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Effect of recycling flux on performance and characteristics of activated sludge hydrolytic–aerobic recycling process in degradation of 2,4-dichlorophenol

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ABSTRACT

The degradation of a model molecule, 2,4-dichlorophenol (2,4-DCP), was studied using an activated sludge hydrolytic–aerobic recycling process (HARP). 2,4-DCP and chemical oxygen demand (COD) total removal efficiency in the recycling process was 98% and 96% at the recycling flux of 15 mL/min after 24 h degradation, respectively. With the recycling flux increasing, the peak values of the concentration of volatile fatty acid (VFA) declined. Polysaccharide and protein contents in EPS were dramatically increased as recycling flux increasing from 5 to 15 mL/min in the HARP. There is obviously fit to the linear correlation between the PN/PS ratios and recycling flux. The zeta potentials decreased with recycling flux increasing. As the recycling flux increasing at a certain degree, the increase in polysaccharide and protein contents of EPS could more favor the stability of the HARP.

1. Introduction

Chlorophenols are xenobiotic contaminants that are often found in waste discharges from many industries including petrochemicals, refineries, pesticides, pulp and paper, plastic, biocides, wood preservers and insulation materials [1–4]. Due to their high toxicity, strong odor emission and persistence in the environment and suspected carcinogenicity and mutagenity to living organisms, chlorophenols pose a serious ecological problem as environmental pollutants [3]. Some chlorophenols have been listed as priority pollutants [4]. Hence, the removal of chlorophenols from wastewater is a necessary task to conserve the quality of water resources.

Biodegradation of chlorophenols is more specific and relatively inexpensive [5–7], since they could be mineralized by microorganisms under aerobic or anaerobic conditions [8]. The crucial step in biodegradation of chlorophenols is the removal of the halogen substituents from the aromatic nucleus either by oxidative, reductive or hydrolytic enzyme, or by ring cleavage followed by spontaneous loss of the halide through hydrolysis [9–11]. Although the majority of chlorophenols-degrading microorganisms have the necessary enzymes for aromatic ring degradation, they have a limited capacity for halogen removal [10]. Therefore, the degradation efficiency of these recalcitrant toxic chlorophenols depends mainly on the microbial capacity to remove the halogen groups [12,13]. A large amount of work has been carried out on chlorophenol degradation, particularly on aspects relating to suspended pure culture using different bacteria and fungi such as species of *Pseudomonas*, *Azotobacter*, *Alcaligenes* and *Acinetobacter* [14–18,20–27]. Suspended culture systems usually failed to remove high concentration of chlorophenols from wastewater due to toxic nature of those compounds. Mixed culture biodegradation processes such as anaerobic, aerobic, anaerobic–aerobic combination process were developed by many investigators for the removal of chlorophenols from wastewaters [6,7,16,19]. Because most of anaerobic microorganisms could not use chlorophenols as a sole carbon source for their growth, it is necessary to supply other external carbon sources serving as electron donor for microbial growth and biosynthesis [27–29]. The mineralization of chlorinated compounds could be achieved by the combined activities of anaerobic and aerobic bacteria in which dechlorination occurred primarily under anaerobic conditions and degradation of the less chlorinated intermediates occurred more readily under aerobic conditions [6,12,30–36].

However, the anaerobic treatment has many disadvantages as well. One is that methanogenic bacteria could be inhibited by chloroaromatic compounds and intermediates produced under anaerobic conditions. Another disadvantage is that the control of pH in anaerobic treatment is difficult but necessary because the desired pH range for methanogenic bacteria is rather narrow. Moreover, for high concentration chlorophenols wastewater, most of organics could be converted into volatile fatty acid (VFA), leading to a low pH environment. The accumulation of VFA resulted in that the wastewater was seriously acidified and the activity of

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hydrolytic microorganisms was depressed. Therefore, the process of combining a hydrolysis acidification step with an aerobic treatment (hydrolytic–aerobic combination process) is attracting increasing interests [37].

The objectives of the present work were to examine the effect of recycling flux on degrading 2,4-dichlorophenol (2,4-DCP) in the hydrolytic–aerobic recycling process (HARP). The 2,4-DCP, chemical oxygen demand (COD) total removal efficiencies and the variations of VFA were also evaluated as recycling flux increasing from 5 to 15 mL/min. The contents of polysaccharide and protein in the extracted EPS samples were analyzed for comparison at the different recycling fluxes of the HARP and the stand-alone hydrolytic and aerobic processes. In addition, the relationships among the EPS contents, PN/PS ratio (protein/polysaccharide) and the zeta potential of activated sludge were also discussed to better understand the possible role of polysaccharide and protein in the stability of the HARP.

