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Effect of Nutrient Supply on the Production of Soluble Microbial Products (SMP) in Anaerobic Reactors

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Abstract

Three up-flow anaerobic sludge blanket (UASB) reactors added with different amount of nutrients were compared to investigate the effect of nutrient on the production of soluble microbial products (SMP) in the bench-scale anaerobic reactors. The running performance and the analysis of SMP verified that the nutrient level could greatly affect the treatment performance and the quality of anaerobic effluent. The surplus nutrimental addition would stimulate the bacterial activity and increase the metabolism products, causing the high concentration of effluent organic matter. On the contrary, the nutrient deficiency could keep the microbial activity at a low level, which caused the temporary better anaerobic effluent at the low organic loading rate (OLR), but might lead to a vulnerable microbial system, even system failure when OLR went higher. Molecular analysis revealed that the abundant existence of *Methanosaeta* might be beneficial to the SMP consumption.

Keywords: Anaerobic effluent; Nutrients; Soluble microbial product (SMP); *Methanosaeta*

Introduction

Biological treatment effluent contains various soluble microbial products (SMP), which account for an important part in the total amount of organic compounds [1,2]. Part of the SMP has been identified as humic and fulvic acids, polysaccharides, proteins, nucleic acids, organic acids, amino acids, antibiotics, steroids, extracellular enzymes, siderophores, structural components of cells and products of energy metabolism. Crucially, SMP have been found to comprise the majority of soluble organic material in the effluents from biological treatment processes [3] and their presence is, therefore, of particular importance concerning the discharge criteria of biological oxygen demand (BOD) and chemical oxygen demand (COD). SMP also exhibit several characteristics, such as toxicity and metal chelating properties [4], which have been shown to adversely affect the kinetic activity, the flocculating and settling properties of sludge and, more importantly, the performance of the treatment system. For these reasons, SMP from the biological treatment process have been intensively studied. Many studies have well proved the important effect of operational parameters, such as the influent concentration, the organic lording rate (OLR), the sludge retention time (SRT), the hydraulic retention time (HRT), etc, on the production of SMP in aerobic [5,6] and membrane bio-reactors [1,7-10]. It was clearly found that adjusting the operational parameters could effectively control the production of SMP and thus improve the effluent quality.

In the last years SMP in the anaerobic biological treatment process have also aroused much interest [11,12] because the SMP contents in the anaerobic effluent not only seriously affect the treatment performance of anaerobic reactor, but also directly decide the process selection, the running cost and the energy consumption of the post treatment. The high strength industrial wastewaters, which require anaerobic process as the necessary first-step treatment, are generally characterised with complex composition, uneven quality and quantity, various contents of nutrients and inorganic salts, etc. These characteristics may affect the SMP production greatly. For instance, Aquino and Stuckey [11] have found that the nutrient deficiency caused more SMP production, probably due to the enhanced cell lysis. Some organics might have been deliberately excreted to scavenge metal nutrients or to dump electrons.

Besides the nutrients, trace elements also play a crucial role in the metabolism of microorganisms, especially in anaerobic treatment. Generally the nutrient and trace elements are abundant in domestic wastewater, so their effects on the activity of aerobic systems are not easily observed, but lacking of trace metals and/or sulphide imbalance are very frequently the reasons of the negative results in the anaerobic biotransformation of wastewaters, especially the industrial wastewaters [13]. The macro-nutrients N, P and S are always important cell components in the biological processes. In the anaerobic process, the micro-nutrients such as iron, zinc, cobalt, nickel and magnesium can activate the metabolism of the methane production bacteria. Iron is the important component of enzymes which catalyze the anaerobic bio-reactions. Maganesium participates in the energy metabolism and expedite the production of methane [14]. Nickel is the indispensable element of urease and sulfur takes a considerable ratio in cell materials. However, due to the economic reasons, nutrient supplementation is often not considered practicable in the real application of industrial wastewater treatment, particularly in the developing countries.

Although the important roles of nutrients on the anaerobic process performance have already been well known, the effect of nutrient addition on the SMP was rarely reported. Since the nutrients are of great importance to microbial growth, activity and metabolism, unavoidably they also would affect the SMP secretion, including their components and amount. The objectives of this study were to investigate the effect of nutrient supply on the effluent water quality, especially SMP, and the system stability. Through the connection between microbial activity and effluent COD, the concept of SMP

production and consumption is to be applied in the manipulation of anaerobic treatment, finally to improve the quality of anaerobic effluent and ease the further treatment.

