Diatomite Modified Immobilized Delftia sp. for the Bio-Abiotic Removal of Antibiotics Amoxicillin in the Aqueous System

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Abstract. Diatomite modified sodium alginate (Si/SA) immobilized *Delftia*sp. *A2(2011) (STT01)*was applied to degrade amoxicillin. The immobilized pellets provided a direct and visual probe for the degradation process due to their intrinsic bright colour. The results demonstrated that 100% of amoxicillin and 68.5% of COD_{cr} removal were achieved after 72 h, comparing with the cases of sodium alginate (SA) system (81.2%, 46.9%) and the free cells system (60.5%, 35.5%). The degradation kinetics was in good agreement with Michaelis-Menten equation. The maximum rate (V_m) and Michaelis constant (K_m) were calculated as 9.09 mg L^{-1} h^{-1} and 228 mg L^{-1}, respectively. The results further revealed that diatomite not only acted as immobilization support to improve the mechanical strength and lifetime of the pellets but also as absorbent to promote the treatment efficiency. Therefore, both enzymatic catalysis and chemisorption were responsible for the removal of amoxicillin.

1. Introduction

Even though antibiotics has been widely used for treating and preventing diseases in humans and veterinary practice since 1940s, not until 2010, did it attract an increasing worldwide concern in the increasing potential risk for ecosystem and the bacterial resistance [1]. It has been reported that the rates of antibiotics-resistant isolates are increasing over time, not only in hospital wastewaters and animal production wastewaters, but also in sewage, wastewater treatment plants, and even in drinking water [2-4]. Therefore, the ecological risk of antibiotics in the aquatic environment should be urgently estimated.

A variety of techniques have been applied to pre-treat or directly degrade antibiotics wastewater, such as ozonation or O_3/H_2O_2 [5], Fenton or Fenton-like process [6], biological process [7] and combined chemical and biological oxidation process [8,9]. However, the direct biotic process is more attractive because the microbial or enzymatic degradation is an eco-friendly and cost-competitive alternative to chemical decomposition process, in addition, any pretreatment would increase the cost as well as the energy consumption.

Recently, diatomite has been applied to handle a number of wastewater due to the high chemical stabilities and specific surface areas [10-12]. These studies have demonstrated the promising outlook

of diatomite that directly used as an absorber for wastewater treatment. Yet, up to date, few studies have been focused on the utilization of diatomite to immobilize *Delftia* sp. for the treatment of antibiotics wastewater.

Therefore, the objectives of this study are to identify the feasibility of using Si/SA system as an alternative to SA system and the free cells system for the direct degradation of antibiotics. As one of the most commonly used antibiotics in China, amoxicillin was selected as the model contaminant. The comparisons among these systems were established with amoxicillin concentration and COD_{cr} removal efficiency. The Si/SA process was further characterized by scanning electron microscopy (SEM), infrared spectra (IR), and Michaelis-Menten equation.

2. Experimental

2.1. Materials

Amoxicillin, NaOH, H_2SO_4(AR), diatomite with a surface area of ca. 100 m^2 g^{-1} (AR), ammonium acetate and acetonitrile (HPLC grade) were purchased from Sinopharm Chemical Reagent Co., Ltd., China. Reagents used for the bacterial media were purchased from Sigma. Deionized water was provided by a Milli-Q system (Millipore). Bacterium *STT01* was self-isolated from the activated sludge of urban sewage treatment plant by our research group in 2011.

2.2. Cells immobilization

The process of immobilization of bacteria (*STT01*) was according to the previous research [13]. The resulted pellets were soaked into 4% (w/v) $CaCl_2$ and held for 24 h at 4^oC to immobilize completely, and then were washed with sterile water twice and then for degradation experiment. The control experiments were conducted under the same conditions.To achieve the optimal immobilization conditions, the diatomitedose (1.0, 2.0, 3.0, 4.0 and 5.0, g L^{-1}), and the mass ratio of wet cells to immobilization support (C:IS, 1:10, 1:20, 1:30, 1:40, and 1:50, w/v) were investigated.