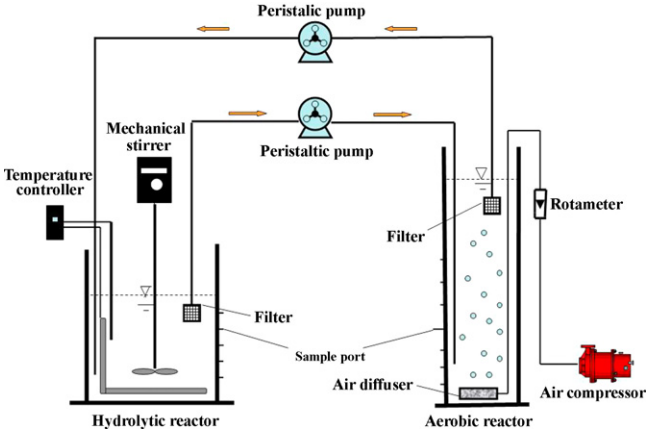

Fig. 1. Bench-scale activated sludge hydrolytic–aerobic recycling process.

2. Materials and methods

2.1. Seed sludge and wastewater

The hydrolytic and aerobic microorganisms were obtained from activated sludge provided by Xiamen Domestic Sewage Treatment Plant, which had little chance to contact with 2,4-DCP. Hydrolytic and aerobic microorganisms were simultaneously cultured in the two sets of reactors, respectively. During the acclimation period, to improve the microorganisms' adaptability to the 2,4-DCP wastewater, 2,4-DCP concentration was stepwise increased from 5 to 20 mg/L in about 1 month with 2000 mg/L of glucose as external carbon source. After acclimation, 2 L of acclimated hydrolytic and aerobic sludge was inoculated into the hydrolytic and aerobic reactor, resulting in mixed liquor suspended solids (MLSS) concentration of 4.6 and 4.8 g/L in the hydrolytic and aerobic reactor, respectively.

The compositions of the simulated 2,4-DCP wastewater in the hydrolytic and aerobic reactor contain (g/L): 2,4-DCP 0.02, NH_4Cl 0.07, KH_2PO_4 0.03, $NaHCO_3$ 1.5. Trace elements contain (mg/L): $CaCl_2{\cdot}6H_2O$ 0.01, $FeSO_4{\cdot}7H_2O$ 1.55, $MnSO_4$ 4.95, $ZnSO_4{\cdot}7H_2O$ 0.71, $CuSO_4{\cdot}5H_2O$ 0.48, $CoCl_2{\cdot}6H_2O$ 0.01. The composition of the trace element solution was prepared according to Sponza et al. [38,39].

For comparison, all experiments were conducted at 2,4-DCP 20 mg/L and glucose 2000 mg/L (COD 2100 mg/L). Before each run, the simulated wastewater was prepared by adding 2,4-DCP and glucose into tap water. The pH of the simulated wastewater was adjusted to approximately 7.5 by NaOH and HCl solution.

2.2. Experimental setup and procedure

The bench-scale activated sludge hydrolytic–aerobic recycling process is presented in Fig. 1. The column hydrolytic reactor is 30 cm in diameter and 60 cm in height. The column aerobic one is 9 cm in diameter and 80 cm in height. Both the hydrolytic and aerobic reactor with working volume of 4 L was operated in a batch mode. The hydrolytic and aerobic reactors were equipped with sampling ports apart along its side. The hydrolytic reactor was maintained at $35 \pm 2\,^{\circ}C$ using temperature controller and the solution in it was mixed by a mechanical stirrer. In the hydrolytic reactor, dissolved oxygen (DO) was below 0.5 mg/L. The solution in the aerobic reactor was aerated using an air compressor with aeration flux 3 L/min at room temperature, where DO was above 2 mg/L.

The HARP was performed by means of two external recirculation peristaltic pumps (BT01-100) in continuous recirculation 24 h at the certain recycling flux (5, 8, 10, 12, and 15 mL/min). Firstly, the simulated wastewater was simultaneously added to the hydrolytic and aerobic reactor. Secondly, turn on the two peristaltic pumps. The effluent of the hydrolytic reactor was circulated into the inlet of the aerobic reactor by a peristaltic pump that drew liquid through filter from the top of the hydrolytic reactor and transferred it to the bottom of the aerobic reactor to ensure good internal mixing of liquid in the reactor. Meanwhile, the effluent of the aerobic reactor was circulated into the inlet of the hydrolytic reactor by peristaltic pump that drew liquid through filter from the top of the aerobic reactor and transferred it to the bottom of the aerobic reactor.

