International Symposium on Water Environment Problem

With Perspective of Global Safety in East Asian Countries, Part II

G-Safety
SCIENCE FOR GLOBAL SAFETY

March 21 – 22, 2014

Tohoku University, Sendai, JAPAN

TOHOKU UNIVERSITY

TSINGHUA UNIVERSITY -1911-

ORGANIZERS

Dr. Yu-You LI (Professor, Tohoku University)

Dr. So KAZAMA (Professor, Tohoku University)

Dr. Osamu NISHIMURA (Professor, Tohoku University)

Dr. Xia HUANG (Professor, Tsinghua University)

Dr. Xianghua WEN (Professor, Tsinghua University)

SECRETARIES

Dr. Adriana LEDEZMA ESTRADA (English and Japanese)
e-mail: adriana@epl1.tohoku.ac.jp

Dr. GADOW Samir (English)
e-mail: gadow@epl1.tohoku.ac.jp

M. Sc. Weikang QI (English and Chinese)
e-mail: qiwk@epl1.civil.tohoku.ac.jp, qiweikang10@163.com

Sponsors

http://www.g-safety.tohoku.ac.jp/

Tohoku University, Main Administration Office Organization for Leading Graduate School Program of Tohoku University, Sendai, Miyagi 980-8578, Japan

http://www.eng.tohoku.ac.jp/

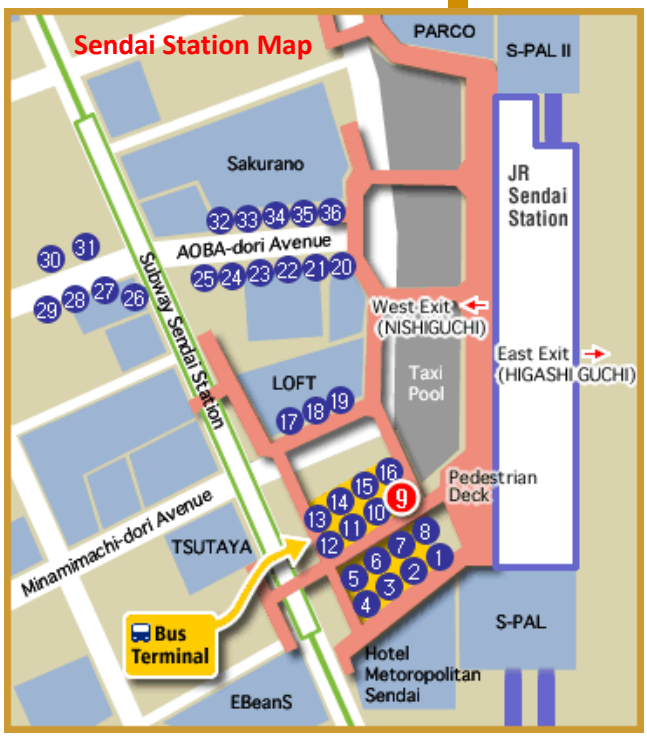
Tohoku University, Graduate School of Engineering, Sendai, Miyagi 980-8579, Japan

http://www.tsinghua.edu.cn/

Tsinghua University, School of Environment, Beijing 100084, PR China

ACCESS TO AOBAYAMA CAMPUS

SCHOOL OF ENGINEERING



Buses leave the bus terminal at the JR Sendai Station (bus stop No. 9) every 30 minutes for the 20-minutes trip to the "**Kogakubu-chuo**" bus stop in front of the School of Engineering.

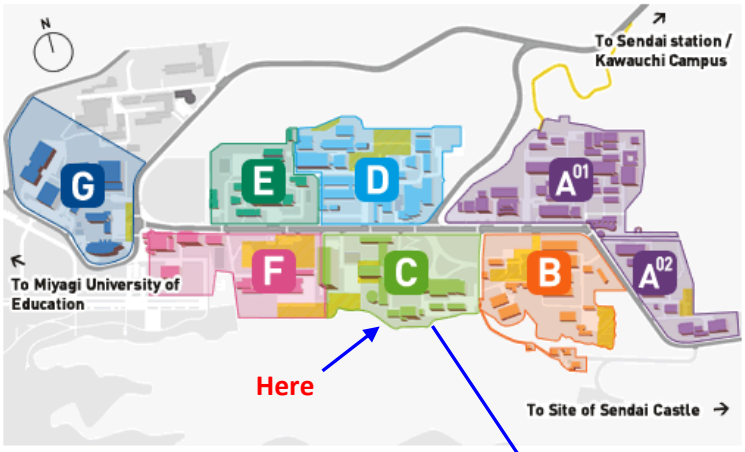
Buses Numbers

S710-715, 719, 750, or 757

Conference venue

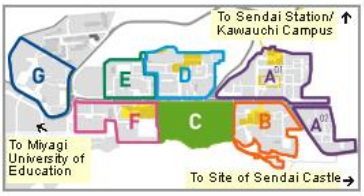
Complex building Room No. 101,
Aobayama campus, Tohoku University

Aobayama Campus Map



- A01 Mechanical and Aerospace Engineering
- A02 Quantum Science and Energy Engineering
- B Materials Science and Engineering
- C Center Square
- D Electrical Engineering and Applied Physics
- E Applied Chemistry, Chemical Engineering and Biomolecular Engineering
- F Civil Engineering and Architecture
- G Various Area

Complex building Room No. 101



Program

March 21, 2014 Oral presentation

9:30 – 9:40 **Opening speech** (Prof. Yu-You Li, Tohoku University)

Session I | **Nitrification, denitrification and anammox**

Chair: Dr. Adriana (Tohoku University)

9:40 – 9:55	Operation and control of partial nitritation in a CSTR reactor **Yuan Liu**
9:55 – 10:10	The Start-up and Inhibition Analysis of Anammox Process in a UASB Reactor Using Mixed-Culture Sludge **Yanlong Zhang**
10:10 – 10:25	GeoChip-based analysis of the microbial community of a combined nitritation-anammox reactor treating digestion supernatant **Jian Zhao**
10:25 – 10:40	Enrichment of denitrifying methanotrophic bacteria from Taihu sediments by a membrane biofilm reactor **Qing Wu**
10:40 – 10:55	Carbon Source Recovered from Sludge Hydrolysis Do have impacts on the Performance and Microbial Community Structure of Denitrifying Reactor in an Enhanced Nitrogen Removal Process **Bo Shen**

10:55 – 11:15 | **Coffee break**

Session II | Antibiotics on the environment

Chair: Dr. Guangyin Zhen (Tongji University)

11:15 – 11:30	Effect of antibiotics in water environments and its removal from wastewater by means of electrochemical process: electro-Fenton **Adriana Ledezma Estrada**
11:30 – 11:45	Effect of erythromycin on nitrification during batch and continuous experiments **Jingru Du**
11:45 – 12:00	A microfluidic platform for inhibitory test of antibiotic on nitrifier and denitrifier **Bing Li**

12:00 – 13:00 | Lunch time

	Lunch at the main cafeteria in Aobayama Campus

13:00 – 13:30 | Technical visit

	Visit to the laboratory, explanation of the experiments and the reactors.

Session III | Renewable energy and environmental security

Chair: Prof. Xianghua Wen (Tsinghua University)

13:30 – 13:45	Characterization and 16s rRNA gene sequences analysis of the cellulolytic and hydrogen-producing activities of three different anaerobic mixed microflora **Gadow Samir**
13:45 – 14:00	Kinetics of complex organic waste degradation under anaerobic thermophilic and mesophilic process **Qian Li**
14:00 – 14:15	Improving MFC performance at low COD concentrations by adsorptive anode. **Shijia Wu**
14:15 – 14:30	Thermophilic anaerobic digestion of spent coffee grounds and coffee liquid with sludge and milk waste as the co-substrate **Shofie Mohammad**
14:30 – 14:45	The VFA & alcohols production from mixed culture anaerobic fermentation: effect of pH **Yuanyuan Wu**

14:45 – 15:00 | Coffee break

Session IV | Wastewater treatment technologies

Chair: Dr. Gadow Samir (Tohoku University)

15:00 – 15:15	Effect of COD/SO_4^{2-} Ratio on UASB treatment of Synthetic Organic Chemical Industrial Wastewater **Yong Hu**
15:15 – 15:30	Upgrading of anaerobic digestion of waste activated sludge by temperature-phased two-stage process and the introduction of a recycle system **Lijie Wu**
15:30 – 15:45	Application of model and simulations in wastewater treatment process **Weikang Qi**
15:45 – 16:00	Combined electrical-alkali pretreatment to increase the anaerobic hydrolysis rate of waste activated sludge during anaerobic digestion **Guangyin Zhen**

16:00 – 16:10 **Closing speech** (Prof. Xia Huang, Tsinghua University)

March 22, 2014 Fieldwork

Minami-Gamo Sewage Treatment Plant

Tsunami was hitting Sewage Facilities

Effect on the wall of pump station

Session I: Nitrification, denitrification and anammox

Operation and control of partial nitritation in a CSTR reactor

○Yuan Liu[1], Shilong He[2], Toshimasa Hojo[2], Yu-You Li[1,2]*

[1]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[2]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

This study investigated partial nitritation achievement and maintenance in a CSTR reactor. The reactor was operated at temperature range of 25~33℃ and pH between 7.8-8.7. Under the comprehensive effect of FA and FNA, nitrite accumulation and partial nitritation was achieved successfully. The effect of variation of DO concentration in the reactor on the efficiency of NH_4-N conversion to NO_2-N and ratio of NO_2-N/NH_4-N in the effluent was evaluated. 50% of partial nitritation was achieved and maintained for 24 days when DO concentration was set as 0.07 mg/L and collapsed as a result of the inhibition of FA to AOB activity. When DO concentration was increased to 0.21 mg/L, the ratio of NO_2-N/NH_4-N was consequently enhanced to 2.1 due to the excess DO for 50% of partial nitritation. On the condition of DO concentration at 0.12 mg/L and 0.16 mg/L, the ratio of NO_2-N/NH_4-N was achieved to 1.3 and 1.4 for 55 days and 14 days, respectively. It was assumed that combined DO was an important and sensitive parameter for partial nitritaion achievement and maintenance under the experimental condition.

Keywords: Partial nitritaion; low C/N ratio; eutrophication; Anammox

1. Introduction

The nutrients (such as nitrogen (N) and phosphorus (P)) over-enrichment of freshwater and coastal ecosystems, or eutrophication has emerged as a leading and ever-growing water quality problem. In the world, the number of eutrophication-impacted coastal areas stands at over 500 until 2009, including Lake Erie (United States), Lake Victoria (Tanzania/Uganda/Kenya), and Tai Lake (China). As a result of global trends in agriculture practices, energy uses, and population growth indicates that eutrophication will be an ever-going problem. To avoid harmful algal bloom and eutrophication, N and P removal from sewage is necessary.

The discovery of Anammox process in the early 1990s that convert NH_4^+ and NO_2^- to N_2 with the absence of oxygen and organic carbon sources has arisen great expectations regarding elimination of N high-loaded wastewaters containing low biodegradable organic carbon to nitrogen (C/N) ratio. Over the last decade, the cumulative nitrogen load increased almost 60-fold, indicating that from an economic perspective, the Anammox-based processes are becoming increasingly widespread. Currently, the Anammox process is successfully implemented for full-scale ammonium-rich wastewater treatment at mesophilic temperatures. However, certain previous 50%~60% of partial nitritation (PN) of NH_4^+ is necessary in order to supply Anammox process with just NO_2^-/NH_4^+ ratio of about 1:1.32. Two different strategies are used: NO_2^- can be either produced in a separate aerated reactor and subsequently be fed into an anoxic Anammox reactor or accumulated in the same reactor with Anammox prcocess. With respect to the separate reactor strategy named as PN-Anammox process, it is a key factor to accumulate nitrite successfully and stable in PN process. The purpose of this study was to explore a practical operation for long-term about 50% of partial nitritation maintenance.

2. Materials and Methods

2.1. Experiment configuration and operation

As shown in Fig. 1, the partial nitritation was carried out in a lab-scale continuous stirred-tank reactor (CSTR), which is made of a transparent PVC plastic rectangular reactor with working volume of 8 liters and settling zone volume of 2L.The temperature in the reactor were set and keep in the range of 25~33℃ by a heater with temperature adjuster set in the reaction zone. Dissolved oxygen (DO) concentration was controlled in low concentration, and adjusted to a series of concentrations. The hydraulic retention time (HRT) was kept as 12h, nitrogen loading rate in the operation period was 0.5kgNH_4-N/m3・d.

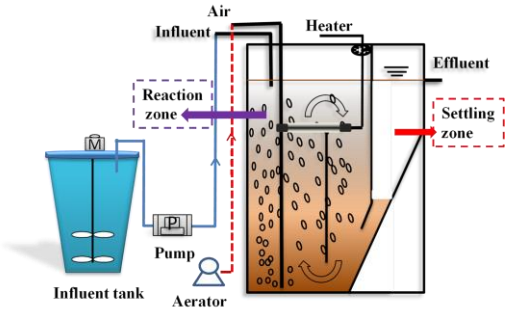


Fig. 1. Schematic diagram of partial nitritation reactor

2.2. Synthetic wastewater and seed sludge

During the whole operation period, the reaction was feeding with synthetic wastewater and trace element shown in Table 1, in which the NH_4-N concentration was 250 mg/L. pH in the substrate was adjusted in a range of 7.8~8.7 by adding of buffer agents consist of $NaHCO_3$, KH_2PO_4 and K_2HPO_4. The seed sludge was obtained from S Municipal wastewater treatment plant and was cultivated by full-and-draw technique before transferring it into the

reactor. The initial mixed liquor suspended solid (MLSS) concentration of the reactor was set at 2000 mg/L.

Table 1 Characteristic of the substrate for PN reactor

Synthetic Wastewater (g/L)	NH_4HCO_3	$NaHCO_3$	KH_2PO_4	$Na_2HPO_4 \cdot 12H_2O$	$CaCl_2 \cdot 2H_2O$	$MgCl_2 \cdot 6H_2O$
	1.414	1.500	0.081	0.214	0.036	0.051
Trace Element (g/L)	$FeSO_4 \cdot 7H_2O$	$ZnSO_4 \cdot 7H_2O$	$CoCl_2 \cdot 6H_2O$	$MnCl_2 \cdot 4H_2O$	$CuSO_4 \cdot 5H_2O$	$NaMoO_4 \cdot 2H_2O$
	5.000	0.215	0.120	0.495	0.125	0.110
	H_3BO_4	$NiCl_2 \cdot 6H_2O$	Na_2SeO_4	$Na_2 \cdot EDTA \cdot 2H_2O$		
	0.007	0.095	0.078	8.304		

2.3. Analytical methods

The MLSS were determined according to the Standard Methods (APHA, 2002). Ammonium, nitrite and nitrate concentrations were detected by capillary electrophoresis of Agilent 7100 (M. Vilas-Cruz, 1994). DO concentration and pH were measured by a portable DO and pH meter.

3. Results and discussion

3.1. Achievement of nitrite accumulation

The changes of nitrogen concentrations in the PN reactor during the operation period are shown in Fig.2a. During the whole operation period, no matter DO concentration (illustrated in Fig.3b) was at high or low level, the FA (free ammonia) concentration (shown in Fig.3c) in the reactor was still higher than Anthonisen et al. reported initial inhibition concentration of FA to NOB activity, which is a range of 0.1~1.0 mg FA/L. Accumulation of nitrite and little nitrate in the effluent were obviously observed at the start-up of the reactor due to the inhibition of FA to NOB (nitrite oxidizing bacteria). O the relatively higher operation pH between 7.8~8.7 and temperature of 25~33 ℃, the FNA (free nitrous acid) levels remained depressed to almost zero (illustrated in Fig.3c). Therefore, FA inhibition on the activity of NOB was considered to be the main factor for the satisfactory nitrite accumulation obtained in this study.

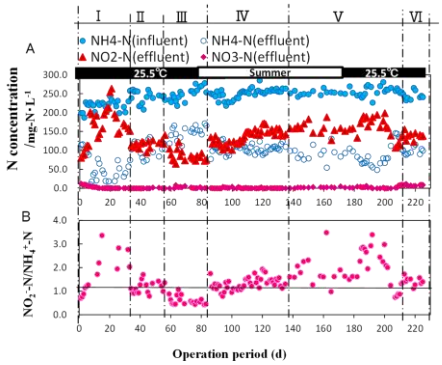


Fig.2. Characteristic of the substrate for PN reactor

3.2. Achievement and maintenance of partial nitritation

During the first 24 days in Phase Ⅰ, due to the nitrifiers were acclimated in the reactor by high concentration of ammonium and sufficient DO in the first week, the efficiency of NH_4-N conversion to NO_2-N was accelerated from 40% to 99% gradually. With the acceleration of nitritation process, the concentration of NH_4^+ decreased and NO_2^- became the domain production of nitrification. In addition, the pH in the reactor was also decreased from 8.35 to 6.90 because alkalinity consumption is proportional to the efficiency of nitrifying. As a result, the FA concentration in the reactor depressed from 16.74mg/L to 0. However, FNA concentration was improved to 0.245mg/L on day 21, which was higher than Jimenez et al. reported initial inhibition concentration of FNA to AOB (0.0043 mg FNA/L) activity. Furthermore, the DO concentration was lowered down to insufficient for nitritation. The efficiency of NH_4-N conversion to NO_2-N fell down until 47.65% on day 34 which standed for the achievement of approximately 50% partial nitritaion in the reactor.

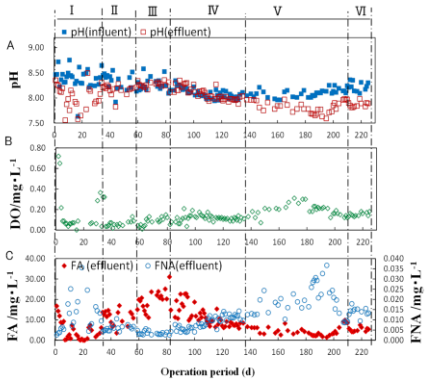


Fig.3. Variation of pH, DO FA and FNA concentrations in the effluent during the operation period

3.3. Effect of fluctuation of DO concentration on partial nitritation

The effect of variation of DO concentration in the reactor on the efficiency of NH_4-N conversion to NO_2-N and ratio of NO_2-N/NH_4-N in the effluent was evaluated. As shown in Table 2, 50% of Partial nitritation was achieved in Phase Ⅱ for 24 days when DO concentration was set as 0.07 mg/L and collapsed for the inhibition of FA to AOB. When DO concentration was increased to 0.21 mg/L, the ratio of NO_2-N/NH_4-N was consequently enhanced to 2.1 due to the excess DO for 50% of Partial nitritation. On the condition of DO concentration at 0.12 mg/L and 0.16 mg/L, the ratio of NO_2-N/NH_4-N was achieved to 1.3 and 1.4 for 55 days and 14 days, respectively.

Table 2 Operation parameters and performance during each operation phases

Phase	pH	DO	FA	FNA	Efficiency of NH_4-N conversioin to NO_2-N	NO_2-N/NH_4^+-N
	–	mg/L	mg/L	mg/L	%	–
Ⅰ	7.90	0.24	4.86	0.031	74.5	12.6
Ⅱ	8.16	0.07	11.21	0.006	48.5	1.1
Ⅲ	8.26	0.08	19.71	0.003	33.5	0.6
Ⅳ	8.08	0.12	11.41	0.009	54.3	1.3
Ⅴ	7.80	0.21	4.06	0.020	65.3	2.1
Ⅵ	7.87	0.16	5.21	0.014	56.3	1.4

4. References

1. World Resources Institute, 2012. World Resource Institute. Eutrophication and Hypoxia: Nutrient Pollution in Coastal Waters. (2012) URL: http://www.wri.org/project/eutrophication
2. Anthonisen A.C., Loehr R.C., Prakasam T.S., et al. Inhibition of nitrification by ammonia and nitrous acid. J. Water Pollut. Control. Fed. 48, 835-852, 1976.
3. E. Jimenez, J.B. Gimenez, A. Seco, et al. Effect of pH, substrate and free nitrous concentration on ammonium oxidation rate. Bioresouce Technology. 124:478-484, 2012.

The Start-up and Inhibition Analysis of Anammox Process in a UASB Reactor Using Mixed-Culture Sludge

○Yanlong Zhang[1], Shilong He[2], Haiyuan Ma[1], Qigui Niu[1], Yu-You Li[1,3]*

[1]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
[2]School of Environment Science and Spatial Informatics, CUMT, Xuzhou, 221116, China
[3]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

The Anammox system was started up with 2L of activated sludge and 3L of anaerobic digestion sludge in a UASB reactor. After 140 days enrichment, the Anammox system was successfully started up. The ammonium removal efficiency reached to near 99.5%. HRT was shortened from 24h to 3h step by step to increase the NLR to 1.68g/L/d. During the steady state, the NRR reached to 1.46g/L/d, and the consumption ratio of NH_4^+ to NO_2^- was in the range of 1.0~1.1. Substrate concentration shock was also investigated in the experiment. When the concentration of FA and FNA was higher than 20mg/L and 7μg/L, the Anammox system was 60% inhibited. The recovery process took 40 days long time by decreasing the TN concentration to 210mg/L. When the nitrogen removal efficiency was totally recovered, NLR was increased to 2.35g/L/d, the Anammox system maintain stable.

Keywords: Anammox, HRT, inhibition, free ammonium (FA), free nitrite acid (FNA), recovery

1. Introduction

As an innovative and sustainable biotechnology for nitrogen removal with the advantage of low-cost and low sludge production , anaerobic ammonium oxidation (Anammox) process has been increasingly valued in the wastewater treatment with low carbon to nitrogen (C/N) ratios. In this process, Planctomycete type bacteria combine ammonium and nitrite to generate nitrogen gas under anaerobic condition without organic carbon source. Eq. (1) describes the nitrogen removal reaction by Anammox process.

$$NH_4^+ + 1.32NO_2^- + 0.066HCO_3^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 0.066CH_2O_{0.5}N_{0.15} + 2.03H_2O \quad \cdots\cdots\cdots\cdots (1)$$

The reaction characteristics of Anammox process results in 60% reduction of energy consumption as well as 90% reduction of CO_2 emission that minimize the carbon footprint in wastewater treatment plant. However, challenges in fast start-up and steady operation of Anammox system limited its application and industrialization. The long doubling time (10d~14d) and low cell yield ($0.11gVSSg^{-1}NH_4^+$-N) of Anammox bacteria results in a quite long startup period of Anammox reactor. Both substrates can exert inhibitory effects on Anammox process, high substrate concentration can easily cause the decrease of Anammox activity and the loss of the stable operation.

The aim of this study focused on the evaluation of the start-up process in UASB reactor. Substrate inhibition and recovery process was also studied in the experiment.

2. Materials and Methods

2.1. Experimental facilities

As shown in Fig.1, UASB reactor with 5L working volume was used in the experiment. The reactor had a height-diameter ratio of 8:1 and made with strong plexiglass. Water jacket was used to control the temperature in the range of 33±1℃. The reactor was covered with light-weight shading fabric to avoid the growth of photosynthetic microorganism which would produce oxygen. Influent pumps were controlled by timer

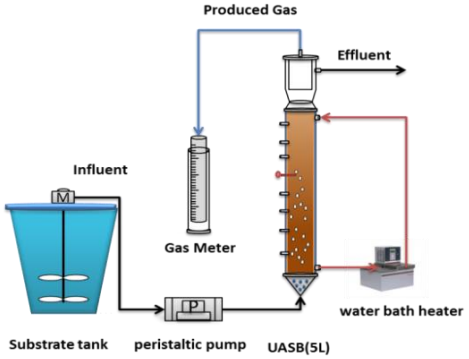


Fig. 1 Experimental setup

(on 1min, off 1min) to intermittently provide substrate into the reactor. Gas production was measured with homemade Gas-holder.