Experimental

Experiment setup

The bench scale experiment was carried out in three identical UASB reactors. Each of the reactors consisted of a cylindrical glass tube with a total effective volume of 16.1 L, height of 1.7 m and inner diameter of 110 mm. The reactors were kept in mesophilic (35°C) surroundings and each was inoculated with 9 L of digested sludge (MLSS: 21 g/l; MLVSS: 11 g/l) from the municipal wastewater treatment plant. The synthetic glucose solution was used as the substrates and its composition is shown in Table 1.

	Reactor A	Reactor B	Reactor C
Glucose	9.4 (g/l)	9.4 (g/l)	9.4 (g/l)
$NaHCO_3$	3.0 (g/l)	3.0 (g/l)	3.0 (g/l)
K_2HPO_4	3.0 (g/l)	3.0 (g/l)	3.0 (g/l)
Liquor A	2.0 (ml/l)	1.0 (ml/l)	1.0 (ml/l)
Liquor B	10.0 (ml/l)	5.0 (ml/l)	0
Liquor C	1.0 (ml/l)	0.5 (ml/l)	0
Total	1 L tap water	1 L tap water	1 L tap water

In which

Liquor A	$(NH_4)_2HPO_4$	350.0 (g/l)
Liquor B	KCl	75.0 (g/l)
	$MgCl_2{\cdot}6H_2O$	81.0 (g/l)
	NH_4Cl	85.0 (g/l)
	$MgSO_4{\cdot}7H_2O$	25.0 (g/l)
	$FeCl_3{\cdot}6H_2O$	42.0 (g/l)
	$CoCl_2{\cdot}6H_2O$	1.8 (g/l)
	$NiCl_2{\cdot}6H_2O$	1.8 (g/l)
Liquor C	$CaCl_2{\cdot}6H_2O$	150.0 (g/l)

Table 1: Composition of the synthetic wastewater (10,000 mgCOD/l).

Liquor A, B and C contains the necessary nutrients such as N, P, S, as well as the trace elements such as Mg, Fe, Co, Ni, Ca, etc. Different amount of the liquor A, B and C were added into the substrate solution according to the purpose of this study. The effluent was collected and measured every day and the produced biogas was removed of hydrogen sulphide and counted with the wet style gas meters.

For the convenience of description, the three reactors were named as A, B and C. Different amount of nutrients were fed to the three reactors as shown in Table 1. Reactor A received the most amounts of nutrients and trace elements, half amount was provided for reactor B, and no trace elements was added to the feeding of reactor C. Each reactor was run twice to verify the reoccurrence. Because the tap water in this city was acidic, a relatively high dosage of $NaHCO_3$ and K_2HPO_4 was added, adjusting the pH from 6.1 to 7.4. Therefore the phosphorus content in the raw water was always sufficient in this study. On the other hand, the tap water contained 90-100 mg/l of sulfate and about 0.2 mg/l of Zn so the microbial need of S and Zn could be mostly met. According to Speece [13], the minimum COD/N ratio for highly loaded system is about 100:2. The ratio of COD:N in the influent A, B and C was 100:3.70, 100:1.85 and 100:0.74, supplying the three reactors with surplus, barely enough and deficient nutrients, respectively. Correspondingly, the trace elements in UASB A, B and C were also surplus, barely enough and deficient.

Running Conditions

The three reactors were running under the same operational conditions as shown in Table 2. Increase of OLR was implemented by raising influent concentration or flow rate. Flow rate increase was carried out step by step to avoid the shock load. And in the last running stage (Table 2), the OLR was abruptly decreased from 20 kg COD/m^3.d to 5 kg COD/m^3.d to evaluate the simulative effect of starvation on the SMP production.

Time (d)	Inf. COD (mg/l)	Flowrate (l/d)	HRT (d)	OLR (kg COD/m^3·d)
1-9	5000	6.1-8.1	2.0-2.4	2.1-2.5
10-23	5000	9.3 → 16.2*	1.7 → 1.0	2.9 → 5.0
24-31	10000	7.5-9.0	1.8-2.2	4.6-5.6
32-46	10000	10.8 → 16.0	1.6 → 1.0	6.7 → 9.9
47-53	20000	7.9-8.5	1.8-2.0	9.9-11.3
54-70	20000	10.0 → 15.9	1.6 → 1.0	10.4 → 20.2
71-74	20000	16.0-17.5	0.9-1.1	18.5-21.7
75-88	5000	5.7-6.0	2.7-2.8	1.7-1.9

* "→" Indicates that the parameter changed gradually from the left value to the right one.

Table 2: Operation parameters.

Analytical Method

UASB effluents were sampled and analyzed every day for COD and VFA. Analysis of COD was performed according to the standard method (11). Gas chromatography apparatuses (GC-8APF/FID and GC-8APF/TCD, Shimadzu, Japan) were used for VFA and biogas analysis, respectively. The effluent SMP was calculated according to the equation as follows [15].