Samples (2 mL) were withdrawn and centrifuged at 12,000 rmp for 10 min to remove biomass from the liquid phase. The samples were kept at $4\,^{\circ}C$ in a freezer before analysis. Whenever operational parameters were changed with batch, the reactors were operated for at least five batches until the reactor performance reached a new steady-state condition.

2.3. Calculation methods

The total removal efficiency of 2,4-DCP ($R_d(t)$) that of COD ($R_c(t)$) at t moment during the HARP were calculated according to the following equation:

$$R_d(t)\ (\%) = \left(1 - \frac{C_{ad}(t)V_a + C_{hd}(t)V_h}{C_{adi}V_a + C_{hdi}V_h}\right) \times 100 \quad (1)$$

$$R_c(t)(\%) = \left(1 - \frac{C_{ac}(t)V_a + C_{hc}(t)V_h}{C_{aci}V_a + C_{hci}V_h}\right) \times 100 \quad (2)$$

where C_{adi}: the initial concentration of 2,4-DCP in aerobic reactor, mg/L; C_{hdi}: the initial concentration of 2,4-DCP in hydrolytic reactor, mg/L; $C_{ad}(t)$: the residual concentration of 2,4-DCP in aerobic reactor at t moment during degradation, mg/L; $C_{hd}(t)$: the residual concentration of 2,4-DCP in hydrolytic reactor at t moment during degradation, mg/L. C_{aci}: the initial COD in aerobic reactor, mg/L; C_{hci}: the initial COD in hydrolytic reactor, mg/L; $C_{ac}(t)$: the residual COD in aerobic reactor at t moment during degradation, mg/L; $C_{hc}(t)$: the residual COD in hydrolytic reactor at t moment during degradation, mg/L. V_a: the effective volume of aerobic reactor, L; V_h: the effective volume of hydrolytic reactor, L.

2.4. Analytical methods

Measurements of MLSS, COD were conducted in accordance with the standard methods [40]. The 2,4-DCP concentration was determined by the 4-aminoantipyrene colorimetric method [40]. VFA concentration in the hydrolytic reactor was determined using titration method [41]. The EPS yields in the extracts were represented by protein and polysaccharide concentrations. The protein content was determined using the modified Lowry

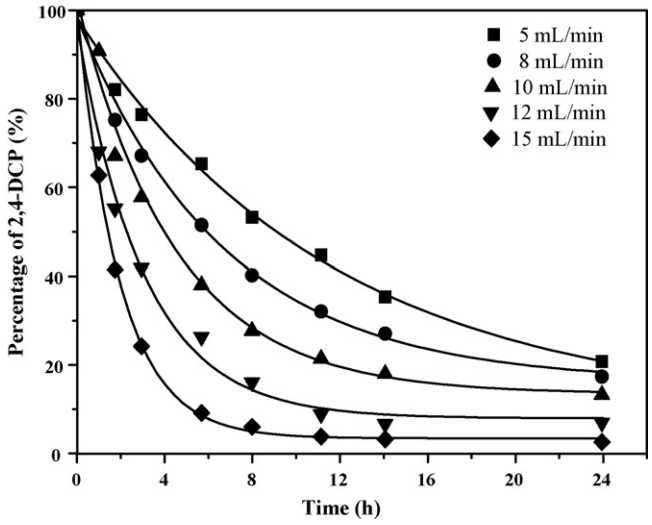

Fig. 2. Profiles of 2,4-DCP at different recycling fluxes.

method [42], with bovine serum albumin as the standard in the range of 0–250 mg/L. Polysaccharides were determined using the phenol–sulphuric acid method and glucose was used as the standard in the range of 0–50 mg/L [43]. The surface zeta potential distribution of bacterial cells was measured using a Zeta-potential Analyser (Zetasizer Model Nano-ZS, Malvern, UK). Then, the zeta potential values were calculated from the measured distribution using Smoluchowski's equation. The pH value was measured by a pH meter (PHS-3C). The DO value was measured by SJG-203A dissolved oxygen meter (Shanghai Fine Victory Scientific Instrument Limited Company, China). The samples were analyzed in triplicates with less than 3% standard deviations from the average.