2.2 Experimental procedures

3L of nitrification sludge and 2L of anaerobic digestion sludge were inoculated into the reactor. Effluent pH was controlled in the range of 7.6~8.5.The reactor was fed with synthetic wastewater, the initial HRT was set at 24 hours. NLR increased gradually by changing HRT at a constant substrate concentration of 210mg-N/L, and finally reached to 1.68 g/L/d. Then, the influent TN concentration was doubled without change of NLR. After the TN removal efficiency decreased to 40%, the influent TN concentration was returned to the initial level of 210mg-N/L to study the effects of substrate shock. When the Anammox reaction was totally recovered, TN concentration was increased to 315mg/L to raise the NLR to 2.35g/L/d.

3. Results and Discussion

3.1. Start-up phase

As shown in Fig.2, N_2 gas production rate and TN removal efficiency began to increase from 100d. Gas component analysis indicated that over 98% of the produced gas was N_2. The theoretical N_2 gas production was basically consistent with the measurement value that confirmed the Anammox reaction. Accompany with the occurrence of Anammox activity, pH also increased due to the consumption of HCO_3^-

which was provided as inorganic carbon source and buffer agent. It took around 40 days to achieve the highest ammonium removal efficiency which around 99% and maintain stable.

Accompany with the change of HRT, gas production maintained stable growth. Anammox bacteria further enriched in the reactor. Fig.3 shows the successfully enriched Anammox granular with an average diameter of 1-2mm.

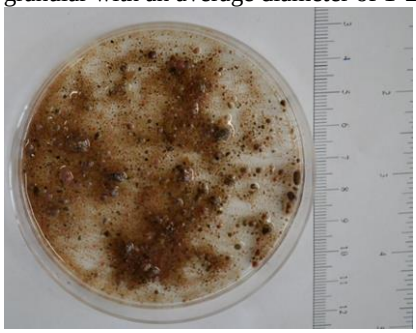


Fig. 3 Enriched Anammox granular sludge

3.2. Inhibition and recovery phase

After doubling the TN concentration at 327d, pH increased to 9.0. Then, 2 times of buffer was added in order to decrease the pH to the optimum range. Gas production rate and TN removal efficiency rapidly decreased to 420mL/L/d and 40% respectively. As a result, effluent NH_4^+-N and NO_2^--N reached to 100 mg/L and 120 mg/L respectively. The average results obtained from previous studies to evaluate the effects of substrate on Anammox process are presented on the basis of the Free Ammonia (FA) and Free Nitrite Acid (FNA) concentration, due to the fact that FA and FNA are considered to be the true inhibitor compounds. In this study, FA increased to 20 mg/L, FNA decreased during the first 10 days and then increased rapidly to 7μg/L due to the change of pH and NO_2^- concentration (Fig.4). The results showed that Anammox system was inhibited by high TN concentration. 40 days later after decreasing the TN concentration to 210mg/L, nitrogen removal efficiency was totally recovered.

To evaluate the ability of the recovered Anammox system, influent TN concentration was increased to 315mg/L from 543d, correspondingly, the NLR increased to 2.35g/L/d. The nitrogen removal efficiency decreased with the change of influent TN concentration but soon recovered to previous level, the results that indicated that the nitrogen removal potential of the Anammox system was further improved.

4. Conclusions

(1) Anammox system was successfully started up in 140 days from a mixture of nitrification sludge and anaerobic digestion sludge. TN removal efficiency reached to 90%.
(2) The system was sensitive to the substrate shock. FA, FNA and pH were the factors that affecting the stability of Anammox system.
(3) The inhibition is restorable. Recovery took a long time around 40 days.

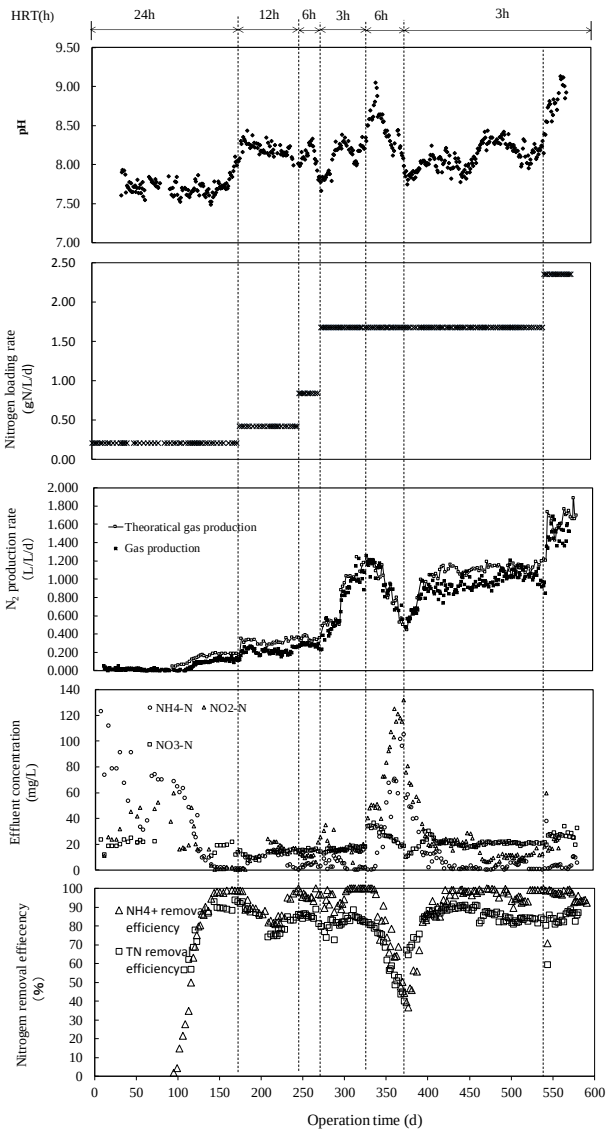


Fig. 2 Time course of Anammox reaction during continuous operation

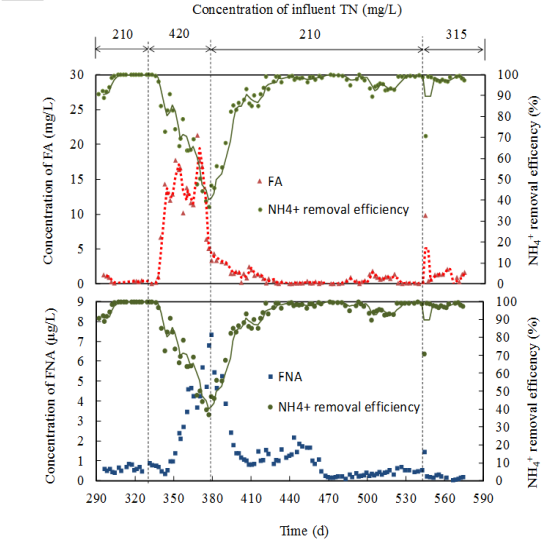


Fig. 4 Effects of FA and FNA

GeoChip-based analysis of the microbial community of a combined nitritation-anammox reactor treating digestion supernatant

○Jian Zhao[1], Jiane Zuo[1]*, Xiaolu Wang[1]

[1] *State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, China*
*E-mail: *jiane.zuo@tsinghua.edu.cn*

Abstract

A combined nitritation-anammox reactor was established to treat anaerobic digestion supernatant. The reactor achieved a nitrogen load rate of 0.5 kg N/(m^3 d) and total nitrogen removal efficiency of 85% after 140 days operation. GeoChip 4.0 was subsequently used to examine the microbial community functional structure under different percentage of supernatant. The results indicated that the microbial community of reactor which presented stable performance still revealed significant difference under varied environmental press. The genes diversity and the total abundance of functional genes were both depressed by the increasing percentage of the supernatant. In addition, it was revealed that nitrogen compounds, C/N ratio in the influent and operation parameters were the key variables shaping the microbial community, counted for 76.64% of the variance of the reactor.

Keywords: Geochip 4.0, microbial community, combined nitritation-anammox, anaerobic digestion supernatant

1. Introduction

The combined nitritation-anammox process has been considered as a promising alternative to conventional nitrogen removal because of its cost-saving. This process has been applied to treat the wastewater with high concentration of ammonia and relative low COD, such as anaerobic digestion supernatant, landfill leachate and optoelectronic industrial wastewater (Daverey et al. 2013). The mechanism that the reactor treating anaerobic digestion supernatant could operate stably and efficiently was complicated. This process is primarily related to the nitrogen cycling such as nitritation, nitrification, denitrification, and anammox. Furthermore, microbial genes related to carbon cycling, metal resistance and organic remediation and so on should also be examined because of the relative high concentration of TOC, heavy metals and organic pollutants. Therefore, the purpose of this study is to elucidated the underlying mechanisms the efficiency and stability for combined nitritation-anammox treating anaerobic digestion supernatant and find out the key environmental variables shaping microbial community.

2. Materials and methods

2.1 Reactor setup and Operational Conditions

A SBR with a working volume of 5 l was used for the combined nitritation-anammox process. The reactor was made by polymethyl methacrylate with inner diameter of 10 cm. Sponges were used as the biomass carrier and the packing rate was 40% (v/v). The temperature was kept at 32±1 °C during the whole experiment by a thermostat water jacket. The exchange volume rate of the SBR was kept at 40%. Compressed air was supplied via a diffuser at the bottom of the reactor to keep the dissolved oxygen around 2mg/L.

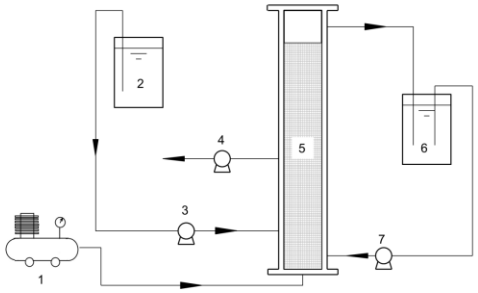


Fig. 1. Schematic of SBR for combined nitritation-anammox. (1) Air compressor; (2) Influent tank; (3) Influent pump; (4) Effluent pump; (5) SBR; (6) Water bath; (7) Circulating pump.

2.2 DNA Extraction, Purification, Labeling and Hybridization.

DNA was extracted using freeze-grinding method (Lu et al. 2012)and then quantified by a NanoDrop ND-100 Spectrophotometer (NanoDrop Technologies Inc., Wilmington, DE, USA). DNA was labeled with the fluorescent dye Cy-5 using3 a random priming method and then purified with QIA quick purification kit(Qiagen,

Valencia, CA, USA) according to the manufacturer's instructions and dried at 45°C for 45min (ThermoSavant, Milford, MA, USA). Subsequently, labeled DNA was resuspended in 120μl hybridization solution and hybridizations were performed with a MAUI hybridization station (BioMicro, Salt Lake City, UT, USA) according to the manufacturer's recommended method. Microarray was scanned by a NimbleGen MS200 scanner(Roche, Madison, WI,USA) and signal intensities were subsequently quantified.

3.Results and discussion

3.1 Reactor Performance

The reactor showed the capacity of nitrogen removal after inoculation immediately. From the 48th day, NLR was kept at 0.5 kg N/ (m^3 d) for about 100 days. The percentage of the supernatant (Lin et al. 2011) was gradually increased so that the ammonia from the supernatant contributed 20%, 40%, 60%, 80%to 100% of the total ammonia of the influent, respectively. Nevertheless, stable NH_4^+ and TN removal efficiencies were maintained around 99% and 85%, respectively. There was no significant organic removal in phase P20, P40, P60 and P80, and occasionally very mild TOC increase in the effluent could be detected.

3.2 Overview of functional gene diversity and structure of microbial community

DCA (Detrended Correspondence Analysis) was conducted to illustrate whether the structure of microbial community changed significantly through different phases. Samples were grouped by phases and samples from different phases were well separated from each other. A total of 28575 gene probes were detected across all samples, ranging from 19910 to 23498 probes in each sample, and the probes detected in P100 were less than the probes in P40 significantly ($P<0.05$). Shannon Index and Simpson Index were calculated to express the functional diversity across the samples. the Shannon Index and Simpson Index was significantly smaller in final phase compared to the initial phase ($P<0.05$) and kept relatively stable through P40 to P80, then fell down to the lowest in P100 finally. An average of 73.25% (ranging from 68.68% to 79.05%) of probes were shared among the phases, and 16517 probes (57.80% of all detected probes) presented in all phases, in which genes involved in organic remediation (3916 probes), stress (3308 probes), metal resistance (1894 probes), carbon cycling (1863 probes), and nitrogen cycling (1322 probes) consisted of the majority of probes detected in all phases.

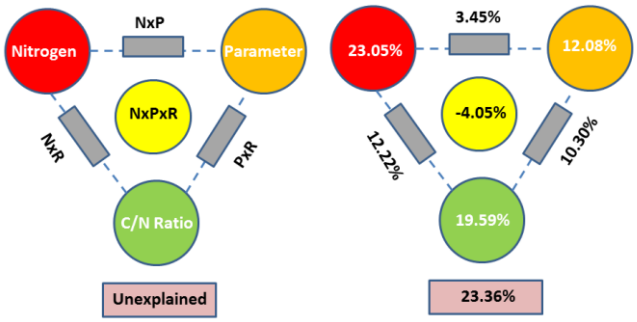


Fig. 2. Variation Partitioning Analysis based on CCA that partitions relative influence of environmental variables on microbial community structure. Environmental Variables are divided into groups of Nitrogen (ammonia, nitrite and nitrate in the influent), Parameters (DO and temperature) and C/N Ratio (TOC in the effluent and C/N ratio). The circles represent the specific variables group by partitioning out the effects of other groups. The squares represent the joint effect of the circles on the both sides of the square. The portion unexplained by any of the tested variables is represented by the square at the bottom of the figure.

3.3 Relationship between Community Functional Structure and Reactor Performance

Variance partition analysis (VPA) was subsequently performed to dissect the contribution of nitrogen compounds in the influent, operation parameters and C/N ratio to the microbial community structure, as shown in Fig.2. A total of 76.64% of community variations could be explained by these selected variables. Nitrogen compounds, C/N ratio and operation parameters contributed to 23.05%, 19.59% and 12.08%, respectively. Furthermore, C/N ratio and nitrogen compounds together, C/N ratio and operation parameters together contributed extra 12.22% and 10.30% of the total variance, indicating that there were considerable interaction within C/N ratio and nitrogen compounds, C/N ratio and operation parameters, respectively.

Reference

1. Daverey, A., Su, S.H., Huang, Y.T., Chen, S.S., Sung, S.W. and Lin, J.G. (2013) Partial nitrification and anammox process: A method for high strength optoelectronic industrial wastewater treatment. Water Research 47(9), 2929-2937.

2. Lu, Z.M., He, Z.L., Parisi, V.A., Kang, S., Deng, Y., Van Nostrand, J.D., Masoner, J.R., Cozzarelli, I.M., Suflita, J.M. and Zhou, J.Z. (2012) GeoChip-Based Analysis of Microbial Functional Gene Diversity in a Landfill Leachate-Contaminated Aquifer. Environmental Science & Technology 46(11), 5824-5833.

3. Lin, J., Zuo, J.E., Gan, L.L., Li, P., Liu, F.L., Wang, K.J., Chen, L. and Gan, H.N. (2011) Effects of mixture ratio on anaerobic co-digestion with fruit and vegetable waste and food waste of China. Journal Of Environmental Sciences-China 23(8), 1403-1408

Enrichment of denitrifying methanotrophic bacteria from Taihu sediments by a membrane biofilm reactor

Shenghui Wang, ○Qing Wu, Ting Lei, Peng Liang, Xia Huang*
State Key Joint Laboratory of Environment Simulation and Pollution Control
School of Environment, Tsinghua University, Beijing, 100084, P.R. China
*E-mail: xhuang@tsinghua.edu.cn

Abstract

Denitrification coupled to anaerobic methane oxidation (DAMO) process was recently reported to be an energy-efficient way for nitrogen polluted wastewater treatment. This process was performed by putative denitrifying methanotrophs, which could use methane as sole electron donor to drive denitrification. However, it is difficult to obtain DAMO cultures due to the slow growth rate. In this study, a membrane biofilm reactor was designed to enrich the DAMO microorganisms from Taihu sediments. After 13 months of enrichment, the obtained maximum of denitrification rate was 0.54 mM/d for nitrate and was 1.06 mM/d for nitrite in the reactor.

Keywords: NC10 phylum; denitrification; anaerobic methane oxidation; membrane biofilm reactor

1. Introduction

Municipal wastewater with low carbon/nitrogen ratio is a common problem in China [1]. Conventional biological denitrification requires an external carbon source such as methanol and acetate, or other electron donors such as hydrogen and elemental sulfur for treatment [1], which may cause some problems such as excess biological growth, increased operating cost and residual organic carbon in the effluent. Denitrification coupled to anaerobic methane oxidation (DAMO) is a recently discovered process which can use methane as carbon source for denitrification in the absence of oxygen. Simultaneously, DAMO provides a new way for greenhouse gas utilization.

However, the main obstacle for the application of this process is the low growth rate of the involved microorganisms, a bacterium affiliated with the NC10 phylum and an archaea distantly related to anaerobic methanotrophic archaea (ANME-II) [2].The slow mass transfer of methane is likely to be an explanation for the slow growth rate.

In this study, methane is supplied from the interior of a membrane to the biofilm growing on the membrane surface and the carrier. A DAMO culture was successfully enriched by the membrane biofilm reactor (MBfR), using Taihu sediments in China as inocula.

2. Materials and Methods

2.1. Reactor configuration and operation

The 3.6 L cylindrical MBfR consisted of a polyvinylidene fluoride (PVDF) hollow fiber membrane module (Fig.1). Methane was continuously sparged through the membranes.

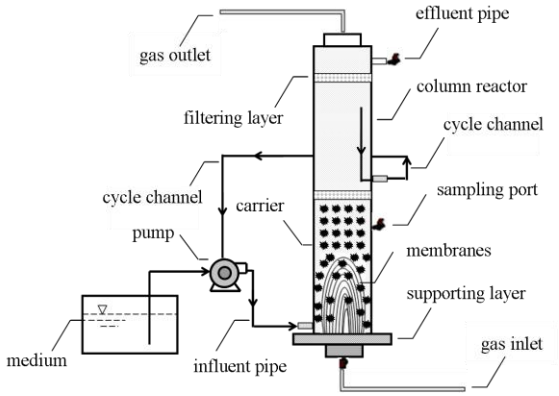


Fig. 1. Schematic of the constructed membrane biofilm reactor (MBfR)

Samples for inoculation were collected from sediments of Taihu. The synthetic medium used to enrich the DAMO culture contained (g/L): 0.722 KNO_3; 1.0 $MgSO_4 \cdot 7H_2O$; 0.27 $CaCl_2 \cdot 2H_2O$; 0.0091 $FeSO_4 \cdot 7H_2O$; 2.0 mL of phosphate buffer; 1.0 mL of trace mineral solution. The phosphate buffer contained (g/L): 24.4 KH_2PO_4; 10.2 Na_2HPO_4. The trace mineral solution contained (g/l): 2.486 $FeSO_4 \cdot 7H_2O$; 0.5 $MnCl_2 \cdot 4H_2O$; 0.05 $ZnCl_2$; 0.101$NiSO_4 \cdot 6H_2O$; 0.05 $CoCl_2 \cdot 6H_2O$; 0.026 $Na_2MoO_4 \cdot 2H_2O$; 0.05 H_3BO_3; 0.31$CuSO_4 \cdot 5H_2O$; and 35% HCl 5 mL. The pH was adjusted to 7.0-7.2. The medium was boiled and sparged with N_2 to remove dissolved oxygen before it was added to the reactor. To increase the internal mass transfer in the reactor, the medium was recycled with a peristaltic pump. The effluent nitrate was measured 3-5 times per week, and the nitrate was supplemented if less than 1 mg/L. This reactor was operated under steady-state denitrification for 10 months prior to the batch experiments.

2.2. Anaerobic activity batch tests

Inocula with volume of 20 mL was transferred from the MBfR to 120 mL serum bottles. Each bottle was added with 60 mL fresh medium. The headspace was filled with 20 mL CH_4 and 20 mL N_2. These bottles were incubated in a shaker at 30 ℃, 180 rpm. Methane and nitrite (nitrate) consumption rates were determined by periodic measurements.

3. Results and discussions

3.1. Enrichment of DAMO cultures from Taihu sediments

The reactor was operated for 13 months which was divided into 4 stages (I: day 0-60; II: day 61-120; III: day 12-210; IV day 211-410.). The average nitrate removal rate was 1.27, 2.36, 0.35, 0.54 mM/d respectively on stage I-IV. Due to the addition of an interior cycle, Stage II had a higher nitrate removal rate than stage I. The depletion of residual organics contained in the inocula might led to the decrease of removal rate in stage III. However, the nitrate removal rate rose in stage IV, which was higher than the maximum rates reported by most other studies[2]. This might be ascribed to the enrichment of denitrifying methanotrophic bacteria. These results suggested that the use of hydrophobic hollow-fiber microporous membranes might enhance the gas-liquid mass transfer of methane, and promote the utilization efficiency of methane, thus improve denitrification activities.

The relative abundance of NC10 bacteria was obtained by fluorescence in situ hybridization (Fig. 2). There was no clear hybridization signal with NC10 specific probes in the initial inocula (Fig. 2A). After 9 months, NC10 bacteria could be detected and the relative abundance reached 55% (Fig. 2B), and they accounted for about 73% at the thirteenth month (Fig. 2C). These results indicated that the biomass of NC10 bacteria became more abundant after 13 months of enrichment and denitrification coupled to anaerobic methane oxidation was the major denitrification process in the reactor.

