Bioresource Technology 102 (2011) 10221–10229

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Microbial fuel cell based biosensor for *in situ* monitoring of anaerobic digestion process

Zhidan Liu [a,b,c], Jing Liu [a,*], Songping Zhang [b], Xin-Hui Xing [c], Zhiguo Su [b]

[a] Department of Biotechnology, Lund University, Lund SE-221 00, Sweden
[b] National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, Beijing 100190, China
[c] Department of Chemical Engineering, Tsinghua University, Beijing 100084, China

ARTICLE INFO

Article history:
Received 13 March 2011
Received in revised form 10 August 2011
Accepted 11 August 2011
Available online 1 September 2011

Keywords:
Anaerobic digestion
Biogas
Microbial fuel cell (MFC)
Microbial biosensor
Electroactive biofilm

ABSTRACT

A wall-jet microbial fuel cell (MFC) was developed for the monitoring of anaerobic digestion (AD). This biofilm based MFC biosensor had a character of being portable, short hydraulic retention time (HRT) for sample flow through and convenient for continuous operation. The MFC was installed in the recirculation loop of an upflow anaerobic fixed-bed (UAFB) reactor in bench-scale where pH of the fermentation broth and biogas flow were monitored in real time. External disturbances to the AD were added on purpose by changing feedstock concentration, as well as process configuration. MFC signals had good correlations with online measurements (i.e. pH, gas flow rate) and offline analysis (i.e. COD) over 6-month operation. These results suggest that the MFC signal can reflect the dynamic variation of AD and can potentially be a valuable tool for monitoring and control of bioprocess.

1. Introduction

Anaerobic digestion (AD) is an efficient and versatile biological approach for organic wastewater/waste treatment, due to its advantages, such as production of bioenergy, conservation of ammonia type fertilizer, and low sludge yield (van Lier et al., 2001). AD, which organic matter is converted into biogas, is a complex process typically consisting of four major steps: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Gerardi, 2003). As a consequence, various microbial communities with different physiological characters are involved. The interdependence of the different microbial communities can therefore cause the instability of AD and difficulty in the process control by traditional method (Gujer and Zehnder, 1983). In fact, many full-scale biogas plants are operated far below the maximum design capacity in order to keep a big safety margin and eliminate the risk of system overload. This is, however, performed at the cost of process efficiency, degree of capital investment, and operational cost as a whole (Liu et al., 2003). Lack of reliable process dynamic information and robust on-line sensors is one of big problems for the operation of AD system. To achieve more stable and efficient operation, there are increasing demands on advanced monitoring and control of AD system using reliable sensors (Liu, 2003; Spanjers and van Lier, 2006; Ward et al., 2008; Boe et al., 2010). Although various process parameters have investigated to be measured on-line, such as BOD monitoring based on biosensor (Liu et al., 2004b), VFA monitoring based on headspace GC (Boe et al., 2007) and substrate quality analysis based on spectroscopy principle (Jacobi et al., 2011), these sensors are still not commercially available for industrial application. Based on process information provided by sensors, knowledge-based (Scherer et al., 2009; Vaiopoulou et al., 2011) and model-based (Morel et al., 2007; Méndez-Acosta et al., 2010) control strategies had been developed via the control of loading rate, temperature or pH. Among the process parameters, VFAs, particularly acetate and propionate, as the key intermediates of AD system, are suggested as an important process indicator due to the reason that system imbalance is accompanied with accumulation of VFAs (Spanjers and van Lier, 2006; Boe et al., 2007, 2010; Ward et al., 2008; Méndez-Acosta et al., 2010).

Microbial fuel cell (MFC) is a device where electrical signals are directly conducted from chemical energy stored in organic matter (e.g. acetate and glucose) via microbial catalysis (Potter, 1911; Logan et al., 2006; Pant et al., 2010). Due to its novel transform mechanism, MFC has received considerable interests and a number of potential applications based on its concept have been proposed, particularly in environmental and energy field (Bond et al., 2002; Liu et al., 2004c; Rabaey and Verstraete, 2005; Moon et al., 2006; Logan, 2008; Virdis et al., 2010). MFC, in principle, could also be considered as a biosensor, where microorganisms in the anode compartment act as biological recognition element whereas electrodes and proton exchange membrane (optional) serve as a transducer (Kumlanghan et al., 2007). In comparison with other existing sensors (i.e. pH, temperature, and gas flow meter) in AD,

* Corresponding author. Tel.: +46 46 2228347; fax: +46 46 2224713.
E-mail address: Jing.Liu@biotek.lu.se (J. Liu).

0960-8524/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2011.08.053

microorganisms are involved as bio-catalyst in the measurement of target substrate. MFC biosensor works like a mini-bioreactor with high selectivity (Spanjers and van Lier, 2006). MFC based biosensor was first reported in 1977 (Karube et al., 1977), where pure culture *Clostridium butyricum* was positioned as sensing element to transfer electrons using hydrogen as electron acceptor. Compared with other biosensors, MFC biosensor using electroactive biofilms as sensing element has the advantage of long-term stability (Gil et al., 2003; Chang et al., 2004), which prolongs the lifetime of sensing element and minimize the replacement of sensing elements. Furthermore, biodegradable organic matters, such as acetate could be directly converted to electricity via MFC, and MFC itself is an integration of signal generator and transducer, which reduces the cost for external transducers. Due to these above characters, MFC biosensor has received attractive attentions in the last few years (Chang et al., 2004; Tront et al., 2008; Di Lorenzo et al., 2009). Kim and Chang group (Chang et al., 2004) firstly used MFC for BOD analysis with the linear limit up to 100 mg ml^{-1}. The accuracy was further enhanced by the inhibitions of other competitive electron acceptors (Chang et al., 2005). A packed bed MFC biosensor using carbon cloth anode was developed to further increase the linear range to 350 mg BOD ml^{-1} (Di Lorenzo et al., 2009). Besides the mixed culture used for sensing element, pure culture *Geobacter sulfurreducens* was employed in a packed bed MFC (Tront et al., 2008), the system was used for the measurement of acetate concentration and microbial respiration.

All these studies focus on the performance of sensor itself. In this study, we focus our study on design of MFC sensor and its application in AD process. It is known that the process information from the liquid phase, particularly VFAs and other fermentation intermediates, plays a great role in the operation of AD system. Therefore it will be of great interest to apply the concept of state-of-the-art MFC based sensor to the monitoring of liquid-phase process information of the AD system.

In a previous study, we have designed a MFC based H-type biosensor for rapid estimation of content of organic matter, where bio-receptors were renewed for each analysis by the replacement of the anaerobic consortium (Kumlanghan et al., 2007). In that case, the consortium in the suspended solution served as biocatalyst. The purpose of running an anaerobic digester was to provide the biocatalyst periodically. A glucose solution was used as fuel by external dosing to MFC anode chamber.