3. Results and discussion

3.1. Effect of recycling flux on 2,4-DCP degradation

2,4-DCP removal at different recycling fluxes is shown in Fig. 2, which indicated that 2,4-DCP total removal efficiencies and removal rate were dramatically increased as recycling flux increasing from 5 to 15 mL/min during the whole recycling process. 2,4-DCP total removal efficiencies could be up to 47%, 60%, 72%, 84% and 94%, respectively in the initial 8 h when recycling flux increased from 5 to 15 mL/min. After that, 2,4-DCP total removal efficiencies increased slowly at different recycling fluxes. 2,4-DCP total removal efficiencies could be up to 79%, 83%, 87%, 93% and 98%, respectively after 24 h degradation as recycling flux increasing from 5 to 15 mL/min.

It is known that the reductive dechlorination process under the anaerobic condition is of environmental importance because anoxic condition in soils, as well as bottom layers of aquatic sediments and freshwater and marine is often prevailing [44]. Most anaerobic microorganisms had the capability of converting DCP to mono-chlorophenol (MCP) then phenol through the reductive dechlorination process under anaerobic conditions [45]. But anaerobic microorganisms have a limited ability to completely degrade MCP and phenol. The rate of mineralization was much slower than that of the initial dechlorination steps [44]. Therefore, dechlorination is the rate-limiting step in degradation of 2,4-DCP. Wang et al. [22] found that the removal of 20 mg/L 2,4-DCP by *Bacillus insolitus* reached 44% for the 1st day whereas the removal efficiency was only 77% after degradation 480 h.

Comparison of our study with the previous literature abovementioned, it can be inferred that in the HARP, reductive dechlorination is a continuous process. The HARP supplied a possibility of improving 2,4-DCP removal efficiency and rate through continuously circulating the solution between hydrolytic reactor and aerobic reactor. With recycling flux increasing, large value of MCP and phenol in the solution of the hydrolytic reactor could be more quickly transferred into the aerobic reactor and decomposed by aerobic microorganisms in time. The metabolic and kinetic limitations to anaerobic and aerobic microorganisms could be overcome by coupled reductive dechlorination mechanisms in the recycling process as recycling flux increasing.

Another key strategy in degradation 2,4-DCP by the HARP was using dose of glucose as a co-substrate to promote chlorophenol degradation. Similar strategies have been adopted in chlorophenol-degrading biofilm or granule-based reactors to facilitate the biofilm adhesion and co-metabolic transformation of the contaminant. For example, Eker and Kargi [46] added molasses along with 2,4-DCP to ensure an acclimation of the attached biofilm to 2,4-DCP. Hendriksen et al. [47] found that the addition of glucose was beneficial to the substrate utilization, stimulated the dechlorination, and maintained sufficient biomass in an upflow anaerobic sludge bed reactor treating pentachlorophenol. Bali and Sengul [7] reported that in co-metabolic transformation of 4-chlorophenol, phenol was a good primary substrate since it could not easily induce the mono-oxygenase required for 4-chlorophenol transformation. Phenol oxidation could also efficiently regenerate consumed NADH. Strong competitive inhibition between phenol and 4-chlorophenol could inhibit 4-chlorophenol transformation significantly. It had been found that 4-chlorophenol was transformed rapidly only after phenol was almost fully depleted [48]. In this case, the co-metabolic enzymes required for 4-chlorophenol transformation were most probably induced by 4-chlorophenol. As for the NADH required for 4-chlorophenol transformation, which could be efficiently formed through the oxidation of glucose [7]. With the rapid oxidation of glucose, NADH is quickly regenerated, consequently facilitated the transformation of 4-chlorophenol.

Therefore, the HARP could effectively improve the total removal efficiency and rate of 2,4-DCP through increasing recycling flux to a certain degree and using the key strategy of adding co-substrate to promote chlorophenol degradation. Meanwhile, the HARP could enhance transformation of co-metabolic intermediate products through continuously circulating the solution between hydrolytic reactor and aerobic reactor with the recycling flux increasing.

3.2. Effect of recycling flux on COD removal

COD removal at different recycling fluxes is presented in Fig. 3. COD total removal efficiencies and rate were dramatically increased as recycling flux increasing from 5 to 15 mL/min in initial 3 h. COD total removal efficiencies could be up to 68%, 74%, 84%, 91% and 93%, respectively in the initial 3 h with recycling flux increasing from 5 to 15 mL/min. After that, the removal efficiencies and rate of COD was increased slowly. COD total removal efficiencies could be up to 80%, 85%, 91%, 93% and 96%, respectively after degradation 24 h as recycling flux increasing from 5 to 15 mL/min.

