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Enhanced volatile fatty acids production of waste activated sludge under salinity conditions: Performance and mechanisms

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Volatile fatty acids (VFAs) are essential for removing biological nitrogen and phosphorus in wastewater treatment plants. The purpose of this work was to investigate whether and how the addition of NaCl could improve the production of VFAs from waste activated sludge (WAS). Sludge solubilization was efficiently improved by the addition of NaCl. Both protein and carbohydrate in the fermentation liquid increased with the dosage of NaCl, and it provided a larger amount of organic compounds for the production of the VFAs. NaCl had inhibitory effects on the production of methane and a high dosage of NaCl could severely suppress the growth of methanogens, which decreased the consumption of the VFAs. Consequently, the production of VFAs was significantly enhanced by the addition of NaCl. The maximum production of VFAs was achieved with the highest dosage of NaCl (3316 mg (COD)/L at the NaCl dosage 0.5 mol/L; 783 mg (COD)/L without the addition of NaCl). Therefore, this study indicates that using NaCl could be an efficient method for improving the production of VFAs from WAS.

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The rapid urbanization in China has necessitated the development of wastewater treatment plants (WWTPs), leading to the production of large amounts of waste activated sludge (WAS). On one hand, WAS is a pollutant that should be reduced, since it can occupy large land areas and release harmful chemicals into the environment. On the other hand, WAS is a useful resource because its components are mainly proteins and carbohydrates. Anaerobic treatment is the preferred technology for WAS treatment. Using this technology not only reduces the amounts of sludge to be disposed but also produces reliable resources of volatile fatty acids (VFAs) and methane (1).

In recent years, researchers have started paying more attention to the production of VFAs in the fermentation process of WAS (2). The VFAs produced in this way can be used for nitrogen and phosphorus removal or for the synthesis of the biodegradable polyhydroxyalkanoates plastics (3,4). The following four steps are typically involved in the WAS fermentation process: sludge solubilization, hydrolysis of soluble compounds, acidification of hydrolyzates, and the production of methane from VFAs and H_2. If the first step is enhanced and the last step is prevented, the VFAs will accumulate in large quantities (5). Numerous methods have been reported in the literature for improving the production of VFAs from WAS. These methods include controlling the pH values at alkaline conditions (6,7), adding surfactants (5), adding enzymes (8), and applying ultrasonication to break up the cell walls (9).

Sodium chloride (NaCl) is widely used in the chemical and food industries and in fisheries, resulting in high salinity (1%–5%) wastewater being produced. The saline wastewater are often discharged into the sewer systems and subsequently transported to WWTPs for treatment. NaCl has been found to decrease the activity of the microorganisms, including nitrifiers and methanogens (10,11), in the wastewater treatment process. Moreover, NaCl has been found to deteriorate the settleability and dewaterability of WAS (12). Recently, it was found that NaCl had a negative effect on the production of VFAs during the fermentation of kitchen waste (13). However, the effects of NaCl addition on the production of VFAs during the fermentation of WAS have not been investigated to date. The purpose of this work was to investigate whether and how NaCl could improve the production of VFAs from WAS. The solubilization of WAS, release of proteins and carbohydrates, release of nitrogen and phosphorus, and the production of VFAs and methane were monitored during the fermentation of WAS, at different NaCl concentrations. The abundance of methanogens in the fermented sludge was also determined. The mechanisms responsible for the improvement of the production of VFAs under high NaCl concentrations are discussed.

MATERIALS AND METHODS

Sludge source The WAS used was withdrawn from a pilot-scale sequencing batch reactor (SBR) (8.8 m^3) in our laboratory that performs the task of biological organic carbon and nitrogen removal from domestic wastewater. The

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characteristics of the domestic wastewater were as follows: soluble chemical oxygen demand (SCOD) 201–332 mg/L, NH_4^+–N 29–100 mg/L, total nitrogen (TN) 48–110 mg/L, PO_4^{3-}–P 2–9 mg/L, and pH 6.5–7.5. The characteristics of the WAS were as follows: pH 6.86 ± 0.01, total suspended solids (TSS) 12,727 ± 250 mg/L, volatile suspended solids (VSS) 11,207 ± 232 mg/L, soluble chemical oxygen demand (SCOD) 58 ± 1 mg/L, total chemical oxygen demand (TCOD) 16,946 ± 69 mg/L, soluble proteins 12 ± 1 mg/L, soluble carbohydrates 12 ± 1 mg/L, calcium ion (Ca^{2+}) 64 ± 5 mg/L, magnesium ion (Mg^{2+}) 25 ± 2 mg/L, and VFAs 0 mg/L. The values were measured by using the analytical methods described below, and the errors of the values were standard deviations calculated from three measurements.

