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High efficiency of batch operated biofilm 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 four biofilm processes: stand-alone hydrolytic process, stand-alone aerobic process, hydrolytic–aerobic in-series process (in-series process) and hydrolytic–aerobic recycling process (recycling process). The overall removal efficiency of 2,4-DCP was far higher in the recycling process than in the stand-alone hydrolytic process, the stand-alone aerobic process and the in-series process. 2,4-DCP removal efficiency in the recycling process was 99% with the recycling rate being 10 mL/min in 12 h, while those in the stand-alone hydrolytic, stand-alone aerobic and the in-series process were 96%, 82% and 89%, respectively. COD removal efficiency could reach 91% in the recycling process in 4 h whereas those were only 23%, 69% and 25% in the stand-alone hydrolytic, stand-alone aerobic and the in-series process, respectively. In the recycling process, the concentrations of volatile fatty acid (VFA) gradually increased to 3.5 mmol/L in first 5 h and then declined to below 3 mmol/L, and the pH values were all around 7.5 during the whole process. The alkalinity of the solution in the recycling process was apparently higher than that in both the stand-alone processes and in-series process within 12 h. Moreover, the ratios of VFA/alkalinity were all less than 0.8 in the recycling process, which indicated the activity of hydrolytic microorganisms was not inhibited and the process maintained a stable condition. Therefore, the recycling process could successfully solve the problem of over-acidification and effectively enhanced the removal efficiencies of 2,4-DCP and COD.

Keywords: Biodegradation; 2,4-DCP; Recycling; Hydrolytic; Aerobic

1. Introduction

Effluents of some chemical industries such as petrochemicals, refineries, pesticides, pulp and paper contain toxic chlorophenol compounds which make physico-chemical treatment of such effluents much cost or difficult [1]. Biodegradation of chlorophenols is more specific and relatively inexpensive [2–4]. The number and position of chlorine groups on the aromatic ring strongly affect biodegradability and toxicity of chlorinated compounds. Usually, biodegradability decreases but toxicity increases with the number of chlorine groups increasing [5].

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 [6–8]. Although the majority of chlorophenols-degrading microorganisms have the necessary enzymes for aromatic ring degradation, they have a limited capacity for halogen removal [7]. Therefore, the degradation efficiency of these recalcitrant toxic chlorophenols depends mainly on the microbial capacity to remove the halogen groups [9,10].

Three different approaches have been attempted to improve chlorophenols removal. Firstly, genetically engineered microorganisms with modified dehalogenases are used [11]. However, this approach requires substantial genetic knowledge and is not easily performed even in laboratory. Secondly, some investigations on biodegradation of chlorophenols were focused on suspended pure culture using different bacteria and fungi such

Abbreviations: 2,4-DCP, 2,4-dichlorophenol; MCP, mono-chlorophenol; VFA, volatile fatty acid; COD, chemical oxygen demand

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as species of *Pseudomonas*, *Azotobacter*, *Alcaligenes* and *Acinetobacter* [12–20]. Suspended culture systems usually failed to remove high concentrations of chlorophenols from wastewater due to toxic nature of those compounds. Thirdly, mixed culture biodegradation processes such as anaerobic, aerobic, anaerobic–aerobic combination process were developed by many investigators for removal of chlorophenols from wastewaters [3,4,14]. 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 [21–23]. 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 [3,5,9,24–29]. In order to overcome toxic effects of chlorophenols, immobilized cell or biofilm reactors were used recently [30–33]. Microorganisms in biofilm reactors were more resistant to high concentrations of chlorophenols because of high biomass concentrations and diffusion barriers within the biofilm for the toxic compounds.

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 VFA, leading to a low pH environment. The accumulation of VFA resulted in that the wastewater was seriously acidified and the activity of hydrolytic microorganisms was depressed. Therefore, the process of combining a hydrolysis acidification step with an aerobic treatment (hydrolytic–aerobic combination process) was proposed and attracted much attention [34].

Although chlorophenols were used in many industries such as production of pesticides, glue, paint, leather and pulp materials, there has not been any published research regarding the biodegradation of 2,4-DCP containing wastewaters by batch operated biofilm hydrolytic–aerobic recycling process. Therefore, it is interesting to investigate the feasibility and biotransformation fate using the hydrolytic–aerobic recycling process to improve the removal efficiency of 2,4-DCP. The objectives of the present work were to examine the performance of the hydrolytic–aerobic recycling process in degrading 2,4-DCP in wastewater and compare it with the stand-alone hydrolytic and aerobic processes and the hydrolytic–aerobic in-series process. The COD removal efficiencies and the variations of VFA, pH, alkalinity and the ratio of VFA/alkalinity in the four processes were also evaluated.