With increasing recycling flux of the solution, the residual COD of the solution in the hydrolytic reactor can be in time transferred into the aerobic reactor and some of them consumed by aerobic microorganisms more promptly in the latter reactor, which improved COD removal efficiency and rate for the whole process.

The COD removal is benefit from the "cooperative metabolism" between hydrolytic and aerobic microorganisms, which lied in the exchange of metabolites between the hydrolytic and aerobic reactor. "Cooperative metabolism" enhanced the biodegradability of hydrolytic and aerobic microorganisms simultaneously. The exchange of metabolites mentioned here could be compared to the metabolite exchange at interfaces between the anaerobic and aerobic zones of natural eco-process (sediments, bacterial colonies,

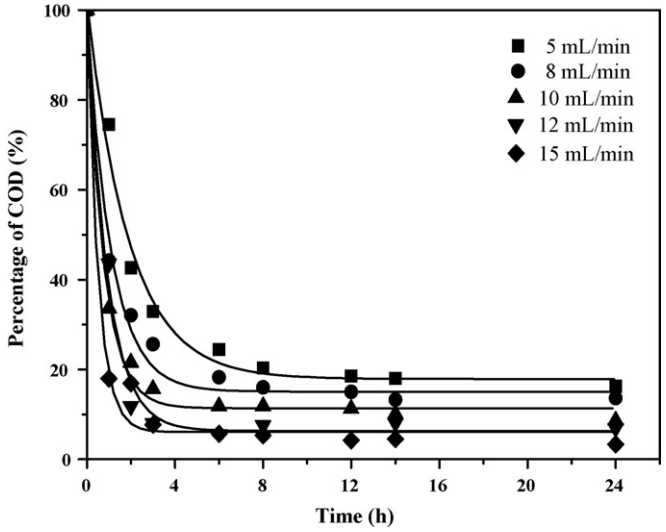

Fig. 3. Profiles of COD at different recycling fluxes.

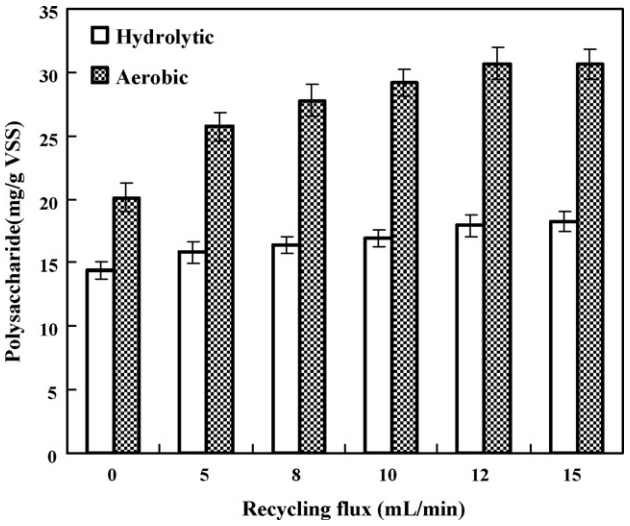

Fig. 5. Profiles of polysaccharide content in EPS at different recycling fluxes.

stratified lakes and seas, microbial mats, biofilm, etc.). Hydrolytic microorganisms were effectively protected against oxygen damage by aerobic microorganisms, and aerobic microorganisms consumed the metabolism products of hydrolytic microorganisms. The resistance to mass transfer across the hydrolytic–aerobic "interface" between the reactors of the recycling process was much lower than that in natural process due to the recycling [18].

3.3. Effect of recycling flux on VFA

The concentrations of VFA in the solution after 24 h degradation at different recycling fluxes are shown in Fig. 4. As could be seen, at the recycling flux of 5 mL/min the concentration of VFA gradually increased from 2.7 to 14.4 mmol/L in first 8 h. After that it declined to 3.6 mmol/L after 24 h degradation. At the recycling flux of 8 mL/min the concentration of VFA increased to 11.9 mmol/L in first 6 h and then gradually declined. At the recycling flux 10 mL/min the concentration of VFA increased to 7.5 mmol/L in first 4 h. The similar change tendencies were shown at recycling flux of 12 and 15 mL/min, the maximal concentration of VFA could be up to 6.6 and 4.6 mmol/L in first 2 h, respectively. The results indicated that recycling flux had great effect on the change of VFA concentration. The peak values of the concentration of VFA were declined from 14.4 to 4.6 mmol/L with the recycling flux increasing from 5 to 15 mL/min. Moreover, the time for VFA reaching the maximal concentration was shortened from 8 to 2 h with the recycling flux increasing from 5 to 15 mL/min.

