Performance and granulation in an upflow anaerobic sludge blanket (UASB) reactor treating saline sulfate wastewater

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Abstract An upflow anaerobic sludge blanket reactor was employed to treat saline sulfate wastewater. Mesophilic operation (35 ± 0.5 °C) was performed with hydraulic retention time fixed at 16 h. When the salinity was 28 g L^{-1} , the chemical oxygen demand and sulfate removal efficiencies were 52 and 67 %, respectively. The salinity effect on sulfate removal was less than that on organics removal. The methane productions were 887 and $329 \text{ cm}^3 \text{ L}^{-1}$ corresponding to the NaCl concentrations of 12 and 28 g L^{-1} , respectively. High salinity could stimulate microbes to produce more extracellular polymeric substances (EPSs) and granulation could be performed better. Besides, with the high saline surroundings, a great deal of Na^+ compressed the colloidal electrical double-layer, neutralized the negative charge of the sludge particles and decreased their electrostatic repulsion.

The repulsion barrier disappeared and coagulation took place. The maximum size of granules was 5 mm, which resulted from the coupled triggering forces of high EPSs and Na^+ contents. Sulfate-reducing bacteria (SRB) were dominant in the high saline surroundings while the methane-producing archaea dominated in the low saline surroundings. The SRB were affected least by the salinity.

Keywords Anaerobic processes · Biodegradation · Biotransformation · Fluorescence in situ hybridization (FISH) · Granulation · Waste-water treatment

Abbreviations

UASB	Upflow anaerobic sludge blanket
COD	Chemical oxygen demand
EPS	Extracellular polymeric substances
SRB	Sulfate-reducing bacteria
FISH	Fluorescence in situ hybridization
MPA	Methane-producing archaea
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
HRT	Hydraulic retention time
ORP	Oxidation-reduction potential
FITC	Fluorescein isothiocyanate

Introduction

The biological treatment of saline wastewater has become a topic of increasing importance in the

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industrialized world. Lefebvre reported that the treatment of saline and hypersaline wastewater could represent as much as 5 % of worldwide effluent treatment requirements (Lefebvre et al. 2007). This concerns seawater-based food industries, such as fish processing, as well as concentrated wastewaters coming from chemical industries and distilleries. Besides, as an economic and sustainable alternative water resource, Hong Kong has developed the world's largest seawater supply system for toilet flushing since 1950s, which provides $750,000 \text{ m}^3 \text{ day}^{-1}$ for 80 % of the inhabitants, saving 22 % of valuable freshwater imported from Guangdong Province of China and subsequently generates about $750,000 \text{ m}^3 \text{ day}^{-1}$ of saline sewage (Lu et al. 2012). However, high salinity could cause osmotic stress on the bacterial cells and inhibition of reaction pathways in substrate degradation processes (Pollice et al. 2000). High salinity was known to significantly reduce the treatment efficiency of anaerobic processes in mesophilic UASB reactors (Vallero et al. 2004; Gomec et al. 2005) and thermophilic UASB reactors (Vallero et al. 2002). Vyrides et al. highlighted the positive effect of the membrane in preventing the presence of high molecular weight organics in the effluent while also retaining biomass inside the reactor so that they could rapidly acclimatize to salinity (Vyrides and Stuckey 2009). A previous study had indicated that the methanization could be inhibited by sodium in mesophilic UASB reactors (Vallero et al. 2004). When salinity abruptly increased to $11.7 \text{ g Na}^+ \text{ L}^{-1}$ in a mesophilic UASB reactor, VFA-removal efficiencies declined sharply to 10 %. Methane production also decreased sharply and then stopped. The inhibition of methanization by sodium had also been reported in thermophilic UASB reactors (Vallero et al. 2002).