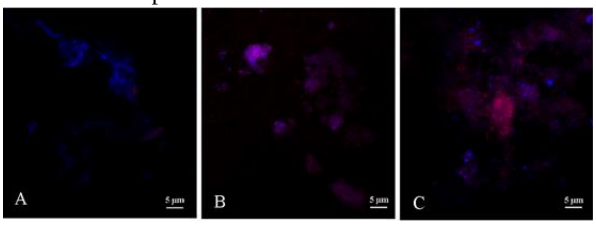


Fig. 2. Fluorescence in situ hybridization of biomass from the MBfR at different times. A: initial sample; B: after 9 months enrichment; C: after 13 months enrichment. The cells were hybridized with the probes S-*-DBACT-1027-a-A-18 specific for the NC10 phylum (Cy3, red) and S-D-Bact-0338-a-A-18 that target most bacteria (Cy5, blue). "NC10" bacteria appear pink due to double hybridization with both specific and general bacterial probes. Scale bar = 5 μm

3.2. Analysis of substrate consumption

After 13 months of enrichment, the anaerobic methane oxidizing activity was 15 μM/d and nitrite removal activity was 26.76 μM/d (Fig. 3A). The observed stoichiometry of methane consumption versus nitrite conversion was 3 CH_4 : 10.6 NO_2^-, which was close to the theoretical value 3:8 (Eq. 1)[2]. Simultaneously, the similar result was obtained in nitrate-depended methane anaerobic oxidation (Fig. 3B). The anaerobic methane oxidizing activity was 14.73 μM/d and nitrate consumption activity was 15.8 μM/d, and the molar conversion ratio of CH_4 : NO_3^- was 5:10.7, which was similar to the theoretical value 5:8 (Eq. 2)[2].This further indicated a successful enrichment of the denitrifying methanotrophic bacteria.

$$3CH_4 + 8NO_2^- + 8H^+ \rightarrow 3CO_2 + 4N_2 + 10H_2O \quad \text{(Eq.1)}$$

$$5CH_4 + 8NO_3^- + 8H^+ \rightarrow 5CO_2 + 4N_2 + 14H_2O \quad \text{(Eq.2)}$$

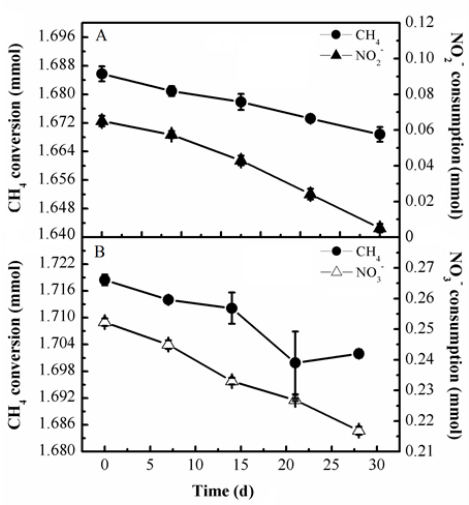


Fig. 3. Methane and nitrite (nitrate) conversion of the enrichment culture on day 387. A: NO_2^- as the electron acceptor; B: NO_3^- as the electron acceptor.

4. Conclusions

In this research, methane was supplied to the DAMO microorganisms by hollow fiber membrane in an MBfR. The increase of nitrate removal rate and abundance of NC10 proved the feasibility of MBfR in DAMO culture enrichment.

References

1. Park, J. and Y. Yoo, Biological nitrate removal in industrial wastewater treatment: which electron donor we can choose. Applied Microbiology and Biotechnology, 2009. 82(3): p. 415-429.
2. Raghoebarsing, A.A., et al., A microbial consortium couples anaerobic methane oxidation to denitrification, 2006, Nature Publishing Group. p. 918-921.

Carbon Source Recovered from Sludge Hydrolysis Do have impacts on the Performance and Microbial Community Structure of Denitrifying Reactor in an Enhanced Nitrogen Removal Process

○Bo Shen, Fang Zhao, Xianghua Wen*

Environmental Simulation and Pollution Control State Key Joint Laboratory, School of Environment, Tsinghua University, Beijing 100084, China
*E-mail: xhwen@tsinghua.edu.cn

Abstract

In this study, the treatment performance and microbial community of denitrifying unit in a novel enhanced nitrogen removal process composed of adsorption/nitrification/denitrification/sludge-hydrolysis (ANDH) were investigated. For comparison, methanol and the hydrolyzed sludge was used as carbon source for the denitrification Operating data indicated that the denitrification unit can produce steadily qualified effluent. The specific denitrification activity measurements showed that the acclimatized sludge with the hydrolyzed sludge as carbon source instead of methanol had a high denitrification rate. Both clone library and 454 pyro-sequencing analysis results suggested a more diversified evenly distributed microbial structure in stable phase but the two results showed different variation tendency in the microbial phylum. Results of 454 pyro-sequencing also demonstrated that variation in *Betaproteobacteria, Bacteroidete, Planctomycetes* and other newly-presented phylum may be the potential contributors to the excellent performance of the denitrifying unit.

Keywords: hydrolyzed sludge; denitrification; microbial community; 454 pyro-sequencing; wastewater treatment

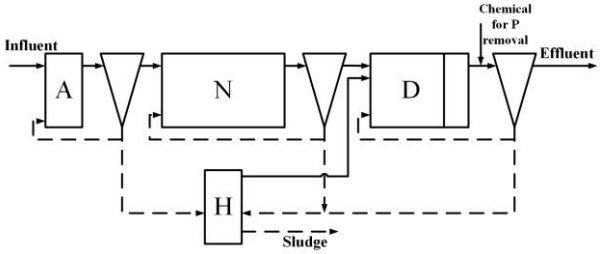
1. Introduction

To solve the problems of most wastewater treatment plants in Southern China such as low C/N ratio of sewage, carbon shortage of denitrification, instable nitrification under low temperatures and high costs of sludge disposal, we proposed an enhanced nitrogen removal process called ANDH characterized by a combination of biological adsorption and sludge hydrolysis (see Fig. 1). A pilot-scale ANDH system was built in Chengbei municipal wastewater plant, Wu Xi city. The ANDH was successfully operated after parameter optimization and can provide qualified effluent. Among the four units in the system, the denitrification unit may be the most susceptible when hydrolyzed sludge is used as external carbon source. Both the process and microbes would go through an adjusting period to achieve stability. Therefore, following our previous studies, our objective of this work was to evaluate the carbon source dependent performance and microbial community of the denitrification unit.

2. Materials and Methods

2.1. Operation of the pilot ANDH process

After optimization of the operational parameters of each units, we stepwisely raised the ratio of hydrolyzed sludge over methanol as external carbon source for the denification unit in order to make the microbes adapt to the hydrolyzed carbon source gradually. The total amount of carbon source added was controlled to 375gSCOD/d. After methanol was completely replaced, the system was operated steadily for 310 days from June, 2010 to April, 2011. Sludge samples were taken from the denification tank before using hydrolzed sludge (initial phase) and on the 296th day of stable operation (stable phase).



A: Adsorption N: Nitrification D: Denitrification H: Hydrolyzation

Fig. 1. The ANDH process

2.2. Cloning analysis

6 clone libraries targeting 16S rRNA of total bacteria, NirS and NirK gene of DNB were constructed. The amplification of 16S rRNA was performed with the primers 8F and 1492R, NirS gene with the primers NirS 1F and NirS 6R and NirK gene with the primers NirK 1F and NirK 5R. Sequencing was done by Chinese National Human Genome Center (SinoGenoMax).

2.3. 454 pyro-sequencing

454 pyro-sequencing analysis was applied to the bacterial 16S rRNA gene of the same samples of clone library. Primers used for PCR is as the previous (Reference 2). Sequencing was also done by SinoGenoMax.

3. Results and discussion

3.1. Performance of the denitrification unit

During the 310 days of operation, the ANDH system achieved good performance in removal of pollutants.

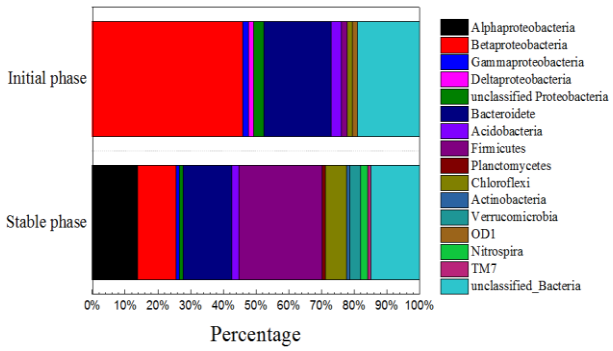


Fig. 2. Classification of phylum of clone library targeting 16S rRNA

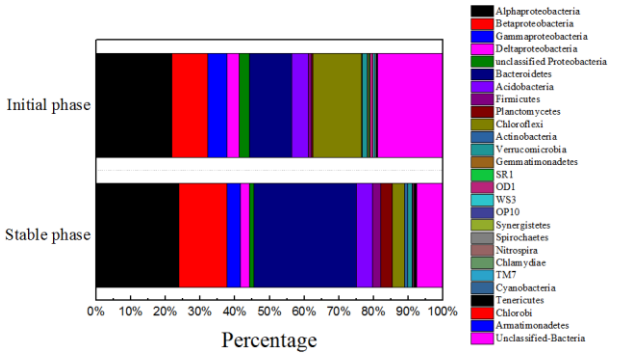


Fig. 3. Classification of phylum of 454 pyrosequensing targeting 16S rRNA

Although the influent loading rate fluctuated largely, the concentrations of pollutants in the effluent of the denitrification reactor were stable and met with the requirements of national discharge standard A of China. The hydrolyzed sludge had an average COD of 1529.7mg/L with C/N atio of around 15, which was proven to be satisfactory carbon source for denitrifiaction resulting in qualified effluent.

3.2. Clone library and 454 pyro-sequencing of 16S rRNA

The result of clone library showed that 40 and 81 OTUs were detected from 98 and 94 clones of samples of initial and stable phase separately, which indicated that the microbial community were more diversified. The classification of phylum (see Fig. 2) indicated that in both phases the dominant phylum were *Proteobacteria* and *Bacteroidete*. The greatest changes of bacterial phylum from initial phase to stable phase in percentage include the decrease of *Alphaproteobacteria* and *Bacteroidete* and the increase of *Betaproteobacteria* and *Firmicutes*.

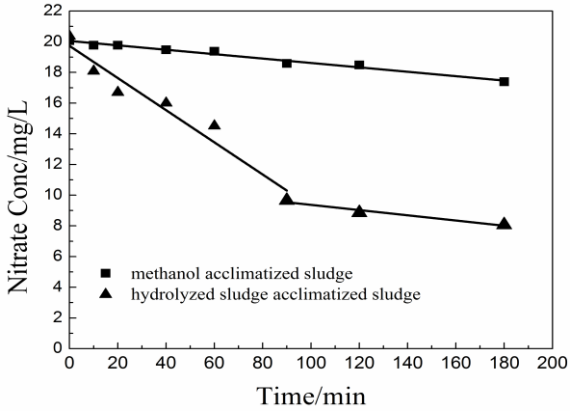


Fig. 4. Specific denitrification activity measurements

Both samples harboured more than 4700 OTUs based on 454 pyro-sequencing results, showing little difference in number of OTUs. Much more species were found in the classification of phylum (see Fig. 3). Comparing to the clone library data, similarly, *Proteobacteria* and *Bacteroidete* were the dominant phylum in both samples as well. However, there existed great discrepancy on variation tendency of several main phylum: *Alphaproteobacteria* and *Firmicutes* did not change much in both phases, *Bacteroidete* was significant increased and *Chloroflexi* was greatly decreased in the stable phase.

The huge differences between clone library and 454 pyro-sequencing may be due to inadequate coverage of clone library. 454 pyro-sequencing can provide more comprehensive and precise information as a high-throughput technology. In spite of these differences, the results of both technologies showed that the microbial community structure changed vastly in the stable phase. When hydrolyzed sludge was added for long-term, the microbial community showed more diversity and evenness.

3.3. Sludge denification activity and its microbes

The sludge specific denitrification activity (SDA) of the sludge acclimated with methanol (Sm) and hydrolyzed carbon (Sh) were measured by using hydrolyzed sludge as carbon source at the temperature of 14.6 ^{o}C with a MLVSS concentration of 2.4 g/L. Calculated from the decreasing rate of nitrate concentration, the SDA was 8.2mg/g·d for Sm, 62.88 mg/g·d in the early stage for Sh and decreased to almost the same level as Sm (see Fig. 4). Therefore, through acclimation, the sludge can have very high denitrification rate when using hydrolyzed carbon.

Clone library analysis of NirS and NirK genes showed that *Alphaproteobacteria* and *Betaproteobacteria* increased noticeably in the stable phase, many common denitrifying bacteria are associated with which. According to 454 pyro-sequencing, *Bacteroidete* increased as well apart from *Betaproteobacteria* in the stable phase, which is said to be capable of sulfide-dependent autotrophic denitrification. *Planctomycetes* were supposed to be an anommox bacteria. Some undetected bacteria emerged in the stable phase, which may also be potential contributors to the good performance of denitrification.

4. References

1. Liu, H.B., Zhao, F., Mao, B.Y., Wen, X.H. (2012). Enhanced nitrogen removal in a wastewater treatment process characterized by carbon source manipulation with biological adsorption and sludge hydrolysis. Bioresource Technology, 114, 62-68.
2. Hu, M., Wang, X.H., Wen, X.H., Xia, Y. (2012). Microbial community structures in different wastewater treatment plants as revealed by 454-pyrosequencing analysis. Bioresource Technology, 117, 72-79.

Session II:
Antibiotics
on the environment

Effect of antibiotics in water environments and its removal from wastewater by means of Advanced Oxidation Processes: electro-Fenton

○Adriana Ledezma Estrada[1], Yu-You Li[1,2,*]

[1]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[2]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
*E-mail: adriana@epl1.civil.tohoku.ac.jp

Abstract

The presence of antibiotics in water environments is a growing concern due to the threat that it represents for both health and environment whether promoting multi-antibiotic resistance bacterial strains or affecting the environment. A high incidence of antibiotics in the environment usually occurs in areas where antibiotics are indiscriminately used or/and where the wastewater treatments are not efficient to remove them. An alternative to completely eliminate them from the wastewater are the Advanced Oxidation Processes (AOPs), which are based on the production of highly oxidizing species, like the electro-Fenton process (EFP). The EFP is based on the reaction $H_2O_2 + M^+ \rightarrow M^{(n+1)} + (^{\bullet}OH) + HO^-$, where M is the catalyst. This study deals with the use of different transition metals as the catalyst, Co, Cu, Mn and Fe, in order to enhance the efficiency. The TOC removals were as follows: 81% for Co, 65% for Cu, 49% for Fe, and 40% for Mn.

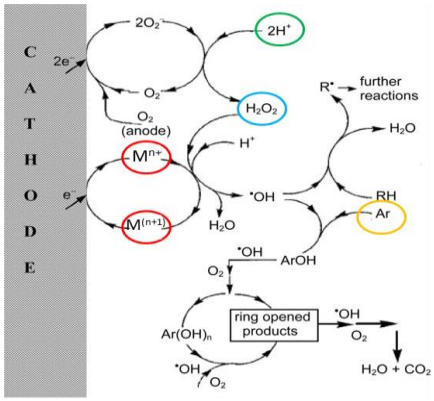
Keywords: antibiotics, antibiotic resistance, Advanced Oxidation Processes, electro-Fenton process, biodegradability

1. Introduction

The use of antibiotics has significantly increased during the last decades. They are used in the farming industry to promote animal growth as well to prevent and treat diseases generated by the bad conditions were they are farmed. Additionally, the antibiotic use in humans is very high and in some countries it is not even regulated. Usually antibiotics are not completely metabolized, thus they and their active metabolites are excreted by the organism. As a consequence, they can be detected in the wastewater. It has been reported that wastewater promote multi-antibiotic bacterial resistance.

The common wastewater treatments are inefficient to remove them for several reasons like they are bio-recalcitrant, in low concentrations and their chemical structure is very diverse[1].

A feasible alternative is the AOPs, which can mineralize hazardous pollutants or increase the biodegradability of the wastewater. The EFP is a commonly used AOP that produces $^{\bullet}OH$ by the general reaction **Eq. 1**. $H_2O_2 + M^+ \rightarrow M^{(n+1)} + {}^{\bullet}OH + HO^-$ [2]. The reaction mechanism is showed in Fig.1. The classic reaction uses Fe^{2+} as the catalyst; however, it has been reported that the reaction could be improved if other transition metals are used as the catalyst ions. The most used after the Fe^{2+} is the Cu^+ ion. Nevertheless, cobalt and manganese could also be used and get higher efficiencies [2]. Also, to decrease the operational cost an option is to combine the AOP with a biological process. Thus, the AOP will increase the biodegradability for a further biological treatment that would be able to remove the pollutant, in this case the antibiotic cefalexin (CLX)[3]. We select CLX because a lot of resistant bacteria strains have been reported at hospitals.



$$H_2O_2 + M^{n+} \rightarrow M^{(n+1)} + {}^{\bullet}OH + OH^-$$

Fig. 1. Reaction mechanism of the EFP

2. Material and methods

A cefalexin solution of 200mg/L was electro-oxidized by the eletro Fenton process. In an undivided glass reactor of 500 mL with a RuO_2/Ti mesh used as the anode and an activated carbon fiber (ACF) was used as the cathode. The experiments were done in batch for 8h and sampled at different times. The catalyst used concentration in all the electro-Fenton processes was of 1mM, the salt used were $FeSO_4$, $MnSO_4$, $CoSO_4$ and Cu_2SO_4 analytical grade from WAKO. For the Anodic oxidation with H_2O_2 generation, any catalyst was used. The oxidation of CLX was measured by the standard methods COD and TOC. The biodegradability was evaluated by the BOD controller OxiTop®-1020WTW, for 5 days. The bacteria seed used for such measurement was obtained from the Sendai Wastewater Treatment Plant. All with an O_2 input of 01L/min and stirred.

3. Results and discussion

3.1 Electro-Fenton process catalyzed by different metals: Fe, Cu, Mn and Co

The oxidation efficiency of each electro-Fenton process was assessed in terms of the TOC removal. Then, the results were compared with the Anodic oxidation with electrogenerated H_2O_2 in order to remark the importance of the effect of the catalyst in the electron-Fenton process. Fig 2**.** shows the behavior and the percentage of TOC removal during 8h of process. As can be noticed, when no catalyst is used the CLX oxidation is very low, only of 7%. But when catalyst is used the oxidation considerably increases. The highest oxidation was with the Co ion, 81%, then with the Cu ion, 65%, and the lower were with Fe and Mn ions with 49% and 40% respectively. These results are in accordance of what other authors have been reported [2]. The difference among the efficacies seems to be the electronic configuration of the ions, which allows freeing its electrons easier than the other, in other words, their electro-negativity. This property refers to the property of an element to at track electrons.

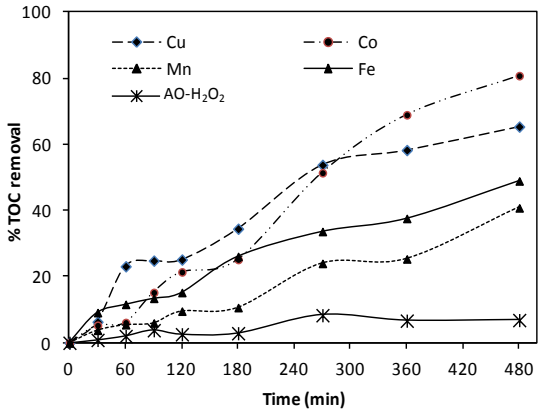


Fig. 2. TOC removal percent as a function of time for the EFP catalyzed with different ions and the AO-H_2O_2 process

All of these results indicated that the use of different catalyst is able to enhance the oxidation efficiency of the electro-Fenton process.

3.2 Biodegradability enhancement of the cefalexin by the EF processes

The biodegradability is also another important parameter to analyze the oxidation efficiency of the AOPs since sometimes they do oxidize the pollutant molecule but produce even more bio-recalcitrant compounds. Thus, the biodegradability was measured in terms of the BOD_5/COD relation. Fig. 3 depicts the biodegradability of each electro-Fenton process as well of the Anodic Oxidation with electrogenerated H_2O_2. The results are in accordance with the TOC removal percentages. However, it is important to note that the biodegradability when Co ions as well of Cu ion were used it is significantly increased. To consider a wastewater biodegradable, the minimum value of BOD_5/COD ratio is of 0.3 and the optimal is of 0.5. As shown in Fig. 3 the biodegradability for AO-H_2O_2 is of 0.05, for the EFP-Fe is of 0.34, EFP-Mn is of 0.29, EFP-Cu is of 0.65 and for the EFP-Co is of 0.62. It is interesting to notice that the difference in the biodegradability between EFP catalyzed with the Co and the Cu ion is not markedly different, only of 5% while the difference in the oxidation process is about 20%. This could be explained by the mechanism reaction, that suggests the formation of different by products during the EFP[3].

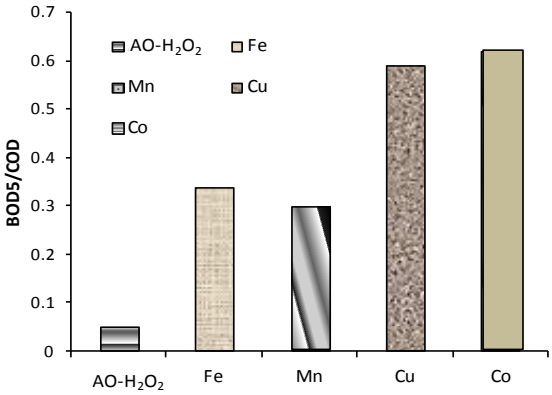


Fig. 3. Biodegradability (BOD_5/COD) of each process

The difference between the Co and the Cu ions could be an important consideration for the further treatment, since cobalt is more toxic than copper.

4. CONCLUSIONS

This study demonstrated that efficiency of the Electro-Fenton process can be enhanced by changing the catalyst ion. The catalyst ions could be manganese (Mn^{2+}), cupper (Cu^{2+}), Cobalt (Co^{2+}), or the classical ferrous ion (Fe^{2+}). A higher oxidation is possible with Co > Cu > Fe and > Mn.

The electro-Fenton process could be used as a pretreatment for a biological one. If the catalysts are Co or Cu ions less than 8 hours of process is enough to enhance the biodegradability.

On the other hand, when the electro-Fenton process is catalyzed by either Mn^{2+} or Fe^{2+} ions the oxidation and the biodegradability are notably than with other two ions.

4. References

1. Zhang, Y., Marrs, C.F., Simon, C., and C. Xi. Wastewater treatment contributes to selective increase of antibiotic resistance among Ancinetobacter spp., Science of the Total Environment, 407, 3702-3706, 2009.

2. Marcio Pimentel, Nihal Oturan, Marcia Dezotti, and Mehment Oturan. Phenol degradation by advanced oxidation process electro-Fenton using a carbon felt cathode. Appl. Catalyst. B. Environ. 83 (2008) 140-149.

3. Adriana Ledezma Estrada, Amin Wang, Yu You-Li, Biodegradability enhancement of wastewater containing cefalexin by means of the electro-Fenton process. Journal of Hazardous Mat. 227-228 (2012) 41-48.

Effect of erythromycin on nitrification during batch and continuous experiments

○Jing-Ru Du[1], Yu-You Li[1,2]*

[1]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[2]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

The influence of erythromycin (ERY) on ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) was investigated using batch and continuous experiments. The specific ammonia-oxidizing activity (SAA) of a control was 0.44 gN/(gVSS•d) and at an ERY concentration of 400 mg/L the SAA was 56% inhibited. The specific nitrite-oxidizing activity (SNA) was higher than SAA with 0.64 gN/(gVSS•d) and 25% was inhibited with 400 mg/L ERY on the first, and 59% on the third day. Adding 1mg/L ERY continuously led to a 60% reduction of ammonia removal efficiency, while 64% of nitrite was not converted to nitrate in the presence of 5 mg/L. This adverse effect disappeared after days 96 with 10 mg/L ERY and the reactor performance recovered to a stable condition. 1 mg/L ERY with the influent also led to the reduction of nitrifier activity: 63% and 17% reduction for AOB and NOB respectively which suggested AOB was more sensitive to ERY than NOB. A foam layer was formed immediately after adding ERY so that 79% and 37% MLVSS left at ERY concentration of 1 mg/L and 10 mg/L respectively. More than 36% ERY was removed by adsorption by sludge and over 30% of COD was eliminated with it.