SMP=Soluble COD-1.07(HAc)-1.51(HPr)-1.82(HBu)-1.07(Glu)

Where, HAc: measured acetic acid, mg/l; HPr: measured propionic acid, mg/l; HBu: measured iso- and n-butyric acid, mg/l; and Glu: measured glucose, mg/l.

In SMP analysis, SMP was roughly divided into two parts according to the molecular weight (MW). Low MW SMP were analyzed qualitatively and semi-quantitatively with GC-MS. Polysaccharide and protein, which are regarded as important parts of SMP materials, were

analyzed to represent the high MW part of SMP. Polysaccharide was determined with sulfuric acid-anthrone method [16,17] and protein was analyzed according to the Lowry Folin method [18]. For the GC-MS analysis, the 50 ml water samples were first extracted with dichloromethane, then dehydrated with dry sodium sulphate, condensed into 100 µl, finally analysed with GC-2010/GCMS-QP2010 (Shimadzu, Japan). The chromatograph was equipped with a HP-1 nonpolar capillary column, 30 m length, 0.25 mm internal diameter and 0.25 µm film thicknesses. The chromatographic elution was temperature programmed as follows: isothermal at 60°C for 2 min, then from 60 to 260°C at a rate of 10°C/min, and isothermal held at 260°C for 15 min. The carrier gas was helium with a constant flow of 3.0 ml/min. The split/splitless injector was used in splitless mode and its temperature was maintained at 270°C. 1.0 µl sample was injected each time. Mass spectra were acquired under electron ionisation mode (EI) at 70 eV and recorded from m/z 33 to 500 at one cycle/s after 4 min solvent delay. The chromatographic peaks were identified either by direct analysis of the mass spectrum or/and comparison with two reference mass spectral libraries, US national institute of standards and technology, NIST 147 and NIST 27.

Molecular analysis was carried out for the sludge samples to clarify the microbial community in the reactor. DNA was extracted from 0.3 g filtered sludge samples following the benzyl chloride-SDS protocol described by Kageyama et al. [19] and Zhou et al. [20]. With a MJ Mini^{TM} thermal cycler (Biorad, US), the extracted DNA template was amplified with the specific primers to detect the Archaebacteria (Forward primer, AR21F: TTC CGG TTG ATC CYG CCG GA; Reverse primer, AR958R: YCC GGC GTT GAM TCC AAT T) [21,22]. For PCR amplification, the total volume of 25 µl of reaction mixtures contained 2.5 µl template, 400 nM of each primer, 1.25 units of rTaq DNA polymerase (Sangon, Shanghai China), 200 µM dNTP mixture (Takara, Dalian China) and 1 × PCR buffer (Sangon, Shanghai China). Amplification conditions for the reaction included an initial denaturation at 95°C for 10 min, 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 45 s; with the last cycle followed by a 10-min extension at 72°C. The amplicons were examined by electrophoresis for 30 min at 100 V in 1.5% agarose (Sangon, Shanghai China) gel. Then the gel was stained with ethidium bromide and photographed under ultraviolet light.

In order to detect the variation of microbial community, the PCR amplicons were analyzed with denatured gradient gel electrophoresis (DGGE) using the Dcode universal mutation detection system (Biorad, US) [23]. Electrophoresis was performed on 0.8-mm-thick polyacrylamide (PAGE) gels (8% acrylamide/bisacrylamide, 37.5:1(v/v)), with a denaturing gradient ranging from 20% to 60% (100% corresponding to 7 M urea and 40% formamide), at a constant temperature of 60°C in 1 × TAE buffer for 270 min at 200 V. After electrophoresis, the gels were stained in 10 µg/ml ethidium bromide solution for 30 min and visualized under UV light.

The specific DGGE bands were excised from the PAGE gel with sterile scalpels. DNA fragments were extracted from the gel using an EZ spin column PAGE gel DNA extraction kit (Bio Basic Inc., Canada). 2.5 µl of the DNA eluted from each DGGE band was used for re-amplification. The amplicons were further purified with EZ spin column PCR product purification kit (Bio Basic INC., Canada), then sent to Shanghai Sangon Biotech Co. LTD (Shanghai, China) for sequencing. The sequences obtained were compared with those deposited in the gene banks (http://www.ncbi.nlm.nih.gov/ and http://blast.ddbj.nig.ac.jp/top-e.html).

Results and Discussions

Running performance

During the experiment each reactor was run twice and good reoccurrence was observed. The running performance of UASB A is shown in Figure. 1.

