Optimized Operational Parameters for the Biodegradation of the β-lactam Antibiotics and Intermediates in Treating High-strength Organic Wastewater Treatment

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Abstract: Many attentions have been devoted to the fate of antibiotics such as β-antibiotics in the soil and water, especially in the developing countries. The problems of their fate in the environment have to be clearly identified to prevent any environmental pollution. Therefore, it's necessary to research the biodegradation law of β-lactam antibiotics and intermediates if the antibiotics entered the sewage treatment plants (SATS), and to estimate the removals of SATs. Ceftriaxone sodium, cefoperazone sodium, ampicillin sodium, amoxicillin, 6-APA and 7-ACA, typical β-antibiotics, were chosen as the analytes. Typical biologic treatment active sludges, which has been widely used in the developing countries, was transformed in experimental at laboratory-sized in this study to measure the β-lactam antibiotics and intermediates' removal efficiency during the biologic treatment on different carbon sources, temperature, dissolved oxygen and pH value conditions. When using glucose as plus carbon source, the effect of β-lactam antibiotics and intermediate was best, and the optimum mass ratio of glucose and analytes was 1:8. Considering the treatment effect and the processing cost factors, then gained a relatively appropriate condition: controlling temperature at the range from 25 to 35 ℃, pH value in neutral and dissolved oxygen at 2.5 mg/L.

1 INTRODUCTION

In recent years, there are many reports confirmed that different concentration level of drugs was found in surface water, groundwater, drinking water, sludge, soil, biological body and the others kinds of environmental medium (Magda 2011, Halling-Sùren, 1998). And its influences to environment and human health has caused the international social attention. Antibiotics was harmless to human cells, however, while the antibiotics abusing in the developing countries is common, which will produce terrible consequences, that build the pathogenic bacteria resistant ability to antibiotics. At the same time, the antibiotic chemical structure is so complicated with strong bacteriostatic and sterilization effect, belongs to the difficult biodegradable material. Beta-lactam antibioticcontain typical beta lactam ring structure, occupy an important position in antibiotic use. Beta-lactam antibiotic's mechanism of action is to inhibit synthesis of cell wall, it has bactericidal broad-spectrum and very good antibacterial, a good curative effect and hypoallergenic with low adverse (Buynak 2016, Calamari 2003, Bel 2009).

So, antibiotics once polluted the environment, will leave a long-term serious influence on the environmental micro-ecological. Antibiotic substances enter municipal sewage and sewage treatment plants (STPs). If they are not eliminated during sewage treatment, they are emitted into surface water and may reach drinking water (Edward Turos, 2007). As a consequence, the occurrence and fate of pharmaceutically active compounds in the natural environment has been recognized as one of

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the emerging issues in environmental chemistry. Knowledge of pharmaceuticals in the environment is little.

Emad researched that UV/ZnO photocatalysis can be used for amoxicillin, ampicillin and cloxacillin degradation in aqueous solution (James 2010). Besides, they also examined degradation of the antibiotics amoxicillin, ampicillin and cloxacillin in aqueous solution by the photo-Fenton process.

Using the application of sonolysis for the degradation of fluoroquinolone antibiotic ciprofloxacin (Kümmerer 2013), the pH effect on the degradation rate is studied and changes in biodegradability.

Besides chemical and photochemical degradation (Kummerer 2003), the biodegradability of antibiotic released into the environment is an important aspect of their partial or complete decomposition by microorganisms (Chaudhuri 2009), which is the major progress in the sewage treatment plants. Thus, it is important to study on the biodegradation of antibiotic substances in the active sludges. As antibiotics are considered to have an adverse effect on microorganisms (Brosillon 2013).

Using the Closed Bottle test (CBT) (Emad 2010), the biodegradation of some clinically important antibiotic drugs was investigated as the first step of an environmental risk assessment. The CBT was performed according to test guidelines in the dark room temperature (20±1℃) was described elsewhere in detail. The standard test period of the CBT is 28 days. The CBT conditions are low bacterial density and low concentration of organic carbon i.e. test compound. As a result, the antibiotics tested were not biodegraded in the CBT. The results indicate that the various antibiotics were active against different groups of bacteria present in wastewater.