Table 1
Principal physical characteristic of the biofilm hydrolytic and aerobic reactors

Physical characteristics[a]	Value	
	Hydrolytic reactor	Aerobic reactor
Total volume of reactor (L)	2.9	2.4
Working volume of reactor (L)	2.4	2.3
Effective volume of reactor (L)	2.0	2.0
Number of soft fibre carrier	15	15
Soft fibre carrier density (g/cm^3)	0.91	0.91
Soft fibre carrier height (m)	0.25	0.25
Packing dry weight (g)	0.525	0.525
Specific surface area of the carrier (m^2/m^3)	5.563	5.563
Total surface areas of the carrier (m^2)	3.4	3.4

[a] Determined before the beginning of the biological process.

2. Materials and methods

2.1. Microorganism and medium

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. To improve the microorganisms' adaptability to the 2,4-DCP wastewater, 2,4-DCP concentrations were increased stepwise from 5 to 20 mg/L for about 1 month with glucose as external carbon source.

Feed medium to the hydrolytic and aerobic reactor contains (g/L): 2,4-DCP 0.02, $C_6H_{12}O_6 \cdot H_2O$ 2.0, NH_4Cl 0.07, KH_2PO_4

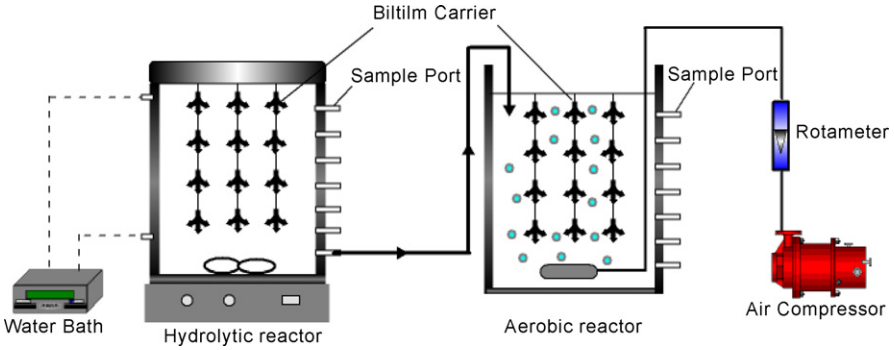

Fig. 1. Bench-scale biofilm hydrolytic–aerobic in-series process.

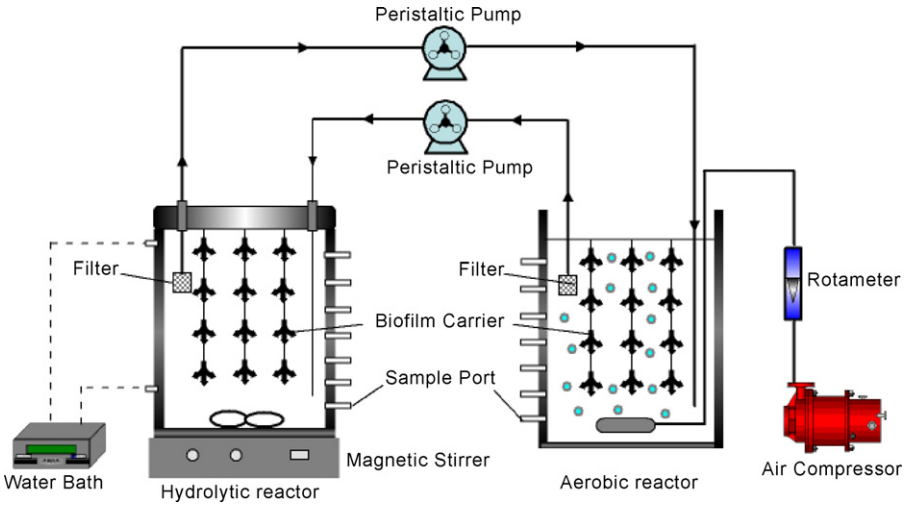

Fig. 2. Bench-scale biofilm hydrolytic–aerobic recycling process.

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.

2.2. Biofilm carrier

The hydrolytic and aerobic reactors were filled with D-2 soft fibre, provided by Xiamen Hengli Ltd., whose principal physical characteristics are presented in Table 1. The carrier was swung from the top of the reactors to the part 5 cm above the bottom of the reactors.

2.3. Experimental setup and procedure

The bench-scale biofilm hydrolytic–aerobic in-series process and recycling process are presented in Figs. 1 and 2, respectively. The hydrolytic reactor is 11 cm in diameter and 30 cm in height. The aerobic one is 10 cm in diameter and 30 cm in height. The hydrolytic and aerobic reactors were equipped with seven sampling ports spaced 3 cm apart along its side. The highest one was 5 cm below the top, and the lowest one was 5 cm above the bottom. The hydrolytic reactor was covered with a plastic cover unsealed where there were two holes to allow the tubes to insert. The hydrolytic reactor was maintained at 35 °C using water bath and the solution in it was mixed by a magnetic stirrer. The solution in the aerobic reactor was aerated using an air compressor with aeration rate 3 L/min at room temperature, where DO (dissolved oxygen) was above 2 mg/L. The simulated wastewater was added from the top of the hydrolytic and aerobic reactor. The operational conditions for both reactors are presented in Table 2.