2.3. Optimal conditions investigations

All degradation experiments were conducted in 250-mL tapered bottle and statically incubated in an adjustable illumination incubator (250D, Jiangsu Fuhua). As for the Si/SA system, based on large investigations, the basic operation conditions were chosen as follows: amoxicillin 600 mg L^{-1} ($COD_{cr,0}$ = 825 mg L^{-1}); pH 7.0, illumination intensity 3000 lx, temperature 30°C, DO2.0 mg L^{-1} and treatment time 72 h. Here were focused on the dosage of diatomite and the ratio of C: IS, which play the prerequisite role in the work. During the degradation, samples were collected at certain intervals for analyses. Selected samples had been repeatedly analyzed in order to validate the produced results and they were found within acceptable analytical error (±5%). The results were the means of triplicate determinations.

2.4. Analysis

The samples used for COD_{cr} test were collected by centrifuging at 10000 rpm for 20 min to remove bacteria cells. COD_{cr} was tested according to APHA Standard Methods (1995) [14]. IR spectrum was performed on Perkin Elmer FTIR spectrophotometer (USA) in KBr pellet. HPLC-MS were carried out on Thermo Finnigan LCQ-Advantage (USA) according to the methods presented by Nägele and Moritz [15].

2.5. Calculations

The removal efficiency (R_e) was calculated by the following equation:

$$Re = \frac{(C_0 - C_e)}{C_0} \times 100\% \quad (1)$$

where C_0 is the initial concentration of amoxicillin or COD_{cr}, and C_e is the detected concentration of amoxicillin or COD_{cr} in the removal process.

3. Results and discussion

3.1. Optimal parameters

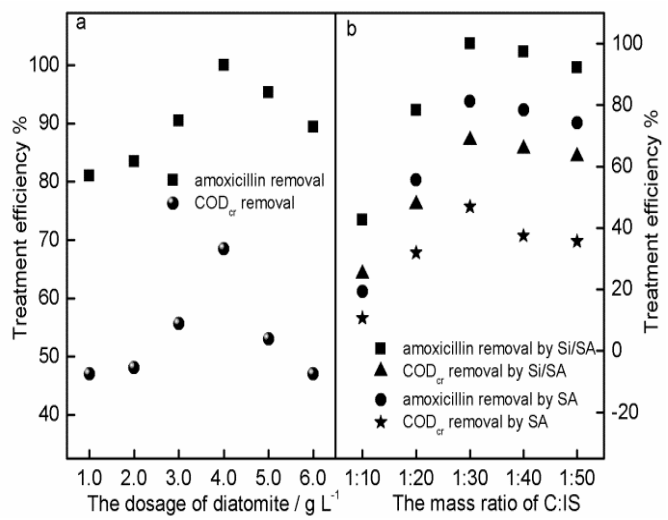

Figure 1. The treatment efficiency with varying dosages of diatomite (a) and mass ratios of C: IS (b) in the Si/SA and SA system correspondingly. Noted: C: IS is the mass ratio of the wet cells to immobilization support.

It was suggested that the appreciable treatment efficiencies were achieved at the dosage of diatomite of 4.0 g L^{-1} (Fig. 1a) and the mass ratios of C: ISof 1:30 (Fig. 1b). When the load of diatomite was more than that of 4.0 g L^{-1}, it made the immobilization support too hard to be pelletized; whereas when the load of diatomite was less than that of 4.0 g L^{-1}, which was found to do little effect on the improvement of treatment efficiencies (Fig. 1a). Similarly, when C: IS was increased to 1:20 or 1:10, higher cell loads might be an effect of substrate and/or light limitation of the cells inside the pellets, decreasing the degradation efficiencies. In addition, the less mass ratio of C: IS couldn't immobilized the cells completely that a great amount of cells was shed off in $CaCl_2$ and then led to lower biomass of *STT01*. On the contrary, the excess of Si/SA or SA load would bring up a rich nutrition environment, which had an inhibitory effect on cells growth, reducing the efficiency. The results might be ascribed that the proper dosages and mass ratio of C: IS could facilitate the preferential mass transfer, sufficient biomass and high light harvest, which was in favour of the performance of photo biological ability of *STT01*[13]. Under the optimal condition, 100%of amoxicillin and 68.5% of COD_{cr} removal efficiency were carried out in the Si/SA system compared with the cases of SA (81.2%, 46.9%), and free cells (60.5%, 35.5%). Additionally, we also found that the biodegradability of immobilization cells were not readily inhibited by the variations of external conditions, which might be due to the preferential retention of bioactivity and the avoidance of product inhibition [16].