Batch experiments The effects of the addition of NaCl on the hydrolysis and acidification of the WAS were assessed by batch experiments. These batch experiments were carried out in wide-mouth reagent glass bottles, with a volume of 1 L. In each batch experiment, 0.9 L WAS was transferred to the bottles. NaCl was added to the reactors at dosages of 0, 0.05, 0.2, 0.3, and 0.5 mol/L, respectively. Results obtained by Rinzema et al. (14) and Vallero et al. (15) indicated that methanogenic activity could be inhibited completely when the concentration of Na^+ was in the range of 0.43–0.61 mol/L. Consequently, a concentration of 0.5 mol/L was chosen as the highest concentration to be tested. A duplicate experiment was prepared for each NaCl dosage level, as one was used for liquid sampling and the other for gas sampling. The bottles were purged with nitrogen gas for 2 min and then sealed by rubber stoppers before fermentation. All the bottles were placed in an incubator, with the temperature controlled at 25°C. Each bottle was mixed with a magnetic stirrer at 100 ± 10 rpm. The duration of the fermentation was 32 days, while the interval for sampling was 4–7 days. SCOD, protein, carbohydrate, VFAs, NH_4^+–N, PO_4^{3-}–P, Ca^{2+}, and Mg^{2+} in the fermentation liquid and methane, which was collected in a gasbag, were analyzed. Sludge samples on day 10 were drawn from the reactors for fluorescence *in-situ* hybridization (FISH) analysis (16).

Analytical methods Sludge samples from the reactors were centrifuged at 4000 rpm for 20 min and subsequently filtrated by using a 0.45 μm cellulose membrane. The filtrate was analyzed for SCOD, proteins, carbohydrates, Ca^{2+}, Mg^{2+}, VFAs, NH_4^+–N, and PO_4^{3}–P. The filter was analyzed for TSS and VSS. The analyses of TCOD, SCOD, TSS, and VSS were conducted in accordance with the standard method (17). The pH was monitored with a WTW pH/oxi340i meter (WTW Company, Germany). Proteins were measured by the Lowry–Folin method, with bovine serum albumin as the standard (18). Carbohydrates were measured by the phenol-sulfuric method, with glucose as the standard (19). NH_4^+–N and PO_4^{3}–P were analyzed by a QC850 flow injection analyzer (Lachat, USA). To analyze the VFAs, the filtrate was acidified with 10% H_3PO_4 in a 2 ml gas chromatography (GC) vial. An Agilent 7890A GC, with a flame ionization detector (FID) and a 30 m × 530 μm × 1 μm Agilent DB-WAXetr column, was used to analyze the composition of the VFAs. The temperatures of the injection and the detector were 220 and 250°C, respectively. Nitrogen gas was the carrier gas. The GC oven was programmed to raise the temperature to 180°C. The sample injection volume was 2.0 μL Ca^{2+} and Mg^{2+} were measured with a 761 Compact Ion Chromatograph (Metrohm, Switzerland).

The gas produced in the WAS fermentation process was collected by gasbags. An amount of 250 μL gas from the gasbags were sampled by an injector, and the percentages of methane, carbon dioxide, and nitrogen in the gas samples were subsequently determined by an Agilent 7890 N GC, with a thermal conductivity detector (TCD) and a 30 m × 0.32 mm GS-CarbonPLOT column. The temperatures of the injection, column, and the detector were set at 100°C, 35°C, and 200°C, respectively. Helium was used as the carrier gas, at a flow rate of 12 ml/min.

The following probes were used for FISH analysis: EUB_{mix} (equimolar mixture of probes EUB338, EUB338-II, EUB338-III), targeting most of the bacteria, and ARC915 targeting most of the archaea. Oligonucleotides were synthesized and fluorescently labeled with FITC for EUB_{mix}, and Cy3 for ARC915. Therefore, all the bacteria appeared green, and the methanogens appeared red. The fixation and hybridization of the sludge sample (drawn on day 10) were carried out according to the method previously described by Zhang et al. (20). The images of the FISH samples were obtained by an OLYMPUS-BX61 fluorescence microscope (Japan). Fifteen to twenty randomly selected fields of each sludge sample were used to quantify, by using the Image-Pro Plus 6.0 software.