Keywords: nitrification, erythromycin, inhibition, acclimation, macrolides

1. Introduction

ERY act as inhibitors of bacterial protein synthesis and are among the most important antibacterial agents used in human medicine. It can resist degradation in the wastewater treatment process by the lack of the beta-lactam ring. It is frequently detected in the aquatic environment, especially in hospital wastewater. The concentration range could be around μg/L which is higher than urban sewage.

Nitrification is an essential link during the treatment of pharmaceutical wastewater in order to remove nitrogen. However most of the previous studies with ERY consider its effect on nitrification involved by the activity sludge system but the fast growing heterotrophic bacteria usually occupy the major population than the autotrophic nitrifying bacteria[1]. Meanwhile the existing literature, there are only a limited number of studies which have investigated the influence of ERY on nitrification by long-term experiments, but short-term exposure experiments cannot exhibit the accumulation of intermediate products which can undeniably affect nitrification performance.

In this study therefore, the influence of ERY at pharmaceutically relevant concentrations on the nitrification system (cultivated without organics) was investigated. The specific ammonium-oxidizing activity (SAA) of AOB (ammonia-oxidizing bacteria) and specific nitrite-oxidizing activity (SNA) of NOB (nitrite-oxidizing bacteria) were detected via batch experiments at different concentration of ERY. A continuous experiment was carried out to evaluate the effect of long-term exposure to ERY. The shift of sludge structure was analyzed by biomass reduction.

2. Materials and Methods

The previously reported activity measurement was utilized for batch experiments. The specific ammonium-oxidizing activity (SAA) and the specific nitrite-oxidizing activity (SNA) for AOB and NOB were respectively measured below completely aerobic conditions (initial DO≈7.5 mg/L). Sludge was taken from the reactor and incubated in a 500 mL beaker at 30˚C in a shaking water bath (Yamato BT200) with a sample interval of 30 minutes.

Fig. 1 shows the experimental schematic diagram of the reactor which is abundant of nitrifying bacteria. The effluent of the aeration tank was connected with a sedimentation tank, and returned the sludge back to the reaction zone every day in order to maintain the biomass. After becoming stable, added ERY into the reactor with the substrate. Increasing its concentrations by five steps and after the last stage becoming stable then the next one will start.

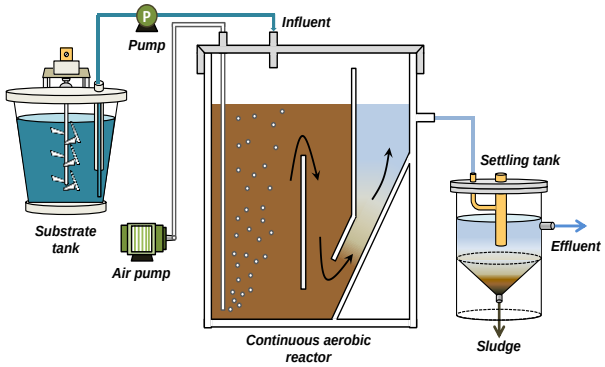


Fig. 1. Schematic diagram of the nitrification reactor

3. Results and discussion

3.1. Batch experiments

Relative SAA and SNA at different concentration of ERY were measured and the result is shown in Fig. 2. The SAA of control exhibited 0.44 gN/(gVSS•d) on the first day while after adding ERY, there appears to obvious inhibition in the activity of the AOB. The inhibition to SAA within three days was almost same. The lowest percentage of SAA below different ERY concentrations is about 44%. On another hand, SNA of control was 0.64 gN/(gVSS•d) and that NOB has a faster reaction rate than AOB. The inhibition for NOB was not as obvious as AOB and the relative SNA was 75%, 54% and 41% at ERY concentration of 400 mg/L in three days. This result illustrates that the inhibition increase with the days rising.

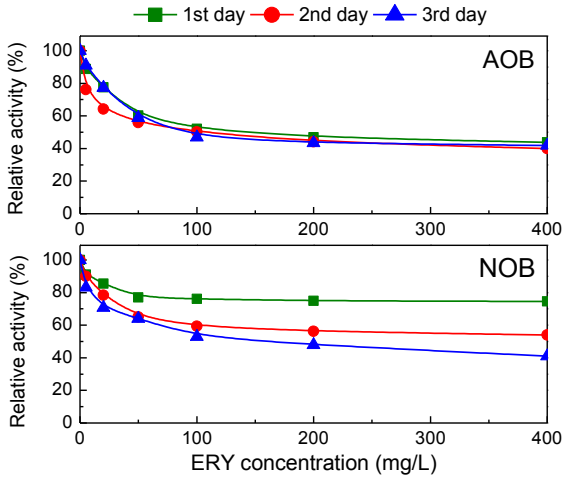


Fig. 2. Relative SAA and SNA at different concentration of ERY

3.2. Continuous experiments

The concentration of ammonia, nitrite and nitrate was detected in the effluent showed in Fig. 3. In the presence of 1 mg/L ERY, ammonia removal efficiency decreased to 40% while no obvious nitrite accumulation was found. At ERY concentration of 5 mg/L, 59% ammonia transformed to nitrite and 16.4 mg/L nitrate was in the effluent. After adding 10 mg/L ERY, with higher concentration of ERY the reactor stayed at a stable stage and the nitrifiers had adapted to ERY. In general, the adverse effect on NOB would appear within a long-term exposure which was consistent with the results of the activity test. These results demonstrated that AOB and NOB had different susceptibility to ERY, and AOB is more sensitive than NOB. This was corresponding with a previous research via PhyloChip-analysis of microbial communities in SBR reactor [2]. It suggested that ERY inhibited nitrite oxidation (18-61%) less significantly than ammonium oxidation (56-95%). This acclimation and distinction between AOB and NOB could be caused by the less diverse nitrifiers selection by ERY and gram-negative nitrifiers were left to resist ERY.

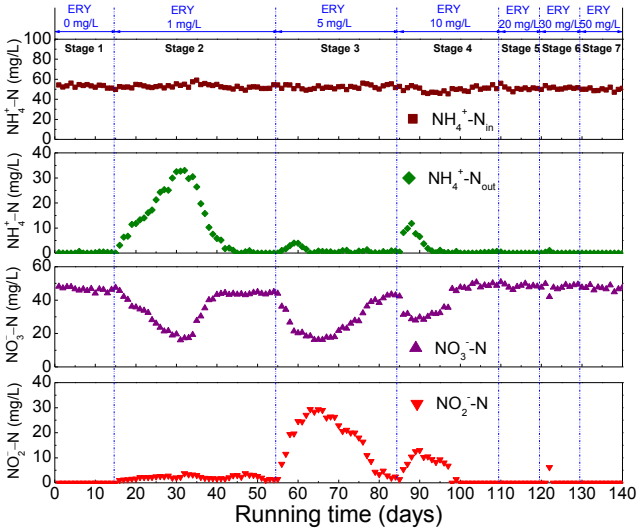


Fig. 3. Concentration change of ammonia, nitrate and nitrite at different stages of the reactor.

As soon as spiking with ERY to the reactor, a foam layer was observed on the liquid surface. It was composed of sludge adhered by micro-bubble and lead to a mass of biomass reduction. Fig. 4 shows the residual MLVSS in the reactor at different concentration of additive ERY. At 1 mg/L ERY, 79% MLVSS left while only 38% MLVSS was found in the reactor at 10 mg/L ERY. However this transfer did not exert more intensely at higher antibiotic concentrations. Thus, ERY can alter the sludge performance and this potency of ERY could change the settleability of sludge and increases the risk of bacteria dissemination and spread antibiotic resistance genes in the environment [3].

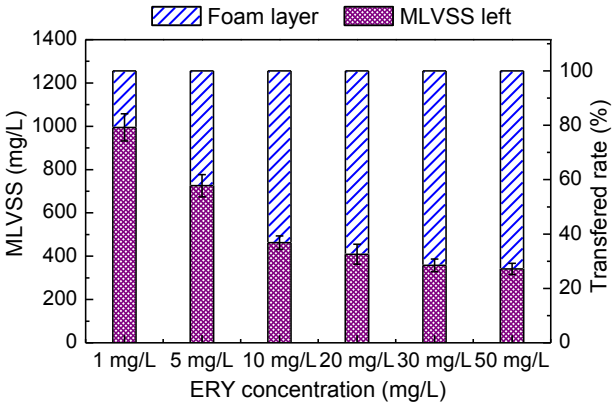


Fig. 4. Reduction of MLVSS at different stages of the reactor.

4. References

1. Roberts, P.H. and K.V. Thomas, The occurrence of selected pharmaceuticals in wastewater effluent and surface waters of the lower Tyne catchment. Science of the Total Environment, 2006. 356(1-3): p. 143-153.
2. Fan, C.A., et al., Influence of trace erythromycin and erythromycin-H2O on carbon and nutrients removal and on resistance selection in sequencing batch reactors (SBRs). Applied Microbiology and Biotechnology, 2009. 85(1): p. 185-195.
3. Joss, A., et al., Biological degradation of pharmaceuticals in municipal wastewater treatment: Proposing a classification scheme. Water Research, 2006. 40(8): p. 1686-1696.

A microfluidic platform for inhibitory test of antibiotic on nitrifier and denitrifier

○Bing Li[1], Yong Qiu[1], Hua-bing Yin[2], Han-chang Shi[1]*

[1] School of Environment, Tsinghua University, Beijing 100084, China
[2] Department of Electronics and Electrical Engineering, University of Glasgow, Glasgow G128QQ, U.K.
*E-mail: hanchang@mail.tsinghua.edu.cn

Abstract

Bacterial growth inhibition tests have become a standard measure of the adverse effects of inhibitors for a wide range of applications, such as toxicity testing in the medical and environmental sciences. However, conventional well–plate formats for these tests are laborious and provide limited information (often being restricted to an end-point assay). In this study, we have developed a microfluidic system that enables fast quantification of the effect of an inhibitor on bacteria growth and survival, within a single experiment. This format offers a unique combination of advantages, including: long term continuous flow culture, generation of concentration gradients, and single cell morphology tracking. Our methods can provide additional information, over and above that of the standard well-plate assay, including kinetic information on growth inhibition and measurements of bacterial morphological dynamics over a wide range of inhibitor concentrations. We illustrate this using *Nitrosomonas europaea* and *Comamonas denitrificans*, which are two kind of important baceteria for the removal of nitrogen in the wastewater, and show the inhibition effect of amoxicillin on them.

Keywords: microfluidics , bacteria inhibitory test; amoxicllin; concentration gradient

1. Introduction

Antibiotics have been detected in wastewater treatment plants (WWTPs). It is especially important to determine if pharmaceuticals have an adverse impact on functionally important microorganisms since WWTPs rely so heavily on microbial activity in biological processes. Nitrifier and denitrifier are key groups of microorganisms in wastewater treatment for the removal of nitrate. Considering the batch tests are time consuming and couldn't get information of bacteria morphology, we developed a microfluidic platform for inhibitory test, it can achieve long-term culture, single cell tracing and chemical gradient on a small chip. In the present study, we use this microfluidic platform to investigate the inhibitory effect of amoxicillin on the bacteria of *Nitrosomonas europaea* and *Comamonas denitrificans*, which are common bacteria in wastewater treatment plants.

2. Materials and Methods

2.1. Microfluidic chip for single-layer cell culture

The PDMS Chip was made by standard photolithography and soft lithography techniques, and assembling of individual components with an acrylic mechanical clamp for seal. The structure of the chip was shown as Fig. 1.

It contains an assembly of a coverslip glass, a thin agarose gel membrane and a PDMS chip (Fig. 1). A monolayer of bacteria were trapped and grew between the agarose membrane and glass. The PDMS chip was made by replica moulding. Briefly, a mixture of PDMS oligomer and curing agent (Sylgard 184, Dow Corning) at ratio of 10:1 was casting against a master to give a chip thickness of ~ 5 mm. This was cured at 70℃ overnight. The chip consists of two parallel channels at a distance of 1 mm apart. The length, width and depth dimensions of each channel are 13 mm × 500 μm × 500 μm.

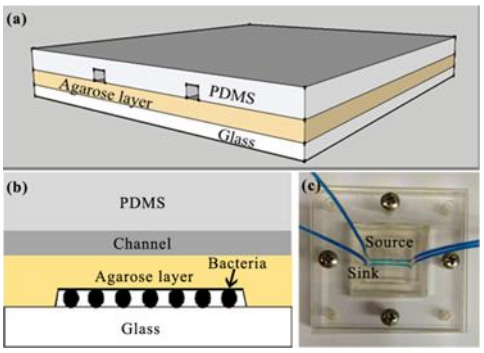


Fig. 1. Configuration of the three-layered microfluidic device.

2.2. Bacterial strains and Chemicals

Nitrosomonas europaea (ATCC25978) and *Comamonas denitrificans* (ATCC700936) were purchased from ATCC. Before loaded into the microfluidic device, bacteria were cultured in the incubator to the exponential phase. After harvested, the bacteria were washed by PBS and resuspended in fresh nutrient broth. Then the bacterial suspension was diluted to make the OD_{600} reach about 0.1 for the inhibition test.

Amoxicillin (MW=419.46 Da, Sigma-Aldrich) was prepared as a 2.0 g/l stock solution in deionised water and then diluted in LB broth or nutrient broth to the required concentration.

2.3. Image and data processing

Time-lapse image acquisition was carried out using an inverted fluorescence microscope (Zeiss AxioObserver Z1) equipped with an automated stage and a 40x objective lens. Images were captured using a CCD camera (Photometrics Cascade II). Image processing and data acquisition was done by using software Image J.

3. Results and discussion

3.1. Characterization of Concentration Gradients

The concentration gradient of amoxicillin in the device was estimated by collecting fluorsecence images when fluorescein was used in place of amoxicillin in the source channel (note, amoxicillin and fluorescein have similar molecular weights, and consequently similar diffusion characteristics). Due to the porous structure and high water content (98%) of agarose gel membranes, diffusion leads to the concentration gradient of fluorescein (or amoxcillin) at the membrane/coverslip interface being rapidly established, as shown in Fig. 2.

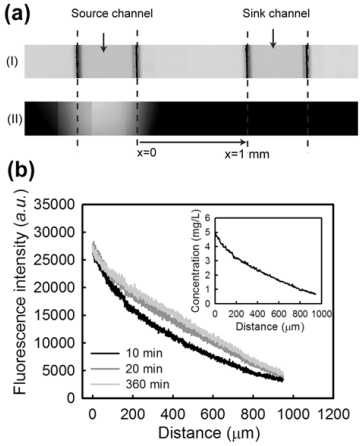


Fig. 2. Concentration gradient formed under the agarose layer in the microfluidic chip. Inset shows the concentration gradient versus distance at time 20 min.

3.2. Cell growth on the chip

The continous flow culture formed in the microfluidic chip provided constant nutrient supply and removal of metabolic waste. Given the abundance of nutrients, bacterial cells were able to grow exponentially within the monolayer.

Time-lapse images of *Comamonas denitrificans* cells were used to track their growth during 7h as shown in Figure 3..While time lapse tracking of the growth of N. europaea on-chip was carried out for 2 days as shown in Fig. 4. For the chip can continuously remove nitrite which inhibit the growth of *N. europaea*, so this bacteria can grow quickly on the chip.

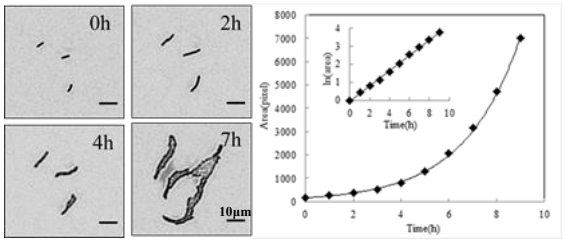


Fig. 3. The growth of *Comamonas denitrificans* on the chip

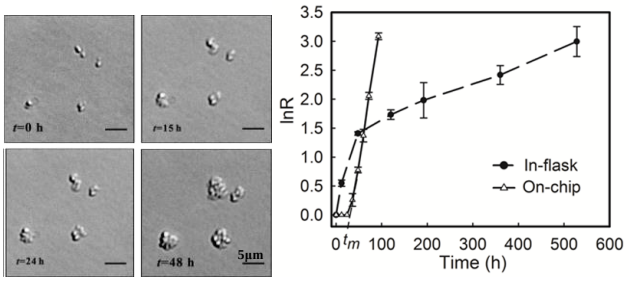


Fig. 4. The growth of *N. europaea* on the chip.

3.3. Bacterial inhibition test on the chip

Time-elapse images of the bacterial monolayer in the microfluidic chip demonstrated the inhibitory effects on *Comamonas denitrificans* in 7 hours by the antibiotic in gradient, as shown in Fig. 5. While *Nitrosomonas europaea* grow much slower than *Comamonas denitrificans* , we took 40h to track the growth of nitrifier as shown in Fig. 6.

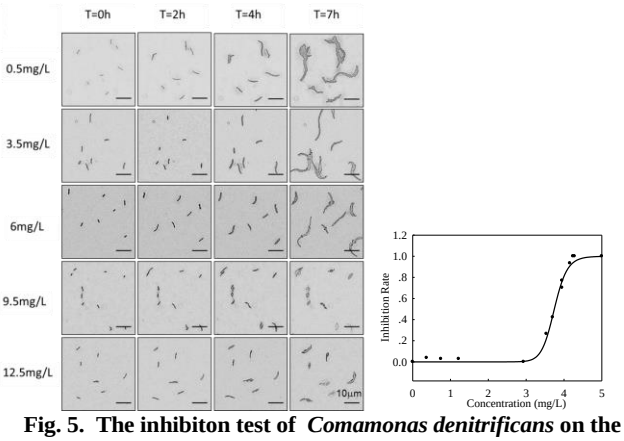


Fig. 5. The inhibiton test of *Comamonas denitrificans* on the chip

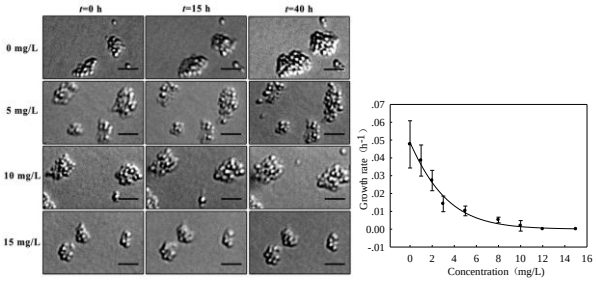


Fig. 6. The inhibiton test of *N. europaea* on the chip.

The IC50 and MIC of amoxicillin on *Comamonas denitrificans* are 3.8mg/L and 5mg/L respectively. While the IC50 and MIC for *N. europaea* are 2mg/L and 10mg/L respectively.

4. References

1. Kim, K. P.; Kim, Y. G.; Choi, C. H.; Kim, H. E.; Lee, S. H.; Chang, W. S.; Lee, C. S. Lab Chip 2010, 10, 3296-3299.
2. Chen, C. H.; Lu, Y.; Sin, M. L. Y.; Mach, K. E.; Zhang, D. D.; Gau, V.; Liao, J. C.; Wong, P. K. Anal. Chem. 2010, 82, 1012-1019.
3. Luo, C. X.; Jiang, L. L.; Liang, S. B.; Ouyang, Q.; Ji, H.; Chen, Y. Biomed. Microdevices 2009, 11, 981-986.
4. Sun, P.; Liu, Y.; Sha, J.; Zhang, Z. Y.; Tu, Q.; Chen, P.; Wang, J. Y. Biosens. Bioelectron. 2011, 26, 1993-1999.

Session III:
Renewable energy
and
environmental security

Characterization and 16s rRNA gene sequences analysis of the cellulolytic and hydrogen-producing activities of three different anaerobic mixed microflora

○Gadow S.I[1,2], Hongyu Jiang[1], Yu-You Li[1,3]*

[1]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[2]Agric. & Biol. Res. Division, National Research Center, Dokki, Cairo, 12311, Egypt
[3]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

The effect of temperature variation on the hydrogen production from cellulose and glucose by mesophilic, thermophilic and hyper-thermophilic hydrogen producing anaerobic mixed microflora was evaluated in standardized batch experiments. Analysis of 16S rRNA sequences showed that the cellulolytic bacteria were close to *Enterobacter* genus in mesophilic culture and *Thermoanaerobacterium* genus in thermophilic and hyper-thermophilic cultures. The mesophilic mixed culture was able to utilize the cellulose to produce methane gas within a temperature range between 25 to 45 ^{o}C and hydrogen gas from 35 to 60 ^{o}C. By contrast, the thermophilic and hyper-thermophilic cultures produced only hydrogen gas at all temperature ranges. The activation energy is estimated using regression (R^2, 0.98 and 0.92) to be 118.7 and 86.8 kJ/mol by mesophilic culture, (R^2, 0.93 and 0.91) to be 103 and 98.8 kJ/mol by Thermophilic culture and (R^2, 0.93 and 0.94) to be 133.2 and 117.7 kJ/mol by hyper-thermophilic culture for cellulose and glucose, respectively..

Keywords: Bio-hydrogen; Cellulose; Dark Fermentation; Temperature effect, 16S rRNA, Activity test, thermodynamic

1. Introduction

Burning of fossil fuels as the main source of our energy cause of some serious environmental issues such as greenhouse effect, ozone layer depletion and acid rains has long been recognized. The cellulosic-dark hydrogen fermentation is getting more importance because around 9.2 x10^{11} tones cellulose produced at the annual rate of 0.85 x10^{11} tones per year , which consider an important source for biomass energy technology we need to believe. A variety of factors are important in the fermentative production of hydrogen from organic substrates. Among these factors, the temperature is the most important key factor for bacterial growth. Based on our previous study, the reaction rate increased with temperature however, this can be adapted to microbiological processes in limited temperature ranges. Therefore, the purpose of this study was to evaluate the maximum activities of three different hydrogen producing anaerobic mixed cultures using cellulose or glucose as sole of carbon source, and their activation energies with 16S rRNA sequences analysis.

2. Materials and Methods

2.1. Activity test experiments

Seventy-eight batch experiments were performed in 120 ml serum bottles. Each bottle with a working volume of 80 ml was added 16 ml of inocula from running reactor (see Table 1) to 64 ml of synthetic medium containing cellulose or glucose carbon sources (10 g/l). The serum bottles were placed in the water bath at temperature range from 25 to 85^{o}C.

2.2. 16S ribosomal RNA gene cloning analysis

Table 1 Comparative characteristics of three different anaerobic acidogenic bioreactors for continuous cellulosic-hydrogen fermentation

	Parameter	Units	Temperature of reactor (°C) 37±1	55±1	70±1
	pH		5.2	4.89	5.5
Biogas	Volume	L/L/d	0.38	0.56	0.47
Biogas	H_2 concentration	%	1.6	43.3	33.7
Biogas	CH_4 concentration	%	23	ND	ND
Liquid Phase	Soluble COD	g/l	3.1	2.6	2.11
Liquid Phase	VFA	g/l	2.7	2.5	1.83
Solid Phase	VSS	g/l	4.2	3.81	4.11
Solid Phase	Cellulose	g/l	3.21	3.38	3.82
Solid Phase	Proteins	g/l	0.46	0.42	0.48

The microbial community was analyzed by 16S rRNA gene cloning and sequencing. The amplification of 16SrRNA was performed with the primers EUB8F, and UNIV1500R. Sequence reactions were carried out at TAKARA BIO dragon genomics center (Yokkaichi, Japan).