Due to properties of long term stability for biofilm system, distinguished from our previous work (Kumlanghan et al., 2007), here we used mature electroactive biofilms other than suspended consortium as biocatalyst, and immobilized it into a new designed wall-jet MFC sensor for the monitoring of AD process by the real time detection of acetate based intermediates. The AD process was simulated by establishing an UAFB system fed with artificial wastewater. The MFC sensor was placed *in situ* in the recirculation loop of an upflow anaerobic fixed-bed (UAFB) reactor. External disturbances to the AD were added on purpose by changing feedstock concentration, as well as process configuration. The purposes of this study are: (1) to design a portable, robust, and cost-efficient MFC sensor and assess its feasibility for AD monitoring; (2) to compare MFC based sensor for the reflection of the dynamic variation of the AD process with other on-line and off-line conventional process parameters, i.e. pH, biogas flow, VFAs, etc.; (3) to develop a new process viable for on-line monitoring and control of AD process.

2. Methods

2.1. MFC sensor design

Fig. 1 showed the structure of wall-jet type MFC, which consisted of cathodic (A-part) and anodic compartment (B-part). A cathodic electrode roll (a), a plastic net (f), a proton exchange membrane (g) and a plastic net (f) were sandwiched between these two parts by two pairs of screws in opposite sides. The graphite roll (Spektralkolstav, ISOAB, Germany) served as electrodes. The anodic electrode had an effective area of 6.28 cm^2, whereas the cathodic roll contained holes to keep the ion transfer smoothly from catholyte to proton exchange membrane and had an effective area of 5.78 cm^2. New electrodes were pretreated by soaking in 1 mol l^{-1} HCl to eliminate possible metal ion contamination. The membrane (inner diameter: 2 cm, Nafion TM117, Dupont Co., Wilmington, USA) was incubated in 1.45 g l^{-1} NaCl for 2 h prior to use. Each chamber has a media volume of approximately 1.6 ml after being equipped with an electrode.

2.2. Cultivation of electroactive biofilms

Electroactive biofilms was collected from an H-type MFC as previously described (Liu et al., 2009). The MFC was inoculated using anaerobic sludge and had been running for more than 1 year. The feeding substrate is a synthetic wastewater based on a receipt from OECD (Organization for Economic Cooperation and Development, 2001): 16.0 g l^{-1} peptone, 11.0 g l^{-1} meat extract, 3.0 g l^{-1} urea, 3.6 g l^{-1} glucose, 2.9 g l^{-1} NaCl, 0.4 g l^{-1} $CaCl_2$, 0.2 g l^{-1} $MgSO_4{\cdot}2H_2O$, 2.8 g l^{-1} K_2HPO_4, 10 ml l^{-1} trace vitamin solution and 10 ml l^{-1} trace mineral solution and tap water (pH 7.0). This stock solution had a total COD of 35 ± 0.25 g l^{-1} and was diluted with tap water for desired experiment. The collected biofilms were transferred to MFC biosensor and served as bio-receptors.

2.3. Experimental design

2.3.1. Optimization procedure of MFC biosensor

In order to maximize the MFC signal, the optimization of working parameters (flow rate, electrolyte, substrate concentration, and external resistor) was carried out in order.

Six different flow rates i.e. 1.07, 2.48, 5.12, 7.74, 15.55, and 23.11 ml min^{-1} were firstly tested for the anodic compartment. During the optimization, the operational conditions of MFC were 7.28 ml min^{-1}as the cathodic flow rate, phosphate buffer (60 mM) with NaCl (50 mM) as the electrolyte for anodic and cathodic chamber, 200 mg l^{-1} OECD wastewater as substrate, and 800 Ω as the external resistor. Six different flow rates i.e. 1.14, 2.53, 7.28, 14.35, and 21.44 ml min^{-1} were tested for the cathodic compartment while keeping the anodic flow rate under optimized condition. The electrolyte, substrate and resistor were the same as that for the optimization of anodic flow rate. The tested

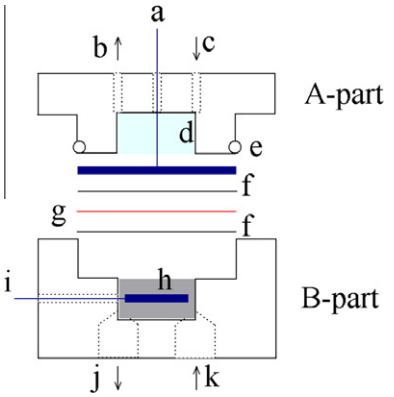

Fig. 1. Diagram of wall-jet flow cell type MFCs. (a) cathodic electrode, (b) cathodic effluent, (c) cathodic influent, (d) cathodic compartment, (e) O-ring, (f) plastic net, (g) proton exchange membrane, (h) anodic compartment, (i) anodic electrode, (j) anodic effluent, and (k) anodic influent.

electrolytes include phosphate buffer (60 mM), phosphate buffer (60 mM) with NaCl (50 mM), HCl solution (1 mM), distilled water, and NaOH (1 mM). During the optimization, substrate was 200 mg l^{-1} OECD wastewater; the external resistor was 800 Ω while the flow rate was kept at the optimal value.

OECD wastewater with concentrations ranging from 0 to 8000 mg COD l^{-1} was tested to investigate the effect of substrate concentration. During the experiment, the external resistor was 800 Ω, while the flow rate and the electrolyte were kept at their optimal value. In order to optimize the resistor, external resistor was designed to be adjustable in order to plot power curves at different resistor loadings. This was performed by simply using a resistor regulator from 11 to 6000 Ω. The main reason for optimizing the external resistor is to minimize the ohmic losses of MFC sensor system resulted from for example low ionic conductivity of MFC electrolyte During the experiment, substrate was 200 mg l^{-1} OECD wastewater, while the flow rate and the electrolyte were kept at their optimal value.

2.3.2. Monitoring of AD

An upflow anaerobic fixed bed (UAFB) reactor was used here as a model AD system according to Liu et al. (2003). This reactor has been running for over 3 years. To create an adapted stable microbial community of AD for monitoring, UAFB was fed with OECD wastewater with increased loading. A stable state of UAFB with a organic loading rate (OLR) of 3.5–4 g COD l^{-1} d^{-1} was characterized by following parameters: methane content, 70%; gas production rate: 1.5–2.0 l d^{-1}, partial alkalinity: 2500 mg $CaCO_3$ l^{-1}, total alkalinity: 3000–3500 mg $CaCO_3$ l^{-1}, pH: 7.2–7.5.

As illustrated in Fig. 2, the reactor system consists of a cylindrical column made from jacketed glass, and a gas–liquid separator. The reactor was kept at 36.5 ± 0.2 °C by heated water bath. The carrier materials for biofilm are polyethylene tubes (10 mm long and 8 mm in diameter) with internal longitudinal walls, which formed a cross inside the carrier and longitudinal fins on the outside to form a large specific area (0.39 m^2 l^{-1}). A working volume of around 1 l remained after filling the reactor with plastic carriers and attached biomass. The leachate and biogas flowed from an up outlet of the reactor and were separated in the gas–liquid separator.

