane (35% of methane converts to electricity) [3, 37]. Compared with these studies, the conversion of organics to electricity of MFC was 6.63 times more efficient than the thermophilic anaerobic process.

3.3 Microbial community and diversity

Fig. 6 describes microbial community in different cycles. At phylum level, *Proteobacteria* was the most abundant (84%), followed by *Bacteroidetes* (8%) and *Firmicutes* (0.8%) in the first cycle (Fig. 6A). It has been reported that *Proteobacteria* is the important microorganism in anaerobic hydrolysis and most of highly efficient electrogenesis belong to *Proteobacteria* [38, 39]. It was noted *Proteobacteria* was reduced from 83% to 34% due to the change of feed from acetate acid to FL (Fig. 6A).

In contrast, *Bacteroidetes* and *Firmicutes* increased to 27% and 22%, respectively. The results suggest although *Proteobacteria* decreased along the operation time, it still occupied the major phyla which contributed to power generation (Fig. 5). *Firmicutes* is well known to produce proteases, cellulases, lipases, and other extracellular enzymes that are closely involved in organic compounds degradation [40]. *Bacteroidetes* was always notable in various sewage treatment processes for metabolizing a variety of complex carbohydrates [41]. Clearly, the long-term operation with FL as feed improved the abundance of population for organic compounds degradation. Such degradation may continue in the system even the power generation was slowed down (Fig. 5, Table 3).

The presence of *Alphaproteobacteria* were reported to be beneficial for the degradation of carbohydrate and electrons generation [42, 43]. After the first cycle, *Alphaproteobacteria* decreased from the high abundance of 56% to 16% and remained stable, due to the adaptation of electrogenesis to the FL feed. Nevertheless, *Bacteroidia* increased from 7% to 27% indicating the improvement of microbial degradation ability for protein and polysaccharide [44].

At genus level, *Rhodopseudomonas* and *Geobacter*, as exoelectrogenic bacteria, displayed high abundance of 42% and 19% in the first cycle and sharply dropped to 7% and 1% respectively once the feed was changed (Fig. 6B). However, other exoelectrogenic bacteria such as *Desulfovibrio* was not affected by the change of feed and stayed with the 2% abundance. The ammonia oxidizing bacterium *Nitrosomonas* was found on the anode electrode with 5% abundance. Meantime, anaerobic denitrification species *Alcaligenes* was found to be 8% in the anode. The presence of above two types of nitrogen converting bacteria explains the ammonium loss from the

anode chamber due to nitrification and denitrification. But most of the ammonium was lost to cathode chamber across through the membrane [15].

Notably, *Rhodocyclaceae* included *unidentified_Rhodocyclaceae* (0.2%), *Thauera* (0.2%), *Azovibrio* (0.4%) and *Dechlorobacter* (0.002%) were also found in the anode. These species have been reported as the highly efficient phosphate remover [40, 41]. Even though *Rhodocyclaceae* was not the dominant population, its function for phosphate removal and polyphosphate accumulation was observed (Fig. 1A and Fig. 2). *Aquamicrobium*, which is a chemoheterotrophic microorganism, was the subdominant population in this system. Organic carbon and nitrogen may be used as carbon source and nitrogen source for their growth and reproduction, as such they may involve in organic matter and nitrogen removal as well [45].

The microbial community results suggest that the exoelectrogenic microbes remained as the dominant community and played an important role for extracting and transferring electrons from organic substrates in FL. Meanwhile, large number of functional microorganisms that were favored for ammonium and phosphate removal were also enriched with the FL as feed. At this stage, it is hypothesized that phosphorus stored in the biomass can be released and recovered in an off-line set-up since the biomass was withdrawn from the system regularly. In order to develop a matured nutrients removal/recovery system, further efforts should be made. First of all, captured phosphorus should be recovered. Phosphorus can be released and concentrated in-situ by forcing the phosphorus released back into solution via shock conditions. Secondly, the lowest concentration level of phosphorus that the system can achieve should be also investigated. Finally, ammonium in the cathode can be recovered using the air stripping method. The role of the organic matters that can potentially improve the power generation will be investigated further. In summary,

this integrated system described feasibility of nutrients removal/recovery in a MFC. However, to further estimate the potential economics of the proposed technology, the experiment needs to be performed in a larger scale system.

4. Conclusions

This study, for the first time, reports an integrated system for energy and nutrients recovery from the pretreated waste sludge. Long-term performance of the system, key DOMs transformation and microbial community change were closely monitored. The main conclusions of this study are as follows.