3. Results and discussion

3.1. 16S rRNA analysis

The cloning results of the mesophilic mixed culture show that a total of 37 OTUs were detected from a total 100 clones. According to analysis results, the 52% of the microbial community were able to hydrolyze the cellulose effectively such as *Enterobacter cloacae* and *Pedobacter* sp., *T. thermosaccharolyticum* and *Bacteroides*. Several bacteria such as *Clostridium*, *Bacillus*, *Enterobacter* and *Pseudomonas* produce cellulases; *Enterobacter cloacae* produced carboxymethyl cellulase (CMCase),

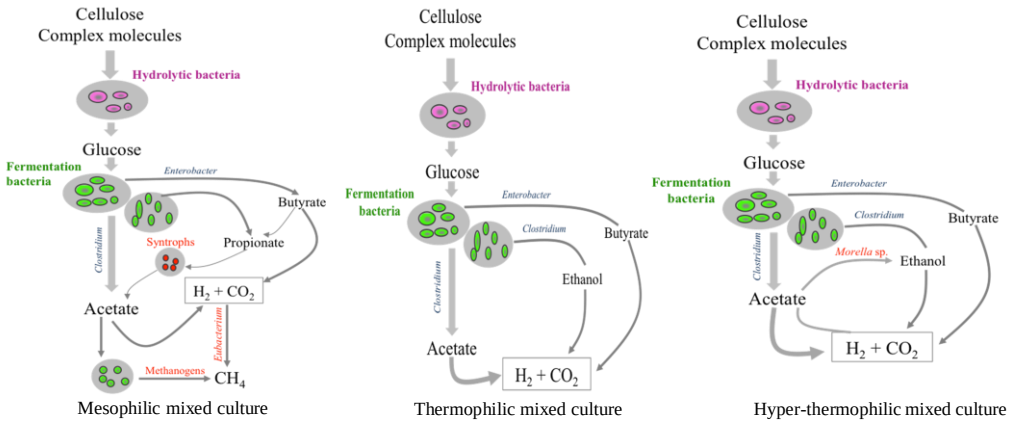


Fig. 1. Effect of temperature on microbial community dynamics in the cellulosic-hydrogen fermentation

which is an important enzyme in the cellulase enzyme system (see Fig. 1). It also plays an important role in hydrolyzing cellobiose to fermentable glucose (see eq. 1, 2, and 3). While the cloning results of thermophilic culture show that a total of 21 OTUs were detected from a total 104 clones. The 70% of the microbial community were able to hydrolyze the cellulose effectively in particular; *T. thermosaccharolyticum* generates hydrogen from cellulose effectively because it has β-Glucosidase, which is an important enzyme in the cellulase enzyme system. As for the hyper-thermophilic culture, the cloning results show
that a total of 6 OTUs were detected from a total 100

clones. Eighty five clones of cellulose-degrading mixed culture were composed of microbes closely affiliated to genus *clostridium*. The dominant cellulolytic bacterium was *T thermosaccharolyticum*.

3.2. Effect of temperature variation

A total of twenty-six batch experiments were performed to evaluate the effect of temperature on the activity of each inoculum (see Fig.2). The results of mesophilic inoculum show that the anaerobic mixed microflora was able to utilize the cellulose to produce

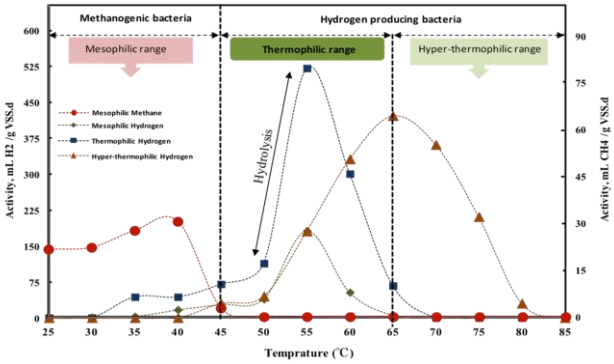


Fig. 2. Effect of temperature variation on mesophilic, thermophilic and hyper-thermophilic cellulosic hydrogen mixed culture activity using cellulose as sole of carbon source

methane gas within a temperature range between 25 to 45 °C and hydrogen gas from 35 to 60 °C. It should be noted that the bacteria started to produce hydrogen gas without methane gas at 50 °C with the maximum activity obtained at 55 °C. Our results indicated that the thermophilic temperature range (45-65 °C) could effectively inhibited methanogenic reaction by mesophilic mixed microflora as well as pH. Another important consideration the hydrogen production started at 35 °C and at the same time methanogenic activity was higher, however at 45 °C the methane reaction was completely stopped and hydrogen gas reaction was started to increase. For thermophilic inoculum, the experiment results show that the cellulosic-hydrogen mixed microflora utilized the cellulose in a wide range of temperature from 35 to 65 °C, with a sharp increase was found between 50 and 55°C and maximum activity of 521.4mL H_2/g VSS d at

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 4H + 2C\,H\,O + 2CO \quad \ldots\ldots\ldots\ldots\ldots\ldots(1)$$

$$C_6H_{12}O_6 \longrightarrow 2H_2 + C_4H_8O_2 + 2CO_2 \quad \ldots\ldots\ldots\ldots\ldots\ldots(2)$$

$$C_6H_{12}O_6 + 2H_2O \longrightarrow 2H_2 + C_2H_4O_2 + C_2H_5OH + 2CO_2 \quad \ldots\ldots\ldots\ldots(3)$$

55 °C. However, the performance was negatively affected by increasing fermentation temperature after 55 °C and completely inhibited at 70 °C. As for hyper-thermophilic inoculum, the results show that the anaerobic mixed microflora was able to utilize the cellulose and glucose within a wide range of fermentation temperatures (45-80 °C).

3.3. Thermodynamic analysis

According to the Arrhenius equation, the relationship between bio-reaction rate (r) and temperature is as follows:

$$r = A \,.\, \mathrm{Exp}\,(-E_a/R\,.T) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots(4)$$

where A is a constant, E_a is activation energy (kJ/mol), *R* is ideal gas constant (8.314 J/mol-K) and T is the absolute temperature (K).

When r is substituting r with HPR into equation (1), the activation energy is estimated using regression (R^2, 0.98 and 0.92) to be 118.7 and 86.8 kJ/mol by MC, (R^2, 0.93 and 0.91) to be 103 and 98.8 kJ/mol by TC and (R^2, 0.93 and 0.94) to be 133.2 and 117.7 kJ/mol by HC for cellulose and glucose, respectively. Since this activation energy is higher than the hydrogen production from glucose, it can be concluded that more energy is consumed by hydrogen production from cellulose than from glucose

4. References

1. Gadow, S. I., Jiang, H., Hojo, T.,Li, Y. Y. (2013). Cellulosic hydrogen production and microbial community characterization in hyper-thermophilic continuous bioreactor. International Journal of Hydrogen Energy, 38(18), 7259-7267.
2. Gadow, S. I., Li, Y. Y., Liu, Y. (2012). Effect of temperature on continuous hydrogen production of cellulose. International Journal of Hydrogen Energy, 37(20), 15465-15472

Kinetics of complex organic waste degradation under anaerobic thermophilic and mesophilic process

○Qian Li[1,2], Wei Qiao[1,3], Yu-You Li[1,4]*

[1]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
[2]Key lab of northwest water resource, Environment and Ecology, MOE, Xi'an University of Architecture and Technology, Xi'an 710055, China
[3]State key laboratory of heavy oil processing, China University of Petroleum, Beijing, China
[4]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

The kinetics of complex organic waste degradation was evaluated in anaerobic batch experiment under thermophilic and mesophilic conditions. Due to the accumulation of VFA in thermophilic anaerobic digestion, four stages were introduced to describe anaerobic digestion. With the reaction processes being speeded up by high temperature, the CH_4 conversion ratio decreased from 75.8% in mesophilic digestion to 64.4% in thermophilic digestion for coffee ground, but increased from 36.3% in mesophilic digestion to 47.2% in thermophilic digestion for dewatered activated sludge. The decrease of CH_4 conversion ratio may due to the accumulation of propionic acid and the inhibitory compounds generated under thermophilic condition. Compared the value of R_{max} in each stage under different temperature, it was found that hydrolysis was the rate-limiting step in anaerobic digestion of sludge. For coffee ground, acetogenesis and acidogenesis could be considered as the rate-limiting step under thermophilic condition and mesophilic condition, respectively. Anaerobic digestion kinetic showed well relevance to the exponential model and the modified Gompertz model.

Keywords: anaerobic digestion; kinetics; thermophilic; mesophilic, coffee waste, dewatered activated sludge

1. Introduction

Thermophilic and mesophilic are two conventional operation temperature levels for anaerobic digestion. Under thermophilic condition, the greater pathogen reduction and faster reaction rate could be achieved. However, the increase in TVFA (especially propionic acid) level in the long-term thermophilic anaerobic digestion process will lead to system failure. Acetogenesis is an important step in anaerobic digestion process. Therefore, traditional three-stage process used to describe the anaerobic digestion of complex organic waste was replaced by four-stage process involves hydrolysis, acidogenesis, acetogenesis and methanogenesis. Hydrolysis was always considered as the rate-limiting step. However, in thermophilic process, due to the high temperature, (1) if the high temperature could enhance the hydrolysis process and lead it no longer to be the rate-limiting step, (2) if the accumulation of propionic implied the acetogenensis would be the rate-limiting step. Maybe the answer will be different for different complex organic waste. The kinetics of different substrates depend on their physicochemical properties. In this study, two different substrates were used to evaluate the kinetics characteristics of complex organic waste degradation under anaerobic thermophilic and mesophilic process.

2. Materials and Methods

2.1. Batch experiments

Batch experiments were conducted in 120 ml serum bottles with 100mL seed sludge took from CSTR reactors under thermophilic (55℃) and mesophilic (35℃) conditions respectively, after long-term domestication. Coffee ground and dewatered activated sludge was used as two different substrates. Coffee ground contained higher levels of carbohydrate (0.59g/g-TS) and lower levels of protein (0.24g/g-TS) and lipid (0.24g/g-TS). Dewatered activated sludge had a high protein component (0.69g/g-TS) and a lower component of carbohydrate (0.31g/g-TS) and lipid (0.02g/g-TS).

2.2. Kinetic models

Three kinetic models used in this study are shown as follow:

(a) The Michaelis-Menten model

$$\frac{dS}{dt} = V_m \frac{S}{K_m + S} \quad (1)$$

where S is the substrate concentration, K_m is the half-saturation rate coefficient, and V_m is the maximum reaction rate.

(b) The exponential model

$$P = P_0[1 - e^{-k(t - t_0)}] \quad (2)$$

where P is the product concentration, P_0 is the production potential, k is a constant, and t_0 is the lag time of reaction.

(c) The modified Gompertz model

$$P = P_0 \exp\{-\exp[\frac{R_{max} e}{P_0}(t_0 - t) + 1]\} \quad (3)$$

where P is the product concentration, P_0 is the product concentration, R_{max} is the maximum production rate, t_0 is the lag time of reaction and e=2.718281828.

3. Results and discussion

3.1. Effects of temperature on kinetic parameters

The four stages of anaerobic digestion was expressed as: cumulative hydrolysed COD (methane+soluble COD), cumulative acidified COD (methane+VFA), cumulative acetified COD (methane+acetate) and methane COD. As

shown in Fig.1 and Table.1, the effects of temperature were shown in two aspects: production potential and production rate in anaerobic process. R_{max} of each stage indicated that high temperature could speed up the anaerobic process. For coffee, no acetate acid accumulated and $R_{max(hydrolysis)} > R_{max(acidogenesis)} > R_{max(acetogenesis)} = R_{max(methanogenesis)}$ under thermophilic condition. It suggested that acetogenesis may be the rate-limiting step. While, for sludge, the production potential of each stage were almost same. Although in the first half of thermophilic anaerobic digestion, VFA could be detected, it was utilized fast in the latter half and hydrolysis became the rate-limiting step. Under mesophilic condition, no VFA could be detected in digestion process for both coffee and sludge. In another word, generated VFA could be converted to CH_4 fast, mesophilic system was stable than thermophilic system. Hydrolysis was the rate-limiting step for sludge and acidogenesis was the rate- limiting step for coffee ground under mesophilic condition.

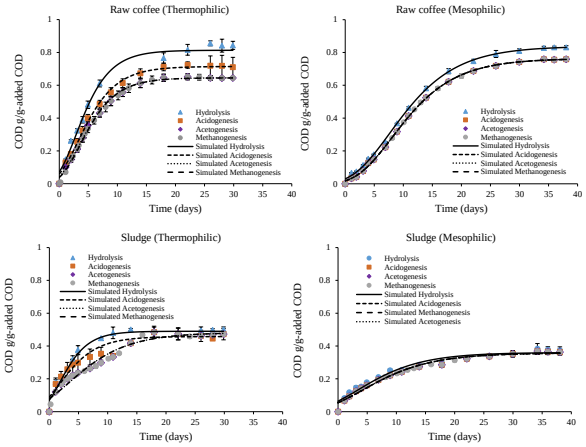


Fig. 1. Four stages of anaerobic digestion expressed as accumulated COD under thermophilic condition and mesohpilic condition. Symbols refer to the experimental data, lines to the simulation of Model 3.

3.2. Kinetics of different substrates degradation

The main component of ground coffee and dewatered activated sludge are carbohydrate and protein, respectively. The R_{max} of each stage in coffee ground digestion was higher than dewatered activated sludge digestion, because the hydrolysis of protein under anaerobic conditions is slower than the hydrolysis rate of carbohydrate. In thermophilic coffee ground digestion, although the reaction rate was higher, but the CH_4 conversion ratio was lower than under mesophilic conditions. There may be two reasons: (1) the accumulated propionic acid could not be converted to acetate acid timely, and led acetogenesis to become the rate-limiting step, (2) some inhibitory compounds generated under thermophilic conditions. The higher hydrolysis rate, the lower conversion ratio, but the phenomenon could not be observed under mesophilic condition. For sludge, protein was difficult to be degraded, higher temperature was beneficial for its degradation. Although the production potential and production rate could be accelerated by high temperature, the total CH_4 conversion ratio was still lower than coffee ground.

3.3. Sensitivity of the three kinetic model

The kinetic constants of the exponential model (Model 2) and the modified Gompertz model (Model 3) were estimated by a non-linear fitting program using the Original 8.5. The value of constant K_m and V_m of The Michaelis-Menten model was obtained by a linear regression. Fitting the experimental data utilizing the three models, showing anaerobic digestion kinetic well relevance to the Model 2 and Model 3. Michaelis-Menten equation was the basic kinetic model, however, not all the kinetic parameters estimated by this model could be used to describe the four stages of anaerobic digestion due to the negative value of K_m as shown in Fig. 2. The V_m was also higher than which obtained from Model 2 and Model 3. The Michaelis-Menten could not be fit for simulating every stages of anaerobic digestion for different substrates.

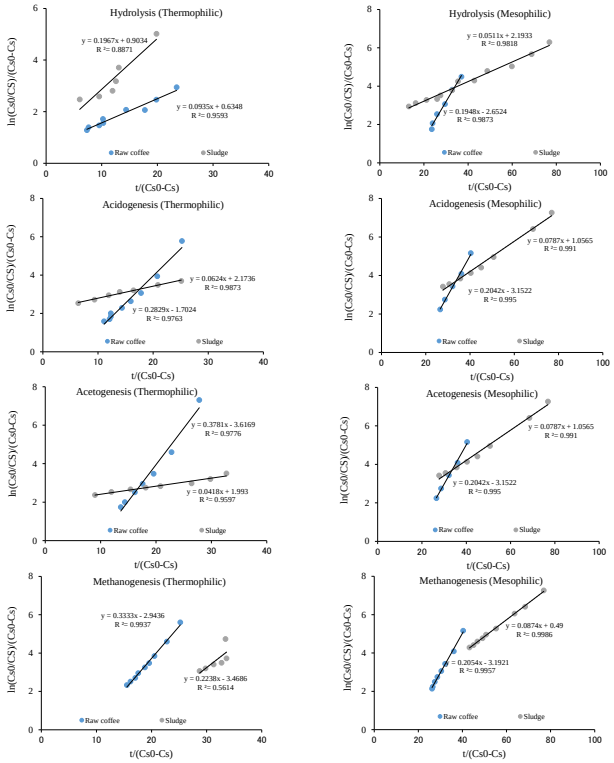


Fig. 2. Kinetics of four stages in anaerobic digestion under thermophilic and mesophilic conditions obtained from Model 1

Table 4. The kinetic parameters of four stages in anaerobic digestion simulated by Model 3

$P = P_0 \exp\{-\exp[\frac{R_{max} e}{P}(\lambda - t) + 1]\}$		Thermohilic (55°C)				Mesophilic (35°C)			
		Hydrolysis	Acidogenesis	Acetogenesis	Methanogenesis	Hydrolysis	Acidogenesis	Acetogenesis	Methanogenesis
coffee	P_0 (g-COD)	0.813±0.019	0.715±0.009	0.645±0.008	0.645±0.007	0.838±0.006	0.763±0.004	0.763±0.004	0.763±0.004
	R_{max} (g-COD/d)	0.091±0.011	0.072±0.004	0.067±0.003	0.068±0.003	0.048±0.001	0.046±0.001	0.046±0.001	0.046±0.001
	λ (d)	-0.39±0.470	-0.36±0.264	-0.06±0.218	0.115±0.194	1.288±0.164	1.950±0.122	1.950±0.122	1.950±0.122
	R^2	0.978	0.992	0.994	0.996	0.999	0.999	0.999	0.999
sludge	P_0 (g-COD)	0.490±0.012	0.458±0.017	0.482±0.020	0.482±0.020	0.361±0.012	0.358±0.010	0.358±0.010	0.358±0.010
	R_{max} (g-COD/d)	0.066±0.009	0.047±0.009	0.031±0.004	0.031±0.004	0.021±0.003	0.020±0.002	0.020±0.002	0.020±0.002
	λ (d)	-0.92±0.506	-1.82±0.899	-2.39±0.983	-2.389±0.983	-2.82±1.009	-2.459±0.811	-2.46±0.811	-2.459±0.811
	R^2	0.962	0.925	0.95	0.951	0.955	0.972	0.972	0.972

Improving MFC performance at low COD concentrations by adsorptive anode.

○Shijia Wu, Peng Liang, Xia Wang*

State Key Joint Laboratory of Environment Simulation and Pollution Control, School of Environment, Tsinghua University, Beijing 100084, PR China
*E-mail: xhuang@tsinghua.edu.cn

Abstract

In this study, granular activated carbon (GAC) packed bed anodes were developed in Microbial fuel cells (MFCs) and compared with granular graphite (GG) packed anodes to evaluate the adsorptive effect of anode materials on MFC performance. GAC packed MFC (GAC-MFC) produced higher electric currents and had lower internal resistances than GG packed MFC (GG-MFC) at low substrate COD concentrations (< ~200 mg/L). The electric current generation as a function of COD concentration was well described by Monod-type model, and the half-saturation constant (K_s) of GAC-MFC was about half as much as that of GG-MFC at all external resistances tested. EIS analysis demonstrated that lower mass diffusion resistance was the main reason why GAC-MFC outperformed GG-MFC at low COD concentrations. It was possible that the adsorption capacity of GAC helped to create a kind of "microenvironment" that contained higher concentrations of substrate than bulk solution, from which the electrochemically active bacteria on GAC could directly take in the nourishment, thus facilitating mass transfer process in GAC-MFC.

Key words: Microbial fuel cells (MFCs); Adsorptive effect; Electric current generation; Half-saturation constant (K_s); mass diffusion resistanc

1. Introduction

Microbial fuel cell is a kind of device that utilizes bacteria as catalysts to degrade organic materials in water and convert its chemical energy into electricity[1]. Its potential prospect to be used in sewage plant to recover energy in the process of wastewater treatment makes MFC a promising technology of both economic and environmental benefits. However, the high capital costs and low yields of power generation have been hindering its practical applications and make it uncompetitive with other conventional anaerobic wastewater treatment technology. Anodes have been one of the key components in deciding the performance and cost of MFC. So far, most of anode-related researches mainly focus on its electrical conduction, specific surface area and catalytic oxidation properties. Various Strategies, such as coating some highly conductive materials like CNTs (carbon nanotubes) on the anodic surface, or treating anode thermally with some chemical acid or gas, are developed to improve anode performance[2]. However, few studies have ever investigated the adsorptive property of anode materials on MFC performance. Therefore, the purpose of this study was to throw light on how the adsorptive capacity of anode materials affected MFC performance at different anolyte COD concentrations.

2. Materials and Methods

2.1. Construction and operation of MFCs

Several double-chamber flat rectangle MFCs were constructed in the experiment. Granular activated carbon and granular graphite with the same size and shape were respectively filled in the anode compartment of MFCs. Sodium acetate and potassium ferricyanide with phosphate buffer were respectively used as anode substrate and catholyte. All MFCs were operated in fed-batch mode.

After successful start-up of all MFCs, sodium acetate was added to the anode with different concentrations to determine electric current generation of MFCs as a function of substrate COD concentrations. At each COD concentration, various external resistances (100 Ω, 50 Ω, 30 Ω, and 20 Ω, respectively) and different circulation flow rate (2mL/min and 20mL/min, respectively) were applied to examine the difference of electricity generation performance between granular activated carbon (GAC) packed MFC and granular graphite (GG) packed MFC.

2.2. Analysis and calculation

The internal resistance R_{int} was obtained from the slope of polarization curves by changing external resistance. In order to investigate different components of internal resistance, including ohmic resistance R_s, charge transfer resistance R_{ct} and diffusion resistance R_d, electrochemical impedance spectroscopy (EIS) tests were carried out.

3. Results and discussions

3.1. Impact of external resistances on electric current generation

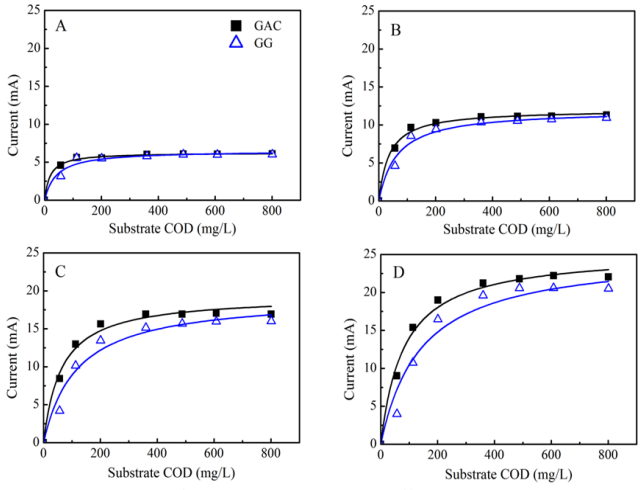


Fig. 1. Changes of current generation with different substrate COD concentration in granular activated carbon (GAC) packed MFC (■), and granular graphite (GG) packed MFC (▲) at external resistances of (A) 100 Ω, (B) 50 Ω, (C) 30 Ω, and (D) 20 Ω.

Table 1. Monod Fitting results of granular activated carbon packed MFC (GAC-MFC) and granular graphite packed MFC (GG-MFC) at different external resistances.