Hybrid processes (Reddy 2006) involving a physical-chemical and pre-treatment like photocatalysis coupled to a biological treatment have been considered for antibiotics' removal. Using photocatalysis as pre-treatment as prior to a biological treatment, an irradiation time of 2h ensure a significant residual organic content for the biological treatment. A decrease of the residual amount of antibiotics contained in the irradiated solution s was recorded, which can be related to an inherent biodegradation since these residual concentrations were below their inhibitory thresholds, 18 and 9 mg/L for TC and TYL.

So, the biodegradation of the beta-lactam antibiotics in the environment, especially in the SATs, is important to the antibitics problems. This study, therefore, investigated the biodegradability of some typical beta-lactam antibiotics in the actives on lab-scales: ceftriaxone sodium, cefoperazone sodium, ampicillin sodium, amoxicillin, 6-APA and 7-ACA to observe the existing conventional biologic treatments ability to remove the antibiotics.

2 EXPERIMENTAL

2.1 Materials

This context selected four beta lactam antibiotics (ceftriaxone sodium, cefoperazone sodium, ampicillin sodium, amoxicillin) and two intermediates (6 - APA ,7 - ACA) as the research object, with a purity of > 99 % determined by HPLC according to the Chinese Pharmacopoeia 2005 (ChP 2005). But methanol (MeoH), formic acid, disodium hydrogen phosphate (Na_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4) (purchased from Tianjin Chemical Reagent Co., China) were of analytical-reagent grade. The water used was domestic sewage. They were filtered by 0.45 μm filter membrane before experiment. Pencil core aerator was used to control the DO of the system, and electric-heated thermostatic water bath was used to control the temperature.

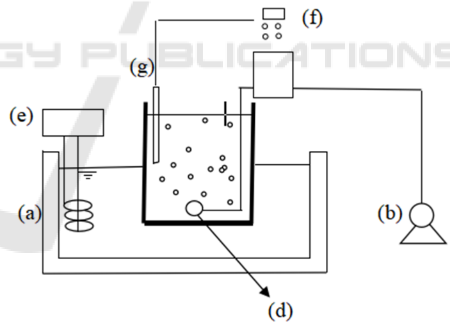


Figure 1: Schematic view of the active sludges. (a) thermostatic bath; (b) air pump; (c) tank; (d) pencil core aerator; (e) thermostatic devices; (f) pH, DO and detector; (g) electrode.

2.2 Characterization

This context selected four beta lactam antibiotics (ceftriaxone sodium, cefoperazone sodium, ampicillin sodium, amoxicillin) and two intermediates (6 - APA 7 - ACA) as the research object. The experimentally characterization for the research object are summarized in Table 1. An Agilent Technologies 1200 HPLC system with an

UV detector and an Eclipse XDB-C18 column (150 mm × 4.6 mm×5 µm) were employed to study the degradation process (Yahiat 2011).

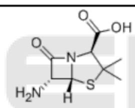
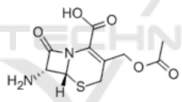
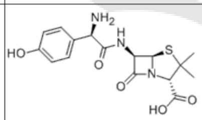
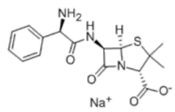
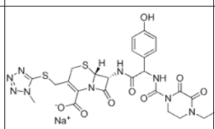
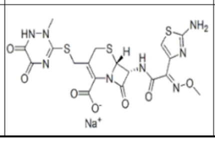
2.3 Degradation Experiments

Activated sludge was collected from municipal wastewater treatment. The basic culture medium contains the following mineral supplementation: K_2HPO_4 (34g/L), $CaCl_2$ (18g/L), $MgSO_4$ (25g/L).

Firstly, biodegradation in different carbon source (regarded as co-metabolism substance) including glucose, sugar, starch and was investigated at 298 K and almost neutral pH with the initial respective concentration of 80 mg/L, and the check test was not added in the culture medium. Selected glucose as the carbon source used in co-metabolism action and was investigated at 298K and almost neutral pH and maintianed DO at 2.5 mg/L, tested biodegradation in series of glucose concentration/respective target contaminants concentration (1:1, 1:3, 1:5, 1:8, 1:10 and 1:15) system.