3.2. Visual degradation efficiency

Even though amoxicillin could inhibit the growth of isolated free cells of *STT01*, yet it seems to be negligible to *STT01* in Si/SA system due to the increasing bright colour and cell density of the pellets (Fig. 2). It was noted that the removal of amoxicillin and COD_{cr} was not appreciable since the process of hydrolysis might require longer duration for digestion and the feed of amoxicillin influent was more toxic for *STT01* at the first stage, with the time on, the bacteria were gradually accommodated to it and the biodegradability was increased correspondingly. The appreciable treatment efficiencies were

gained in various treatment time, i.e. 100% amoxicillin removal and 68.5% COD_{cr} removal were achieved in 72 h, 48 h, 48 h, 72 h, and 84 h when the pellets were reused five times, respectively. The decreasing treatment time could be ascribed to the increasing biomass and bioactivity of the pellets, leading to higher degradability in shorter time, whereas, when the amount of cells was increased to the maximum, it was potential to lead to a poor nutrition environment in the pellets, and the endogenous metabolism of cells might occur, resulting in the prolonged treatment time. As shown in Figs. 2e-f, the relatively darker colour of the pellets indicated that some cells were autolysis to death, the pellets gradually dissolved out of the pellets, which was also a hint that it was the time to add new active biomass for the continuous degradation.

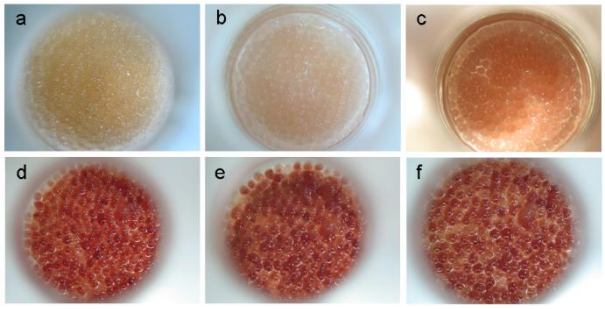

Figure 2. Optical images of the pellets immobilized with Si/SA under various treatment time of before treatment (a), once (b), twice (c), three times (d), four times (e), and five times (f).Noted: Images were taken from the top side when the effluents were emptied out after treatment

3.3. Mechanisms investigation

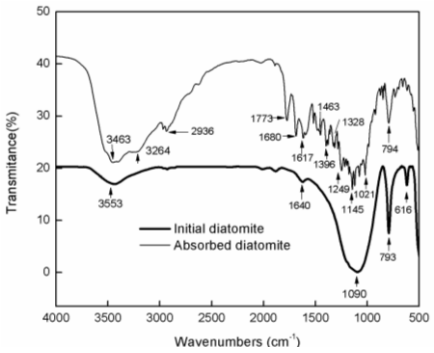

Figure3. IR spectra of the initial and absorbed diatomite

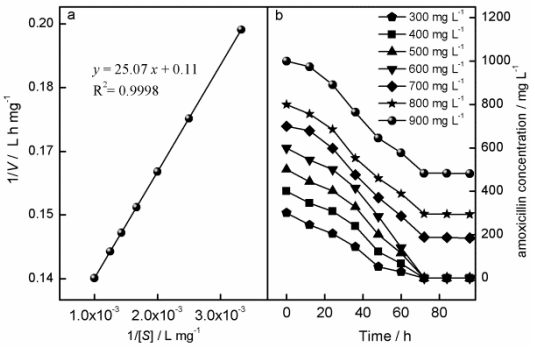

Figure4. Degradation kinetics ofamoxicillin: Lineweaver-Burkplot of 1/V and 1/[S] (a), various initial concentration (b).

3.3.1. Diatomiteabsorption mechanism. As well known, the surface of diatomite is covered by silicon hydroxyl groups with hydrogen bonds. These silicon hydroxyls the surface activity and adsorption properties of the diatomite. Adsorption ability is influenced by the action between adsorbent and absorbate in solution, as well as the pore structure [11, 12, 17].