After biomass formation, the in-series process and recycling process were operated in intermittent mode to study the 2,4-DCP biodegradation. 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.

Table 2
Operational parameters of the hydrolytic and aerobic reactors

Operational conditions[a]	Value	
	Hydrolytic reactor	Aerobic reactor
2,4-DCP concentration (mg/L)	20	20
Glucose concentration (mg/L)	2000	2000
Hydraulic residence time (h)	24	24
Recycling rate (mL/min)	10	10
pH value	7.5	7.5
Aeration rate (L/min)	–	3.0
Temperature (°C)	35	Room temperature

[a] Established during the biological process. See Section 2 for further explanation.

For the two stand-alone processes, the simulated wastewater was independently biodegraded by hydrolytic and aerobic microorganisms for 24 h in the hydrolytic and aerobic reactor, respectively.

For the hydrolytic–aerobic in-series process, the simulated wastewater was firstly added to the hydrolytic reactor. After hydrolytic degradation 12 h, the solution in the hydrolytic reactor was collected and poured into the aerobic reactor for further aerobic degradation 12 h.

For the hydrolytic–aerobic recycling process, it was performed by means of two external recirculation peristaltic pumps (BT01-100) in continuous recirculation 24 h with the same recycling rate 10 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 peristaltic pump that drew liquid though 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 though filter from the top of the

aerobic reactor and transferred it to the bottom of the aerobic reactor.

The main parameters such as COD, VFA, pH and alkalinity were measured after centrifugation of the samples collected at the sample ports of the hydrolytic and aerobic reactor at different time intervals. The samples were kept at 4 °C in a freezer before analysis. After each run of the experiments, all the wastewater in the reactor was discharged and the reactor was washed twice with tap water to remove detached biomass. 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.

2.4. Calculation methods

In the two stand-alone processes, the removal efficiencies of 2,4-DCP (S_d) and COD (S_c) were calculated according to the following formulation:

$$S_d = \frac{C_{id} - C_{ed}}{C_{id}} \times 100\% \quad (1)$$

$$S_c = \frac{C_{ic} - C_{ec}}{C_{ic}} \times 100\% \quad (2)$$

where C_{id}: the concentration of 2,4-DCP in the influent, mg/L; C_{ed}: the concentration of 2,4-DCP in the effluent, mg/L. C_{ic}: the COD in the influent, mg/L; C_{ec}: the COD in the influent, mg/L.

In the in-series process, the removal efficiencies of 2,4-DCP (I_d) and COD (I_c) in the hydrolytic stage (I_{dh}, I_{ch}) and aerobic stage (I_{da}, I_{ca}) were calculated according to the following formulation:

$$I_{dh} = \frac{C_{idh} - C_{edh}}{C_{idh}} \times 100\% \quad (3)$$

$$I_{da} = \frac{C_{ida} - C_{eda}}{C_{ida}} \times 100\% \quad (4)$$

$$I_{ch} = \frac{C_{ich} - C_{ech}}{C_{ich}} \times 100\% \quad (5)$$

$$I_{ca} = \frac{C_{ica} - C_{eca}}{C_{ica}} \times 100\% \quad (6)$$

where C_{idh}, C_{ida}: the concentration of 2,4-DCP in the influent of hydrolytic and aerobic reactors, mg/L; C_{edh}, C_{eda}: the concentration of 2,4-DCP in the effluent of hydrolytic and aerobic reactors, mg/L. C_{ich}, C_{ica}: the COD in the influent of hydrolytic and aerobic reactors, mg/L; C_{ech}, C_{eca}: the COD in the effluent of hydrolytic and aerobic reactors, mg/L.

In the recycling process, the removal efficiencies of 2,4-DCP (R_d) and COD (R_c) at t moment were calculated according to the following formulation:

$$R_d = \left(1 - \frac{C_{ad}(t)V_a + C_{hd}(t)V_h}{C_{adi}V_a + C_{hdi}V_h}\right) \times 100\% \quad (7)$$

$$R_c = \left(1 - \frac{C_{ac}(t)V_a + C_{hc}(t)V_h}{C_{aci}V_a + C_{hci}V_h}\right) \times 100\% \quad (8)$$

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.

2.5. Analytical methods

Samples (2 mL) were withdrawn for analysis and centrifuged at 12,000 rpm for 10 min to remove biomass from the liquid phase. The supernatants were analyzed for 2,4-DCP content by the 4-aminoantipyrene colorimetric method [35]. VFA concentration and alkalinity in hydrolytic reactor were determined using titration method [36]. Biomass concentrations were determined by filtering the samples through 0.45 μm Millipore filter and dried in an oven at 105 °C until constant weight [37]. The pH value was measured by a pH meter (PHS-3C). The samples were analyzed in triplicates with less than 3% standard deviations from the average.

3. Results and discussion

3.1. Formation of biofilm

In order to promote the sufficient immobilization of biomass onto the carrier, the reactors have been operated in batch mode for 24 h. The structure and appearance of the biofilm carrier in the hydrolytic and aerobic reactor are shown in Fig. 3. The soft fibre carrier possessed lots of micro-pores and cavities, which offered high specific surface and were beneficial to microbial immobilization. The adhesion of microorganisms occurred on the surface of soft fibre carrier during batch feeding of the simulated wastewater containing 2,4-DCP and glucose as carbon and energy source. The steady-state formation of biofilm reached after 60 days' culture in the hydrolytic and aerobic reactor, which was determined as described by Ascon-Cabrera et al. [37].

3.2. 2,4-DCP degradation

The 2,4-DCP degradations in the stand-alone process, in-series process and recycling process are presented in Fig. 4. The removal rate of 2,4-DCP in the stand-alone hydrolytic process was much faster than in the stand-alone aerobic process in the first 4 h. After degradation 12 h, 2,4-DCP removal efficiency reached 96% and 82% in the stand-alone hydrolytic and aerobic process, respectively. Among the two stand-alone processes, in-series process and the recycling process, the recycling process attained the highest 2,4-DCP removal efficiencies within the same degradation time. The removal efficiency of 2,4-DCP was up to 98.8% after degradation 12 h, which suggested that 2,4-

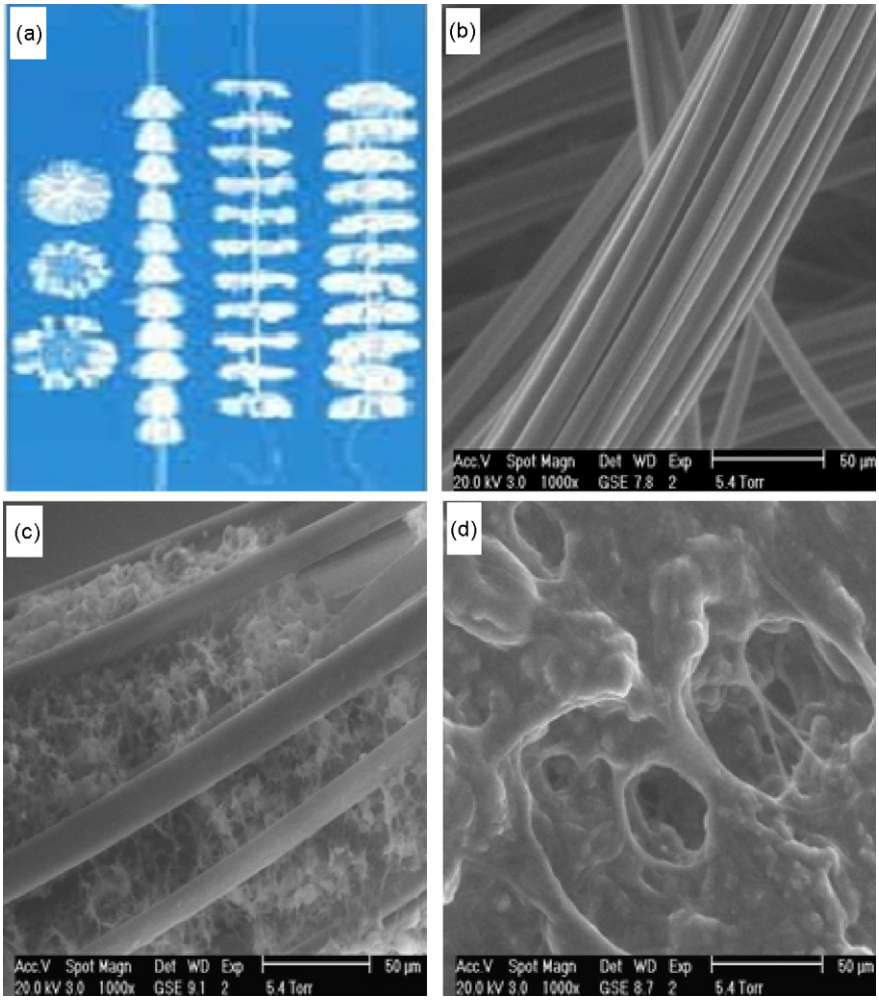

Fig. 3. Structure and appearance of the biofilm carrier: (a) the photo of biofilm carrier in trim size; (b) SEM of biofilm carrier before attaching the biomass; (c) SEM of biofilm carrier after attaching the biomass in hydrolytic reactor; (d) SEM of biofilm carrier after attaching the biomass in aerobic reactor.

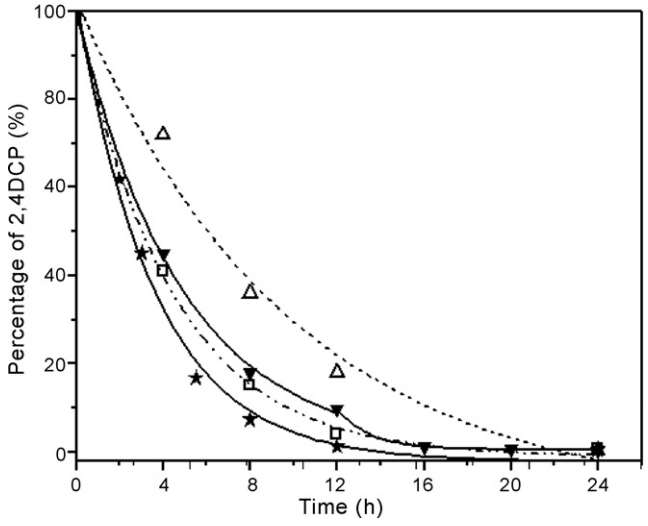

Fig. 4. 2,4-DCP removal in the stand-alone, in-series and recycling process: (□) stand-alone hydrolytic; (△) stand-alone aerobic; (▼) in-series process; (⋆) recycling process.

DCP could be effectively decomposed and mineralized in the recycling process.

It is known that the reductive dechlorination process under the anaerobic condition is of environmental importance because anoxic conditions in soils, as well as bottom layers of aquatic sediments and freshwater and marine are often prevailing [37]. Most anaerobic microorganisms had the capability of converting DCP to mono-chlorophenol (MCP) then phenol through the reductive dechlorination process under anaerobic conditions [38]. 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 [37]. Therefore, dechlorination is the rate-limiting step in degradation of 2,4-DCP. Wang et al. [19] 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 20 days. A long period of time is required to achieve high removal efficiency in the stand-alone anaerobic and aerobic degradation.

In the hydrolytic–aerobic in-series process, hydrolytic microorganisms carried out the initial reductive dechlorination step, after which dechlorination products were decomposed

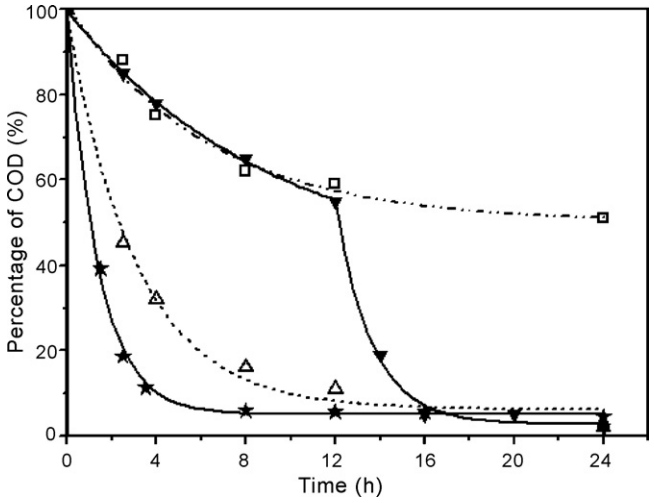

Fig. 5. COD removal in the stand-alone, in-series and recycling process: (□) stand-alone hydrolytic; (△) stand-alone aerobic; (▼) in-series process; (⋆) recycling process.

by aerobic microorganisms. In the hydrolytic–aerobic recycling process, reductive dechlorination is a continuous process. The metabolic and kinetic limitations to anaerobic and aerobic microorganisms could be overcome by coupled reductive dechlorination mechanisms in the recycling process. Therefore, the removal efficiency and rate of 2,4-DCP could effectively be improved in the recycling process.

3.3. COD removal

As shown in Fig. 5, COD removal was more efficient in the stand-alone aerobic process than in the stand-alone hydrolytic process. The COD removal efficiency was 90% in the stand-alone aerobic process after degradation 12 h whereas it was only 41% in the stand-alone hydrolytic process. It suggested that the intermediate products could not completely decompose in the stand-alone hydrolytic process, leading to large value of residual COD in the solution.

In the hydrolytic–aerobic in-series process, the residual COD in the solution after hydrolytic degradation was removed in the subsequent aerobic stage. The COD removal efficiency could be up to 98% within 24 h. The results indicated that aerobic microorganisms were capable of utilizing the intermediate products of hydrolytic degradation. It supplied a possibility of improving the COD removal efficiency through continuously circulating the solution between hydrolytic reactor and aerobic reactor.

In the hydrolytic–aerobic recycling process, after degradation 4 h, the COD removal efficiency could reach 91% whereas those were only 69% and 25% in the stand-alone aerobic process and the in-series process, respectively. The COD removal efficiency was far better than those in the two stand-alone processes and the in-series process at the same degradation time. The COD removal benefited from the "cooperative metabolism" between hydrolytic and aerobic microorganisms, caused by the exchange of metabolites between hydrolytic and aerobic reactors. "Cooperative metabolism" improved the biodegradability of hydrolytic and aerobic microorganisms simultaneously. The exchange of metabolites could be compared to the metabolite exchange at interfaces between the anaerobic and aerobic zones of natural eco-process (sediments, bacterial colonies, stratified lakes and seas, microbial mats, biofilm, etc.). Hydrolytic microorganisms were apparently 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 in reactors of the recycling process was much lower than it was in natural process [37].

3.4. Change of VFA

The changes of VFA in the two stand-alone processes, in-series process and recycling process are shown in Fig. 6. In the stand-alone hydrolytic process, VFA concentration sharply increased from 2.2 to 11.1 mmol/L in the first 3 h and then maintained at a high level. The concentrations of VFA were all below 1.5 mmol/L in aerobic reactor. 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 conditions. The degradation of 2,4-DCP into VFA was much faster than further conversion into methane, which resulted in accumulating of a quantity of acid [21]. 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 totally restrained.

In the hydrolytic–aerobic in-series process, aerobic microorganisms were capable of utilizing the intermediate products of hydrolytic degradation. It was shown that the concentrations of VFA were rapidly decreased from 12 to 1.3 mmol/L after 12 h aerobic treatment. Therefore, aerobic stage was a necessary step followed by hydrolytic stage to further decompose accumulated VFA.

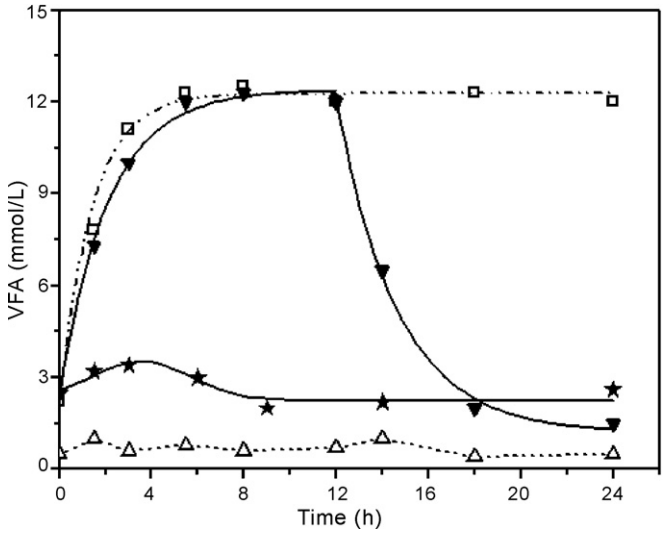

Fig. 6. Changes of VFA in the stand-alone, in-series and recycling process: (□) stand-alone hydrolytic; (△) stand-alone aerobic; (▼) in-series process; (⋆) recycling process.

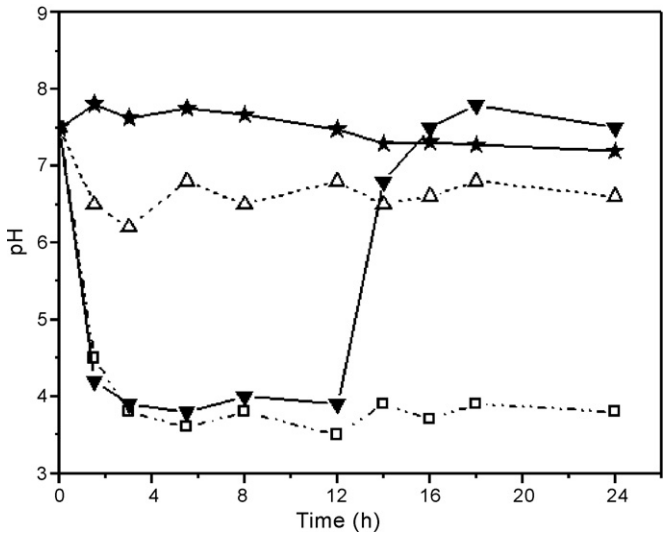

Fig. 7. Changes of pH in the stand-alone, in-series and recycling process: (□) stand-alone hydrolytic; (△) stand-alone aerobic; (▼) in-series process; (⋆) recycling process.

In the hydrolytic–aerobic recycling process, the concentrations of VFA gradually increased to 3.5 mmol/L in first 5 h after that it declined to below 3 mmol/L, which indicated that there had not been VFA accumulation in the both reactors. By circulating the wastewater between the hydrolytic reactor and the aerobic reactor continuously, VFA produced in the hydrolytic reactor could be consumed by aerobic microorganisms in time, thus over-acidification would not happen, and the organic matter could go through the hydrolysis acidification step more smoothly and completely. Therefore, circulation of the wastewater between these two reactors will simultaneously intensify both the hydrolytic process and aerobic process.

3.5. Change of pH

The changes of pH values in the two stand-alone processes, in-series process and recycling process are shown in Fig. 7. In the stand-alone hydrolytic process, with VFA concentration increasing up to 11.1 mmol/L, pH value decreased from 7.5 to 3.8 in the first 3 h. The reason is that 2,4-DCP and glucose could be decomposed into relatively simple intermediate products like VFA resulting in the decreasing of pH value in the solution. In the stand-alone aerobic process, the pH value decreased from 7.5 to 6.2 in first 3 h and then maintained at a stable level during the degradation period.

In the hydrolytic–aerobic in-series process, aerobic microorganisms were capable of utilizing the VFA produced by hydrolytic microorganisms. Therefore, when the solution of the hydrolytic reactor was poured into the aerobic reactor, the pH value of the solution dramatically increased and reached 7.5 after further aerobic degradation 12 h.

In the hydrolytic–aerobic recycling process, the pH values were all around 7.5. It indicated that there was no VFA accumulation and the solution maintained a neutral condition in this process, which was beneficial to hydrolytic and aerobic microorganisms.

3.6. Change of alkalinity

Fig. 8 depicts the variations of the alkalinity in the two stand-alone processes, in-series process and recycling process. In the stand-alone hydrolytic process, with the concentration of VFA increasing, the alkalinity of the solution decreased from 10.5 to 2.7 mmol/L in the first 5 h and then maintained at a low level, which indicated that the buffering capability of solution was greatly declined. In the stand-alone aerobic process, the alkalinity also decreased from 10.5 to 4.3 mmol/L in the first 5 h and then maintained at a stable level during the degradation period.

In the hydrolytic–aerobic in-series process, the change of alkalinity in the hydrolytic stage showed a similar trend as in the stand-alone hydrolytic process, whereas in the aerobic stage, alkalinity gradually increased from 3.2 to 6.8 mmol/L after aerobic degradation 4 h.

In the hydrolytic–aerobic recycling process, the alkalinity of the solution decreased from 10.5 to 6.2 mmol/L in the first 5 h. The alkalinity of the solution in this process was apparently higher than in the both stand-alone processes. It suggested that the buffering capability of the solution was obviously increased.

3.7. Inhibition level in the four processes

Inhibition level could be assayed through measuring the ratio of VFA/alkalinity in the reactor, which was a good indicator of process performance [39]. Grady et al. suggested that VFA variations could be accommodated when VFA/alkalinity was less than 0.4. But when the ratio of VFA/alkalinity was higher than 0.4, the performance of the process deteriorated due to lack the buffering capacity. When the ratio of VFA/alkalinity was higher than 0.8, the performance of the process was in a severely imbalance. Thus, the ratio of VFA/alkalinity could benefit to us a good understanding of the microbial inhibition level and give us a clear estimation of the unstable condition to better control the process [40].

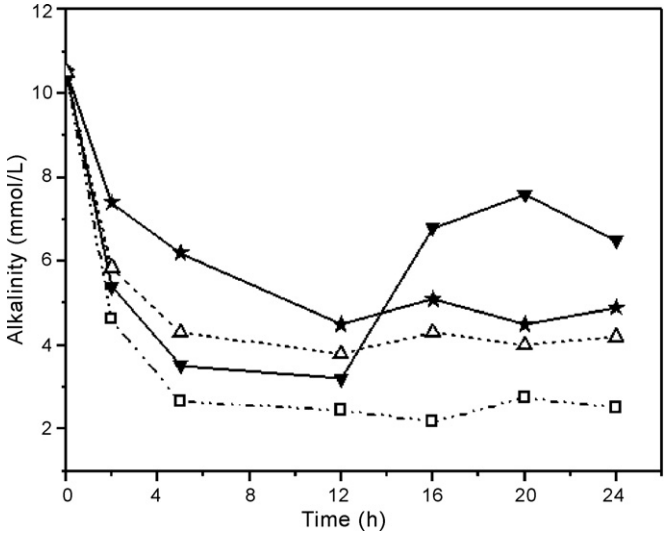

Fig. 8. Changes of alkalinity in the stand-alone, in-series and recycling process: (□) stand-alone hydrolytic; (△) stand-alone aerobic; (▼) in-series process; (⋆) recycling process.

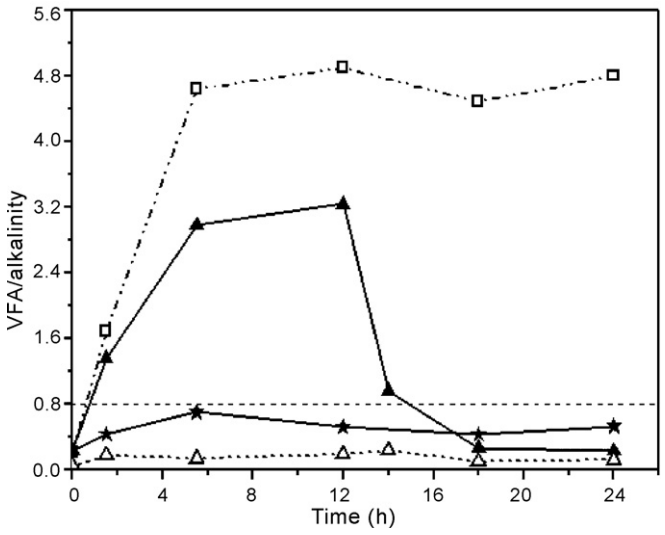

Fig. 9. Changes of VFA/alkalinity in the stand-alone, in-series and recycling process: (□) stand-alone hydrolytic; (△) stand-alone aerobic; (▼) in-series process; (⋆) recycling process.

The changes of VFA/alkalinity in the two stand-alone processes, in-series process and recycling process are presented in Fig. 9. In the stand-alone hydrolytic process, the ratio of VFA/alkalinity could be up to 4.5 within the first 5 h. In the stand-alone aerobic process, the ratios of VFA/alkalinity were all less than 0.4.

In the hydrolytic–aerobic in-series process, the ratio of VFA/alkalinity in the hydrolytic stage increased from 0.2 to 3.2 within 12 h, whereas in the aerobic stage, the ratio of VFA/alkalinity decreased from 3.2 to 0.3 after aerobic degradation 12 h. An explanation for the inhibition is that the high VFA concentration resulted in the sharp dropping in pH as well as the inhibition of metabolic activity and methane production of hydrolytic microorganisms.

In the hydrolytic–aerobic recycling process, the ratios of VFA/alkalinity were all less than 0.8, which indicated the activity of hydrolytic microorganisms had not been inhibited and the process maintained a stable condition. The significance of recycling solution continuously between the two reactors in the recycling process seemed to be attributed to the two ways. The first one was the trace amounts of oxygen transported by recycling solution from aerobic reactor to the hydrolytic reactor could be enhanced the activity of hydrolytic microorganisms [37]. Michael et al. thought that dechlorination efficiency and rate by hydrolytic microorganisms increased under microaerobic conditions [41]. The other one was that hydrolytic and aerobic conditions favored a change in the recalcitrance order of different chloride isomers [42].

4. Conclusions

The present study suggested that 2,4-DCP could be successfully and completely degraded in the hydrolytic–aerobic recycling process. The removal efficiencies of 2,4-DCP and COD were far higher in the recycling process than in the two stand-alone processes and in-series process within the same degradation time. 2,4-DCP removal efficiency in the recycling process was 99% with the recycling rate being 10 mL/min in 12 h, while those in the stand-alone hydrolytic, stand-alone aerobic and the in-series process were 96%, 82% and 89%, respectively. COD removal efficiency could reach 91% in the recycling process whereas those were only 23%, 69% and 25% in the stand-alone hydrolytic, stand-alone aerobic and the in-series process in 4 h, respectively. In the recycling process, there was no VFA accumulation and the solution maintained a neutral condition in both reactors. Meanwhile, pH values were all around 7.5 during the whole process, which was beneficial to hydrolytic and aerobic microorganisms. The alkalinity of the solution in the recycling process was apparently higher than in the both stand-alone processes, which suggested that the solution had more buffering capability. Moreover, the ratios of VFA/alkalinity were all less than 0.8 in the recycling process during the whole process, which indicated the activity of hydrolytic microorganisms had not been inhibited and the process maintained a stable condition. The results suggested that the biodegradation of 2,4-DCP was a good example of a process that benefited from the combination of reductive and oxidative degradation mechanisms and cooperative metabolism. The metabolic and kinetic limitations of aerobic and hydrolytic microorganisms could be overcome in the recycling process. Therefore, the biofilm hydrolytic–aerobic recycling process perfectly solved the problems of over-acidification and inhibition in the hydrolysis process and effectively enhanced the removal efficiencies of 2,4-DCP and COD.

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