In the glucose concentration/respective target concentration contaminants (1:8) system, the influence of the pH on the degradation of target contaminants was determined at 5, 7, 9 and with the initial concentration of 400 mg/L, with glucose (50 mg/L) and primary nitrogen source ammonium (32.5 mg/L NH_4Cl) at 298 K and DO at 2.5 mg/L, each condition was aerated for 20h, deposited for 1h per day and run for 20 days. The temperature effect on the degradation of target contaminants was determined at 288, 298 and 308K with the initial concentration of 400 mg/L under pH around 7 and DO around 2.5 mg/L, and also each condition was aerated for 20h, deposited for 1h per day and run for 20 days. Finally the DO effect on the degradation was determined at 1, 2.5 and 4 mg/L with initial concentration of 400 mg/L.

Table 1: Overview of study β-lactam antibiotics.

Antibiotics name	Molecular structure	Molecular weight	Character	Dissolved situation
6-aminopenicilanic acid		216	white crystal	Slightly soluble in water, insoluble in general organic solvent
7-aminocephalospranic acid		272	White crystalline powder	Slightly soluble in water, insoluble in general organic solvent
amoxicillin		419	White crystalline powder	Slightly soluble in water, insoluble in the ethanol
Ampicillin Sodium		371	White powder	soluble in water,slightly soluble in the ethanol,insoluble in the ether
Cefoperazone Sodium		667	White crystallin e powder	soluble in water
Ceftriaxone Sodium		554	White crystallin e powder	soluble in water

3 RESULT AND DISCUSSION

3.1 Degradation of Target Contaminants in Different Carbon Sources System

Fig.2 demonstrates the temporal evolution of the concentration during the temporal evolution of in different carbon sources solvent systems. It shows that, on the basis of the degradation in the out-added carbon sources, the introduction of different carbon sources increased the degradation rate of the target contaminants, the possible reason is that the out-added carbon sources were oxidative decomposed by microorganisms, which used the release of large amounts of energy in decomposition, for the synthesis of their own cellular material, so that the microorganism bloomed, and also letted key enzyme of the β-lactam antibiotics and intermediates's decomposition keep active, and it was conducive to the further degradation (Heberer 2002). And this process is called Co-Metabolism (Smital 2004). Among the three out-added carbon sources, glucose has the monosaccharide structure, and sucrose and starch has disaccharides and polysaccharides structure, so their molecular structure is more complex compared with glucose, the reactions required more kinds of enzymes involved, not as directly and quickly as glucose. Obviously all the target contaminants had the best removal efficiency in carbon sources system of glucose.

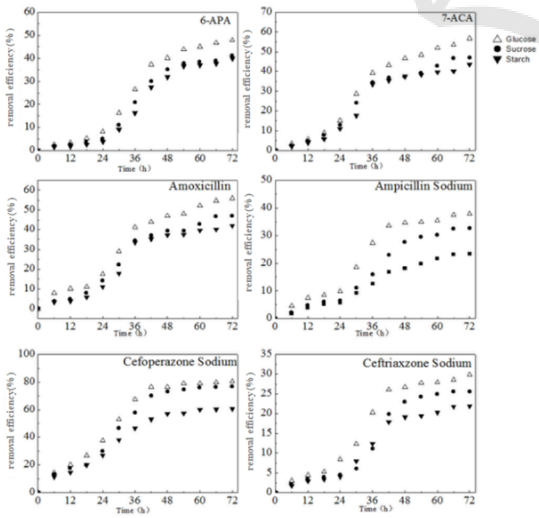


Figure 2. Different target contaminants' removal efficiency in different carbon system.