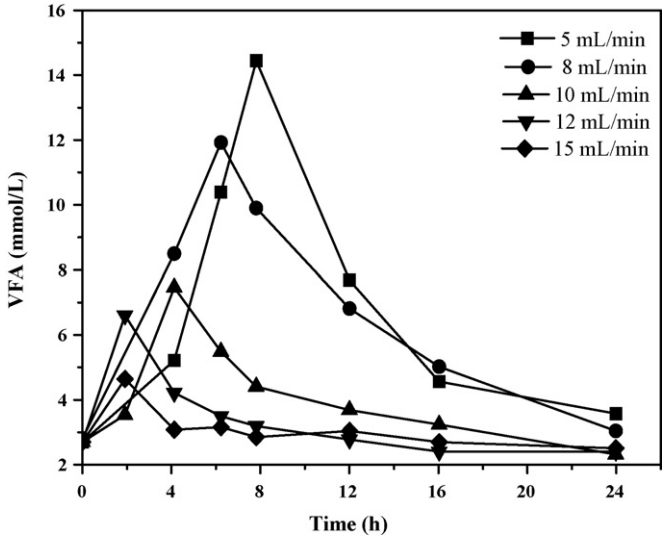

Fig. 4. Profiles of VFA at different recycling fluxes.

It was known that 2,4-DCP and glucose could be decomposed into relatively simple intermediate products like VFA, mainly in the form of acetate and propionate in anaerobic condition. The degradation of 2,4-DCP into VFA was much faster than further conversion into methane, which resulted in the accumulation of a quantity of acid [28]. Therefore, the accumulation of VFA resulted in that the wastewater was seriously acidified and the activity of hydrolytic microorganisms was depressed. Accordingly, the whole hydrolytic process was restrained. In the HARP, by increasing recycling flux at a certain degree, the VFA produced in the hydrolytic reactor could be consumed by aerobic microorganisms more quickly, thus over-acidification in the hydrolytic reactor would not happen, and a quantity of acid organic could go through the hydrolysis acidification step more smoothly and completely. Therefore, the HARP could simultaneously intensify both the hydrolytic and aerobic process to solve the problem of over-acidification in the hydrolytic reactor, which favored the stability of the hydrolytic process.

3.4. Effect of recycling flux on polysaccharide and protein content in EPS

Polysaccharide and protein contents in EPS at different recycling fluxes are presented in Figs. 5 and 6. Polysaccharide contents in EPS in the seed hydrolytic and aerobic sludge were 14.4 and 20.1 mg/g VSS, respectively. It was found that recycling flux had a great effect on polysaccharide content in the HARP. Polysaccharide contents in EPS were dramatically increased as recycling flux increasing from 5 to 15 mL/min in the HARP. Polysaccharide contents in EPS in the hydrolytic and aerobic sludge were 18.6 and 32.6 mg/g VSS, respectively, which were 1.2 and 1.6 times as much as polysaccharide content in EPS in the seed hydrolytic and aerobic sludge.

There was the similar change tendency of protein content in EPS in the hydrolytic and aerobic sludge. Protein contents in EPS in the seed hydrolytic and aerobic sludge were 31.2 and 32.6 mg/g VSS. When recycling flux increasing from 5 to 15 mL/min, protein content in EPS was gradually increased. Protein contents in EPS in the hydrolytic and aerobic sludge were 66.4 and 88.6 mg/g VSS, which

were 2.1 and 2.7 times as much as polysaccharide in EPS in the seed hydrolytic and aerobic sludge.

Comparison of protein and polysaccharides content in EPS both hydrolytic sludge and aerobic sludge, it indicated that protein content in EPS became more abundant than polysaccharides content as recycling flux increasing in the HARP. The increase in protein content of EPS could enhance neighboring microbial cells and form a cross-linked network by the attraction of organic and inorganic materials [49].

The similar results had been found in some studies. Garnier et al. [50] reported that the molecular weight of extracellular protein extracted from activated sludge varied from small (10 kDa) to large (600 kDa) sizes, while all polysaccharides were smaller than 1 kDa by high-pressure size-exclusion liquid chromatography. McSwain et al. [51] quantified the EPS composition for aerobic flocs and found protein to be the largest fraction. Su and Yu [52] also found that among the EPS of aerobic granular sludge, protein predominated, and the protein concentration increased, while the carbohydrate content remained almost unchanged during aerobic granulation. In fact, for a variety of biofilm and anaerobic granule types and extraction methods, proteins have been reported more abundant than carbohydrates in EPS [51,53,54]. Some researchers also found that EPS in biofilm, aerobic sludge and granules were composed mainly of carbohydrates [49,55]. This may imply that the composition of extracellular polymers is variable, and is related to microbial species, type of limiting substrate (carbon, nitrogen and phosphorous), oxygen limitation, culture temperature, and so on [56].