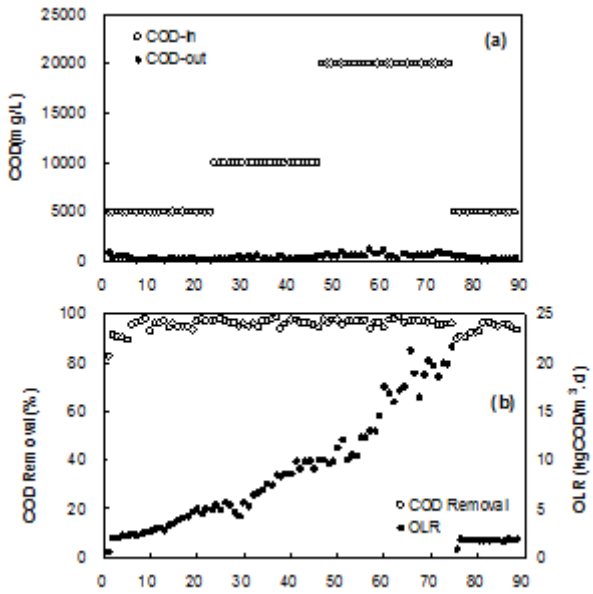

Figure 1: Running performance of UASB A. a) Influent and effluent COD; b) OLR and COD removal.

The reactor was started up at the influent concentration of 5,000 mg/l (Figure 1 (a)), HRT of about 2d and OLR of 2.5 kg COD/(m^3·d). The effluent COD decreased to 200 mg/l from the 9th day with the COD removal of over 97% (Figure 1 (b)). The effluent VFA also dropped quickly. Methane content in the biogas increased to 50%. All these indicated that the UASB reactor fed with glucose wastewater was started up fast. Later when the influent COD gradually increased from 5,000 mg/l to 20,000 mg/l and the OLR from 5 to 20 kg COD/(m^3d), the effluent COD varied from 100 mg/l to 800 mg/l and the COD removal fluctuated from 94% to 98%, with the mean value of 96% approximately. Throughout the whole period of experiment, the effluent VFA remained over 10-200 mgCOD/l, mostly under 50 mgCOD/l, even at the OLR of 20 kgCOD/(m^3·d). When the OLR dropped from 20 to 2.5 kgCOD/(m^3d), the average COD removal remained about 95%, although the biomass at this stage was much higher than the start-up period. Briefly, with the surplus nutrients and trace elements, UASB A ran smoothly and could sustain the high OLR of 20 kgCOD/(m^3·d). The effluent bore the color of jasmine tea. The average effluent VFA and COD removal were approximately 50 mgCOD/l and 95%, respectively.

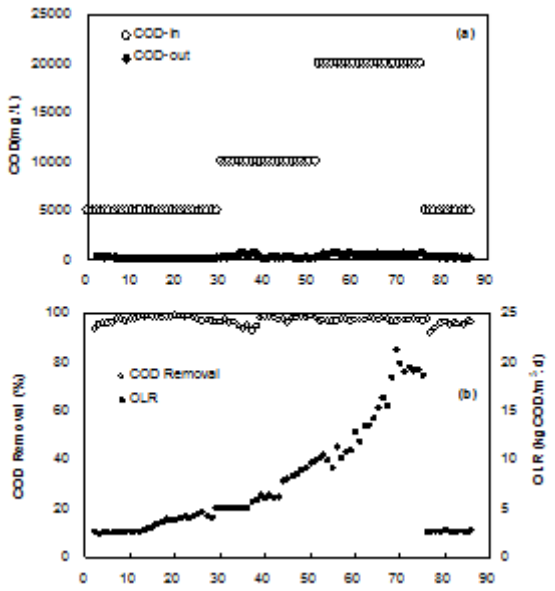

Figure 2: Running performance of UASB B. a) Influent and effluent COD; b) OLR and COD removal.

Figure 2 shows the treatment performance of UASB B in which half amount of N and trace elements, taking UASB A as comparison base, were added. UASB B showed much similarity in the start-up period. However, an temporary performance drop was detected from day 33 when the influent COD was quickly increased to 10,000 mg/l and the OLR to 5 kgCOD/(m^3.d), indicating the weaker resistibility against higher organic load. After recovery, the effluent VFA went under 20 mg/l, mostly undetectable, and the effluent COD was much lower than UASB A as well. At the influent COD of 10,000 mg/l, the effluent COD was about 200 mg/l, with the COD removal of 97-98%. And when the influent COD was 20,000 mg/l, the effluent COD went up to 400-500 mg/l, still remaining the COD removal of about 96-97%. The effluent also bore the color of jasmine tea, but much lighter than that of UASB A. Briefly, although UASB B was provided with less N and trace elements, the VFA degradation was more complete, the effluent was of better quality, and the higher COD removal efficiency was obtained.