3.2 Degradation of Target Contaminants under Different Cgc: Ctc (Gc: Glucose Concentration, Tc: Respective Target Contaminants Concentration)

Fig.3 presents removal of target contaminants under different Cgc: Ctc. Under the constant target contaminants concentration condition, between 1:15 and 1:8 the co-substance increased with the increase of removal efficiency of all the target contaminants, and it had a maximum value of all with Cgc at 1/8 of Ctc. When Cgc: Ctc was above 1:8, the removal efficiency decreased with the increase of the co-substance glucose concentration, excessive growth of substance would appear. Therefore, refractory organic matters were disadvantageous for contesting enzymes, resulting in the decreased removal efficiency.

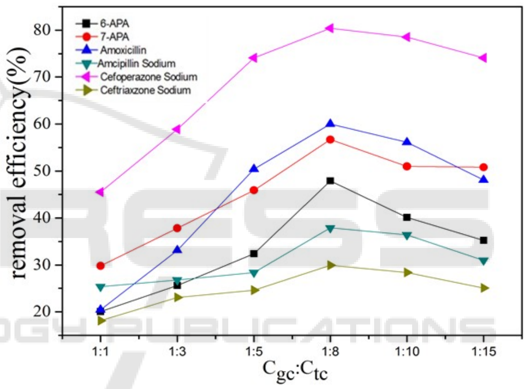


Figure 3: Removal of target contaminants under different Cgc:Ctc.

3.3 pH-Dependent Degradation in the Glucose System

The concentration of target contaminants was monitored during the degradation under different pH condition ranging from 5 to 9, as shown in Fig.3. It shows the process of the active sludges cultivated under different pH with time series. And the average removal efficiency and effluent concentration of all target contaminants was shown in Fig.4. From Fig.4 and Fig.5, it can see that under acidic conditions, degradation is relatively low. And with pH increased, ceftriaxone sodium and ampicillin sodium's degradation efficiency increased with a little margin compared with pH=7, the removal efficiency of 6-APA almost constant, but removal efficiency of cefoperazone sodium, amoxicillin and 7-APA decreased. Considered the appropriate pH of water treatment plants' flows is ranging from 6.5 to 8.5. Fig.3 or Fig.4 showed that when under pH was around 7 (neuter), removal efficiency of all the target contaminants was better considered the relative conditions. Adjusting the pH around 7 for degradation will be helpful to improve the removal efficiency.

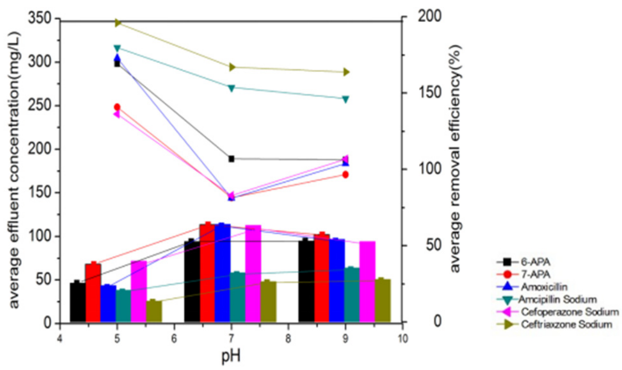


Figure 4: Different contaminants' average removal efficiency and average effluent concentration under different pH.