The biogas flow was then monitored by an in-house developed gas flow meter (Liu et al., 2004c). An external recirculation was created for both coupling to the liquid–gas separation unit and serving as sampling ports for the MFC sensor and pH (Fig. 2). Two suits of MFCs were employed: one contained an anode with immobilized electroactive biofilms (biofilm MFC). The biofilm consortium was transferred from an H-type MFC fed with OECD wastewater over a 1-year period (Liu et al., 2009). The biofilm transfer was performed by scraping the attached bacteria from the anode surface of H-type MFC; another MFC served as control with the blank anode without any pre-immobilized bacteria (control MFC). Dissolved oxygen (DO) meter (Inolab Oxi level 2, Wissenschaftlich Technische Wellcstäffen, Germany) was also installed in cathodic compartment of MFCs to monitor the dissolved oxygen level of catholyte. Recirculation liquid flow rate was maintained at 17.6 l h^{-1} and hydraulic retention time (HRT) of UAFB reactor was as short as 4 min. In this way, fermentation liquid within UAFB reactor and in the recirculation loop was considered consistent. MFC, pH meter, and gas flow meter were implemented as *in situ* sensors.

A hydrolysis reactor was also included when potato was used as raw substrate instead of OECD wastewater. This hydrolysis reactor consisted of a cylindrical column made from jacketed glass with a total volume of 0.2 l. In this case, the hydrolysis reactor and UAFB reactor were operated simultaneously. Fresh potato waste was hydrolyzed and converted into volatile fatty acids and other intermediates in the first stage. The effluent from this stage was used as feeding substrate of the second stage (UAFB reactor). Organic content of the effluent from the hydrolysis reactor would vary a lot due to the nature of hydrolysis process of potato waste. The two stage experiment was designed on purpose to simulate the dynamic variation of feedstock stream in practice. This experiment was running for up to 17 days until biogas production in UAFB reactor was significantly decreased, and the potato had been completely degraded in the hydrolysis reactor.

2.4. Chemicals and materials

All chemicals used in this study were analytical reagent grade. Anaerobic sludge was collected from a local wastewater treatment

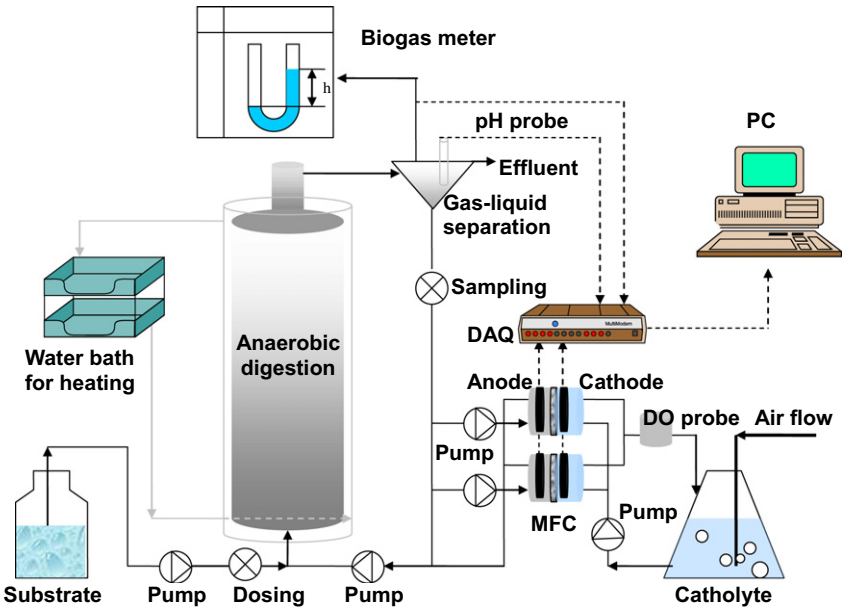

Fig. 2. Scheme of anaerobic biodegradation system integrated with MFC based sensor. An upflow anaerobic fixed-bed (UAFB) reactor fed with OECD wastewater, which was equipped with data acquisition hardware, data record and analysis software and senor units for real time on-line monitoring, containing electrical signal via MFC based sensor, biogas signal via in-house developed gas flow meter and proton signal via commercial available pH meter.

plant (Ellinge, Sweden) and served as inoculums for the hydrolysis of potato. Fresh potatoes were cut into smaller pieces using a kitchen blender and 70 g was loaded into the hydrolysis reactor. The potato has a total solid (TS) content of 16% and volatile solid (VS)/TS is 94%. 119 ml water was added, together with 21 g anaerobic sludge inoculums. Anaerobic sludge has a TS content of 2.3% and VS/TS is 54%.

2.5. Process parameters of AD system

2.5.1. On-line parameters

The closed-circuit potential (U) between the anode and cathode was measured through an external resistor. During the experiment of sensor optimization, the external resistor is 800 Ω except when the polarization study was carried out using different resistors (0–6000 Ω). For the online sensor signal recording, the optimized resistor was defined as 200 Ω. Current (I) was calculated according to Ohm's law: $U = I \times R$. Coulombic yield was obtained by integrating current over time. pH of UAFB was continuously monitored with a commercial available pH probe and meter (Inventron AB, Sweden). Biogas flow rate and biogas volume production were monitored in real-time using an in-house developed volumetric gas meter (Liu et al., 2004c). Electrical potential of the MFC sensor, pH and gas flow rate of biogas process were recorded in a selected sampling frequency using an in-house developed computer data acquisition system.

2.5.2. Off-line analysis

Chemical oxygen demand (COD), TS and VS were determined according to standard methods (APHA, 1985). COD removal was calculated as the ratio between the removed COD and influent COD. Gas composition was off-line analyzed by GC (Agilent 6890, CA, USA) equipped with a Haysep N 80/100, Molsieve 5A column and a thermal conductivity detector as previously described (Nges and Liu, 2009). Gas sample was obtained by syringe from butyl rubber sampling port in the position between biogas meter and gas outlet of UFAB reactor. The detected gas compounds were CH_4, CO_2, O_2, and N_2. Alkalinity was evaluated as partial alkalinity (PA) by titration to pH 5.75 and the total alkalinity (TA) by titration to pH 4.3 with 0.1 M HCl using a titrator (Radiometer, Copenhagen, Denmark) and expressed as mg $CaCO_3$ 1^{-1}. Samples for alkalinity measurements were centrifuged at 6000 rpm for 3 min before analysis. Volatile fatty acids (VFAs) were analyzed by HPLC (Varian Star 9000, Walnut Creek, USA), with a Biorad column, 125–0115 (Biorad, Hercules, USA). The samples were acidified, centrifuged, stored at −20 °C. Before analysis, samples were defrosted and filtered to remove particles. The chromatographic column was maintained at 65 °C, and sulfuric acid (1 mmol l^{-1}) was used as mobile phase at a flow rate of 0.8 ml min^{-1}. The VFAs concentration was detected by using UV–vis detector at 208 nm.