The effluent of UASB C, as shown in Figure 3 was of the best quality during the start-up period, at the low OLR of less than 5 kgCOD/(m3•d). When the influent COD was 5,000 and 10,000 mg/l, the treated water appeared almost colorless and transparent, and the effluent COD was mostly under 100 mg/l, which meant that the anaerobic effluent could be directly discharged according to the discharge standard for industrial wastewater (GB8978-1996, level A). However, when the OLR was increased to 8 kgCOD/(m^3.d), the VFA accumulated abruptly, the effluent gave out sour and odor smell and the COD removal quickly dropped. The reactor failed in a few days and could not be recovered. The same experiment was carried out and the same results verified that the system failure was not due to the other occasional causes.

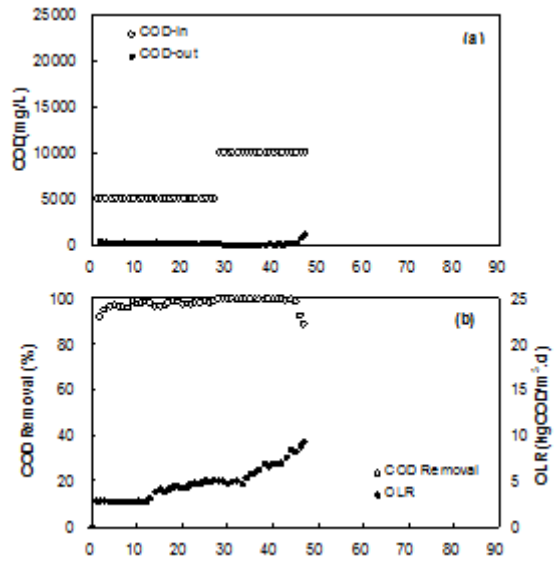

Figure 3: Running performance of UASB C. a) Influent and effluent COD; b) OLR and COD removal.

These results revealed that the nutrient deficiency could keep the microbial activity at a low level, which caused the temporary better anaerobic effluent at the low OLR, but might lead to a vulnerable microbial system, even system failure, when OLR went higher. The variation of the effluent SMP of the three UASB reactors is shown in Figure 4.

The Effect of Nutrient Addition on SMP

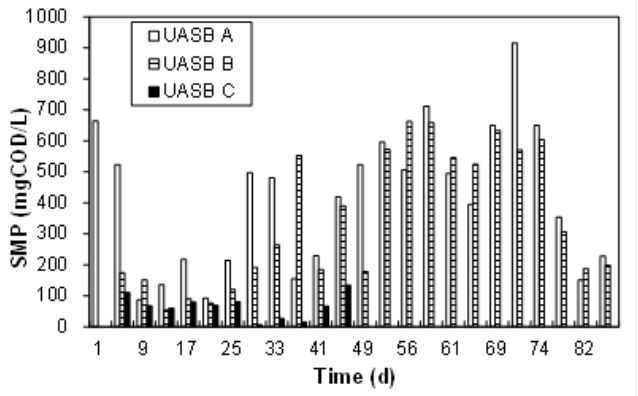

Figure 4: Comparison of the effluent SMP of the three UASB reactors.

For UASB A, the effluent SMP contents were the highest most of the time. This indicated that with the abundant nutrients, the microorganisms were active and their secretion of SMP remained plentiful. SMP from UASB C were the lowest, which implied that the nutrients had become the decisive factor in microbial SMP production in UASB C. The SMP content in the effluent of UASB B was close to

that of UASB C at the low OLR, but became similar to that of UASB A when the OLR rose to 5 kgCOD/m^3.d and above. Combining the facts that the nutrients addition was proportional to COD concentration and that the growth rate of anaerobic bacteria was very low, the variation of SMP in UASB B suggested that the nutrimental level in UAS B was insufficient at low OLR but turned to be sufficient at high OLR, because the biomass in the reactor did not change much but the nutrients had been doubled or tripled. From this point of view, the nutrients may be supplied not according to the influent COD, but according to the biomass in the reactors. On the other hand, the minimum nutrient requirement of anaerobic microorganisms may not be a constant value at the different OLR. The anaerobic reactors running at a relatively high OLR shall be more economical.

The comparison of the treatment performance at the different addition amount of trace elements revealed that the surplus supplementation of nutrients had stimulated the growth of the bacteria, causing more SMP production and higher level of organic matters in the effluent of UASB A. While in UASB C, the poor provision of nutrient and trace elements limited the growth of the microorganisms and the production of SMP, leading to the good quality of the biological effluent at low OLR. However, the low level of nutrients also led to the imbalanced growth between the acidogens and methanogens, the low treatment capacity and weak buffering ability against shock load, finally led to the system failure at the high OLR. This study verified that suitable addition of nutrients would be important to gain the stable operation as well as the good quality effluent.