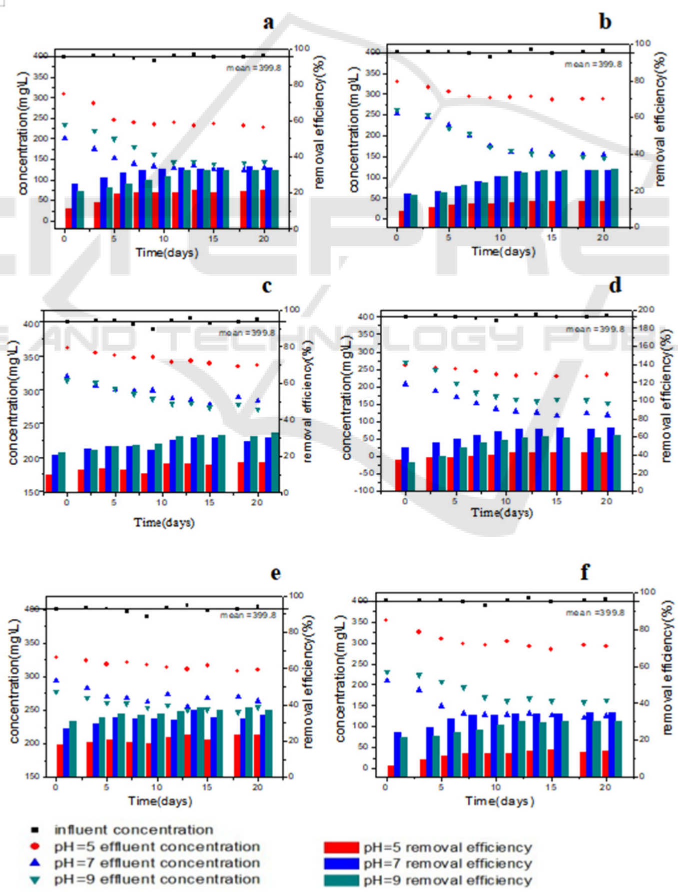


Figure 5: The effluent concentrations and removal efficiency of the different β-lactam antibiotics and intermediates during the 20 days. (a) 7-APA; (b) 6-APA; (c) ceftriaxone sodium; (d) cefoperazone sodium; (e) ampicillin sodium; (f) amoxicillin.

3.4 Temperature Effect on the Degradation of β-lactam Antibiotics and Intermediates

Temperature has a remarkable effect on reaction according to the biodegradation theory. To determine the effect of temperature on target contaminants degradation, the average effluent concentrations and removal efficiency was monitored at 288, 298 and 308K, as shown in Fig.6. The curve lines and histograms in Fig.5 shows the consequent changes of effluent concentrations and removal efficiency in sludge domestication under different temperature. Generally, temperature had a strong effect on degradation, especially under 288K and 298K. It is found that the increase in temperature dramatically accelerates the degradation efficiency, and participated in aerobic biodegradation process microorganism are classified to mesophile microorganism, its optimum growth temperature ranges from 293 to 310K. It also shows that when under 298k or under 308k, the active sludges has similar effluent concentrations and removal efficiency. Considered the energy-consumed, temperature should be controlled under 298K.