3. Result and discussion

3.1. Optimization of the performance of MFC biosensor

Concerning the anodic flow rate, MFC gave a highest signal at 7.74 ml min^{-1} (data not shown). Electrical signal didn't decrease remarkably at the flow rate below 7.74 ml min^{-1}, while it decreased a lot at 23.11 ml min^{-1}(data not shown). This illustrates that flow rate have double effects on MFC signal, due to mass transfer and stability of biofilms. Regarding the cathodic flow rate, the result showed that the higher the flow rate, the higher the signal and dissolved oxygen (DO). However, DO increased slightly from 7.28 to 7.68 mg l^{-1} with the flow rate up beyond 7.28 ml min^{-1} (data not shown). Considering both real oxygen supply needed and the longevity of pump tube, 7.28 ml min^{-1} was chosen as cathodic flow rate. Sensor signal and pH were greatly impacted by the electrolyte type. For either the anolyte or the catholyte, the highest sensor signal was observed for phosphate buffer (60 mM) with NaCl (50 mM). NaOH and HCl resulted in worse signal because they disturbed the acid–alkali balance of cathodic reactions. It should be noted that for the online monitoring experiment, the anolyte will be replaced with the leachate flowing out of AD reactor.

The study of the effect of substrate concentration revealed that sensor signals rose linearly with substrate concentration up to 200 mg COD l^{-1} (data not shown). In the whole tested range, sensor signal had a Monod type equation correlation with substrate concentration. Highest power was achieved at 200 Ω with the external resistors ranging from 11 to 6000 Ω.

To sum up, the optimized conditions were shown as follows: cathodic flow rate, 7.28 ml min^{-1}; anodic flow rate, 7.74 ml min^{-1}; electrolyte, phosphate buffer with NaCl, and external load, 200 Ω.

3.2. Online monitoring of AD process

3.2.1. Operation with discrete loading

The UAFB reactor was fed with the substrate solution every 12 h (Fig. 3a). First loading lasted 6 h and other three ones lasted about 12 h with similar OLR (1 g COD l^{-1} reactor d^{-1}). The HRT was kept at 2 days during the loading periods and zero loading was obtained by simply adjusting the flow rate to zero. As illustrated in Fig. 3b, at the early stage (1 month) of on line monitoring, with each pulse loading, biofilm MFC exhibited clear and positive response from 20–30 to 45–60 mV. This signal change was matched with positive shift of online gas flow rate from 0–3 to 20–30 ml h^{-1} (Fig. 3c). The percentage of methane was also increased from 65% to 75% (Fig. 3d). On the contrary, pH value decreased from 7.65–7.60 to 7.55–7.50 with each loading, indicating the acidogenesis process of AD. The stepwise decrease of alkalinity suggested that buffering system of AD was influenced by the discrete loading. There are two points which should be addressed: First of all, control MFC had also corresponding signal response, but the signal was very poor and had no typical wide peak as exhibited by biofilm MFC. This was because control MFC was equipped with bacteria-free anode and the weak signal was due to electron transfer from the consortia in the recirculation leachate; secondly, as illustrated in Fig. 2, the sampling position for measuring COD was moved a little ahead and close to MFC, therefore the influent COD of MFC could be represented by the COD value measured. The change of Soluble COD (SCOD) in the recirculation loop was not fully correlated with sensor signal change (Fig. 3a and b). It is known that biofilm based MFC quantitatively converts acetate into electricity directly due to electron transfer of the electrochemically active bacteria on the surface of anode (Bond et al., 2002; Logan, 2008), which was the principle mechanism for the function of biofilm MFC biosensor. Here the change of SCOD in the leachate reflected the variation of anaerobic degradation, while the result suggested that the real time electric signal expressed by MFC sensor may provide more sensitive leachate (acetate) information of AD than traditional off-line analysis of COD.

3.2.2. Continuous operation with increased organic loading rate

The UAFB reactor was shifted from fed-batch feeding to a continuous operation with an increased organic load (Fig. 4). The time interval under each loading conditions was as the same as 12 h. The OLR started at zero and increased once every 12 h. As shown in Fig. 4a, four different OLRs have been tested, i.e. 1.04, 1.67, 2.52, and 3.45 g COD l^{-1} reactor d^{-1}. The HRT was maintained at 2 days during the loading period and zero loading was obtained by simply bringing the flow rate to zero. The signal of biofilm

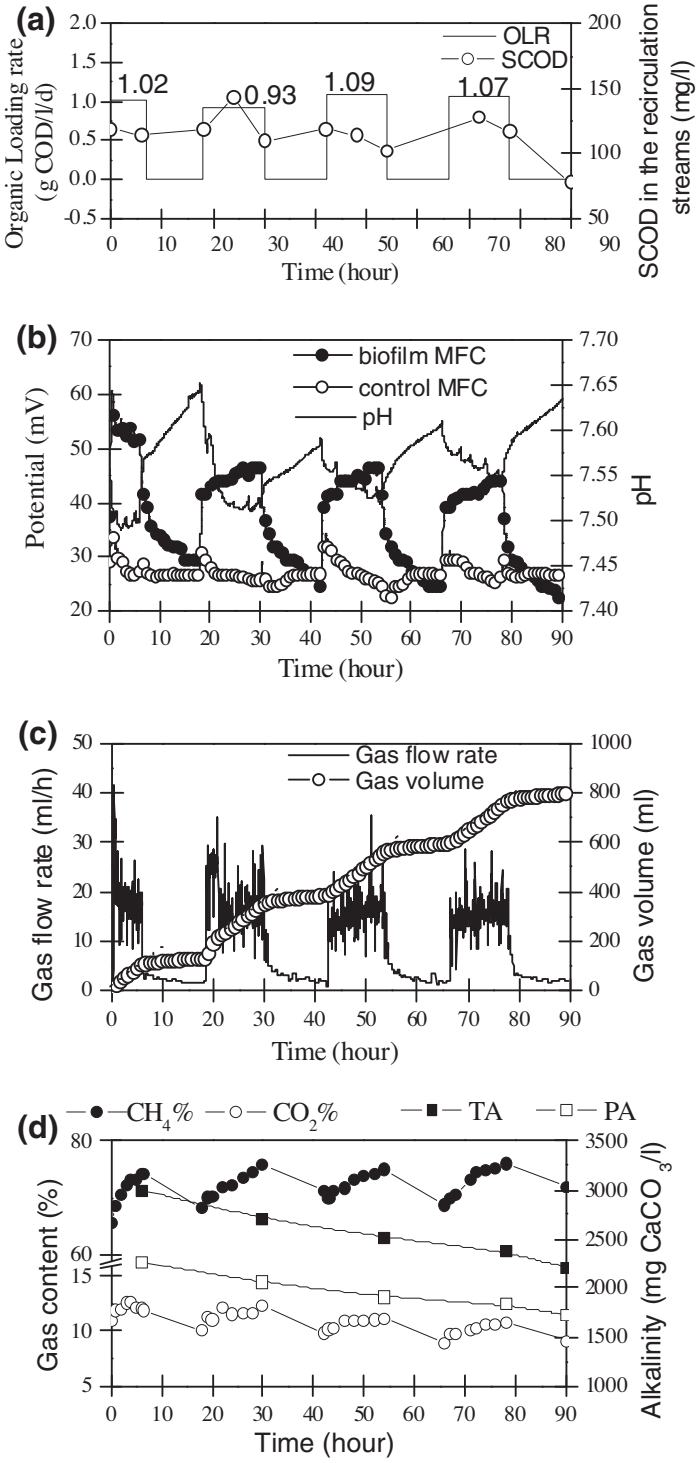

Fig. 3. System performance at discrete loading of OECD wastewater (a). Responses of on-line signals (potential outputs of MFC and pH (b), gas flow rate and gas volume (c)) and off-line analysis (gas content and alkalinity in the recirculation streams (d)).