The damage caused by sulfate emission is indirect, since sulfate is a non-toxic compound. However, high sulfate concentration could unbalance the natural sulfur cycle (Lens et al. 1998). Wastewater containing sulfate is normally treated with physicochemical and biological methods. However, for physicochemical methods, the underlying disadvantages limited their applications, such as separation and appropriate disposal of the solid phase, high cost and energy consumption (Silva et al. 2002). For biological methods, sulfate-rich wastewater was generally treated by anaerobic process (Nusara et al. 2007). In the anaerobic digestion of industrial sulfate-rich wastewater, the

sulfate-reducing bacteria (SRB) can be responsible for the sulfate reduction. Sulfate was the terminal electron acceptor and was reduced to sulfide, with the degradation of organic compound (Nusara et al. 2007). The SRB could suppress methane-producing archaea (MPA) through outcompeting the same substrates such as hydrogen and acetate, and result in a primary inhibition to MPA (Nielsen 1987). Moreover, the SRB can convert sulfate into sulfide which can poison MPA and decrease methane production. The development of molecular biology has improved the detection, quantification, and identification of the microbial communities involved in the wastewater treatment. Fluorescent *in situ* hybridization (FISH) technique had been widely utilized to clarify the microbial community structure and dynamics due to its visual, qualitative, and semi-quantitative characteristics (Zhao et al. 2007). However, the molecular identification of the microbial communities in anaerobic treatment processes were quite limited (Nadai et al. 2010).

Of all the high-rate anaerobic reactors, the UASB reactor has been the most popular by far, with an estimated 80 % of all anaerobic wastewater treatment in the world being done with UASB technology (Abbasi and Abbasi 2012). During the UASB process, the sludge flocs may be transformed into granules through proper operation methods like feed strength, reactor hydraulics and nutrient addition. Granulation in UASB reactors is significant in the treatment of various industrial wastewater containing toxic substances due to their compact structure which can protect the bacteria from inhibitory and toxic pollutants. Moreover, due to the aggregation and compaction of the microbial mass, the granules possess higher density, larger size as well as higher settling velocity than before. So far, there are many models and hypotheses to explain the granulation process, including Capetown's model (Palns et al. 1987), Spaghetti model (Wiegant 1987), multi-layer model (Guiot et al. 1992), ECP bonding model (Schmidt and Ahring 1994), ion-bonding model (Yu et al. 2000) and inert nuclei model (Lettinga et al. 1980). However, the granulation under the condition of high salinity and sulfate is studied little. Not only microbial activity and metabolism are affected by salinity, but microbial granulation is affected by it. Besides, SRB can outcompete MPA with high sulfate concentration. It can result in microbial community modification in granulation.

In this work, anaerobic granular sludge was developed to treat saline sulfate wastewater in a UASB reactor. The performance of simultaneous COD and sulfate removal with different salinities was tested. In addition, the formation and modification of granulation were evaluated, and the SRB and MPA in granules were quantified.

Materials and methods

Wastewater characteristics

The synthetic feed consisted of sucrose ($2,000 \text{ mg L}^{-1}$), acetate ($1,000 \text{ mg L}^{-1}$) and propionate (750 mg L^{-1}) with the general COD around $4,050 \text{ mg L}^{-1}$. This was a typical COD for UASBs. Sulfate was fixed at $1,800 \text{ mg L}^{-1}$ by adding K_2SO_4 . NaCl was chosen as a model compound to regulate the salinity. The whole process was divided into seven stages according to the NaCl concentrations from 4 to 28 g L^{-1} . The next stage did not start until the last one was performed steadily. In order to maintain the population balance of biological system, extra nutrient and mineral elements were added to the wastewater as follows: $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ($0.08 \text{ in mg L}^{-1}$), $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ($0.08 \text{ in mg L}^{-1}$), $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ ($0.05 \text{ in mg L}^{-1}$), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ($0.15 \text{ in mg L}^{-1}$), ZnCl_2 ($0.05 \text{ in mg L}^{-1}$), CuCl_2 ($0.05 \text{ in mg L}^{-1}$), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ($0.05 \text{ in mg L}^{-1}$), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ($0.05 \text{ in mg L}^{-1}$).

Experimental set-up and start-up

Figure 1 shows a flow chart of the UASB reactor used in this study. Continuous operation was carried out during the whole experiment. The working volume of the reactor was 58 L. Temperature control was accomplished by water bath with water recirculation through the reactor's double jacket. The study was conducted at mesophilic condition at $35(\pm 0.5)^\circ\text{C}$. The seed sludge was inoculated from a full-scale UASB reactor treating brewing wastewater. The MLSS concentration in the reactor was maintained at $8,000 \text{ mg L}^{-1}$ by extracting excess sludge, and the HRT was 16 h.

Sample collection and preparation

Samples were withdrawn from the liquid media at the beginning and end of each treatment period. They

were centrifuged at 6,000 rpm for 30 min to remove microorganisms from the liquid medium. Sufficient amounts of HgSO_4 were added to precipitate chloride ions in the form of HgCl_2 in order to avoid their interference to COD measurement. Clear supernatants were analyzed for COD and sulfate contents according to Standard Methods (APHA 1999). Samples were analyzed in triplicate and mean values were recorded. ORP and pH were measured by using the relevant probes and analyzers. Biomass concentrations were determined by filtering the washed salt-free samples through $0.45 \mu\text{m}$ membrane filter and drying in an oven at 105°C to constant weight.

The cooling extraction method, which was described as follows, was applied for the extraction of the extracellular polymeric substances (EPSs) from the sludge. Firstly, 2 mL of sludge was centrifuged. Secondly, the supernatant was removed. Thirdly, 10 mL of NaCl solution (0.85 %) and 60 mL formalin were added. The EPS in this mixed liquor was extracted with ultrasonication for 300 s while being cooled in ice water. After centrifugation at 12,000 rpm for 30 min, the supernatant was analyzed for polysaccharide and protein. These were regarded as the main parts of EPS materials. Polysaccharide was determined by a sulphuric acid-anthrone method and protein was analyzed according to the Lowry Folin method (Lowry et al. 1951).

Measurement of the surface charge was accomplished using the colloid titration technique (Morgan et al. 1990). A small modification was made to measure the surface charge of sludge. After titration, the sludge samples were filtered to measure MLVSS quantities. The colloid charge was calculated and expressed as milliequivalent per liter (for solutions) or

Fig. 1 Schematic diagram of the UASB reactor treating saline sulfate wastewater

milliequivalent per gram of MLVSS (for sludge) of positive or negative charge.

Electron flow calculation

In general, it consumes 0.67 g COD to reduce 1 g sulfate; it consumes 1 g COD to produce 35.5 ml methane (35 °C). Accordingly, we can acquire the $COD_{sulfate}$ and $COD_{methane}$ based on the content of sulfate reduction and methane production. The ratio of $COD_{sulfate}/(COD_{sulfate} + COD_{methane})$ is the electron flow to SRB while the ratio of $COD_{methane}/(COD_{sulfate} + COD_{methane})$ is the electron flow to MPA.

FISH

The samples were fixed with 3.7 % paraformaldehyde solution in PBS for 3 h at 4 °C. After centrifugation, the samples were washed twice with PBS and then stored at −20 °C in a storage buffer consisting of a 1:1 (vol/vol) mixture of PBS and 100 % ethanol. Hybridization was performed according to the standard protocol described by Amann et al. (1990). The oligonucleotide probes used in this study were indicated in Table 1. Probes were labeled with fluorescein isothiocyanate (FITC). For each sample, total microscopic counts were performed by fixed samples stained with 4',6'-diamino-2-phenylindole (DAPI; Sigma, St. Louis, MO, USA) at a final concentration of 0.1 mg L^{−1} for 10 min in the dark at 4 °C. Cells stained with DAPI were defined as total cell counts. Dual staining with DAPI and fluorescently labeled rRNA probes allowed estimation of the proportions of bacteria and different bacterial groups to the total counts. A minimum of 1,000 cells in at least 10 randomly chosen microscopic fields were scored.

Results and discussion

Performance during the whole operating process

Figure 2a presents the COD removal with different salinities in the UASB reactor treating saline sulfate wastewater. The COD removal was affected little when the NaCl concentration was below 12 g L^{−1}. Its removal efficiency could be kept at around 92.5 %, and the effluent COD was less than 317 mg L^{−1}. However, when the NaCl concentration was 16 g L^{−1},

the COD removal efficiency dropped sharply. It arrived at 78 % after several weeks' adaptation, and the effluent COD was 879 mg L^{−1}. With the NaCl added further, the COD removal became worse. When the NaCl concentration was 28 g L^{−1}, the COD removal efficiency and effluent COD reached 52 % and 1,928 mg L^{−1}, respectively.

The COD removal efficiency with high salinity achieved in this work was lower than previous report (Kimata-Kino et al. 2011). On one hand, high salinity affected the microbial activity, which in turn resulted in low treatment efficiency. On the other hand, hydrogen sulfide was produced due to the sulfate reduction. It inhibited the microbes in the reactor. As a result, the degradation of organic matters was affected. In general, the double effects of the high salinity and sulfate led to the poor performance in COD removal.

During the whole process, COD was removed both via methanogenesis and sulfidogenesis. In this work, the influent COD/SO₄^{2−} ratio was fixed at 2.25, and the electron flow to SRB or MPA varied with different salinities. When the NaCl concentration was not more than 12 g L^{−1}, the electron flow to SRB was 36 % and the electron flow to MPA was 59 %. The COD removal through sulfidogenesis was 36 % while the COD removal through methanogenesis was 59 %. However, with the NaCl concentration increased further, the electron flow employed by SRB grew while the electron flow employed by MPA dropped. When the NaCl concentration was 28 g L^{−1}, 57 % of the COD was removed by SRB while 38 % of the COD was removed by MPA. The inhibition resulted from high salinity to MPA was greater than SRB.

Figure 2b presents the sulfate removal with different salinities in the UASB reactor during the whole process. In general, the sulfate removal efficiency, as well as the COD removal efficiency, dropped with the growing salinities. However, the salinity effect on sulfate removal was less than that on COD removal. The sulfate removal efficiency was affected little when the salinity was below 16 g L^{−1}. It could reach around 80 % and the effluent sulfate concentration was about 376 mg L^{−1}. When the NaCl was 28 g L^{−1}, the sulfate removal efficiency decreased to 67 % and the effluent sulfate concentration was 587 mg L^{−1}.

When the NaCl was added from 4 to 28 g L^{−1}, the COD removal efficiency decreased 40.5 % and the sulfate removal efficiency decreased 13 %. The salinity effect on sulfate removal was less than that

Table 1 A list of sequences and hybridization conditions of oligonucleotide probes used in this study

Probe	Specificity	Sequence (5′–3′)	Reference
EUB338	Most bacteria	GCTGCCTCCCGTAGGAGT	Amann et al. (1990)
SRB385	Sulfate reducing bacteria	CGGCGTCGCTGCGTCAGG	Sandaa et al. (1999)
ARC915	Archaea	GTGCTCCCCGCAATTCT	Stahl and Amann (1991)

on COD removal. Early study reported that bacterial sulfate reduction was of great ecological and biogeochemical importance in anoxic hypersaline sediments (Brandt et al. 2001). This indicated that some species of SRB could be salt-adaptive. In this work, after long-term adaptation, the SRB in the reactor belonged to salt-tolerant ones and could perform sulfate reduction in saline surroundings.

Figure 2c presents the methane production with different salinities in the reactor throughout the whole process. When the NaCl concentration was not more than 12 g L^{-1} , methane production was affected little by salinity variation. It could arrive at $887 \text{ cm}^3 \text{ L}^{-1}$. However, with the NaCl added further, methane production dropped sharply. When the NaCl concentration was 28 g L^{-1} , methane production was $329 \text{ cm}^3 \text{ L}^{-1}$. In the high saline surroundings, the salinity inhibition to MPA was more serious than SRB. Still, the SRB could suppress MPA through competition for the same substrates such as hydrogen and acetate. This aggravated the inhibition to MPA. As a result, the SRB could outcompete MPA and predominate in quantity in high saline surroundings (data presented in Part 3.4).

Impact of EPS on granulation

Granulation in UASB reactors is important in the treatment of various wastewaters due to their compact structure which can protect the bacteria from inhibitory and toxic pollutants. Furthermore, the aggregation and compaction of microbial mass results in higher density and larger size of the granule, which has higher settling velocity. Up to now, the important role of EPSs in the process of granulation has already been well established. The EPS is excreted by the microbial cells and exposed at their surfaces under suitable physiological conditions. Because of its position and chemical characteristics, the EPS affects the properties of the bacterial flocs and helps to adhere and connect the flocculent sludge particles together.

In general, the granule size became larger and larger with the increasing NaCl concentrations. When the NaCl concentration was 8 g L^{-1} , the sludge was fine and flocculent shown in Fig. 3a. There was no granule in the reactor. However, granulation was performed step by step when the NaCl content increased. On the 12th week, the granules with sizes between 1 and 2 mm had been formed all over the reactor. With the enhancing salinity, the granules grew further. The sizes varied between 2 and 3 mm when the NaCl was 20 g L^{-1} . When the NaCl was 28 g L^{-1} , the sizes of granules reached 4–5 mm shown in Fig. 3d. All of the granules were black and of irregular and uneven shape. The results found in this work just agreed with Ismail et al. that size distribution of granules fed with $20 \text{ g Na}^+ \text{ L}^{-1}$ were increased compared to granules fed with $10 \text{ g Na}^+ \text{ L}^{-1}$ (Ismail et al. 2010). Salinity increment could favor the size growth of the granule.

During the whole process, the relationship between salinity and EPS content is shown in Fig. 4a. When the NaCl concentration was below 8 g L^{-1} , the EPS content was affected little. It could arrive at about $0.2 \text{ g g}^{-1} \text{ MLVSS}$. Then it grew with NaCl addition and reached $0.55 \text{ g g}^{-1} \text{ MLVSS}$ when the NaCl was 20 g L^{-1} . This agreed with Reid et al. (2006) that high salinity greatly increased EPS content. However, when the NaCl was added further, the EPS content did not increase with enhancing salinity. Early study reported that the EPS of the granules was mainly composed of polysaccharide when the glucose was used as carbon source (Ji et al. 2010). Besides, the polysaccharide played an important role in maintaining the stability of the granule structure (Ji et al. 2010). However, protein dominated the EPS independent of the salinity in this work, and the granules were compact. This indicated that not only polysaccharide but also protein could play an important role in granulation. Our findings also agreed with Claudio et al. (2006) that the EPS was mostly made of protein (protein to polysaccharide ratio varied between 2 and

Fig. 2 Process performance of the UASB reactor treating saline sulfate wastewater; **a** COD removal with different salinities, **b** Sulfate removal with different salinities, **c** Methanogenesis with different salinities, **d** Variation of electron flow with different salinities

Fig. 3 Images of sludge appearance (size of each square: 2×2 mm); **a** Sludge sampled from the UASB reactor on week 8, **b** Sludge sampled from the UASB reactor on week 12,

c Sludge sampled from the UASB reactor on week 21, **d** Sludge sampled from the UASB reactor on week 34

3) and the high content of protein was responsible for the high stability of granules. Eric et al. (2007) had investigated the effect of sulfate and iron on physico-chemical characteristics of anaerobic granular sludge in detail. In their work, the anaerobic sludge fed with glucose and sulfate did not show a significant increase in the protein and polysaccharide content in the EPS compared to the sludge fed solely with glucose. This means that the activity of SRB induced by the presence of sulfate in the feed didn't change the protein/polysaccharide ratio in the EPS. As a result, the high EPS content in this work resulted from the high salinity. In saline surroundings, both the activity and growth of organisms were inhibited. In order to combat the salt inhibition, out of natural response, microbes secreted EPS to protect themselves. EPS made microorganisms adhere to each other tightly, which could resist the salt effect further. High salinity

could stimulate microbes to produce more EPS; as a result, granulation could be performed better. However, the EPS production was not infinite. In this work, when the NaCl concentration was more than 20 g L^{-1} , the EPS content did not increase further. To our surprise, the granules could grow bigger even at the fixed EPS content. In order to explore the phenomena, we investigated the impact of surface charge on granulation.

Impact of surface charge on granulation

Granulation is actually the adhesion of sludge particles. It is known that sludge generally carry negative charges. Surface charges of the sludge were measured and shown in Fig. 4b. When the NaCl were 4 and 28 g L^{-1} , the surface charges of sludge in the reactor were -0.82 and $-0.17 \text{ meq g}^{-1} \text{ MLVSS}$, respectively.

Obviously, the sludge surface charges decreased when NaCl concentrations increased. This revealed that the electrostatic repulsion between the sludge particles was decreasing step by step.

At low NaCl concentration, because the surface charge of sludge was large, the negatively charged sludge particles could not aggregate due to the electrostatic repulsion between each other. Meanwhile, high EPS content was also unavailable. As a result, granule was not formed. With the increase of influent NaCl concentrations, the surface charge of the sludge decreased correspondingly. However, the repulsion barrier may not be reduced enough and thus, the primary minimum adhesion did not take place. Granules formed during this period could be attributed to higher EPS production. EPS bridged the sludge particles together, which revealed that EPS bonding could be independent of electrical neutralization.

Previous reports indicated that the increase of viscosity was the initial phenomenon explaining the

granule formation and the surface charge of granule affected evolution of the parameter “limit viscosity” which in turn affected the granulation (Pevere et al. 2009; Wan et al. 2011). At high NaCl concentration (28 g L^{-1}), a great deal of Na^+ compressed the colloidal electrical double-layer, neutralized the negative charges of the sludge particles and decreased their electrostatic repulsion. Finally, when the negative repulsion decreased to lower than the van der Waals attraction, the repulsion barrier disappeared and coagulation took place. During the process, the NaCl functioned as external electrolyte while the EPS worked as the high molecular flocculants. The electrical neutralization and EPS bonding were the important triggering forces for granulation in this period.

Quantification of microbial community composition

Previous researchers tried to explore the mechanisms of granulation based on the molecular biological tools or microbial morphology. Anjie Li and Xiaoyan Li reported that it was apparent that the dominance of particular bacterial species might not be required for granulation. Rather, granules could be formed from the bacteria ordinarily present in biological wastewater treatment systems (Li and Li 2009). Besides, early studies indicated that filamentous microbes played a crucial role in granulation (Wang et al. 2006; Zhang et al. 2011). Filamentous bacteria appeared to initiate granule formation and generate the structures that supported further granule growth. However, Ahlem et al. (2012) got the conclusion that filamentous bacteria were detrimental to granular sludge properties and performances. In this work, the dynamics of microbial evolution during sludge granulation in terms of species diversity and abundance was explored and characterized.

Fluorescence in situ hybridization (FISH) was employed to quantify the microbial community composition during the process. Sludge was extracted from the reactor on Week 4, 8, 12, 16, 21, 27 and 34. The NaCl concentrations were 4, 8, 12, 16, 20, 24 and 28 g L^{-1} , respectively. The abundances of bacteria, SRB and archaea are shown in Fig. 5. When the NaCl content was below 12 g L^{-1} , the archaea accounted for around 55 %. They were affected little by salinities. However, the abundance of archaea decreased

Fig. 4 Variation of EPS content **a** and surface charge of sludge **b** with different salinities

Fig. 5 Quantification of microbial community composition by FISH

with the increasing NaCl content. It accounted for 8 % when the NaCl concentration was 28 g L⁻¹. Early study reported that archaeal cells, detected by the ARC915 probe, accounted for 77, 84 and 75 % with the COD/SO₄²⁻ ratios of 3.0, 2.0 and 1.6, respectively (Hirasawa et al. 2008). In this work, the COD/SO₄²⁻ ratio was fixed at 2.25, and the abundances of archaea in each salinity stage were lower than the report. Effect of salinity on abundance of MPA was more than that of COD/SO₄²⁻ ratio. The bacteria accounted for 40 % when the NaCl content was below 12 g L⁻¹. Then it grew and peaked at 87 % when the NaCl was 28 g L⁻¹. The ratio of SRB to bacteria was around 39 % with the NaCl below 12 g L⁻¹. When the NaCl was 28 g L⁻¹, the ratio increased to 62 %.

Sulfate-reducing bacteria were predominant in the surroundings with the high salinities while the MPA dominated in the surroundings with the low salinities. Salinity affected MPA greater than that on SRB. Besides, sulfidogenesis mediated by SRB was thermodynamically more favorable than methanogenesis mediated by MPA. This was aggravated in high saline surroundings. As a result, MPA abundance was low due to the double effects of high salinity and sulfate, and the methane production was low. To the contrary, the SRB could be predominant in the high saline surroundings because they could resist the salinity inhibition better. Accordingly, the sulfidogenesis was performed better than the methanogenesis. Furthermore, the ratio of SRB to bacteria was higher in the NaCl concentration of 28 g L⁻¹ than that in 4 g L⁻¹. It indicated that the salt effect on SRB was much less than that on fermentative bacteria. In general, there were three kinds of microorganisms in the reactor treating the saline sulfate wastewater. They were

fermentative bacteria, SRB and MPA. The SRB were affected least by the salinity. The FISH results were also in accordance with the sulfate reduction and methane production presented in Part 3.1.

Conclusions

A UASB reactor was employed to treat saline sulfate wastewater. With the salinity of 28 g L⁻¹, the COD and sulfate removal efficiencies were 52 and 67 %, respectively. The salinity effect on sulfate removal was less than that on COD removal. The greatest size of granules was 5 mm, which resulted from the coupled triggering forces of high EPS and Na⁺ contents. The SRB were predominant in the high saline surroundings while the MPA dominated in the surroundings with the low salinities. In high saline surroundings, the MPA were inhibited by the double effects of salinity and sulfate.

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References

- Abbasi T, Abbasi SA (2012) Formation and impact of granules in fostering clean energy production and wastewater treatment in upflow anaerobic sludge blanket (UASB) reactors. *Renew Sust Energy Rev* 16:1696–1708
- Ahlem F, Angela M, Myriam M, Yolaine B, Beatrice B, Mathieu S (2012) Stability and performance of two GSBR operated in alternating anoxic/aerobic or anaerobic/aerobic conditions for nutrient removal. *Biochem Eng J* 67:10–19
- Amann RI, Krumholz L, Stahl DA (1990) Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. *J Bacteriol* 172:762–770
- APHA (1999) Standard methods for the examination of water and wastewater. American Public Health Association, Washington
- Brandt KK, Vester F, Jensen AN, Ingvorsen K (2001) Sulfate reduction dynamics and enumeration of sulfate-reducing bacteria in hypersaline sediments of the Great Salt Lake (Utah, USA). *Microb Ecol* 41:1–11
- Claudio DI, Roberto R, Antonio L, Roberto P (2006) Influence of hydrodynamic shear forces on properties of granular biomass in a sequencing batch biofilter reactor. *Biochem Eng J* 30:152–157
- Eric DH, Jarno G, Wim VD, Jacques D, Piet NLL (2007) Effect of sulfate and iron on physico-chemical characteristics of anaerobic granular sludge. *Biochem Eng J* 33:168–177