- i. The system can achieve the maximum voltage of 0.477 V and power density of 8.07 W m^{-3} , which is comparable as the systems with acetate acid as feed.
- ii. Both HMW and LMW PNPs and PSSs, and HSs were converted and contributed to power generation. Certain unknown compounds in FL could enhance the power generation by 5.5 times more.
- iii. A total of 90.59% ammonium was removed with majority concentrated in the cathode. Phosphorus was removed and stored in the biomass in the form of poly-P.
- iv. Exoelectrogenic was the dominant community while *Aquamicrobium*, *Nitrosomonas*, *Achromobacter* and *Rhodocyclaceae* were also enriched in the anode with the FL as feed.

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Figure captions

Fig. 1. The performance of organic matter and nutrients removal in the anode of MFC as the function of operation time in one typical cycle (A) concentration of TOC, HSs, PNs, PSs, ammonium and phosphate; (B) concentration of VFAs.

Fig. 2. (A) DAPI staining of polyphosphate granules in the biomass, (B) Nile red stained PHA present in the biomass from the anode of MFC

Fig. 3. (A) Anode and cathode potential change; (B) LC-OCD chromatogram as a function of operation time in one cycle

Fig. 4. (A) Polarization and power curves of FL and VFA fed reactors; (B) Cyclic voltammetry tests carried out on the FL and VFA fed reactors.

Fig. 5. The potential profile of anode and cathode of FL as feed in MFC (phase A, B and C)

Fig. 6. Distribution of bacterial level at phylum (A) and heat map of genera (B). Taxa represented occurrence at >1% frequency in at least one sample under different operation cycles (1, 2, 14, 30 and 45)

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Table 1.

Characteristics of FL

Parameter	Units	FL value
pH		7.0 ± 0.2
Total organic carbon (TOC)	g L^{-1}	1.25 ± 0.04
Ammonium	mg L^{-1}	277.01 ± 69.79
Humic like substance (HSs)	g L^{-1}	1.68 ± 0.14
Soluble Proteins (PNs)	mg L^{-1}	427.63 ± 18.48
Soluble polysaccharide (PSs)	mg L^{-1}	219.19 ± 14.79
Phosphate	mg L^{-1}	695.61 ± 35.17
Acetic	mM	17.18 ± 0.63
Propionic	mM	4.05 ± 0.24
iso-Butyric	mM	0.97 ± 0.02
n-Butyric	mM	1.75 ± 0.25

Table 2.

Quantification of dissolved organic matters (mg L⁻¹)

Operation time	HI DOC	HMW PSs	HMW PNs	Bio polymers	Building blocks	LMW neutrals	LMW acids
Feed	1139.87	76.16	114.23	190.39	422.35	178.9	549.56
24 h	508.78	79.15	89.26	168.41	318.28	99.66	153.6
48 h	535.14	60.93	60.93	121.86	301.41	80.61	109.14
72 h	378.38	76.44	62.54	138.98	293.95	53.93	71.24
96 h	568.92	105	79.21	184.21	275.13	83.13	9.75
120 h	368.92	100.13	112.91	213.04	218.01	40.83	28.00

Table 3

Average removal efficiency of nutrients and some key organic matters in different phases

Phase	Removal efficiency (%)							
	TOC	Phosphate	Ammonium	Acetic	Propionic	HSs	PNs	PSs
A	70.10 ± 6.87	49.00 ± 0.55	85.28 ± 8.21	89.24 ± 3.05	57.15 ± 23.28	60.46 ± 4.31	55.56 ± 12.15	72.51 ± 12.87
B	48.60 ± 17.19	57.71 ± 6.67	87.36 ± 4.93	78.95 ± 19.46	49.50 ± 16.14	27.55 ± 40.77	40.17 ± 17.26	62.12 ± 15.23
C	21.20 ± 3.33	61.56 ± 9.48	69.21 ± 3.62	60.49 ± 8.44	8.12 ± 10.64	24.31 ± 4.02	6.69 ± 2.24	14.17 ± 9.85

Highlights:

1. Maximum voltage of 0.477 V and power density of 8.07 W m^{-3} was produced
2. Humic substances were removed by 48.27%
3. 90.59% of ammonium was removed with majority concentrated in the cathode.
4. Phosphorus was stored in bacteria cells in the form of poly-P
5. Electrogens was dominant community with FL as feed