Therefore, recycling flux had great effect on contents of protein and polysaccharides content in EPS both hydrolytic sludge and aerobic sludge. Protein was contributed more to the bacterial aggregate structure and stability than polysaccharides. As recycling flux increasing at a certain degree, the increase in protein content of EPS could more favor the stability of the HARP.

3.5. Effect of recycling flux on PN/PS in EPS

The PN/PS ratios in EPS of hydrolytic sludge and aerobic sludge at different recycling fluxes were also investigated. As shown in Fig. 7, the PN/PS ratios in EPS in the seed hydrolytic and aerobic sludge was 2.2 and 1.6. When the PN/PS ratios in EPS gradually increased as recycling flux increasing from 5 to 15 mL/min, the PN/PS ratios in EPS in the hydrolytic and aerobic sludge was 3.6 and 2.9, which were 1.6 and 1.8 times as much as those in the seed hydrolytic and aerobic sludge. The results indicated that there is obviously fit to the linear correlation between the PN/PS ratios and recycling flux. The linear equations were calculated according to the following equation (r: recycling flux, mL/min):

$$\text{hydrolytic sludge}: \frac{\text{PN}}{\text{PS}} = 0.1085r + 2.0519, \quad R^2 = 0.9318 \tag{3}$$

$$\text{aerobic sludge}: \frac{\text{PN}}{\text{PS}} = 0.0823r + 1.7465, \quad R^2 = 0.9452 \tag{4}$$

Above results suggested that the PN/PS ratios were linearly increased with recycling flux. The proportion of EPS components (proteins and polysaccharides) was related to recycling flux. An explanation for these results is that when recycling flux increasing from 5 to 15 mL/min, protein and polysaccharides contents in EPS were gradually increased. Moreover, protein content in EPS was obviously abundant than polysaccharides content as the same recycling flux in the HARP. Therefore, by circulating wastewater between the hydrolytic reactor and the aerobic one continuously at the higher recycling flux, the PN/PS ratios increased.

It was known that dispersed bacteria are negatively charged and there is electrostatic repulsion between the cells. It has been found that EPS could decrease the negative charge of cell surfaces, and thereby bridge two neighboring cells physically to each other [57]. Tsuneda et al. [58] investigated the influence of extracellular polymers on electrokinetic properties of heterotrophic bacterial cells, and found that EPS on cell surface could decrease the negative surface charge density around the cell surface. Since protein has a high content of negatively charged amino acids, protein as the largest fraction in EPS, is more involved than sugars in electrostatic bonds with multivalent cations, thus decreasing the negative surface charge density surrounding the cell surface. This suggested that the increase in the PN/PS value could decrease the surface negative charges of bacterial cells, thus reducing the electrostatic repulsion between the cells. Therefore, the PN/PS ratios increased with recycling flux increasing, which resulted in hydrolytic and aerobic sludge of the HARP maintained a stable condition.

3.6. Effect of recycling flux on zeta potential of activated sludge

Zeta potentials of hydrolytic sludge and aerobic sludge at different recycling fluxes were shown in Fig. 8. Zeta potentials of the seed hydrolytic and aerobic sludge were −37.5 and −38.2 mV. With the recycling flux increasing from 5 to 10 mL/min, zeta potentials of the hydrolytic and aerobic sludge decreased to −17.4 and −16.7 mV.

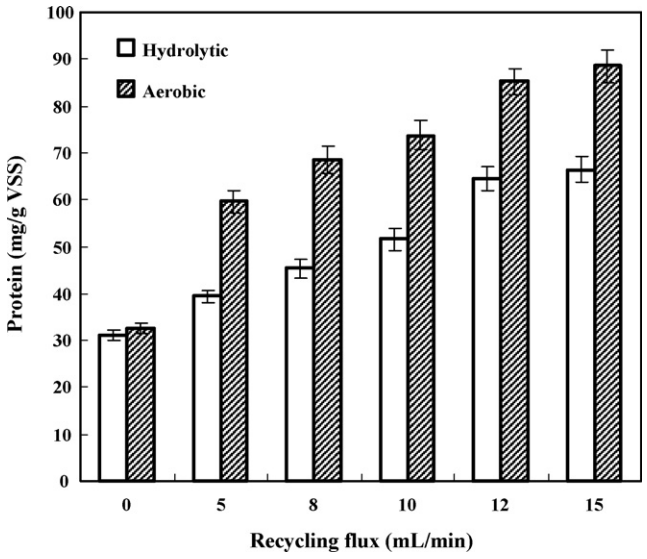

Fig. 6. Profiles of protein content in EPS at different recycling fluxes.