MFC responded positively from 20 to 80 mV with the increase of OLR up to 2.52 g COD l^{-1} d^{-1} (Fig. 4b). Accordingly, gas flow rate increased from 0–3 to 75 ml h^{-1} and pH decreased from 7.6 to 7.3 (Fig. 4b and c). Control MFC did not exhibit the corresponding increase as biofilm MFC. Afterward, biofilm MFC achieved saturated state (Fig. 4b), which was due to the very limited amount of bio-receptors on the anode, and the censoring speed for MFC was slower than the substrate delivery speed. It is therefore important to be aware of this limit when designing MFC biosensor for its applications. Further improved approaches will be discussed in Section 3.2.4. The decrease of sensor signal at higher loading (3.45 g COD l^{-1} d^{-1}) was possibly due to the feedback inhibition of overloaded intermediate or the negative effect from H_2S produced during AD process. The increase of alkalinity in this case (Fig. 4d) was due to the effect of buffering chemicals in AD system ($NaHCO_3$, VFAs and ammonia) (Björnsson, 2000). VFAs resulted from anaerobic degradation weakens partial alkalinity (PA), while the formation of HCO_3^- enhances PA. In this experiment, with

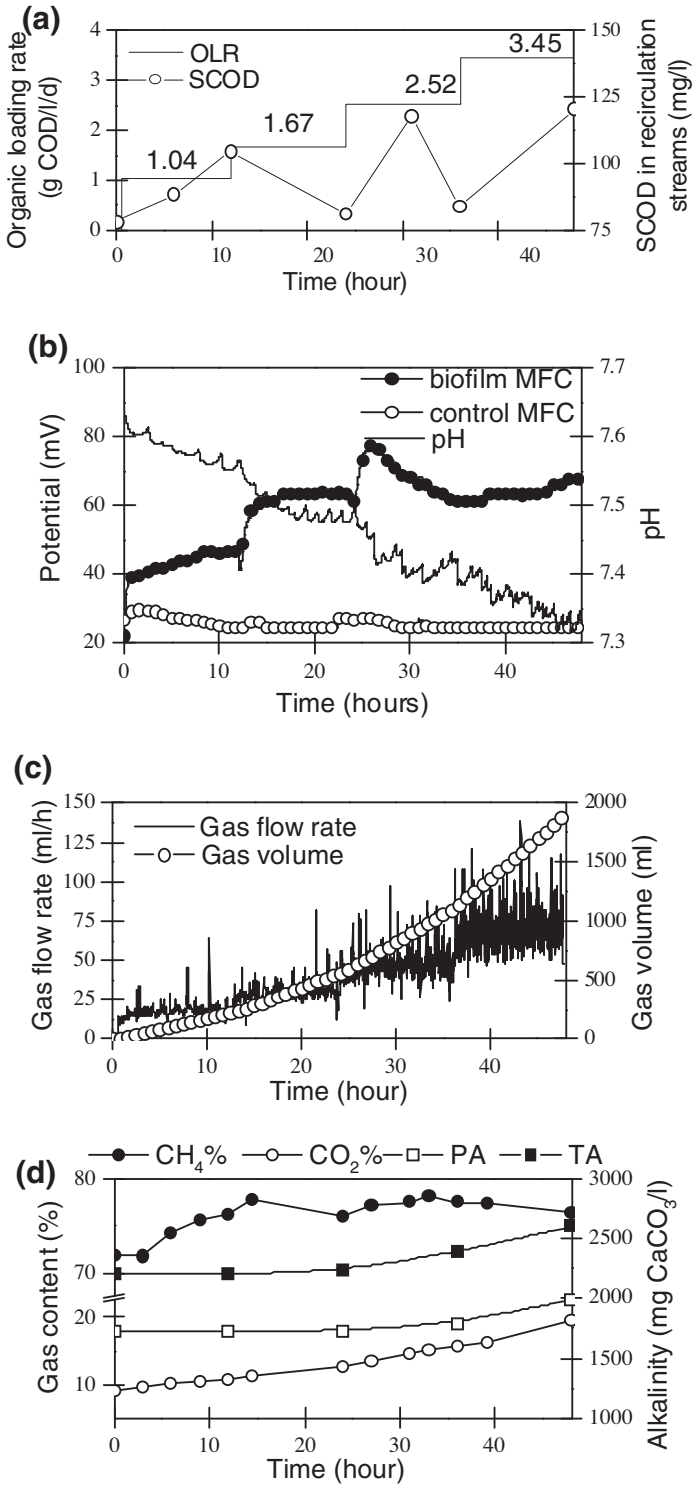

Fig. 4. System performance at increased loading of OECD wastewater (a). Responses of on-line analysis (potential outputs of MFC and pH (b), gas flow rate and gas volume (c)) and off-line signals (gas content and alkalinity in the recirculation streams (d)).

the increased OLR, the positive effect from HCO_3^- on PA was stronger than the negative effect of VFAs, and thus PA was increased.

3.2.3. Continuous operation with decreased organic loading rate

The UAFB reactor was shifted from an increased load of continuous feeding to a decreased load (Fig. 5). The time interval under each loading conditions was the same as 12 h. The OLR started at zero and increased once every 12 h. As shown in Fig. 5a, four different OLRs have been tested, i.e. 3.45, 2.40, 1.66, and 0.93 g COD l^{-1} reactor d^{-1}. The HRT was maintained as two days during the loading period. Zero loading was obtained by simply bringing the flow rate to zero. The sensor signal of biofilm MFC decreased from 68 to 31 mV with the decreased OLR (Fig. 5b). These were the same with the changes for the gas flow rate and gas content (Fig. 5c). pH decreased to below 7.0 in the first 35 h, and increased to over 7.1 later on, whereas alkalinity gave a opposite reply (Fig. 5d). Control MFC appeared consistently at 25 mV. The initial decrease of pH indicated the biological degradation of