External resistance (Ω)	Type	I_{max} (mA)	K_s (mg/L)	R^2 (%)
100	GAC	6.52	19.16	0.996
	GG	6.5	41.4	0.961
50	GAC	12.01	35.49	0.994
	GG	11.98	65.75	0.98
30	GAC	19.36	57.7	0.987
	GG	19.3	118.19	0.971
20	GAC	25.31	80.98	0.989
	GG	25.23	141.51	0.963

It can be seen from Fig.1 that electric current output of MFCs with GAC packed anode was higher than MFCs with GG packed anode at all external resistances, and the discrepancy tended to become greater at lower external resistance. And Fig. 1D further illustrated that at high COD concentrations (>400 mg/L), where electro-microorganisms are perceived to nearly reach their "saturated" exoelectrogenic performance, electric current generation of both MFCs did not vary apparently, nonetheless, at low COD concentrations (<200 mg/L), especially at COD = ~50 mg/L, current output of GAC-MFC (9.02 mA) was superior to that of GG-MFC (4.04 mA). Half-saturation constant K_s of GAC-MFC was about half as much as that of GAC-MFC at all external resistances (Table 1), indicating that GAC-MFC had more affinity for substrate and exhibited higher kinetic performance than GG-MFC. both of MFCs' half-saturation constant K_s increased as external resistance decreased, indicating that at lower external resistances, the electrogenic performance of an MFC was more sensitive to the variation of substrate COD concentration and allow us to better evaluate the differences of electricity generation performance between GAC-MFC and GG-MFC.

3.2. Internal resistance of GAC-MFC and GG-MFC

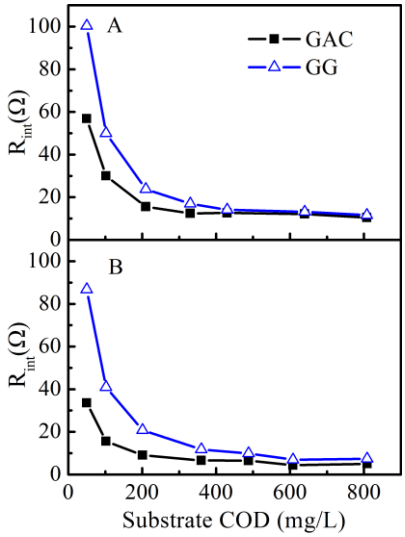


Fig. 2. Interal resistance Rint of granular activated carbon (GAC) packed MFC (■) and granular graphite (GG) packed MFC (△) operating at the inflow rate of (A) 2 mL/min and (B) 20 mL/min in different substrate COD concentration.

GAC-MFC and GG-MFC had similar internal resistances at high COD concentrations (>400 mg/L), while at low COD concentrations, especially when COD = 50 mg/L, R_{int} of GAC-MFC (56.8 Ω) was much less than that of GG-MFC (100.4 Ω) (Fig. 2A). Increasing the circulation rate from 2 to 20 mL/min reduced both MFCs' internal resistances at all COD concentrations. However, more apparent internal resistance reduction was achieved in GAC-MFC, inducing a larger gap between two curves in Fig. 2B.

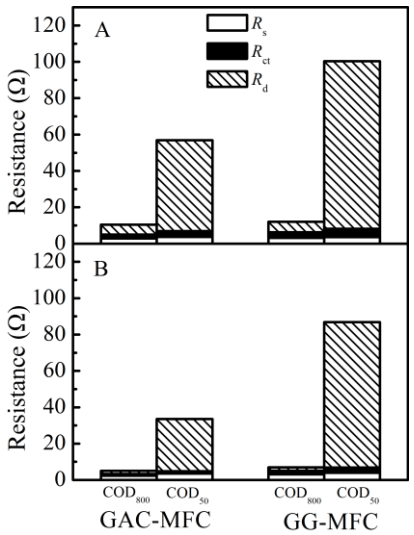


Fig. 3. Individual components of internal resistance at the inflow rate of (A) 2 mL/min and (B) 20 mL/min (R_s: ohmic resistance; R_{ct}: charge transfer resistance; Rd: mass diffusion resistance).

It can be seen from Fig. 3A that mass diffusion resistance R_d accounted for most of total internal resistance (over 85%) at COD = 50 mg/L, with R_d = 49.9 Ω for GAC-MFC, far less than that for GG-MFC (92.2 Ω). Although the ohmic resistance R_s and the charge transfer resistance R_{ct} of both MFCs slightly differed from each other, their proportions in total internal resistance were almost ignorable, indicating that lower mass diffusion resistance R_d in GAC-MFC was the main reason why GAC-MFC outperformed GG-MFC at low COD concentrations.

Because of GAC's superior adsorptive effect on anode substrate, the substrate concentration on the solid surface of GAC might be enriched, thus the exoelectrogenic microorganisms on GAC would grow in a kind of "microenvironment" where substrate concentration was higher than that in bulk solution, and might directly take in the nourishment from such substrate-rich microenvironment rather than from bulk solution. Nevertheless, the exoelectrogenic microorganisms on GG had to utilize the substrate transported by concentration gradient from bulk solution, the process of which would be greatly limited at low substrate concentrations. Thus, the adsorptive capacity of GAC packed anode made bacteria more affinity for substrate, resulting in lower half-saturation constant K_s and lower mass diffusion resistance in GAC-MFC.

4. References

1. Logan, B. E. and K. Rabaey (2012). "Conversion of wastes into bioelectricity and chemicals by using microbial electrochemical technologies." Science 337(6095): 686-690.
2. Wei, J., P. Liang and X. Huang (2011). "Recent progress in electrodes for microbial fuel cells." Bioresour Technol 102(20): 9335-9344.

Thermophilic anaerobic digestion of spent coffee grounds and coffee liquid with sludge and milk waste as the co-substrate

○Mohammad Shofie[1], Wei QIAO[2], Yu-You Li[1,3]*

[1]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[2]College of Chemical Engineering, China University of Petroleum, Beijing, China
[3]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

Long-term anaerobic digestion of spent coffee grounds (SCG) and coffee liquid has been conducted for 160 days, with sludge and milk waste as the co-substrate. A continuous stirred tank reactor (working volume 12L) was operated under thermophilic condition (55±1^{0}C) with HRT 30 and 20 days. The result showed that the steady state condition was started after 40 days under HRT 30 days (OLR 4.2 Kg-COD/m^3/d). During this period, the average of biogas production was 1.54L/L/d (CH_4:CO_2=62%:38%) with biogas conversion efficiency of 66%, while the COD removal efficiency was 55%. The reactor showed unstable condition under HRT 15 days (OLR 8.8 Kg-COD/m^3/d) indicated by unstable biogas production; decreased pH, alkalinity and ammonia-nitrogen; steady increase in VFA especially propionic acid (500 to 2000mg/L). This condition finally led to digester failure for almost 30 days with accumulation of acetic acid, H_2, and steady decrease in methane content. During this inhibition period, the feed was stopped temporarily and buffer solution ($NaHCO_3$) was injected periodically into reactor in order to avoid further accumulation of VFA and to keep the pH within a safe range (7.0-7.5).

Keywords: Anaerobic digestion, co-digestion; coffee grounds, CSTR, milk waste, sludge, thermophilic

1. Introduction

Coffee processing industries are responsible for the generation of large quantities of spent coffee grounds (SCG) with a worldwide annual generation of six million tons. SCG has a high content of polysaccharides, protein, and lipid (40-50 wt%, 10-15wt%, 10-20wt% dry basis, respectively) that can potentially be converted to biogas through anaerobic digestion process. Anaerobic digestion (AD) of SCG has been studied since early 1980's and it has been found that one of the main problems are its long-term digestion instability due to several factors (nutrient insufficiency especially nitrogen and microelements, decreased pH, and accumulation of VFA). The use of dewatered sludge as co-substrate on the AD of coffee grounds has been found to be capable of providing micro elements as well as improving the stability of digestion process (Qiao et al., 2013). The aim of this study was to evaluate the performance of anaerobic digestion of SCG and coffee liquid with sludge and milk waste as the co-substrate under long-term operation time.

2. Materials and Methods

2.1. Materials

Coffee grounds, coffee liquid, sludge, and milk waste were provided by a Japanese company. Anaerobic seed sludge was obtained from Wastewater Treatment Plant in Sendai, Japan.

2.2. Methods

A continuously stirred tank reactor (working vol. 12L) was used as the digester and operated under thermophilic condition (55±1^{0}C). The operation period was divided into two phases with specific operational conditions as shown in Table 1.

Table 1. CSTR operational conditions

	Unit	Start-up	P-I	P-II
TS-inf.	g/L	70	70	70
HRT	days	30	30	15
OLR	kg-COD/m^3/d	4.0	4.2	8.8

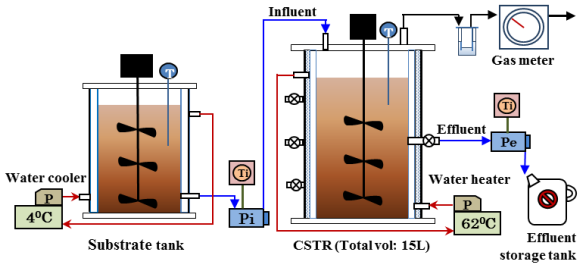


Fig. 1. Reactor configuration using CSTR (Pi: influent pump, Pe: effluent pump, Ti: timer, T: thermometer

3. Result and discussion

3.1. Reactor performance under steady state condition

Reactor started entering steady state condition after 40 days of operation under OLR 4.2 kg-COD/m^3/d (Phase I). This period was maintained for 60 days with average of daily biogas production of 1.54L/L/d (CH_4:CO_2= 62:38%). Reactor efficiency on total solid removal and COD removal was 48% and 55%, respectively. The pH, bicarbonate alkalinity and ammonia-N were maintained at range between 7.4-7.5, 2300-2500mg/L, and 700-800mg/L, respectively. The VFA concentration was kept below 1500mg/L during steady state condition. The long-term reactor performance is shown in Fig.2. Fig. 3 showed the reactor COD mass balance in which ±58% of influent COD is converted to methane.

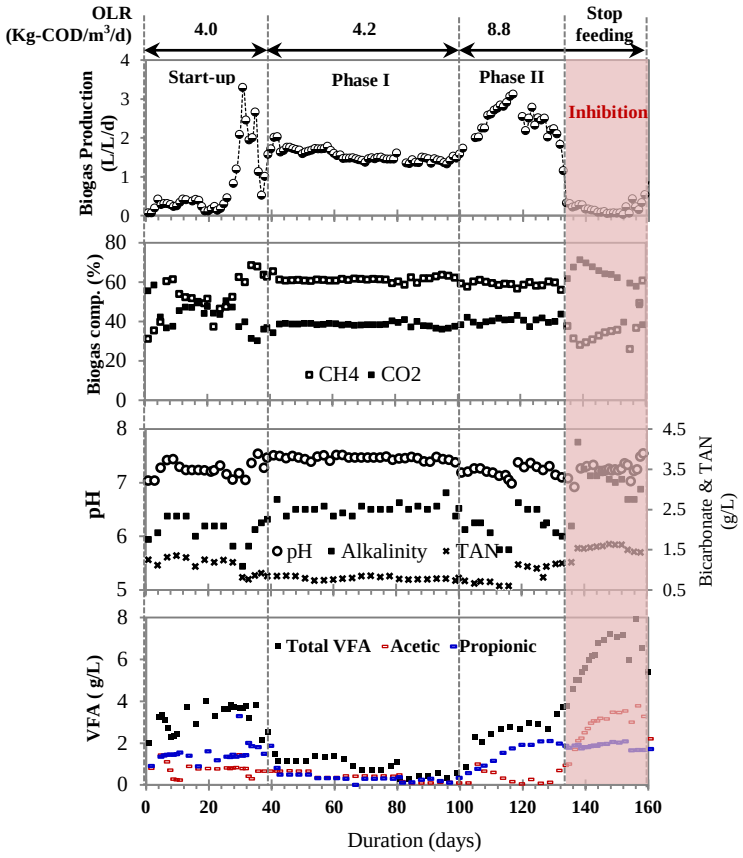


Fig. 2 Reactor performance under long-term operation time (biogas production, biogas composition, pH, alkalinity, TAN, and VFA)

3.2. Reactor perturbation and inhibition

Reactor showed unstable condition during phase II (OLR 8.8 kg-COD/m^3/d). The biogas gradually increased from 2.0 to 3.12L/L/d during the first 20 days, and then started to decrease gradually and drop below 2.0L/L/d. pH, alkalinity, and ammonia-N also showed a gradual decrease along with a steady increase in VFA, especially propionic acid (700 to 2000mg/L) as shown in Fig. 2. This condition indicated that the reactor was under imbalance state where the produced VFA could not be proportionally converted to methane gas by methanogens leading to decreased pH.

The feed was stopped at day 133 due to decrease in pH and methane content. Buffer solution ($NaHCO_3$, 2000mg/L) was injected into reactor to increase the pH. However, the methane content was found to keep decreasing (<40%) although the pH had turned back to normal level (7.2-7.3). A rapid accumulation of VFA was observed during this period, increased from 3700 to 7900mg/L, This condition led to digester failure during which the methane content was kept below 40% and VFA continued to accumulate. During the inhibition period, VFA concentration was monitored daily along with pH and CH_4 in order to provide an appropriate measure for reactor management.

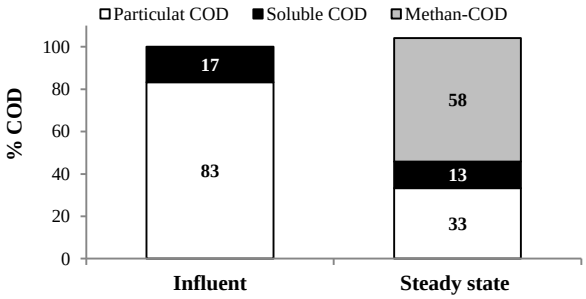


Fig. 3. COD mass balance under steady state condition

3.3. Monitoring reactor stability

Fig. 4 showed a relationship between pH, ratio of intermediate to total alkalinity (IA/TA), and VFA as control parameters for monitoring reactor stability. During phase II, pH, IA/TA, and VFA showed a rapid increase which in turn resulted in reactor failure (inhibition). The ratio IA/TA was maintained at 0.3 during phase I (steady state period) and 0.5 during phase II (unstable period). This value corresponds to that reported by Bernard et al (2001) in which the ratio IA/TA must be less or equal to 0.3 in order to avoid the digester instability.

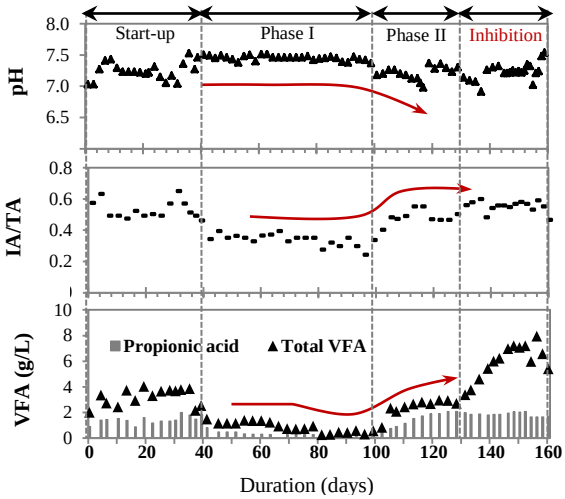


Fig. 4. Key parameters (pH, IA/TA, VFA) for monitoring reactor performance stability

Total VFA was kept at range between 700-1300 mg/L during phase I, with propionic acid <500mg/L; it then increased to 2300-3300mg/L during phase II, with propionic acid >1000mg/L. Accumulation of propionic acid has been reported to be an indicator of digester disturbance (Gallert and Winter, 2008).

4. References

1. Bernard et al. 2001. Advanced monitoring and control of anaerobic wastewater treatment plants: Software sensors and controllers for an anaerobic digester. Wat. Sci. Technol., 43(7).
2. Qiao et al. 2013. Thermophilic anaerobic digestion of coffee grounds with and without waste activated sludge as co-substrate using a submerged AnMBR: System amendments and membrane performance. Bioresource Technology 150 (2013) 249-258. (1
3. Gallert C, Winter J. 2008. Propionic acid accumulation and degradation during restart of a full-scale anaerobic biowaste digester. Bioresource Technology, 99(1): 170–178.

The VFA & alcohols production from mixed culture anaerobic fermentation: effect of pH

○Yuanyuan Wu , Kaijun Wang*

School of Environment, Tsinghua University, HaiDian district, Beijing100084, China

Email: simpleyuan5@gmail.com

Abstract:

Recently, the biorefinery on the basis of VFA & alcohols have been proposed and draws a lot of attention. Therefore, the VFA & alcohols as the objective products instead of methane become the new research point during the mixed culture anaerobic digestion. The effect of different pH (4.0, 5.5, 6.0) and organic load on the acidification of glucose of mixed anaerobic culture fermentation were explored in USAR (upflow sequencing anaerobic reactor) reactors. It was shown that pH had an obvious effect on the acidification products. At pH of 4.0, the percentage of ethanol was high to 86.7% and it was typically the ethanol-type fermentation.

Key words: biorefinery, VFA & alcohols platform, anaerobic acidification, pH, ethanol-type fermentation

1. Introduction

Methane, as the objective products in the AD process has been thought to be an available way to recover the energy in the biomass and helps to reduce global warming. However, methane has a great GWP and it is reported that 4% fugitive emissions of methane produced from AD negates any GHG emission benefit. So different product routes from waste to resource are being researched. Valorization of the organic matter present in the wastewater , sewage sludge and/or other biomass through organic acid, VFA, production by acidogenic fermentation is a key step for valorizing organic matter present in such waste streams. And VFA & alcohols are thought to be one of the key platform chemicals in a biobased economy for the production of a variety of valuable chemicals such as PHA polymers, medium chain fatty acids or electricity that can building blocks for the chemical industry. On the other hand, the acidification process to produce VFA & alcohols is short and the bacteria is relatively compatible to environmental condition.

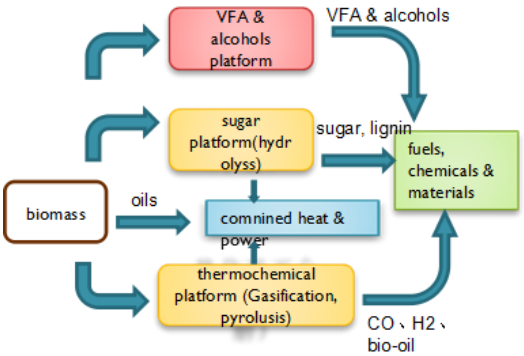


Fig. 1. Three platforms for biorefinery

The acidogenic fermentation can provide a lot of products (Fig. 1). Fluxes in a metabolic network are controlled by kinetic and/or thermodynamic limitations. There are a lot of researches about the acidogenic process with different objective products such as hydrogen gas, ethanol, or lactic acid etc. However, there is still lack of the systemic research.

The aim of this research is to investigate the product spectrum of glucose fermentation as a function of the pH and organic load in a batch reactors.

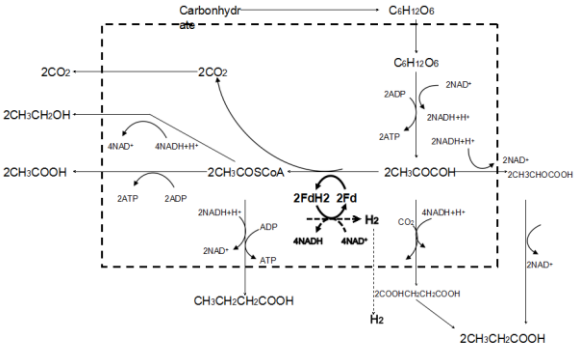


Fig. 2. The metabolic pathways during anaerobic fermentation by mixed cultures

2. Materials and methods

2.1 USAR reactor and medium

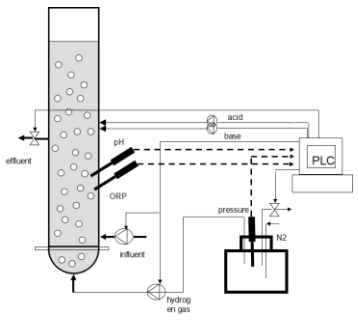


Fig. 3. Schematic representation of the USAR

The experiments were carried out using three USARs (Fig. 2). The reactor consisted of a column with a height of 0.9m and a diameter of 8cm and was operated as a sequenced batch reactor under anaerobic conditions. Operational parameters are presented in Table 1. The operational process includes four phase: feeding phase, airing with nitrogen gas, settling phase and draining phase. And the pH was controlled at 4.0, 5.5, and 6.0. The system was mixed continuously by a nitrogen gas flow (1L/min) for 95min and the gas flow was stopped to let the sludge settle for more than 10min. The next step int he cycle comprised of

pumping out the top half of the volume in the column. Glucose was solar carbon resource and NH_4Cl, KH_2PO_4 and other metal elements were also added.

Table 1 Overview of the operational conditions

Working volume	3.8L
HRT	4-8h
Cycle length	2h
pH setpoint	4.0, 5.5, 6.0
temperature	30℃

2.2 analyses

The sampling and measurements performed during the cycle are presented in Table 2.

measurement	Sample point	method
Soluble COD	effluent	spectrophotometric
Organic acids*	effluent	GC-FID
alcohols	effluent	GC-FID
TSS	effluent	Dry weight
turbidity	effluent	infrared
VSS	reactor	Dry weight

*including: acetate, propionate, butyrate, iso-butyrate, iso-valerate and valerate.

3. results and discussion

The sludge was from the anaerobic digestion reactor for sewage sludge in the Wwtps. At the beginning of the experiments, the sludge was acclimated to resume the activity by long settling time (20 minutes) and relatively low organic load (5.5 gCOD/(L.d)). After 10 days, the TSS in the three reactors was all lower than 31.6mg/L and VFA_{-COD}/COD_{in} of the three reactors (4.0, 5.5, 6.0) were 82.8%, 73.2%, 80% separately.

After start up, the organic loads of three reactors were changed to 11.0 gCOD/(L.d). The VFA & alcohols profiles of three reactors were presented in Fig. 4.

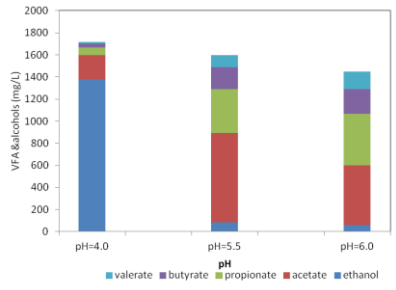


Fig. 4. Organic acids production profile under various pH

As we can see from Fig. 4 and table 2, the VFA_{-COD} decreased from pH=4.0 to pH=6.0. The reason was that the methanogenic bacteria can survive from the pH condition of 6.0 and 5.5 and the VFAs were converted to methane and hydrogen gas also took up some COD.

Effect of pH on acidification types. The ethanol concentration was high to 1378mg/L at pH of 4.0, which account to 86.7% of the acidification products, so it is a typical ethanol type fermentation. According to Ren et al results, at low pH and high organic load benefits the ethanol production, however, the ethanol to acetate ratio (mol/mol) was near 1:1. The reason might be the different pH and organic load and it still need further research.

At pH of 5.5 , the concentrations of HAc, HPr, HBu and ethanol were 810.2, 397.3, 200, and 83.2mg/L. The results were same with Yu, H.Q.(2003).

At pH of 6.0, the concentration of HAc, HPr, HBu , HVc and ethanol were 536.6, 466.4, 223, 163 and 61.4 mg/L. The fermentation type was apt to mixed acid type .