Many studies [5,24] have found that the MW of the SMP shows a bimodal pattern (below 1 kDa or above 10 kDa). These two parts of SMP account for a vast majority of the soluble COD in the biological treatment effluent. In this study, GC-MS analysis was applied for the reactor effluent to detect the variation of low MW SMP. Yet because it is difficult to quantify each detected component, the GC-MS could perform the identification and only semi-quantification of the low MW SMP, by comparing the area of the peaks shown at the same retention time in the chromatograms. The high MW SMP was quantified through protein and polysaccharide analysis, and it should be noted that the amount of polysaccharide unavoidably covers only part of the high MW SMP. In our understanding, the low MW SMP are more possibly related to the metabolism process, i.e., the secretion of viable cells, while the high MW part are mainly cell materials and thus may be more closely related to the cell death and lysis.

The Effect of Nutrient Addition on the Composition of Low MW SMP

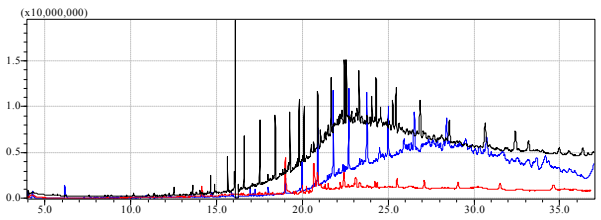

Figure 5: Chromatogram of the effluent from UASB A, UASB B and UASB C.

Figure 5 shows the chromatogram comparison of the effluents from UASB A, B and C under the same running conditions. From the mass spectrum analysis, as shown in Table 3.

UASB A	UASB B	UASB C
Heptadecane	Nonadecane, 9-methyl-	Heneicosane
Nonadecane	Heneicosane	Tricosane
Heneicosane	Tricosane	Octacosane
Tricosane	Tetracosane	Tetratriacontane
Tetracosane	Hexadecanoic acid, 1-methylethyl ester	Tetratetracontane
1,2-Benzenedicarboxylic acid, diisobutyl ester	n-Hexadecanoic acid	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester
1,2-Benzenedicarboxylic acid, mono(2-thylhexyl) ester	9-Octadecenamide, (Z)-	1,2-Benzenedicarboxylic acid, mono(2-ethylhexyl) ester
Palmitic acid	Hexadecanamide	Phenol, 2,4-bis(1,1-dimethylethyl)-
Octadecanoic acid	2-Hexyl-1-octanol	Hexadecanamid
	3-Eicosene, (E)-	Octadecanamide
	(10E)-10-Heneicosene	palmitonitrile
		2-Hexyl-1-octanol

Table 3: The main components in the effluents.

This study proved that the main low MW components were long-chain alkanes, esters and acids, similar to what our former study [25] has revealed, but their amount varied obviously depending on the different nutrient addition. At the same influent COD of 5000 mg/l and OLR of 5 kg COD/m^3.d, the effluent SMP of UASB A, B and C were 143, 160 and 56 mg COD/l, respectively. However, the effluent of UASB A was of the most complicated composition, followed by UASB C and UASB B. The chromatogram of effluent B had the least and lowest peaks, indicating that low MW SMP in UASB B effluent were of the simplest composition and the least amount.

If the low MW SMP, i.e., the long chain alkanes, esters and acids, represent the secretion from the viable cells, the results observed in this study could be explained as follows: In UASB A, as the abundant nutrients could support the growth of more microorganisms, the larger population and the higher viability led to more cell secretion, comparing with UASB B and C. It is still not clear why the effluent of UASB C had the high complexity and amount of low MW SMP, yet since the methanogens are always more sensitive to the surrounding conditions than the acidogens and the growth of acidogens and methanogens was imbalanced in UASB C, the high complexity and amount in UASB C might be due to the abnormal proliferation of the acidogens. In other words, the overgrown acidogens may be responsible for the mass secretion of low MW SMP in UASB C.

The comparison of low MW SMP revealed that the surplus and deficient nutrients would both stimulate the production of low MW SMP, causing the more complicated composition and larger amount of organic matters in the anaerobic effluent.