RESULTS AND DISCUSSION

Sludge solubilization Sludge solubilization could be expressed by the changes of the SCOD concentration of the WAS fermentation liquid (21). The effects of NaCl on the solubilization of the WAS are shown in Fig. 1a. The data show that sludge solubilization could be divided into two phases. From the beginning to day 16 (phase I), the SCOD concentrations in all the reactors increased continuously. After day 16 (phase II), the SCOD concentrations decreased in most cases, especially for the 0.5 mol/L condition. The reason could be that the low pH value in the 0.5 mol/L condition, as shown in Fig. 1f, led to the precipitation of some macromolecular organic compounds, such as protein, in the fermentation liquid (22).

As seen in Fig. 1a, the solubilization of sludge increased with an increased dose of NaCl. Fig. 2a shows that the SCOD on day 16, when SCOD peaked in most cases, had increased linearly with the dosage of NaCl. With the addition of 0.5 mol/L, the efficiency of sludge solubilization was about seven times more than when no NaCl was added. Clearly, sludge solubilization was enhanced by the addition of NaCl. This could be ascribed to the Na^+ released from NaCl exchanging with the divalent cations (e.g., Ca^{2+} and Mg^{2+}) in the extracellular polymeric substances (EPS), which could induce the release of EPS from the WAS (12). High concentrations of Na^+ could also induce plasmolysis. Moreover, it could lyse the cells of microorganisms, which would lead to the release of the intracellular organic compounds (23). In our previous studies, it was found that 40% of the cells in the WAS were lysed by a 20 g/L NaCl solution, while more than 93% of the cells in the WAS were lysed by a 35 g/L NaCl solution (24).

Protein and carbohydrate release Protein and carbohydrate are the main constituents of WAS and are the main substrates for VFAs production (25). In the fermentation process, protein and carbohydrate are first released into the water from the WAS and are subsequently hydrolyzed to amino acids and monosaccharide, which finally are used for the production of the VFAs. Fig. 1b and c show the effects of NaCl on the release of protein and carbohydrate. Protein concentrations increased in the first phase of the fermentation process, while it decreased in the second phase of this process. The carbohydrate concentrations increased during the first four days, and in most cases, remained stable during the rest of the fermentation process. The observed protein decreased at the end of the tests, but the production of VFAs did not increase. This could be ascribed to the protein precipitating with Ca^{2+} and Mg^{2+} at a low pH level, instead of the protein being utilized for the production of the VFAs (12). The observed carbohydrate trends were the net balance of release and degradation, and when the release rate exceeded the degradation rate, the increase of the concentration (6).

As shown in Fig. 1b and c, both the protein and the carbohydrate concentrations increased with the dosage of NaCl, similar to the SCOD concentrations. Additionally, the amounts of protein and carbohydrate released from the WAS with a dosage at 0.5 mol/L were much higher than were those in the other instances. Protein and carbohydrate are the main constituents of extracellular polymeric substances (EPS), which are absorbed onto the surface of microorganisms by divalent cations, such as Ca^{2+} and Mg^{2+} (12). The Na^+ released from NaCl could exchange with divalent cations, thus enhancing the release of protein and carbohydrate (26). Fig. 2b shows the concentrations of Ca^{2+} and Mg^{2+} measured on day 4, the concentrations of both increased with the increased dosage of NaCl on day 4, which proved the occurrence of cation exchange. High concentrations of Na^+ could also induce plasmolysis, and even lyse the cells of microorganisms, which would lead to the release of the intracellular protein and carbohydrate (23). This could have contributed to the increased protein and carbohydrate with the increased addition of NaCl.

VFAs production VFAs can be produced in the fermentation process of WAS. Acetic, propionic, *n*-butyric, *iso*-butyric, *n*-valeric, and *iso*-valeric acids were the main types of acids produced by fermenting WAS (6). The individual acid was converted to COD by multiplying the appropriate conversion factors, and the sum of the individual acids were recorded as the total amount of VFAs (6). Fig. 1d shows the effect of NaCl on the production of VFAs. It was found that the production of VFAs was significantly improved by the addition of NaCl. The maximum production of

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