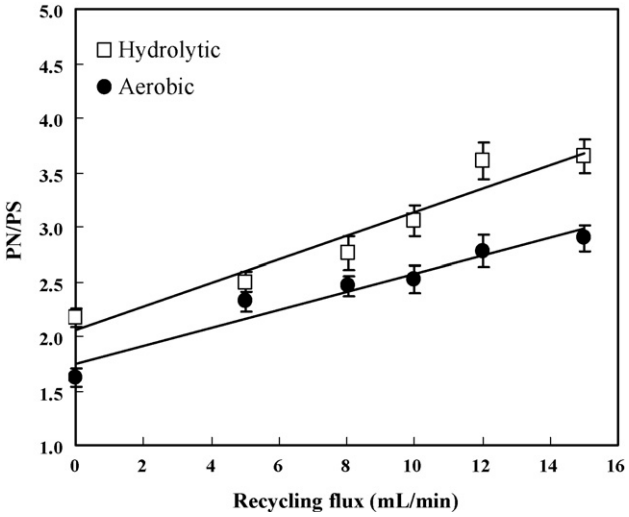

Fig. 7. Profiles of PN/PS ratio in EPS at different recycling fluxes.

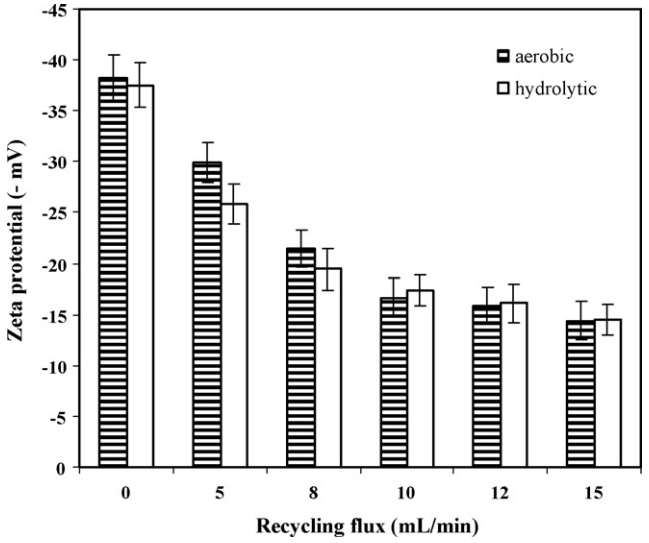

Fig. 8. Profiles of zeta potential of activated sludge at different recycling fluxes.

When the recycling flux was further increased to 15 mL/min, zeta potentials of the hydrolytic and aerobic sludge had not obviously changed.

It was found that the zeta potentials tended to decrease with the recycling flux increasing. The recycling flux was related to the zeta potential of the hydrolytic and aerobic sludge. The hydrolytic and aerobic sludge in the HARP at different recycling fluxes was less negatively charged than the seed aerobic and hydrolytic sludge. The surface charge has been believed to be important in controlling the stability of microbial aggregates. Therefore, the zeta potentials decreased with recycling flux increasing, which favored the stability of the HARP.

4. Conclusions

The present study suggested that 2,4-DCP could be successfully degraded in the HARP. The removal efficiencies of 2,4-DCP and COD were far higher with the recycling process increasing after 24 h degradation. 2,4-DCP and COD total removal efficiencies could be up to 98% and 96% with the recycling flux being 15 mL/min in 24 h, respectively. The recycling flux had great effect on extracellular polysaccharide and protein in EPS, which may play a most important role in controlling the stability of the HARP. With the recycling flux increasing, the increase in protein content decreased the surface negative charge of bacterial cells, thus reduced the electrostatic repulsion between the cells, and tended to decrease the zeta potentials. The results indicated that the biodegradation of 2,4-DCP was a good example of the HARP that benefited from the combination of reductive and oxidative degradation mechanisms and cooperative metabolism. The metabolic and kinetic limitations of hydrolytic and aerobic microorganisms could be overcome in the HARP. The HARP successfully solved the problem of over-acidification and effectively enhance the removal efficiency and rate of 2,4-DCP and COD.

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