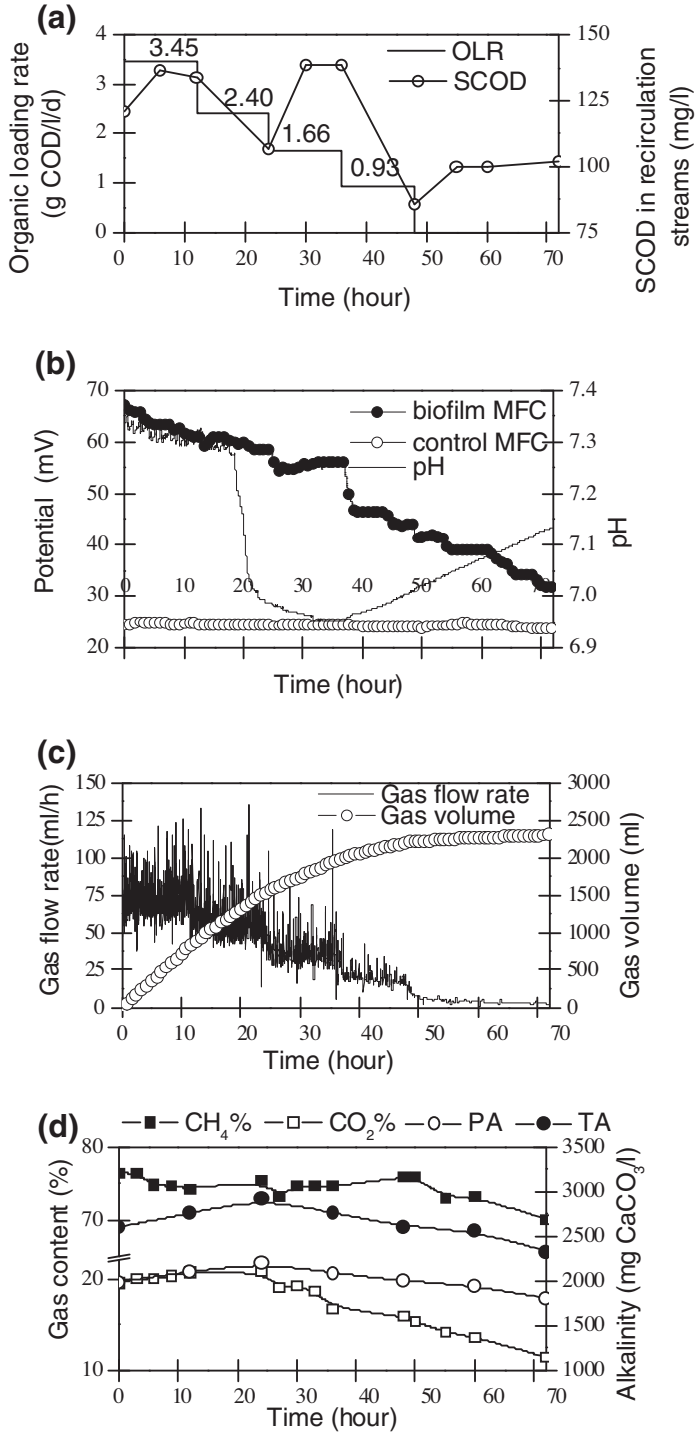

Fig. 5. System performance at decreased loading of OECD wastewater (a). Responses of on-line analysis (potential outputs of MFC and pH (b), gas flow rate and gas volume (c)) and off-line signals (gas content and alkalinity in the recirculation streams (d)).

organic matter, while the increase afterwards indicated the depressed biodegradation due to decreased OLR. It should be noted that the experiment was carried out immediately after the testing in Section 3.2.2, which resulted in the initial increase of alkalinity as shown in Fig. 5d.

3.2.4. Continuous operation with varied organic loading from a hydrolysis reactor

All previous monitoring tests were performed with a feedstock with constant concentration. The variable is either the feeding frequency of pump or the feedstock concentration. After 5 month on-line operation of UAFB and the sensor system, a hydrolysis reactor was introduced to supply varied organic loading conditions. As illustrated in Fig. 6, during 400 h operation, two peaks were found in gas flow rate curves in the time range of 0–30 and 30–140 h, respectively, which were highlighted in red arrows in Fig. 6a. The first peak (MFC potential at 60 mV) was due to high organic concentration loading to UAFB reactor from hydrolysis reactor, which could be explained by the initial high value of COD and VFAs data curves. The biofilm MFC (Fig. 6a) exhibited similar signal peak with VFAs and acetate (Fig. 6d), indicating that electrical signal was able to provide dynamic process information. Control MFC gave a

relative delayed signal peak compared with biofilm MFC. The second peak of MFC sensor curve (30–140 h) had correlations with that of gas flow rate, VFAs and pH of influent to UAFB reactor and even CO_2 content of hydrolysis reactor (Fig. 6b–d). This was probably due to the biodegradation of solid potato. Hydrolysis was almost completed when the pH of hydrolysis reactor went up to over seven and the CO_2 content of hydrolysis reactor decreased to less than 20% (Fig. 6c). In comparison with gas flow meter and control MFC, biofilm MFC displayed a more complex signal variation for the second peak, which may support the multi-step complex biodegradation process of potato.

VFAs had been considered as one of the best and most promising monitoring parameters since they indicate the metabolic status and activity of microbial groups in AD system (Björnsson, 2000; Boe et al., 2007, 2010). System imbalance due to organic overload or inhibition of methane production can lead to the accumulation of VFAs (Spanjers and van Lier, 2006). This experiment clearly shows that the electrical signal from MFC biosensor was highly dependent on the intermediates concentration (mainly acetate) of AD. The metabolic state of AD was monitored by the usage of the biofilm respiration in MFC biosensor. Thus, MFC biosensor could provide important process information for the optimization and management of AD.

One major limit of current biosensor design is that sensor signal reached saturated state when organic loading rate went beyond 3 g l^{-1} d^{-1}, resulting in the over-accumulation of acetate. The high concentration of acetate will limit the microbial activity of MFC anode (as discussed in Section 3.2.2) although acetate is the true substrate of MFC. However, the linearity range of MFC can certainly be enlarged by different approaches, such as design of better electrode materials with increased surface area (Logan, 2008) and improved cathode performance with an air cathode (Di Lorenzo et al., 2009).

Compared with our previous work (Kumlanghan et al., 2007), current MFC biosensor exhibited many improvements and differences. Firstly, in principle, instead of using suspended consortium from an anaerobic digester, current MFC biosensor was established using a mature biofilm catalyst. One dominant advantage of biofilm MFC is its long-term stability (Gil et al., 2003). With regards to the previous work, the experiment on the stability was tested within only 12 days, which was due to the reason that the electrical signal may be variable during the enrichment of consortium (Kumlanghan et al., 2007). The essential difference of microorganisms also influences the performance of sensor. The microbial population of mature biofilm system in this study were relatively stable in comparison with that of suspended consortium, thus leading to better accuracy and repeatability of MFC biosensor. With regards to other sensors based on the electrochemical or physical principles (Spanjers and van Lier, 2006; Jacobi et al., 2011), they will have more stability than MFC biosensor based on the biochemical reactions although current biofilm MFC has the performance of long-term stability due to the nature of biofilm. One effective way is to make calibrations of MFC biosensor regularly e.g. every few months. Furthermore, the detected substrate is different. It is well known that biofilm MFC can consume acetate like fermentation intermediates due to the ability of electroactive biofilm for quantitatively converting acetate into electricity, which was almost impossible for suspended consortium. The MFC in previous work is just like control MFC of current study, which had to be accumulated for a couple of months prior to use for process monitoring. Secondly, it was the first time that MFC concept was reported to be used for online monitoring of AD. Moreover, quite a number of online (pH, gas flow rate) and offline (gas content, VFAs) parameters were measured to justify and evaluate the performance of MFC. Finally, distinguished from previously used H-type configuration, current MFC sensor was designed in a novel flow-through wall-jet architecture. Several key characters including continuous operation, portability, stable cathode electrochemical reactions were taken into account.