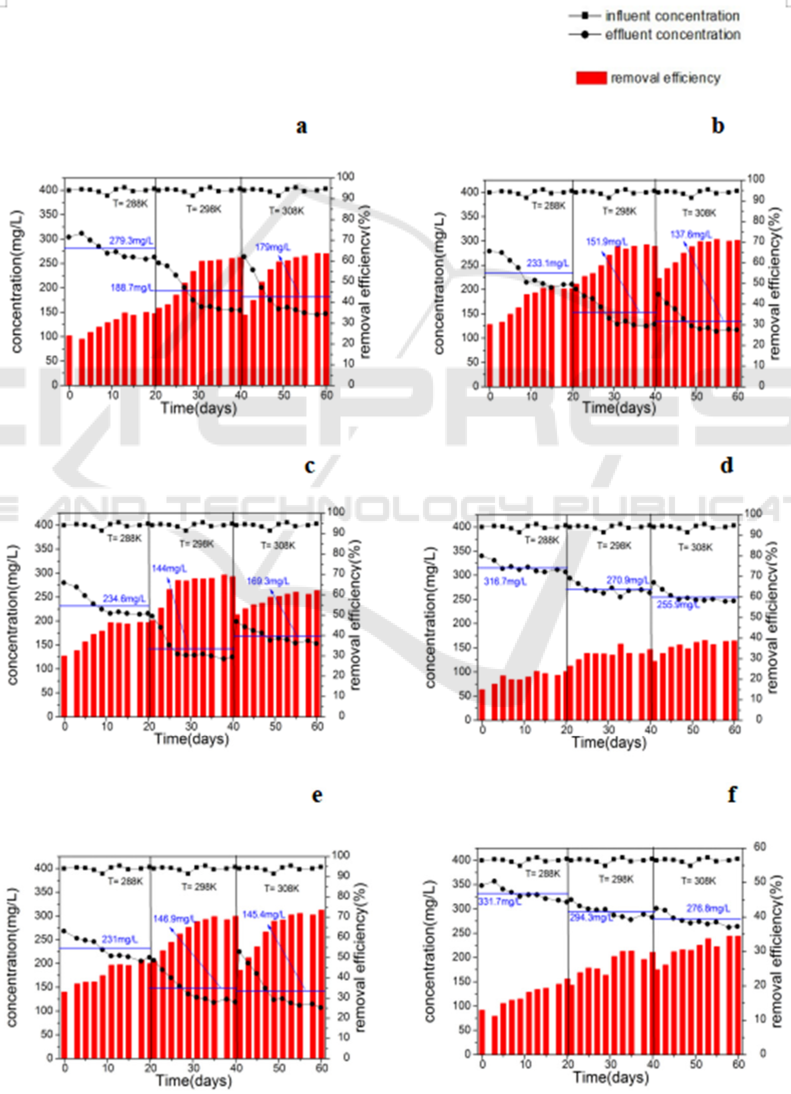


Figure 6: Temperature effect on the effluent concentrations and removal efficiency of the different β-lactam antibiotics and intermediates during the 20 days. (a) 6-APA; (b) 7-APA; (c) amoxicillin; (d) ampicillin Sodium; (e) cefoperazone sodium; (f) ceftriaxone sodium.

3.5 DO Effect on the Degradation of β-lactam Antibiotics and Intermediates

Dissolved oxygen is an important element affecting microbial metabolism. The microorganisms involved in wastewater treatment are mainly based in aerobic respiration. The DO in the reactor must be guaranteed in order to maintain normal aerobic respiration and physiological metabolism of microorganisms in the active sludges. So the low DO concentration has a seriously negative effects, even worse, it decrease the performance of the treatment reactor. While the high DO contents would not only increase the operation costs and reduce the feasibility of the treatment process, but also increase decomposition of pollutants, causing a lack of nutrients for microorganisms, loosely structures of active sludges and poor performance. Average removal efficiency and effluent concentration of the target contaminants during the 20 days' active sludges domestication are show in Fig.6. It shows that with the DO increased, the degradation efficiency increased. There have a little improvement between DO under 2.5 mg/L and under 4.0 mg/L, while removal efficiency of 7-APA decreased when DO contents increased. So considered the more operation costs under a higher DO contents, controlling the DO contents under 2.5 mg/L is more rational.

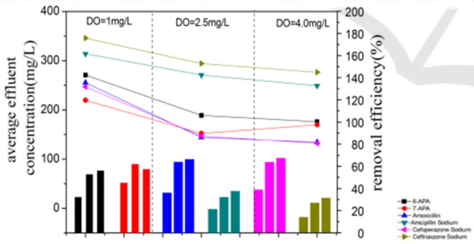


Figure 7: Different contaminants' average removal efficiency and average effluent concentration under different DO (points represented concentration, columns represented removal efficiency).

4 CONCLUSIONS

The degradation of the target contaminants in different systems was determined using HPLC. Out-added carbon sources can accelerate the degradation and out-added-glucose system has higher removal efficiency than sucrose and starch as the carbon sources. And when the initial glucose concentration the initial target contaminants concentration was under 1:8, it has the best removal efficiency.

Investigating the effects of the pH on the degradation of target contaminants in the glucose system revealed that the degradation of 6-APA, amoxicillin and ceftriaxone sodium accelerated generally with the increasing pH, but which was a little improvement compared with under pH=7. So when pH around 7, all the target contaminants had a rational removal efficiency.

The effect of temperature on the degradation of target contaminants is strong. On the basis of the experimental results, when temperature increased from 288K to 298K, the degradation accelerated a lot. While under 298k or 308K, the degradation was similar. So main active microorganism were mesophile.

DO also has a strong effect on the degradation. Experiments results shows that with the DO contents increased, the degradation increased. But there was a small improvement between 2.5 mg/L and 4 mg/L. Considered the operation costing, keep DO under 2.5 mg/L is more practical.

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