Table 2 the distribution of VFA and alcohols at various pH

pH	Eth%	Acet %	Pro %	But %	Val %	COD
4.0	86.7	7.0	3.0	2.0	1.3	3308.6
5.5	5.3	25.6	18.2	11.0	7.3	2225.6
6.0	3.9	17.0	21.3	12.3	11.4	2176.5

*the data was gained from the steady state and the reactors were operated for more than 20 days to ensure stable

The above results showed that pH had an obvious effect on the acidification products. It is the first step in the VFA & alcohols platform establishment and further research is necessary to find out if the product spectrum can be influenced and optimized. For the ethanol-type fermentation, the products were relatively pure and ethanol was an important substance for the chain elongation and also an important liquid energy substance. so how to keep the bacteria quantity and reactor stability is still need further study.

4. Reference

1. Temudo, M.F., Kleerebezem, R. and van Loosdrecht, M.C.M.(2006) Modeling product formation in anaerobic mixed culture fermentations. Biotechnology and Bioengineering 93(3), 592-606
2. Ren, N. Q., Wang, B.Z., Huang, J. (1997) ethnol-type fermentation from carbohydrate in high rate acidogenic reactor. Biotechnology and Bioengineering 54(5), 428-433
3. Zheng, M.Y., Wang, K. J., Zheng, M. X.2012 Volatile fatty acids changes during biomethanization of fruit waste and acidification by overloading. Proceedings: 4thInternational Conference on Engineering for Waste and Biomass Valorisation. Porto, Portugal. 278-281.
4. Chang, H.N., Kim, N.J., Kang, J,. Jeong, C.M. (2010). Biomass-derived volatile fatty acid platform for fuels and chemicals. Biotechnology Bioprocess Engineering 15(1),1-10
5. Yu, H.Q., Fang, H.H.P., (2003). Acidogenesis of gelatin-rich wastewater in an upflow anaerobic reactor: influence of pH and temperature. Water research 37, 55-66

Session IV:

Wastewater treatment technologies

Effect of COD/SO_4^{2-} Ratio on UASB treatment of Synthetic Organic Chemical Industrial Wastewater

○Yong Hu[1], Zhaoqian Jing[1,2], Yuta Sudo[1], Yu-You Li[1,3,*]

[1]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[2]College of Civil Engineering, Nanjing Forestry University, Nanjing 210037, China
[3]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

The effect of the chemical oxygen demand/sulfate (COD/SO_4^{2-}) ratio on the anaerobic treatment of synthetic chemical wastewater containing sulfate, acetate, and ethanol was investigated using a UASB reactor. The experimental results show that at a COD/SO_4^{2-} ratio of 20 and a COD loading rate of 25.2g-COD L^{-1} d^{-1}, the COD removal rate was maintained at as high as 87.8%. However, at a COD/SO_4^{2-} ratio of 0.5 (sulfate concentration 6000 mg L^{-1}), a COD removal rate of 79.2% and a methane yield of 0.20 L-CH_4 g-COD^{-1} were obtained. The conversion of influent COD to methane dropped from 80.5% to 54.4% with a decrease in the COD/SO_4^{2-} ratio from 20 to 0.5. At all the COD/SO_4^{2-} ratios applied, over 79.4% of the total electron flow was utilized by methane-producing archaea, indicating that methane fermentation was the predominant reaction.

Keywords: sulfate; chemical wastewater; COD/SO_4^{2-} ratio; anaerobic treatment; electron flow

1. Introduction

Wastewater streams from chemical industry contain both high concentrations of sulfate and organic compounds[1)]. When this kind of wastewater is treated with anaerobic process, competition between sulfate reducing bacteria (SRB) and methane-producing archaea (MPA) for utilization of carbon sources often leads to decrease of methane production rate and even failure of treatment process. According to the previous study, the influent COD to SO_4^{2-} ratio is an important parameter affecting the competition results[2)].

The aim of this study was to investigate the effect of COD/SO_4^{2-} ratio on anaerobic treatment of synthetic chemical wastewater containing sulfate, acetate and ethanol. For this purpose, a mesophilic UASB lab-scale reactor was operated for 375 days.

2. Material and methods

A cylindrical double wall UASB reactor with a working volume of 6 L and an internal diameter of 0.1m were used in this study (as shown in Fig. 1**)**. The reactor was kept at 35±1 ℃ due to heated water recirculation from a water circulation heater. The synthetic wastewater consisting of 1000 mg L^{-1} acetate and 1000 mg L^{-1} ethanol as model carbohydrate (sole electron donor and carbon source) was pumped from an influent tank with effective volume of 70 L. The concentration of sulfate was added as sodium sulfate into the bioreactors according to the COD/SO_4^{2-} ratios. In experiment 1, the effect of organic loading rate (OLR) on anaerobic sulfate-limited wastewater treatment was investigated. The COD/SO_4^{2-} ratio was maintained at 20 and OLR was increased in stepwise from 1.4 to 36.6 g-COD L^{-1} d^{-1} and the hydraulic retention time (HRT) shortened to 2 h. In experiment 2, HRT was maintained at 6 h, the effect of COD/SO_4^{2-} ratio on anaerobic treatment was investigated by decreasing the ratio from 20 to 0.5 stepwise.

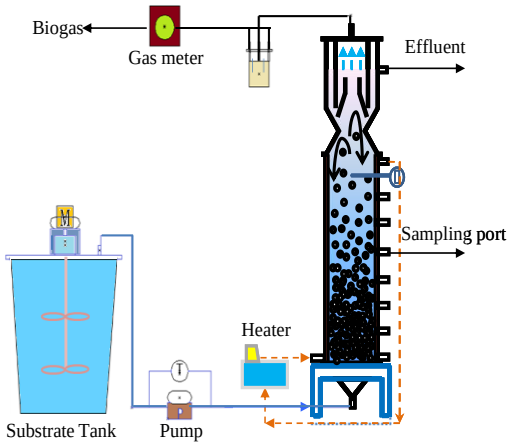


Fig. 1 The UASB reactor set-up

3. Results and discussion

3.1 Overall performance of the UASB reactor

The experimental results were shown in Fig. 2. In experiment 1, at a $NaHCO_3$ dose of 1500 mg L^{-1}, the effluent pH was maintained above 7.3 at an OLR range from 1.4 to 6.0 g-COD L^{-1} d^{-1}. However, the effluent pH decreased sharply with OLR increased to 11.9 g-COD L^{-1} d^{-1}, and resulted in COD removal deterioration. Then, the reactor performance recovered after increasing addition of $NaHCO_3$ to 3000 mg L^{-1}. At a COD/SO_4^{2-} ratio of 20 and organic loading rate (OLR) of 25.2g-COD L^{-1} d^{-1}, the COD and sulfate removal was maintained as high as 87.8% and 97.8%, respectively. High-quality biogas (methane content ≥ 70%) was produced in this experiment. These results indicated that

within a wide range of OLR, a successful anaerobic treatment could be achieved under high COD/SO_4^{2-} ratio condition.

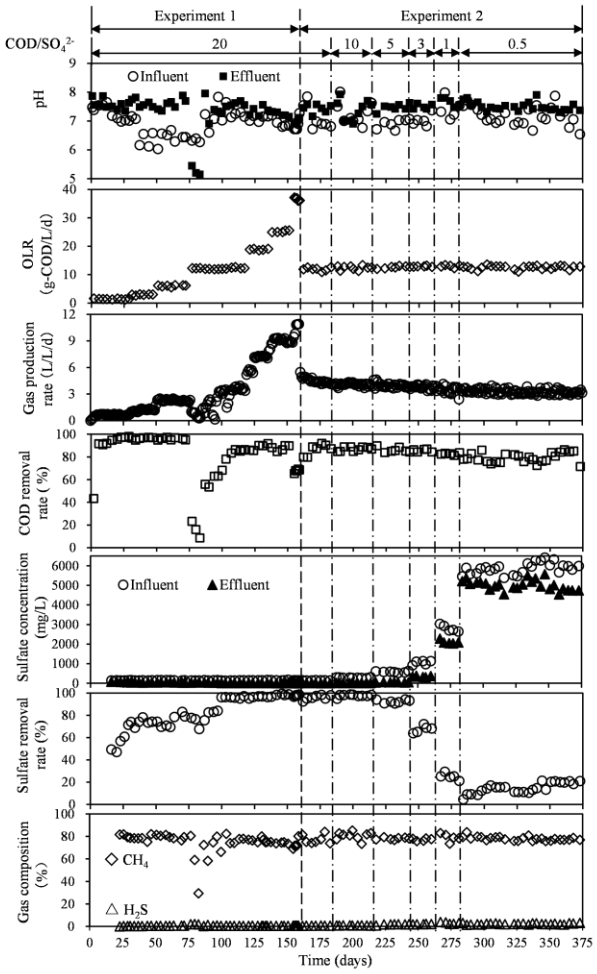


Fig. 2 Operation performance of the UASB reactor

In experiment 2, under the same OLR of 11.9 g-COD L^{-1} d^{-1}, when COD/SO_4^{2-} ratio decreased from 20 to 0.5, COD removal rate dropped from 89.1% to 79.2%. Furthermore, methane production rate decreased from 4.47L L^{-1} d^{-1} to 3.26 L L^{-1} d^{-1}. However, the methane percentage in the biogas was maintained above 70% during the whole experimental period. Ethanol was not detected from the effluent in this study. Consequently, successful operation was achieved at COD/SO_4^{2-} ratio ranging from 20 to 0.5 and a COD loading rate of 12.0 g-COD L^{-1} d^{-1} in the UASB reactor.

3.2 Effect of COD/SO_4^{2-} ratio on electron flow

To quantify the competition between SRB and MPA, the electron flow to SRB and MPA in the UASB reactor was calculated with the following equations:

Electron flow to MPA = CH_4-COD / (CH_4-COD + H_2S-COD) (1)

Electron flow to SRB = H_2S-COD / (CH_4-COD + H_2S-COD) (2)

in which CH_4-COD = moles of CH_4 produced ×64 g, H_2S-COD = moles of sulfide produced in gas and water × 64 g.

Fig. 3 shows the proportions of electrons utilized by MPA and SRB at various COD/SO_4^{2-} ratios were calculated. The percentage of electrons utilized by MPA was 96.5% with COD/SO_4^{2-} ratios of 20. Even at a COD/SO_4^{2-} ratio of 0.5, around 80% of electrons were still utilized by MPA. With COD/SO_4^{2-} ratio in the range of 20-0.5, SRB accounted for 3.5-20.6% of electrons utilization.

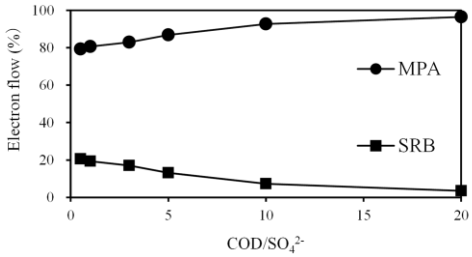


Fig. 3 Electron flow at various COD/SO_4^{2-} ratios

All of these results indicated that methane production was the main reaction under both of high and low COD/SO_4^{2-} ratios in this experiment.

3.3 Effect of COD/SO_4^{2-} ratio on sulfide and methane production

Fig. 4 shows the relationship between methane yields with dissolved sulfide produced by the reduction of sulfate at various COD/SO_4^{2-} ratios. When the COD/SO_4^{2-} ratio dropped from 20 to 0.5, the concentration of dissolved sulfide increased from 29.6 to 205.0 mg L^{-1}, and this was accompanied with a slight decrease in methane yield from 0.28 to 0.20 L-CH_4 g-COD^{-1}. Consequently, there was no obvious sulfide inhibition occurred with a COD/SO_4^{2-} ratio changing from 20 to 0.5. To the best of our knowledge, this is the first report showing a high methane production at a COD/SO_4^{2-} ratio as low as 0.5 by anaerobic treatment.

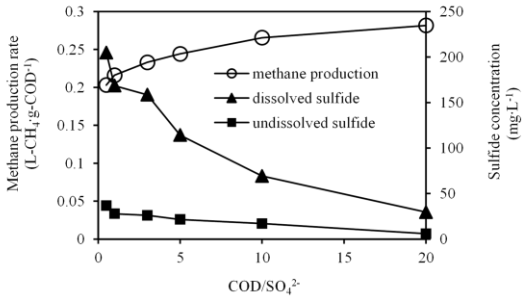


Fig. 4. Methane production rate and dissolved sulfide concentration at different COD/SO_4^{2-} ratios

4. References

1. Jing, Z. Q., Hu, Y., Niu, Q. G., Liu, Y. Y., Li, Y. Y. and Wang, X. C. C. (2013) UASB performance and electron competition between methane-producing archaea and sulfate-reducing bacteria in treating sulfate-rich wastewater containing ethanol and acetate, *Bioresource Technology*, **137**, 349–357.
2. Lopes, S. I. C., Wang, X., Capela, M.I. and Lens, P. N. L. (2007) Effect of COD/SO_4^{2-} ratio and sulfide on thermophilic (55 ℃) sulfate reduction during the acidification of sucrose at pH 6, *Water Res.*, **41**, 2379-2392.

Upgrading of anaerobic digestion of waste activated sludge by temperature-phased two-stage process and the introduction of a recycle system

○Li-Jie Wu[1], Yu Qin[2], Toshimasa Hojo[1], Atsushi Higashimori[1], Yu-You Li[1,2]*

[1]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
[2]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

Low volatile solids destruction and methane production are commonly encountered in the application of conventional mesophilic digestion of WAS. Thermophilic (55℃)/mesophilic (35℃) temperature-phased anaerobic digestion (TPAD) was paid attention due to the advantages combination of thermophilic and mesophilic digestion. However, little attention was paid to WAS, such a single complex substance. With mesophilic digestion as the control, TPAD process was conducted to investigate the upgrading level of WAS. Moreover, a recycle system from the second stage to the first stage was introduced to TPAD, aiming at further upgrading (R-TPAD). With HRT 30 days in the continuous experiment, through above 2 HRTs, the performances of TPAD and R-TPAD were compared with the mesophilic digestion. The results showed that compared with conventional single-stage process, TPAD and R-TPAD were effective in improving the efficiency of reduction and reuse of WAS. The biogas production rate of TPAD and R-TPAD was up to nearly 50% higher than single-stage process in the steady state, and both had higher buffering capacity and stability. The reductions of TS and organic matter, and hydrolysis, and methanogenesis of single-stage process were improved, and dewaterability among the three systems was close to each other.

Keywords: waste activated sludge; anaerobic digestion; mesophilic; temperature phased; volatile solids

1. Introduction

Mesophilic anaerobic digestion is widely used in sludge treatment, but low process efficiency, especially for waste activated sludge (WAS) is an issue. Hydrolysis of bacterial cells and organic solids is a rate limiting step. The cell walls and extracellular biopolymers formed in activated sludge limits the volatile solids (VS) reduction WAS to about 40%.

The temperature-phased anaerobic digestion (TPAD) has gained some interest due to the fact that it combines the advantages of the thermophilic system in higher digestion rate and VS reduction, and the advantages of the mesophilic system in higher process stability and dewaterability. In recent years, a recycle system from the second stage to the first one was introduced to return alkalinity, to further optimize the process.

However, few studies have been published to report the use of TPAD with cycle (R-TPAD) system, even TPAD, to digest WAS. In addition, the quantitative advantages of TPAD and the comparative evaluation of the process with and without cycle haven't also been made.

Based on the information above, this study was designed to evaluate the treatment efficiency and the feasibility of TPAD and R-TPAD, with WAS as substrate.

2. Materials and methods

2.1 Experimental systems

The schematic diagram of the experimental apparatus used in this study was shown in Fig. 1. The apparatus was composed of three series of processes, namely single-stage digestion, TPAD, and R-TPAD (recycle ratio 1:1). The reactors were completed stirred treatment reactors (CSTR), made of plexiglass, with working volume 5L, 3L/12L, 3L/12L, respectively.

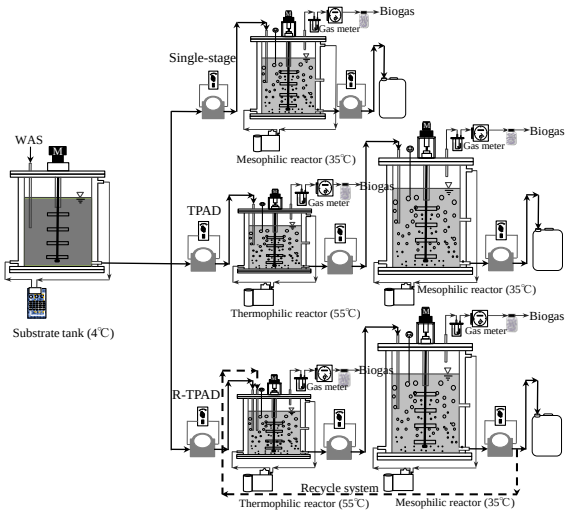


Fig. 1. Schematic diagram of experimental apparatus

Table 1 Characteristics of the WAS

Parameter	Average	Range
TS (%)	4.62	4.55~4.69
VS (%)	3.62	3.56~3.68
TCOD (g/L)	58.5	58.3~58.7
T-Carbohydrate (g/L)	6.5	6.1~6.9
T-Protein (g/L)	20.4	19.3~21.5
T-Lipid (g/L)	4.7	4.4~5.0
NH_4^+-N (mg/L)	550	511~589
Alkalinity (mg $CaCO_3$/L)	940	870~100
VFA (mg COD/L)	1860	1710~2020

2.2 Start up and operation

Reactors were acclimatized by adding the thermophilic and mesophilic seed microorganisms to the corresponding reactors until the working volume. The temperature of

themophilic and mesophilic reactors were controlled to 55±1℃ and 35±1℃ by water jackets and heaters. The feed sludge was thickened WAS, the characteristics of which were shown in Table 1. Reactors were start up by shortening the hydraulic retention time (HRT) from 100d to 50d, then to 30d. Feeding and drawing pumps were operated 6 to 12 times per day.

3. Results and discussion

3.1 Biogas production

Fig. 2 shows the time course of pH, gas production rate, methane content, alkalinity, and NH_4^+-N concentration in the three systems. Throughout the experiment, the pH was maintained at the range between 7 and 8, which is appropriate for methane production. At the beginning, the gas production rate in three systems was close to each other. At the steady state with HRT 30d, the biogas production rate of TPAD and R-TPAD became about 0.6L/L/d, which was higher than that of the single-stage system 0.4L/L/d, demonstrating the superiority of TPAD system. In all reactors, alkalinity went higher than 4000mg $CaCO_3$/L resulting in high buffering ability, which maintained the system pH to be stable. The threshold of NH_4^+-N concentration causing VFA accumulation during mesophilic methanogenesis is about 4000mg/L. NH_4^+-N concentration in every mesophilic reactor was lower than 3000mg/L, almost not inhibitory to the methane fermentation.

3.2 TS, VS and COD reductions

The reduction efficiency of every component at steady state was calculated based on Eq. (1). The results of calculation were shown in Table 2.

$$Reduction(\%) = \left(\frac{Inf.conc. - Eff.conc.}{Inf.conc.}\right) \times 100 \quad \text{.....(1)}$$

Similar to the gas production, the TS, VS and COD reductions in TPAD and R-TPAD were also higher than the single-stage mesophilic system. The TS, VS, and COD reductions increased by about 6%, 8%, and 8%, respectively, which benefits the sludge reduction.

Table 2 TS, VS and COD reduction efficiency

	Single-stage	TPAD	R-TPAD
TS reduction (%)	30.5	40.1	40.5
VS reduction (%)	40.9	49.1	49.7
COD reduction (%)	45.3	53.3	53.8

3.3 Hydrolysis, acidogenesis and methanogenesis

The products of hydrolysis, acidogenesis, and methanogenesis were calculated from a COD balance. The total COD conversion ratios of hydrolysis, acidogenesis, and methanogenesis can be calculated according to the Eq. (2)~(4), using soluble COD, VFA COD and CH_4 COD. The results were shown in Table 3.

$$Hydrolysis\ ratio(COD\%) = \left(\frac{SCOD + COD_{CH_4}}{TCOD_{inf.}}\right) \times 100 \quad \text{.....(2)}$$

$$Acidogenesis\ ratio(COD\%) = \left(\frac{COD_{VFA} + COD_{CH_4}}{TCOD_{inf.}}\right) \times 100 \quad \text{.....(3)}$$

$$Methanogenesis\ ratio(COD\%) = \left(\frac{COD_{CH_4}}{TCOD_{inf.}}\right) \times 100 \quad \text{.....(4)}$$

Table 3 Ratios of hydrolysis, acidogenesis, and methanogenesis

	Single-stage	TPAD	R-TPAD
Hydrolysis (%)	46.3	66.5	65.7
Acidogenesis (%)	38.0	55.6	54.2
Methanogenesis (%)	38.0	55.6	54.2

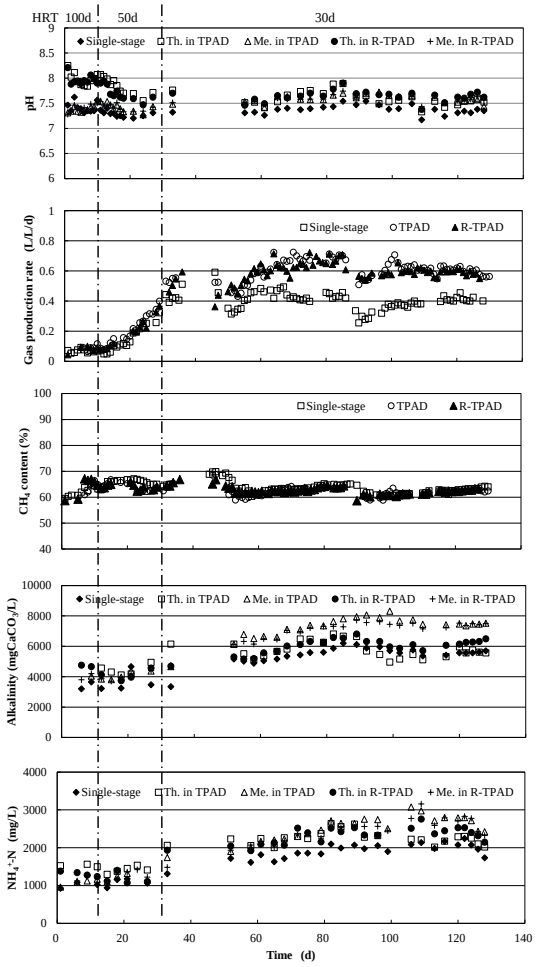


Fig.2. Time course of pH, gas production, CH_4 content, alkalinity, and NH_4^+-N in the three systems

Table 4 Water quality of dewatering filtrate

Parameter	Single-stage	TPAD	R-TPAD
Dosage (mg/g TS)	56	65	65
COD (g/L)	0.46	0.61	0.66
NH_4^+-N (mg/L)	1360	1650	1780
PO_4^{3-}-P (mg/L)	580	566	574
VFA (mg COD/L)	N.D.	N.D.	N.D.

Compared with the single-stage process, the hydrolysis ratios of both TPAD and R-TPAD were improved by nearly 20%, and the acidogenesis and methanogenesis ratios of them were increased by about 17%.

3.4 Dewaterability

The flocculation experiment was done by using polyamidine, a polymer flocculant, and in terms of the reagent adding dosage and water quality of filtrate in the effluents, the dewaterability of every system was evaluated.