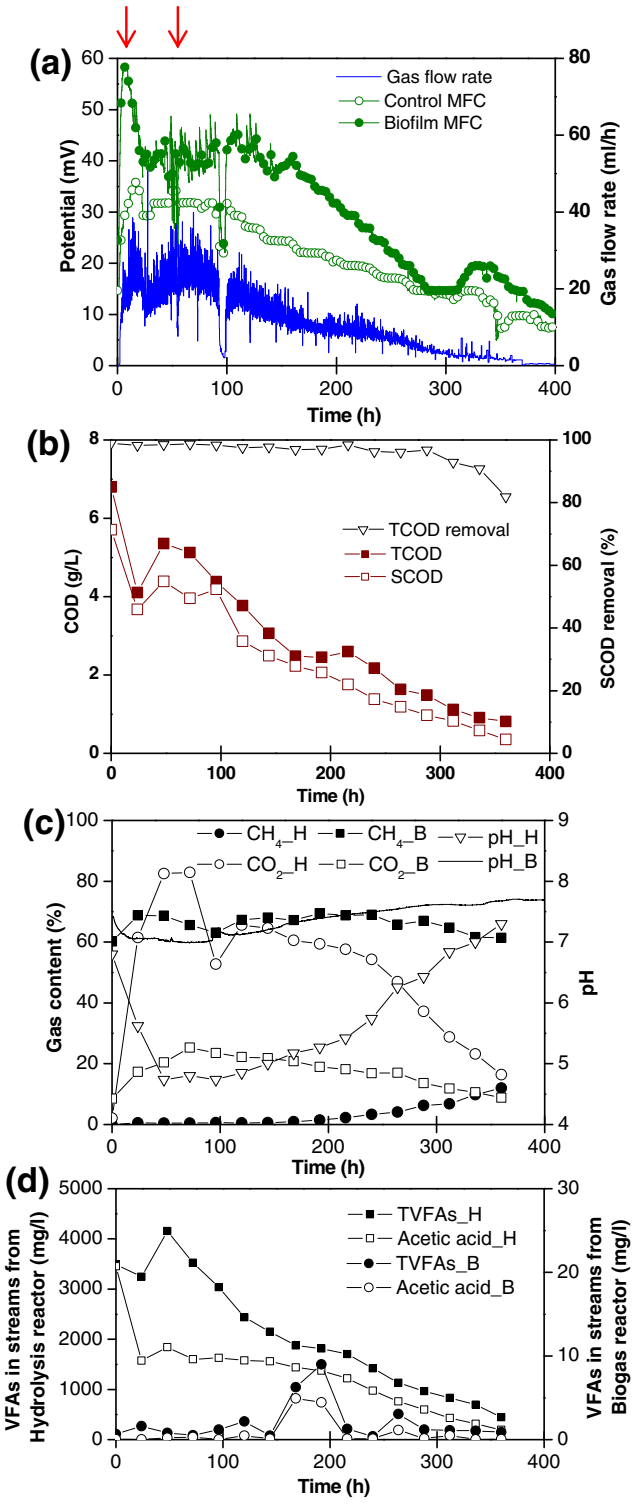

Fig. 6. On-line signals (potential outputs of MFC and gas flow rate (a)) and off-line analysis (COD and COD removal (b), gas content and pH (c), VFAs and acetate acid (d)) in UAFB reactor continuously fed with the leachate from the reactor for hydrolysis of solid potato. H refers to hydrolysis reactor, and B refers to the UAFB biogas reactor. The positions of two peaks in gas flow rate curves were highlighted in red arrows. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

One potential advantage of this MFC sensor is low construction cost. The major cost of MFC comes from proton exchange member (PEM). However, as a sensor device, MFC can be designed as small

as possible, therefore the material cost of PEM can be minimized. Furthermore, different from other biosensors, which normally require proper configuration for setting up sensing element and transducer, these two parts are very well integrated into MFC.

Another advantage is that MFC biosensor is positioned *in situ* in the liquid recirculation loop. The independent design makes it possible to compatible with any type of reactors. Besides this, the MFC biosensor can be set for online monitoring with a sampling frequency of 1 min which is much shorter than many other reported sampling intervals from other online sensors.

Further works are expected by the use of online real time MFC biosensor. First, MFC biosensor can be tested for the monitoring of AD in field. It is possible and promising since current MFC sensor is portable and simple. Second, due to the working principle of current MFC for acetate detection, MFC signals could be used as a process indicator. The anaerobic degradability of organic substrate can also be offline evaluated through a manometric system within 1 week test (Scaglione et al., 2008), which may provide additional substrate support for MFC sensor participated process analysis. Combined analysis based on different sensors had been proved to provide crucial support for the process diagnosis and control of AD (Liu, 2003; Boe et al., 2010). MFC biosensor can be coupled with other measurements, for instance substrate (Jacobi et al., 2011; Scaglione et al., 2008), gas flow (Liu et al., 2004a) and pH, and with different control approaches to seek optimal control strategies for the operation of AD.

4. Conclusion

Current results demonstrated a novel design of MFC biosensor for bioprocess monitoring. A stable electrical signal from MFC was demonstrated to give a comparable result with other commonly used analysis of AD in the operations of different process configurations. The MFC biosensor offers a new approach to reflect the real time microbial activity of bioprocess by the detection of acetate and hence indicates valuable process information. This study introduced MFC concept to the field of monitoring and control of AD, thus expanding the applicability of MFC.

Acknowledgements

The Swedish International Development and Cooperation Agency for Swedish Research Link Programme (SIDA-VR) and The Swedish Institute (Guest Scholarship Programme, No. 05391/2006) were gratefully acknowledged for the financial support. Dr. Songping Zhang acknowledges the support from Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry (No. 2006-331).

References

APHA, 1985. Standard Methods for the Examination of Water and Wastewater, 16th ed. American Public Health Association, Washington DC, USA.

Boe, K., Batstone, D.J., Angelidaki, I., 2007. An innovative online VFA monitoring system for the anaerobic process, based on headspace gas chromatography. Biotechnol. Bioeng. 96, 712–721.