The Effect of Nutrients on the Amount of High MW SMP

Protein and polysaccharide were analyzed for the UASB effluent samples to investigate the variation of high MW SMP under the different running conditions and at the different amount of nutrients. Figure 6, shows the variation of protein and polysaccharide concentration in the effluent of the three UASB reactors. When OLR was lower than 5 kgCOD/m^3.d in the first 30 days, the high MW SMP in all three reactors were stable and those in UASB C were slightly lower than the other two reactors, indicating the smaller population and lower bacterial activity in UASB C at the low OLR. However, when the OLR continually increased, the protein and polysaccharide SMP increased quickly in UASB C, and showed no tendency of recovery till the system failed at the OLR of about 9 kg COD/m^3.d on day 50. In UASB A and B, the high MW SMP increased but kept stable when the influent COD remained unchanged. From day 53 to day 75, the influent COD was increased to 20,000 mg/l and OLR was raised again, the protein and polysaccharide SMP increased abruptly in both reactors, but to a much greater extent in UASB B than in UASB A. With the stabilization of treatment performance, the high MW SMP also stabilized at different levels. These results indicated that the high MW SMP production was stimulated at the high OLR, especially at the high influent COD. If the high WM SMP came from the cell decay and lysis, then the results observed in this study indicated that the high OLR results in higher biomass and more cell death as well. And when the nutrients were limited, cell death and lysis happened more frequently at the high OLR.

Test of starvation started on the 75th day, the OLR and influent COD was suddenly decreased to 2.5 kg COD/m^3.d and 5,000 mg/l, respectively. The protein and polysaccharide SMP also dropped quickly. But those in UASB A were obviously higher than in UASB B, and after a few days, the effluent SMP of UASB A showed a weak tendency of increase, indicating that at the stimulation of starvation, the well-fed microorganisms might have quickly released the cell substances more than the undernourished bacteria.

From the comparison, it may be deduced that the addition of nutrients could not only buffer the VFA variation and pH change, stimulate the microbial metabolism, but also greatly affect the SMP production, including the high MW and low MW SMP. The surplus trace elements will not only enhance the cell growth, but also stimulate the cells to secret large amount of metabolism products, i.e. the low MW SMP. High OLR together with low level of nutrients could lead to a vulnerable microbial system in the anaerobic reactor, along with a relatively high death rate and cytolysis possibility.

SMP-reduction-beneficial microbial environment

In our previous study [25], it has been frequently observed that the SMPs increased quickly at the bottom of the UASB reactors, then decreased dramatically in the upper part. The same results were detected in this study. In order to find out if the SMP consumption was related to any specific microorganisms, the variation of microbial community along the running time and the reactor height was investigated. The sludge samples were taken during the different running stages, and also from the different height of the reactor on day 74, when the microbial system entered a mature status. The sludge samples along the running time were all taken from the sampling pore at the height of 100 cm, while those along height were from the sampling pores at 10, 40, 70 and 110 cm height from the bottom. The molecular biological analysis was focused on the archaebacteria as they are more representative in the anaerobic process. Similar results were observed for UASB A and B, therefore only UASB A is discussed here.

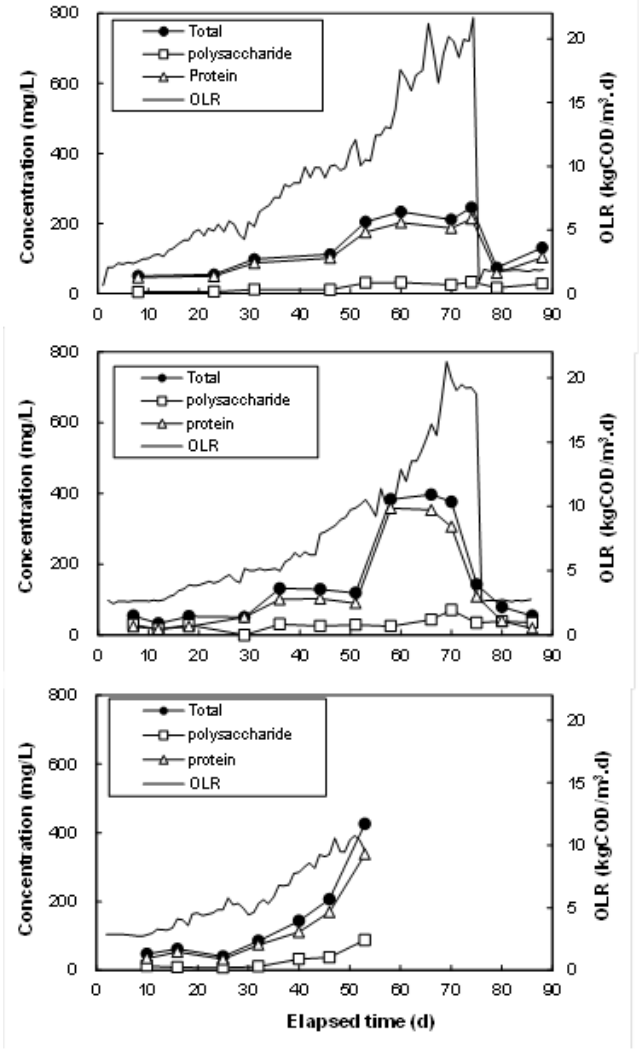

Figure 6: The variation of the protein and polysaccharide SMP along with the OLR in, a) UASB A; b) UASB B and c) UASB C.