- Gomec CY, Gonuldu S, Eldem N, Ozturk I (2005) Behavior of an up-flow anaerobic sludge bed (UASB) reactor at extreme salinity. *Water Sci Technol* 51:115–120
- Guiot SR, Pauss A, Costerton JW (1992) A structured model of the anaerobic granule consortium. *Water Sci Technol* 25:1–10
- Hirasawa JS, Sarti A, Aguila NKSD, Varesche MBA (2008) Application of molecular techniques to evaluate the methanogenic archaea and anaerobic bacteria in the presence of oxygen with different COD: sulfate ratios in a UASB reactor. *Anaerobe* 14:209–218
- Ismail SB, de La Parra CJ, Temmink H, van Lier JB (2010) Extracellular polymeric substances (EPS) in upflow anaerobic sludge blanket (UASB) reactors operated under high salinity conditions. *Water Res* 44:1909–1917
- Ji G, Zhai F, Wang R, Ni J (2010) Sludge granulation and performance of a low superficial gas velocity sequencing batch reactor (SBR) in the treatment of prepared sanitary wastewater. *Bioresour Technol* 101:9058–9064
- Kimata-Kino N, Ikeda S, Kurosawa N, Toda T (2011) Saline adaptation of granules in mesophilic UASB reactors. *Int Biodeterg Biodegrad* 65:65–72
- Lefebvre O, Quentin S, Torrijos M, Godon JJ, Delgenes JP, Moletta R (2007) Impact of increasing NaCl concentrations on the performance and community composition of two anaerobic reactors. *Appl Microbiol Biotechnol* 75:61–69
- Lens PNL, Visser A, Janssen AJH, Hulshoff LW, Lettinga G (1998) Biotechnological treatment of sulfate-rich wastewaters. *Crit Rev Environ Sci Technol* 28:41–88
- Lettinga G, Van Velsen AFM, Hobma SW, De Zeeuw W, Klapwijk A (1980) Use of the upflow sludge blanket (USB) reactor concept for biological wastewater treatment especially for anaerobic treatment. *Biotechnol Bioeng* 22:699–734
- Li A, Li X (2009) Selective sludge discharge as the determining factor in SBR aerobic granulation: numerical modeling and experimental verification. *Water Res* 43:3387–3396
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the folin phenol reagent. *J Biol Chem* 193:265–275
- Lu H, Wu D, Jiang F, Ekama GA, van Loosdrecht MCM, Chen G (2012) The demonstration of a novel sulfur cycle-based wastewater treatment process: sulfate reduction, autotrophic denitrification, and nitrification integrated (SANI) biological nitrogen removal process. *Biotechnol Bioeng* 109:2778–2889
- Morgan JW, Forster CF, Evison L (1990) A comparative study of the nature of biopolymers extracted from anaerobic and activated sludge. *Water Res* 24:743–750
- Nadais H, Barbosa M, Capela I, Arroja L, Ramos CG, Grilo A (2010) Enhancing wastewater degradation and biogas production by intermittent operation of UASB reactors. *Energy* 36:2164–2168
- Nielsen PH (1987) Bio-film dynamics and kinetics during high-rate sulfate reduction under anaerobic conditions. *Appl Environ Microb* 53:27–32
- Nusara S, Sutha K, Winai L, Pramote S, Daniel W (2007) Effect of sulfate on the methanogenic activity of a bacterial culture from a brewery wastewater during glucose degradation. *J Environ Sci* 19:1025–1027
- Palms SS, Loewenthal RE, Dold PL, Marais GR (1987) Hypothesis for pelletization in upflow anaerobic sludge blanket reactor. *Water SA* 13:69–80
- Pevere A, Guibaud G, Goin E, Hullebusch E, Lens P (2009) Effects of physico-chemical factors on the viscosity evolution of anaerobic granular sludge. *Biochem Eng J* 43:231–238
- Pollice A, Rozzi A, Tomei MC, DiPinto AC, Limoni N (2000) Monitoring the inhibitory effect of NaCl on anaerobic wastewater treatment processes by the Rantox biosensor. *Environ Technol* 21:535–544
- Reid E, Liu X, Judd SJ (2006) Effect of high salinity on activated sludge characteristics and membrane permeability in an immersed membrane bioreactor. *J Membr Sci* 283:164–171
- Sandaa RA, Torsvik V, Enger Ø, Daae FL, Castberg T, Hahn D (1999) Analysis of bacterial communities in heavy metal-contaminated soils at different levels of resolution. *FEMS Microbiol Ecol* 30:237–251
- Schmidt JE, Ahring BK (1994) Extracellular polymer in granular sludge from different upflow anaerobic sludge blanket (UASB) reactors. *Appl Microbiol Biotechnol* 42:457–462
- Silva AJ, Varesche MB, Foresti E, Zaiat M (2002) Sulfate removal from industrial wastewater using a packed-bed anaerobic reactor. *Process Biochem* 37:927–935
- Stahl DA, Amann R (1991) Development and application of nucleic acid probes. In: Stackebrandt E, Goodfellow M (eds) *Nucleic acid techniques in bacterial systematics*. Wiley, New York
- Vallero MVG, Hulshoff Pol LW, Lens PNL, Lettinga G (2002) Effect of high salinity on the fate of methanol during the start-up of thermophilic (55 °C) sulfate reducing reactors. *Water Sci Technol* 45:121–126
- Vallero MVG, Sipma J, Lettinga G, Lens PNL (2004) High-rate sulfate reduction at high salinity (up to 90 mS.cm⁻¹) in mesophilic UASB reactors. *Biotechnol Bioeng* 86:226–235
- Vyrides I, Stuckey DC (2009) Saline sewage treatment using a submerged anaerobic membrane reactor (SAMBR): effects of activated carbon addition and biogas-sparging time. *Water Res* 43:933–942
- Wan J, Mozo I, Filali A, Line A, Bessiere Y, Sperandio M (2011) Evolution of bioaggregate strength during aerobic granular sludge formation. *Biochem Eng J* 58–59:69–78
- Wang H, Yu G, Liu G, Pan F (2006) A new way to cultivate aerobic granules in the process of papermaking wastewater treatment. *Biochem Eng J* 28:99–103
- Wiegant WM (1987) The spaghetti theory on anaerobic granular sludge fermentation, or the inevitability of granulation. In: *Proceeding of the granular anaerobic sludge*, pp 146–152
- Yu HQ, Fang HHP, Tay JH (2000) Effects of Fe²⁺ on sludge granulation in upflow anaerobic sludge blanket reactors. *Water Sci Technol* 41:199–205
- Zhang B, Ji M, Qiu Z, Liu H, Wang J, Li J (2011) Microbial population dynamics during sludge granulation in an anaerobic-aerobic biological phosphorus removal system. *Bioresour Technol* 102:2474–2480
- Zhao Y, Wang A, Ren N, Zhao Q, Zadsar M (2007) Impacts of alkalinity drops on shifting of functional sulfate-reducers in a sulfate-reducing bioreactor characterized by FISH. *Chin J Chem Eng* 15:276–280