Bioresource Technology 102 (2011) 4360–4365

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Performance of enhanced biological SBR process for aniline treatment by mycelial pellet as biomass carrier

Si Zhang, Ang Li, Di Cui, Jixian Yang, Fang Ma *

State Key Lab of Urban Water Resource and Environment, School of Municipal and Environmental Engineering, Harbin Institute of Technology, