Study of kinetics of degradation of cyclohexane carboxylic acid by acclimated activated sludge
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ABSTRACT
Activated sludge contains complex microorganisms, which are highly effective biodegrading agents. In this study, the kinetics of biodegradation of cyclohexane carboxylic acid (CHCA) by an acclimated aerobic activated sludge were investigated. The results showed that after 180 days of acclimation, the activated sludge could steadily degrade >90% of the CHCA in 120 h. The degradation of CHCA by the acclimated activated sludge could be modeled using a first-order kinetics equation. The equations for the degradation kinetics for different initial CHCA concentrations were also obtained. The kinetics constant, kd, decreased with an increase in the CHCA concentration, indicating that, at high concentrations, CHCA had an inhibiting effect on the microorganisms in the activated sludge. The effects of pH on the degradation kinetics of CHCA were also investigated. The results showed that a pH of 10 afforded the highest degradation rate, indicating that basic conditions significantly promoted the degradation of CHCA. Moreover, it was found that the degradation efficiency for CHCA increased with an increase in temperature and concentration of dissolved oxygen under the experimental conditions.

Key words | activated sludge, biodegradation, cyclohexane carboxylic acid, kinetics equation

INTRODUCTION
Cyclohexane carboxylic acid (CHCA), a typical naphthenic acid (NA), is a nonbiodegradable substance and is extremely harmful when released into the environment. Studies have shown that CHCA has inhibitory or toxic effects on a variety of animals, plants, and microorganisms (Rogers et al. 2005). CHCA is present in high concentrations in the wastewater from plants producing petrochemicals such as phenol and acetone (Zhao et al. 2015). During the refining of crude oil, toxic acids such as NAs are released into the sewage. Further, CHCA is used commonly in the synthesis of various organic compounds. In addition, CHCA is employed widely in biology and medicine as well as a pesticide for sand flies and mosquitoes. For instance, it can stimulate the growth of streptococci (Barona Salazar 2010). It is also used for synthesizing antipregnancy drugs and the new drug praziquantel for treating blood flukes. Finally, it is also used as a compatibilizer for vulcanized rubber as well as an oil-clarifying agent. Thus, CHCA plays an important role in the modern chemical industry. Hence, it is very important to develop effective techniques for degrading CHCA, in order to render it harmless to the environment.

A number of studies have evaluated different technologies for removing NAs; these include biodegradation (Clemente & Fedorak 2005), advanced oxidation (Scott et al. 2008), coagulation/flocculation (Pourrezaei et al. 2011), and membrane filtration and adsorption (Janfada et al. 2006; Zubot 2010). However, each technology possesses certain limitations and advantages (Quinlan & Tam 2015) For instance, coagulation/flocculation produces a large amount of sludge; this limits its use (Allen 2008). Further, many reports have shown that advanced oxidation processes are suitable for the oxidative degradation of bulkier, polycyclic NAs but cannot degrade the lower-molecular-weight factions (Neyens & Baeyens 2005; Afzal et al. 2012). The main challenges for both membrane filtration and adsorption processes are fouling and poor selectivity (Zubot 2010). Conversely, biodegradation, which involves the use of microbial cultures, has been determined to be an effective technology for biodegrading lower-molecular-weight NAs as well as those with fewer rings and lower degrees of branching. It cannot be used to metabolize higher-molecular-weight NAs (Headley et al. 2009; Zhang

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Thus, biodegradation is being used widely to decrease the concentration of low-molecular-weight NAs, such as CHCA (Kung et al. 2014). Many studies have reported the biodegradation of CHCA under aerobic and anaerobic conditions. Blakley (1978) isolated a bacterial strain for degrading CHCA and found that the degradation process of CHCA was similar to the classical pathway of saturated fatty acid oxidation. That is to say, β-oxidation is required for the participation of the acetyl coenzyme A in the degradation process. Rho & Evans (1975) isolated an aerobic nitrate-negative Acinetobacter from soil that could degrade CHCA. Thereafter, its degradation pathways and degradation cofactor were studied. Hasegawa et al. (1982) isolated a bacterial strain that used CHCA as the sole carbon and energy source. Del Rio et al. (2006) found that the degradation period for single-ring CHCA is approximately 14 days, which is much lower than those of other bicyclic NAs. Kung et al. (2014) identified a pathway for the anaerobic degradation of CHCA in the Fe(III)-reducing protobacterium Geobacter metallireducens. To sum up, most studies on the biodegradation of CHCA have focused on exploring bacteria that can degrade CHCA and on determining its degradation pathways. In contrast, the factors controlling the biodegradation kinetics of CHCA are poorly understood.

In order to understand the aerobic degradation characteristic of CHCA, we used an aerobic acclimated activated sludge to study the kinetics of the biodegradation of CHCA in a research batch reactor. This was done to determine the kinetics constant under different initial conditions and environmental factors and thus establish a model for the reaction kinetics. The results of this study should provide a theoretical basis for treating wastewater containing CHCA as well as serve as a reference for the effective biodegradation of other NAs.

**MATERIALS**

**Materials and instruments**

The CHCA reagent (purity 98%) was purchased from Shandong Qingzhou Aoxing Chemical Co., Ltd. The other equipment used was a PHS-3B Precision acidity meter, a thermostatic water bath, a glass thermometer, an adjustable oxygen pump, a dissolved oxygen (DO) meter (JENCO9010), and an inner loop three-phase fluidized bed reactor, whose effective volume was approximately 10 L.

**Method to determine CHCA concentration**

The concentration of CHCA was measured using an Agilent 1260 high-performance liquid chromatography (HPLC) system equipped with an RP-18 analytical column (5 µm particle size, 100 mm × 4 mm). The analytical column was kept at 40 °C, and the sample injection volume was 60 µL. Gradient elution was used for the analysis. The mobile phase was a mixture of methanol (HPLC grade, Sigma) and ultrapure water (FBZ0520-UP, FLOM, Qingdao). The mobile phase, whose flow rate was 1.5 mL min⁻¹, was 70:30 methanol/water at time zero and 100% methanol at 4 min. The detector was set at 400 nm and the bandwidth was 10 nm (Yen et al. 2004).

**Cultivation and acclimation of activated sludge**

The sludge was acclimated by forming an inoculating biofilm; this method involved both a static stage and a dynamic stage. First, a static culture was grown. The aerobic sludge was obtained from the secondary sedimentation tank of a refinery wastewater treatment plant. The refinery is located in southern China, where highly acidic heavy crude oil was refined. The fresh sludge was exposed to air for 48 h to remove residual organics, and then kept static for another 12 h in a 20 L open-mouthed container to concentrate the sludge. After the supernatant water had been discharged, the sludge was fed into a three-phase fluidized bed sludge reactor for dynamic culturing and acclimation (see Figure 1). During the dynamic acclimation process, the reactor temperature was controlled at approximately 28 °C, the pH at 6.5–8.5, and the concentration of the DO at 3–5 mg L⁻¹. The CHCA concentration in the influent of the reactor was increased at the rate of 5 mg L⁻¹. The acclimation period for each concentration was approximately 20 days; the water flow rate was 0.75 L h⁻¹. In addition, certain amounts of glucose, urea, and potassium phosphate dibasic were added as carbon, nitrogen, and phosphorus sources, respectively. For an influent CHCA concentration of 100 mg L⁻¹, the removal efficiency rates for chemical oxygen demand (COD) and CHCA were more than 85% and 95%, respectively. Once all the indicators, such as the mixed liquor volatile suspended solids (MLVSS), whose value was kept at 1,800–2,400 mg L⁻¹, and the settling velocity index (SVI), whose value was lower than 100 mL g⁻¹, among others, suggested that the sludge had reached a suitable level of acclimation, the acclimation process was considered completed. This step has also been described in our previous study (Wang et al. 2015).

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CHCA degradation experiments

The acclimated sludge was fed into another batch reactor. After being exposed to air for 24 h, the sludge was washed thrice using ultrapure water, in order to eliminate the effects of the residual CHCA and other organics. Batch experiments were carried out in a 3 L reactor containing 2 L of the medium. The degradation research reactor contained the substrate (CHCA) in various initial concentrations (20, 40, and 60 mg L\(^{-1}\)), with the initial pH being approximately 7.0. The reactors were maintained at room temperature (25 °C) and stirred using a magnetic stirrer (250 rpm), in order to achieve sufficient mixing and oxygen transfer. The CHCA concentration and pH value were measured on a daily basis, and the pH was adjusted using 1.0 M HCl and NaOH solutions.

Batch experiments were carried out to assess the effect of the initial pH (5.5, 7, 8.5, 10, and 11), temperature (22, 28, and 32 °C), and DO concentration (2, 3, and 4 mg L\(^{-1}\)) on the activity of the bacterial culture and the biodegradation of CHCA. During these experiments, the initial substrate concentration was 40 mg L\(^{-1}\), while the MLVSS value was 1,500 mg L\(^{-1}\). The pH and CHCA concentration were measured daily.

Equation for degradation kinetics

With regards to the biological treatment of wastewater, the Monod equation is used commonly to describe the reaction kinetics. This equation, which is based on the Michaelis-Menten equation, expresses the relationship between the degradation rate of a substrate and the concentration of the substrate (Gu 1995):

\[
\mu = \frac{\mu_{\text{max}} S}{K_s + S} \quad (1)
\]

where \(\mu\) is the substrate degradation rate (d\(^{-1}\)), \(S\) is the substrate concentration (mg L\(^{-1}\)), \(\mu_{\text{max}}\) is the maximum substrate degradation rate (d\(^{-1}\)), and \(K_s\) is the saturation constant, which is equal to the substrate concentration (mg L\(^{-1}\)) when \(\mu = \frac{\mu_{\text{max}}}{2}\).

When

\[S << K_s, \quad \mu = \frac{\mu_{\text{max}} S}{K_s},\]

then

\[
\frac{dS}{dt} = \frac{\mu_{\text{max}} S}{K_s} = k_d S
\]

The substrate degradation rate and the substrate concentration are proportional; that is, their relationship exhibits first-order kinetics and can be expressed as follows:

\[
\ln S = k_d t + \ln S_0 \quad (2)
\]

where \(S\) is the substrate concentration (mg L\(^{-1}\)), \(k_d\) is the first-order degradation kinetics constant (d\(^{-1}\)), \(t\) is the degradation time (d), and \(S_0\) is the initial substrate concentration (mg L\(^{-1}\)).
RESULTS

CHCA sludge acclimation

During the acclimation process, the sludge, which was dark brown and foul-smelling gradually turned brown. The settling ability of the activated sludge increased as the process progressed, with the SVI reaching approximately 100 mL g\(^{-1}\). The changes in the influent and effluent COD values and the COD removal efficiency over time during the acclimation process are shown in Figure 2. Further, the changes in the CHCA concentration and the CHCA removal rate over time are shown in Figure 3.

Analysis of kinetics of CHCA degradation by activated sludge

The conditions during the test to analyze the degradation kinetics were the following: MLVSS = 1,500 mg L\(^{-1}\), temperature = 25 °C, and pH = 7.0. The degradation rates of CHCA were measured for initial concentrations of 20, 40, and 60 mg L\(^{-1}\). The test results are shown in Figure 4 and Table 1.

Effects of pH on degradation kinetics

The effects of the pH on the degradation process were analyzed during the degradation process. The pH values tested were 5.5, 7, 8.5, 10, and 11. During these tests, the initial CHCA concentration was 40 mg L\(^{-1}\), MLVSS was 1,500 mg L\(^{-1}\), and the water temperature was 25 °C. The CHCA concentration was measured on a daily basis, and the pH values were adjusted using 1.0 M HCl or NaOH solutions. The data from the CHCA degradation tests were analyzed using a first-order kinetics equation. The obtained results are shown in Table 2.

Effects of temperature on degradation kinetics

Next, the effects of the temperature on the degradation kinetics of CHCA were analyzed for temperatures of 20–35 °C. In this case, too, the experimental data were analyzed using a first-order kinetics equation. The initial CHCA concentration was 40 mg L\(^{-1}\), while the initial MLVSS was 1,500 mg L\(^{-1}\). The degradation temperatures tested were 22, 28, and 32 °C. The obtained results are shown in Table 3.

Effects of DO concentration on degradation kinetics

The kinetics of the degradation of CHCA were also analyzed for DO concentrations of 2–4 mg L\(^{-1}\). The experimental data were again analyzed using a first-order kinetics equation. For the tests, the initial CHCA concentration was 40 mg L\(^{-1}\), temperature was 25 °C, pH was 10.00, and MLVSS was 1,500 mg L\(^{-1}\). The results are shown in Table 4.

Figure 2 | Variations in the inlet and outlet COD values and the COD removal rate with time.
DISCUSSION

Acclimation of activated sludge

In the early phase of the acclimation process, the SVI value was above 180 mL g\(^{-1}\), indicating that the sludge exhibited poor sedimentation. As shown in Figures 2 and 3, the removal rates for COD and CHCA remained at 50–60%, because the microbes needed more time to adapt. After 20 days of primary acclimation, the degree of sedimentation in the sludge improved significantly. Further, its antishock capability under pressure was also enhanced. When the CHCA concentration in the influent was increased at the rate of 5 mg L\(^{-1}\), the microbes in the sludge could steadily degrade the CHCA in the system, with the removal rate being as high as 90%. Further, the COD removal rate also
increased to >80%. This indicated that the sludge system was restrained by CHCA in the early stage of domestication, while the acclimated sludge could effectively degrade wastewater containing CHCA.

**Analysis of degradation process**

As shown in Figure 4, CHCA could be degraded quickly by the activated sludge. For an initial CHCA concentration of 40 mg L\(^{-1}\), the CHCA degradation rate reached 90% after 120 h. Further, all of the CHCA was degraded after 240 h. This indicated that the acclimated activated sludge could efficiently degrade CHCA at a certain concentration. However, with an increase in the CHCA concentration, the degradation activity decreased. Table 1 shows that the process of degradation of CHCA by the activated sludge could be expressed by a first-order kinetics equation. The kinetics constant, \(k_d\), decreased with an increase in the CHCA concentration, while the half-life, \(t_{1/2}\), increased with the increase in the CHCA concentration, indicating that a high CHCA concentration lowers the degradation ability of activated sludge and that the degree of inhibition depends on the initial CHCA concentration.

As shown in Table 2, the pH value had a significant impact on CHCA biodegradation. The degree of CHCA degradation was higher when the pH value was 7–11, with a pH of 10 corresponding to the highest degradation rate constant. Therefore, it can be concluded that alkaline conditions are more suitable than acidic ones for the biodegradation of CHCA. Moreover, during the degradation process, the pH value decreased to 7.5–8.2 when the initial pH value within the reactor was higher than 8. A NaOH solution was used to increase the pH value in this experiment. These results are consistent with those reported by Paslawski et al. (2009), who found that the biodegradation of CHCA does not occur when pH of the degrading medium is 5.5 and the concentration of trans-4MCHCA is 500 mg L\(^{-1}\). In this study, we found that CHCA could be degraded at a pH of 6.0; the difference was that the degradation rate was not as high as that under alkaline conditions. This was probably because the acidic environment affected the growth of the microbes in the activated sludge, which, in turn, inhibited the activity of the degrading enzymes.

Table 3 shows the effects of the temperature on the degradation of CHCA by the activated sludge. It can be seen that the rate of degradation of CHCA increased with an increase in the temperature. Thus, the CHCA degradation efficiency, which is related to the activity of the degrading enzymes, could be increased by increasing the temperature.

Table 4 shows that the DO concentration also affected the CHCA biodegradation rate. When the DO concentration was 2–4 mg L\(^{-1}\), the CHCA degradation rate increased and its half-life decreased with an increase in the DO concentration. This was because the amount of oxygen that diffused into the interior of the activated sludge floc was higher at higher DO concentrations. Thus, the internal microbial metabolic activity rate was also high. On the other hand, in this range, the DO concentration was not high enough to cause the rapid decomposition of CHCA and result in the aging of the sludge as well as a loss in its structural integrity, owing to a lack of nutrients for the microbes.

**CONCLUSIONS**

After 180 days of acclimation, the activated sludge could steadily degrade CHCA in a three-phase fluidized bed reactor when the concentration of the influent CHCA was increased at the rate of 5 mg L\(^{-1}\). The degradation of CHCA by the acclimated activated sludge in the batch reactor at low concentrations could be expressed by a first-order kinetics equation. Further, the degradation rate constant decreased with an increase in the initial CHCA concentration, indicating that, at high concentrations, CHCA had an inhibitory effect on the effectiveness of the activated sludge.

The pH value had a significant effect on the degradation of CHCA in the batch reactor. Alkaline conditions were found to be more suitable for CHCA degradation. The maximum degradation rate corresponded to a pH of 10; in contrast, acidic conditions inhibited CHCA degradation. The temperature and DO concentration also affected the degradation of CHCA. For temperatures of 20–55 °C, the CHCA degradation rate increased with the temperature. Finally, for DO concentrations of 2–4 mg L\(^{-1}\), the degradation rate of CHCA was high.

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