COD, NH_4^+-N, PO_4^{3-}-P, and VFA of the dewatering filtrate were listed in Table 4. In terms of the dosage of reagent needed and pollutant content in the filtrate, the dewaterability of the three processes seemed to be close.

4. References

1. Ghosh, S., Buoy, K., Dressel, L., Miller, T., Wilcox, G., Loos, D. (1995). Pilot- and full-scale two-phase anaerobic digestion of municipal sludge. Water Environment Research, 67(2), 206-214.
2. Han, Y., Sung, S., Dague, R.R. (1997). Temperature-phased anaerobic digestion of wastewater sludges. Water Science and Technology, 36(6-7), 367-374.

Application of model and simulations in wastewater treatment process

○Wei-Kang Qi[1,2], Yi-Ling Guo[2], Yu-You Li[1,3]*

[1]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
[2]Institute of Environment and Municipal Engineering, Qingdao Technological University, Qingdao 266033, China
[3]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

Combination of laboratory tests, historical data and experiments modelling, methodology is made up for developing WWTP model which is a significant source for municipal policies in management. For example, WWPT model is intended to evaluate the adequately works ability of receiving particular wastewater treatment. And it could also reflect the relationship of each reactor and used to solve the problem of different correlative process. An innovative anoxic/oxic (A/O) constructed wetlands (CWs) system was applied to remove the nutrient substrate in wastewater, and mathematical modelling for organic degradation, nitrification and denitrification in this system was then developed. A sensitivity analysis was performed for the parameters and operating conditions, and found that the treatment efficiency is affected by the organic loading rate, temperature, and recycling rate. The results indicate that the removal rates of COD and nitrogen are above 95% and 50%, respectively. The flow rate does not affect the removal rate of the substrate, and a higher influent COD or lower ammonia concentration results in a better efficiency in the purification rate. The nitrogen removal rate could be improved by optimizing the interaction between the recycle rate and the influent ammonia.

Keywords: model; simulation; wastewater treatment plant; GHGs; CFD

1. Introduction

Modeling is a powerful tool that researchers and practitioners can use to identify the most promising designs for experimental testing to validate the fundamental mechanisms of wetland formation or performance. In general, mathematical process modeling can be used for two purposes: (1) to better understand processes by testing certain hypotheses using experimental data (which is known as modeling for process analysis) and (2) to optimize system operation, which is accomplished by using well-established models of a system's design. Thus, modeling COD and N removal is standard practice and a valuable tool for the design and operation of wastewater treatment processes. The primary objective of this study is to determine the effects of the influent and then to optimize the treatment method for anoxic/oxic (A/O) submerged constructed wetlands (CWs) system. The results can be used to evaluate wastewater treatment process designs, thereby avoiding the cost, time, and risk associated with building a physical prototype for the process.

2. Materials and Methods

2.1. Experiment configuration and operation

Fig.1 shows the A/O submerged CWs wastewater treatment system. The main experiment apparatus was composed of one upflow anoxic CW and two upflow aerobic CWs. The mixture of influent water and backflow liquid entered the anoxic wetland and then flowed to the first and second aerobic CWs in series. The effluent from the second aerobic wetland was recirculated, primarily to enhance nitrogen removal.

2.2. A/O wetlands system model

The biological removal of carbon and nitrogen from wastewater involves six components: organic soluble substrate (S_S), heterotrophic biomass (X_H), autotrophic biomass (H_A), oxygen (SO), nitrate nitrogen (S_{NO}) and ammonium nitrogen (S_{NH}). The process involves three sequential steps (Fig. 2): aerobic autotrophic growth, in which ammonia and dissolved oxygen are consumed to

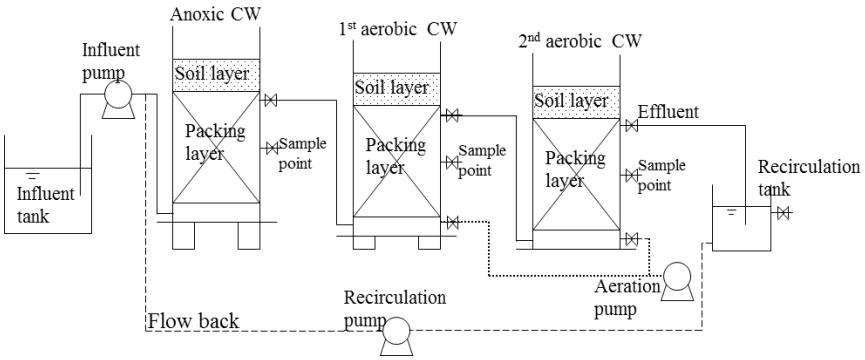


Fig.1. Schematic of A/O wetlands system

produce additional biomass and nitrate in solution; 2) aerobic heterotrophic growth, in which soluble carbon and dissolved oxygen are consumed; and 3) anoxic heterotrophic growth, in which nitrates are used as an oxygen source to produce additional biomass and nitrogen gas. The products of autotrophic and heterotrophic decay were divided into respiration and inactivation products.

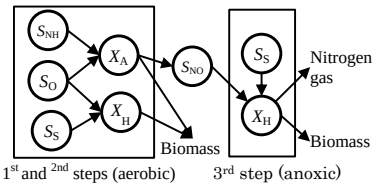


Fig. 2. Model of substrate removal process

3. Results and discussion

3.1. The impact of the input substrate

The effect of the overall substrate flux into the A/O CWs system on the effluent substrate concentration and the bacteria density is evaluated using data obtained at relatively constant SS and SNH values. Fig.3 presents the predicted stable effluent substrate concentrations and the quantity of bacterial under different influent hydraulic load rate (HLR) and S_O values ranging from 1 to 10 $m^3/(m^3 \cdot day)$ and 0.5 to 4 g/m^3, respectively. The effluent S_S and S_{NH} are less than 2.2 g/m^3 and 1.00 g/m^3, respectively, and part of the input ammonia was effectively transformed into nitrate. The effluent S_S and S_{NH} vary slightly with the different HLR values. A short detention time results in unexpectedly high efficiency, whereas longer detention times produce disappointingly small increases in the substrate removal efficiency. The low concentrations of the remaining organics and ammonia indicate that the degradation and nitrification are successful because the amount of bacteria increased with the hydraulic load rate. Organic matter removal is associated with both anoxic and aerobic processes.

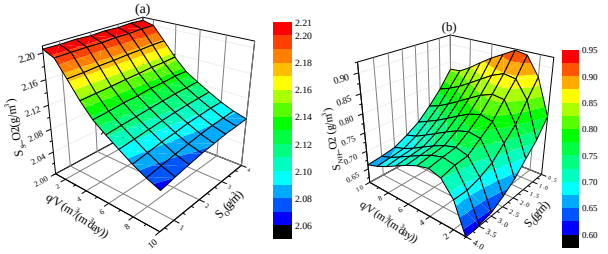


Fig. 3. Effluent substrate concentrations at different hydraulic loadings and oxygen concentrations.

3.2. C/N impact

Fig.4 illustrates the predicted effluent substrate concentrations under different influent S_S and S_{NH}. The effluent ammonia concentration displays little dependence on the influent ammonia nitrogen concentration. The soluble substrate removal rate increases with the influent S_S. However, the S_{NO} increases with the influent S_{NH} and decreases as the S_S increases. As the ammonia and nitrate nitrogen diffuse with the soluble substrate into and through the nitrifying and denitrifying CWs, respectively, the nitrifying and denitrifying wetlands use the ammonia and nitrate nitrogen with glucose for biosynthesis and respiration, such that the nitrifying and denitrifying biomasses increase or decrease with time until the growth rate is balanced by the decay rate. In addition, the high substrate concentration also accelerates the rate of cell death. A high C/N ratio reduces the importance of the nitrifiers, increasing their susceptibility to impacts from heterotrophs and inerts. Consequently, higher percentages of the soluble substrate could be used in the anoxic CW for denitrification. The nutrition concentration clearly impacts the effluent S_{NO} in that higher influent SS or lower influent S_{NH} resulted in lower effluent S_{NO}.

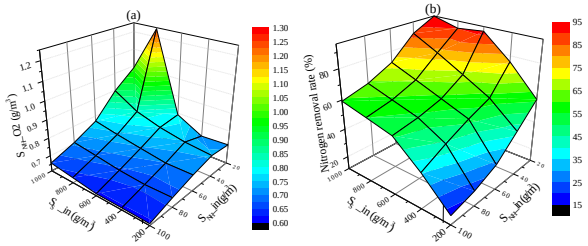


Fig. 4. Contours showing effluent SS, SNO and nitrogen removal rate at different influent conditions.

3.3. Recycle rate

Fig.5 shows the predicted effluent S_{NO} and the nitrogen removal rate for influent S_{NH} ranged from 20 to 100 g/m^3, and the recycle rates ranged from 0.5 to 3. The coupling between the recycle rate and the influent ammonia concentration affects the nitrate removal rate. The effect of the interaction could be classified into two regimes. When the influent S_{HN} is below 60 g/m^3 (i.e., the C/N ratio is above 8.3), the effluent S_{NO} decreases as the recycle rate increases. The corresponding nitrogen removal rate increases from 50% to above 80%. However, for S_{HN} above 60 g/m^3 (i.e., the C/N ratios below 8.3), the recycling has a more complex effect. The effluent S_{NO} varies for recycle rates below 1, but does not change when the recycle rate is increased from 1 to 3. The corresponding nitrogen removal rate does not increase above 60%. When the soluble substrate in the anoxic CW is the limiting factor in denitrification, and the C/N ratio is low, a low S_S value is observed because the soluble substrate is consumed. The process reaction rate is sufficiently low that the recirculated nitrate nitrogen is not completely denitrified. Thus, in this case, increasing the recycle rate above 1 does not clearly enhance the nitrogen removal rate.

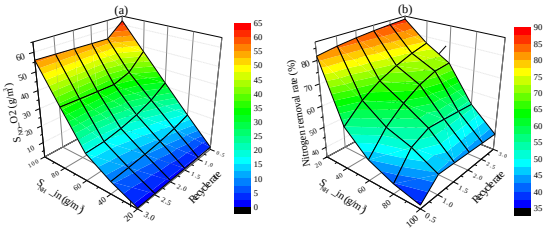


Fig. 5. Effluent S_{NO} (a) and nitrogen removal rate (b) contours at different influent SNH and recycle rates.

4. References

1. Henze, M., 2008. Biological Wastewater Treatment: Principles, Modelling and Design. IWA Publishing, London, UK.
2. Wei-Kang Qi, Yi-Ling Guo, Yu-You Li. An anoxic/oxic submerged constructed wetlands process for wastewater treatment: modelling, simulation and evaluation. Ecological engineering.2013.

Combined electrical-alkali pretreatment to increase the anaerobic hydrolysis rate of waste activated sludge during anaerobic digestion

○Guangyin Zhen[1,2], Xueqin Lu[1], Yu-You Li[1,3,*], Youcai Zhao[2]

[1]Graduate School of Engineering, Tohoku University, Sendai 980-8579, Japan
[2]The State Key Laboratory of Pollution Control and Resource Reuse, Tongji University, Shanghai 200092, China
[3]Graduate School of Environmental Studies, Tohoku University, Sendai 980-8579, Japan
*E-mail: yyli@epl1.civil.tohoku.ac.jp

Abstract

Electrolysis and alkaline (NaOH) solubilization were integrated for promoting WAS anaerobic digestion. Pretreatment effectiveness were investigated in terms of disintegration degree (DD_{SCOD}), the releases of protein (PN) and polysaccharide (PS), and subsequent anaerobic digestion. Electrolysis was able to dissociate extracellular polymeric substances (EPS), crack the sludge cells and release the biopolymers due to the incorporation of alkaline solubilization, enhancing sludge solubilization. Biochemical methane potential (BMP) assays showed the highest methane yield was achieved with 5 V plus pH 9.2 pretreatment with up to 20.3% improvement over the raw sludge after 42 days of operation. In contrast, no discernible improvements on anaerobic degradability were observed for the rest of pretreated sludges. The statistical analysis indicated that increased solubilization induced by electrical-alkali pretreatment increased the first-order anaerobic hydrolysis rate (k_{hyd}), but had no, or very slight enhancement on WAS ultimate methane yield (f_d), demonstrating that increased solubilization of WAS from a pretreatment does not always induce an improved anaerobic digestion efficiency.

Keywords: *Waste activated sludge (WAS); Dewaterability; Electrolysis; Alkaline solubilization; Ultraviolet visible (UV-Vis) spectra*

1. Introduction

Hydrolysis is the rate-limiting step in the anaerobic digestion process because of the complex and rigid floc structure of WAS. In this sense, numerous strategies have been previously studied to enhance the hydrolytic limiting stage[1]. In this study, the combined electrical-alkali process was first applied to pretreat WAS, aiming to enhance sludge disintegration and subsequent anaerobic digestion. Firstly, pretreatment effectiveness was analyzed by comparing the soluble COD, DD_{SCOD}, PN, and PS in pretreated and non-pretreated sludges in order to propose the best solubilization conditions, and also to explore the responsible reasons for enhancing the anaerobic biodegradability. Secondly, the digestion performances of different pretreated sludges were assessed in terms of methane yields via BMP tests.

2. Materials and Methods

2.1Electrical-alkali treatment

Pretreatment experiments were conducted in a 500 mL glass cell by using a pair of Ti/RuO_2 meshes (7 × 10 cm) as the anode/cathode. In each run, a sample of 400 mL was used at its inherent pH. The same operations were conducted when the effect of NaOH on electrically assisted modification of sludge was investigated.

2.3 Biochemical methane potential (BMP) assay

Biogas production of sludges was batch determined in series of 120 mL glass serum bottles. Each bottle was filled with 50 mL of inoculum and 25 mL of substrate. A blank with 75 mL of inoculums was conducted to determine the biogas production from endogenous inspiration. All bottles were placed in a water bath (35±1 °C) under continuous shaking (100±1 rpm) for 42 days. A first-order kinetic model was used for analysis of the BMP data. First-order hydrolysis rate, k_{hyd} (/day) and degradability, f_d (mL CH_4/g COD_{added}) were estimated by fitting the data to Eq. (1).

$$Y_{CH_4} = \frac{V_{CH_4}}{COD_{added}} = f_d(1 - \exp(-k_{hyd}t)) \qquad (1)$$

Where Y_{CH4} is the specific methane yield (mL CH_4/g COD_{added}), V_{CH4} is the volume of methane produced (mL CH_4), COD_{added} is the mass of COD present in the BMP reactors (g COD_{added}) and t is the time since the setup (days).

3. Results and discussion

3.1 Electrical-alkali pretreatment

3.1.1 COD solubilization and SCOD release

Fig. 1 indicates the effects of electrolysis and electrical-alkali pretreatments on SCOD and DD_{SCOD}. It is evident that, for the electrolysis alone, the rising voltage from 5 to 20 V led to a very slight increase in SCOD (from 940.6 ± 17.4 to 1401.7 ± 34.8 mg/L) (Fig. 1a), and the DD_{SCOD} values were calculated as the range of 0.1–8.2% (Fig. 1b).

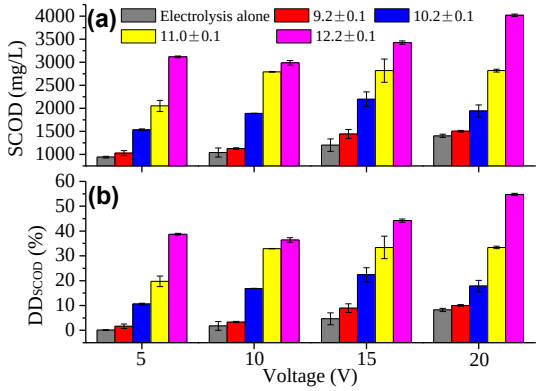


Fig. 1. Effects of electrical-alkali pretreatment on SCOD (a) and DD_{SCOD} (b) (E-alone: electrolysis alone).

Compared with the electrolysis alone, the combined electrolysis and alkalic plays a vital role in improving sludge hydrolysis. DD_{SCOD} progressively jumped from 0.1 ± 0.2% (E-alone) to 1.6 ± 0.9%, 10.6 ± 0.3%, 19.8 ± 2.1% and 38.7 ± 0.3% at pH 9.2, 10.2, 11.0 and 12.2, respectively. Furthermore, higher operational voltages can emphasize the alkaline impact. When at pH 12.2, DD_{SCOD} went up to 54.7 ±

0.5% at 20 V, 43.3% higher compared with that (38.7 ± 0.3%) at 5 V ($F_{observed}$ = 1674.38, F_{crit} = 18.51, $p_{(0.05)}$ = 5.97 × 10^{-4} <0.05) (Fig. 1b). The remarkable increase in DD_{SCOD} achieved by the combined method indicated a clear synergistic disintegration effect.

3.1.2 Soluble protein and polysaccharide releases

By using a combination of electrolysis and alkali, the releases of PN and PS could be enhanced significantly (Fig. 2). Moreover, a higher pH led to the releases of more PN and PS, agreeing well with SCOD behavior (Fig. 1).

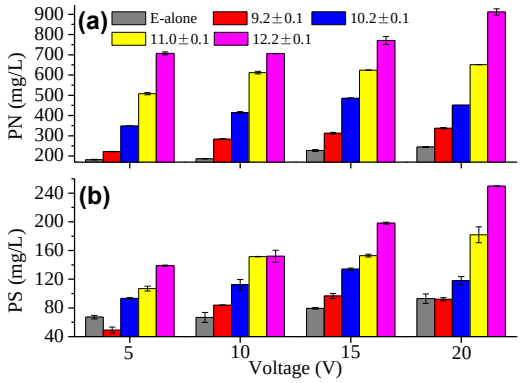


Fig. 2. Effect of electrical-alkali pretreatment on soluble PN (a) and PS (b).

Soluble PN and PS were 182.3 ± 1.6 and 67.2 ± 2.5 mg/L, respectively, at 5 V without the alkali. They were found to be 348.4 ± 1.2 and 93.4 ± 1.1 mg/L at pH 10.2, which further increased to 706.9 ± 7.0 and 139.0 ± 0.8 mg/L respectively at pH 12.2. This was presumably due to the dissociation of acidic groups in the EPS of WAS[2], microbial cells within sludge were liberated and exposed to OH^- ions. At alkaline pHs, the cells could not maintain an appropriate turgor pressure on account of saponification of lipids and hydrolysis of RNA in the cell walls/ membrane[3, 4] and, disrupted completely.

3.2 Batch anaerobic digestion tests

3.2.1 Methane production

The electrical-alkali pretreatment consistently increases anaerobic hydrolysis rate (k_{hyd}) as shown in Fig. 3. The pretreated sludge contained higher levels of soluble organic matters with respect to the raw which were metabolized more rapidly and therefore produced fairly high k_{hyd}. The significantly positive correlations between k_{hyd} and SCOD, DD_{SCOD}, PN as well as PS (R_p = 0.922, $p_{(0.05)}$ = 0.003; R_p = 0.945, $p_{(0.05)}$ = 0.001; R_p = 0.957, $p_{(0.05)}$ = 7.32 × 10^{-4}; and R_p = −0.600, $p_{(0.05)}$ = 0.005, respectively) as illustrated in Fig. 4a also strongly verified this hypothesis. Interestingly, the electrical-alkali pretreatment resulted in an increase in the anaerobic digestion rate but had no, or very slight impact on methane potential. Only the 5 V-pH 9.2 sample achieved discernible improvement in methane yields (149.72 mL CH_4/g COD_{added}), 20.3% higher than the raw sludge (124.39 mL CH_4/g COD_{added}). In the rest of the reactors, however, methane yields were found to be comparable and even slightly lower as compared to the control.

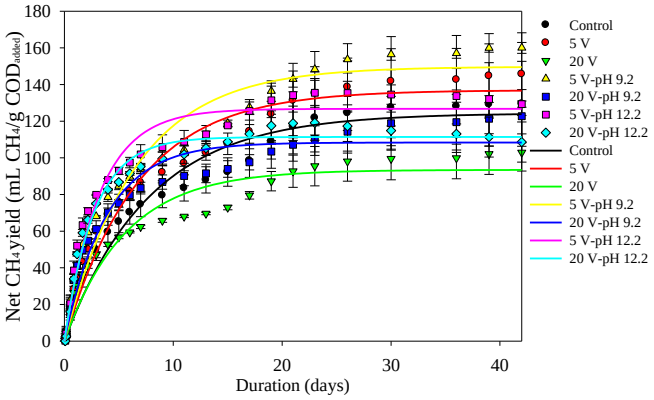


Fig. 3. Net cumulative methane production for different pretreated sludge samples.

In order to provide deep insight into the influence of DD_{SCOD} on anaerobic digestion, the statistical analysis in terms of Pearson's correlation coefficient (R_P) and p-values was thus performed. The results in Fig. 4b showed that there were no clear correlations existing between f_d and SCOD, DD_{SCOD}, PN or PS (R_p = −0.276, $p_{(0.05)}$ = 0.549; R_p = −0.274, $p_{(0.05)}$ = 0.552; R_p = −0.224, $p_{(0.05)}$ = 0.630; and R_p = −0.389, $p_{(0.05)}$ = 0.388, respectively), supporting the finding of Kim et al.[5] that increased solubilization of WAS from a pretreatment does not always induce an improved anaerobic digestion efficiency. From these results, it seems that DD_{SCOD}, SCOD, PN or PS contents achieved are not the only indicators which need to be taken into account for an ideal comparison of pretreatment effectiveness in anaerobic digestion of WAS. Other influencing factors, such as the type and dose of chemical applied, compositions of soluble organics released after pretreatment, etc. should also be attached equal importance to.

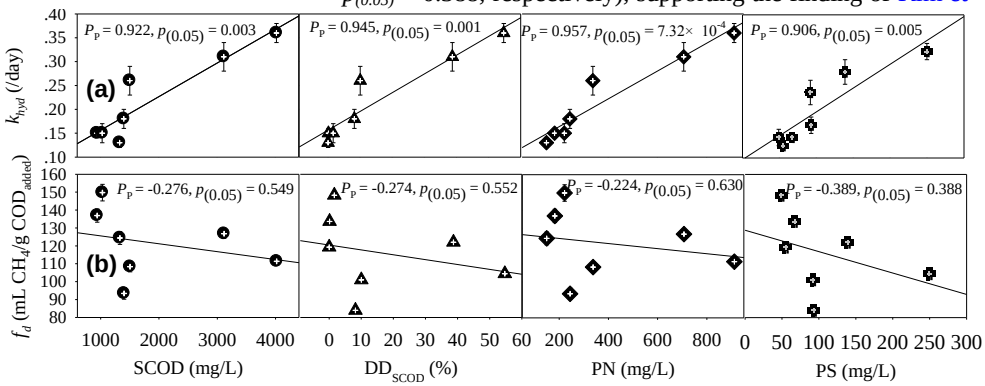


Fig. 4. Pearson's correlation between k_{hyd}, f_d and physical characteristics of different pretreated sludges.

References

[1] Zhen, et al., *Applied energy*, 2014 (revise).
[2] Zhang, et al., *Chemical Engineering Journal*, 2009. 148: 348-353.
[3] Song, et al., *Water Reseach*, 2010. 44: 4371-4378.
[4] Tyagi and Lo, *Bioresource Technology*, 2012. 119: 105-113.
[5] Kim, et al., *Bioresource Technology*, 2013. 143: 660-664.