It could be assumed that diatomite adsorbed amoxicillin in the way that its surface hydrogen bond and surface electric charge reacted with –OH, $–NH_2$, C=O group of amoxicillin to form new hydrogen bonds. To confirm this hypothesis, diatomite was thus alone selected to removal amoxicillin under the same conditions except for the contribution of *STT01*. The IR spectra of the initial and absorbed diatomite were presented in Fig. 3. It was shown that the Si-OH bond of initial diatomite shows bands at 3553$cm^{-1}$and 793 cm^{-1}, Si-O bond at 1090 cm^{-1}, whereas the Si-OH bond of absorbed diatomite took

shift to 3463 cm^{-1} and 794$cm^{-1}$and Si-O bond was changed into 1249 cm^{-1}, 1145$cm^{-1}$and 1021cm^{-1}.Furthermore, the peak around 1773 cm^{-1} might attribute to the C=O of β-lactam ring of amoxicillin; the vibration at 1680 cm^{-1}might assign to the C=O stretching vibration of secondary amido; the peaks at 3463 cm^{-1},3264 cm^{-1}, 2936 cm^{-1},1396 cm^{-1}, 1328 cm^{-1} could assign to the C-H bending or vibration of -CH_2 and -CH_3orN-H of β-lactam ring, and the special absorption at 1617cm^{-1} and 1464 cm^{-1} belong to benzene stretching [19]. The results showed that the physical-chemical adsorption might occur simultaneously on the surface of diatomite. Moreover, 20% of amoxicillin and 18% of COD_{cr} removal achieved in the process further demonstrated that the diatomite was responsible for the improvement of the treatment efficiency. In addition, the silicon hydroxyl is dissociated to ≡Si-O- and H^+ in aqueous solution, so the surface of diatomite carried negative charge with surface ζ potential[11,12], whereas amoxicillin is cationic at neutral condition, so it further deduced that the absorption action could be potentially carried out.

3.3.2. Degradation kinetics. The degradation kinetics were modeled with Michaelis-Mentenequation and were mathematical regression analyzed by Line weaver-Burk method, plotting the inverse of substrate concentration of amoxicillin against the inverse of the initial velocity due to the Michaelis-Mentenequation (2) and (3),

$$V = \frac{V_m[S]}{K_m + [S]} \tag{2}$$

$$\frac{1}{V} = \frac{K_m}{V_m} \bullet \frac{1}{[S]} + \frac{1}{V_m} \tag{3}$$

Where K_m: Michaelis constant/mg L^{-1}, [S]: substrate concentration/mg L^{-1}, V: enzymatic reaction starting velocity/ mg L^{-1} h^{-1}, V_m: enzymatic reaction maximum velocity/mg L^{-1} h^{-1}.

According to the Michaelis-Mentenequation [18], the average degradation velocity within 48 h was selected as the initial velocity of the experiments. The Line weaver-Burk plot (Fig. 4a) was clearly showed that there was a positive relationship between 1/*V* and 1/[S], which suggested the degradation velocity and initial amoxicillin was in line with Michaelis-Mentenequation due to the high R square (0.9998). So, according to Eq. 3, it could be deduced that $1/V_m$ is the intercept of y axis (0.11), and K_m/V_m is the slope (25.07), and thus the maximum degradation velocity (V_m) and Michael is constant (K_m) could be gained as 9.09 mg L^{-1} h^{-1} and 228 mg L^{-1}, respectively. So the enzymatic catalysis was responsible for the removal of amoxicillin in the Si/SA system. In addition, the results demonstrated that the optimal parameters in this experiment are also available for the cases of higher concentrations by scaling up the dosages of diatomite and the amount of the pellets correspondingly (Fig. 4b).

4. Conclusions

This investigation has been confirmed that the immobilized *STT01* is negligible to the toxicity of amoxicillin due to the appreciate removal efficiency. The results further demonstrate that the fast degradation of amoxicillin is due to the synergistic abiotic adsorption of diatomite and the biodegradation of *STT01*. The work opens up a direct visual platform for the degradation process due to the intrinsic bright colour of bacteria, and offers a promising entry for developing novel immobilization techniques of diatomite materials. It is expected to shed a new light on the future investigations on antibiotics-related wastewater.

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