The DNA in the sludge samples was extracted and amplified in PCR with the specific primer set targeting *Archaean* genes. Then the amplicons were separated with DGGE. After staining, the fragment pattern was observed (shown in Fig. 7).

As Figure 7(a) shows, very few specific *Archaean* genes were observed for the sludge sample taken from the bottom of the reactor, implying the absence of methanogens and the predominance of acidogens at the bottom part. At the height of 40 cm, many bands appeared, indicating the predominant existence of *Archaea*. And the bands AR3 and AR4 became the brightest. At the upper part, some of the bands disappeared, but bands AR1-4 were still brightly observed.

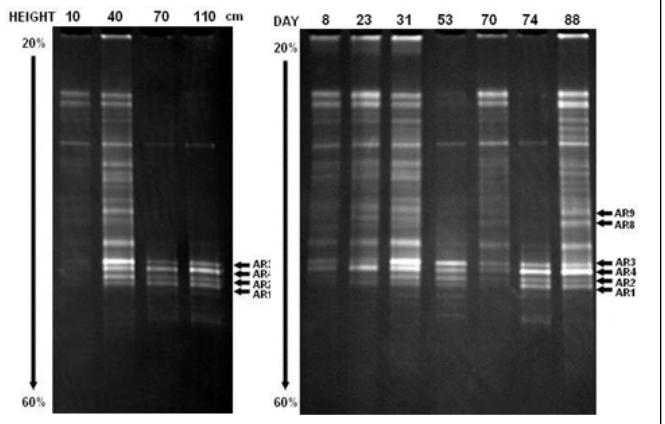

Figure 7: DGGE analysis of DNA amplified with *Archaean* primers, a) along the height of UASB reactor on the 74th d; b) along the running time.

Figure 7 (b) shows the variation of the band pattern of the bacteria at the upper part along the running time. Although due to the great production of the up-flow biogas at the high OLR, the sludge bed was to some degree disturbed and the band pattern varied, the gradual increase of bands AR1-4 could be clearly detected as the time went by.

Bands AR3 and AR4 were cut out and sequenced to identify the most predominant *Archaea* species. For comparison, bands AR1, AR2, AR8 and AR9 were also excised out and sequenced. Finally, bands AR3, AR4, AR8 and AR9 were successfully sequenced and were sent to gene banks for identification. The blast results revealed that band AR3 (650 bp), AR4 (623 bp), Ar8 (688 bp) and Ar9 (672 bp) were all similar to the partial gene of *Methanosaeta concilii*, with the high similarity of 100%. Therefore, molecular analysis revealed that the *Archaea* were missing at the bottom, but predominated at the upper part of the UASB reactor. Meanwhile, during the maturation and stabilization period, *Methanosaeta concilii* become the main *Archaea* species at the upper part of the reactor.

At the bottom of the reactor where acidogens predominate, SMP production was much faster than their consumption, causing the accumulation of SMP. With the VFA being consumed and *Methanosaeta* becoming predominant gradually, SMP production slowed down and was overrun by the consumption, causing the low contents of SMP in the effluent. It may be deduced from these results that the SMP were mainly produced by acidogens but not *Archaea.* According to the previous microbiological studies on *Methanosaeta concilii* [26,27], this species of *Archaea* take only acetate as their sole carbon source and reduce it into methane and carbon dioxide, and they are often found dominant in stable systems with low levels of acetate [28-30]. The *Methanosaeta concilii* could not directly participate in the degradation of SMP, yet their abundant existence may be beneficial to the SMP reduction by other species.

Conclusion

Through this study, the following conclusions could be drawn.

The surplus nutrients stimulated the bacterial activity and increase the metabolism products, causing the high concentration of effluent organic matter in the effluent. The deficiency of nutrients could keep microbial activity at a low level, which led to the better anaerobic effluent, but might jeopardize the running stability of the reactor seriously. Therefore, at a certain OLR, keeping the nutrients addition at a barely enough low level could be helpful to obtain better-quality effluent from the anaerobic treatment.

Molecular analysis revealed that SMP may be mainly produced by acidogenic bacteria and the abundant existence of the *Methanosaeta concilii* might be beneficial to SMP consumption. Raising the OLR more smoothly and allowing the methanogens to grow well would help to improve the treatment efficiency significantly.

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