Boe, K., Batstone, D.J., Steyer, J.P., Angelidaki, I., 2010. State indicators for monitoring the anaerobic digestion process. Water Res. 44, 5973–5980.

Björnsson, L., 2000. Intensification of the Biogas Process by Improved Process Monitoring and Biomass Retention. PhD Thesis, Lund University, Sweden.

Bond, D.R., Holmes, D.E., Tender, L.M., Lovley, D.R., 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. Science 295, 483–485.

Chang, I.S., Jang, J.K., Gil, G.C., Kim, M., Kim, H.J., Cho, B.W., Kim, B.H., 2004. Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor. Biosens. Bioelectron. 19, 607–613.

Chang, I.S., Moon, H., Jang, J.K., Kim, B.H., 2005. Improvement of a microbial fuel cell performance as a BOD sensor using respiratory inhibitors. Biosens. Bioelectron. 20, 1856–1859.

Di Lorenzo, M., Curtis, T.P., Head, I.M., Scott, K., 2009. A single-chamber microbial fuel cell as a biosensor for wastewaters. Water Res. 43, 3145–3154.

Gerardi, M.H., 2003. The Microbiology of Anaerobic Digestion. John Wiley & Sons Inc., New Jersey, USA.

Gil, G.C., Chang, I.S., Kim, B.H., Kim, M., Jang, J.K., Park, H.S., Kim, J., 2003. Operational parameters affecting the performance of a mediator-less microbial fuel cell. Biosens. Bioelectron. 18, 327–334.

Gujer, W., Zehnder, A.J.B., 1983. Conversion processes in anaerobic digestion. Water Sci. Technol. 15, 127–167.

Jacobi, H.F., Moschner, C.R., Hartung, E., 2011. Use of near infrared spectroscopy in online-monitoring of feeding substrate quality in anaerobic digestion. Bioresour. Technol. 102, 4688–4696.

Kumlanghan, A., Liu, J., Thavarungkul, P., Kanatharana, P., Mattiasson, B., 2007. Microbial fuel cell-based biosensor for fast analysis of biodegradable organic matter. Biosens. Bioelectron. 22, 2939–2944.

Karube, I., Matsunaga, T., Mitsuda, S., Suzuki, S., 1977. Microbial electrode BOD sensors. Biotechnol. Bioeng. 19, 1535–1547.

Liu, J., 2003. Instrumentation, Control and Automation in Anaerobic Digestion. PhD Thesis, Lund University, Sweden.

Liu, J., Olsson, G., Mattiasson, B., 2003. Advanced monitoring and control of an anaerobic up-flow fixed bed reactor for high loading rate operation and disturbances rejection. Biotechnol. Bioeng. 87, 43–53.

Liu, J., Olsson, G., Mattiasson, B., 2004a. A volumetric meter for monitoring of low gas flow rate from laboratory-scale biogas reactors. Sens. Actuators B 97, 369–372.

Liu, J., Olsson, G., Mattiasson, B., 2004b. On-line monitoring of a two-stage anaerobic digestion process using a BOD analyzer. J. Biotechnol. 109, 263–275.

Liu, H., Ramnarayanan, R., Logan, B.E., 2004c. Production of electricity during wastewater treatment using a single chamber microbial fuel cell. Environ. Sci. Technol. 38, 2281–2285.

Liu, Z., Liu, J., Zhang, S., Su, Z., 2009. Study of operational performance and electrical response on mediator-less microbial fuel cells fed with carbon- and protein-rich substrates. Biochem. Eng. J. 45, 185–191.

Logan, B.E., Hamelers, B., Rozendal, R., Schröder, U., Keller, J., Freguia, S., Aelterman, P., Verstraete, W., Rabaey, K., 2006. Microbial fuel cells: methodology and technology. Environ. Sci. Technol. 40, 5181–5192.

Logan, B.E., 2008. Microbial Fuel Cell. John Wiley & Sons, New Jersey, USA, ISBN 9780470239483.

Méndez-Acosta, H.O., Palacios-Ruiz, B., Alcaraz-Gonzlez, V., González-Álvarez, V., García-Sandoval, J.P., 2010. A robust control scheme to improve the stability of anaerobic digestion processes. J. Process Contr. 20, 375–383.

Moon, H., Chang, I.S., Kim, B.H., 2006. Continuous electricity production from artificial wastewater using a mediator-less microbial fuel cell. Bioresour. Technol. 97, 621–627.

Morel, E., Tartakovsky, B., Perrier, M., Guiot, S.R., 2007. Temperature-based control of an anaerobic reactor using a multi-model observer-based estimator. Environ. Sci. Technol. 41, 978–983.

Nges, I.A., Liu, J., 2009. Effects of anaerobic pre-treatment on the degradation of dewatered-sewage sludge. Renew. Energ. 34, 1795–1800.

Organisation for Economic Cooperation, Development (OECD), 2001. Aerobic sewage treatment, activated sludge units. OECD Guidel. Testing Chem. 303, 1–38.

Pant, D., Bogaert, G.V., Diels, L., Vanbroekhoven, K., 2010. A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production. Bioresour. Technol. 101, 1533–1543.

Potter, M.C., 1911. Electrical effects accompanying the decomposition of organic compounds. Proc. R. Soc. Lond. B 84, 260–276.

Rabaey, K., Verstraete, W., 2005. Microbial fuel cells: novel technology for energy production. Trends Biotechnol. 23, 291–298.

Scaglione, D., Caffaz, S., Ficara, E., Malpei, F., Lubello, C., 2008. A simple method to evaluate the short-term biogas yield in anaerobic codigestion of WAS and organic wastes. Water Sci. Technol. 58, 1615–1622.

Scherer, P., Lehmann, K., Schmidt, O., Demirel, B., 2009. Application of a fuzzy logic control system for continuous anaerobic digestion of low buffered, acidic energy crops as mono-substrate. Biotechnol. Bioeng. 102, 736–748.

Spanjers, H., van Lier, J.B., 2006. Instrumentation in anaerobic treatment – research and practice. Water Sci. Technol. 53, 63–76.

Tront, J.M., Fortner, J.D., Plötze, M., Hughes, J.B., Puzrin, A.M., 2008. Microbial fuel cell biosensor for *in situ* assessment of microbial activity. Biosens. Bioelectron. 24, 586–590.

van Lier, J.B., Tilche, A., Ahring, B.K., Macarie, H., Moletta, R., Dohanyos, M., Hulshoff, Pol, L.W., Lens, P., Verstraete, W., 2001. New perspectives in anaerobic digestion. Water Sci. Technol. 43, 1–18.

Vaiopoulou, E., Melidis, P., Aivasidis, A., 2011. Process control, energy recovery and cost savings in acetic acid wastewater treatment. J. Hazard. Mater. 186, 1141–1146.

Virdis, B., Rabaey, K., Rozendal, R.A., Yuan, Z., Keller, J., 2010. Simultaneous nitrification, denitrification and carbon removal in microbial fuel cells. Water Res. 44, 2970–2980.

Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. 99, 7928–7940.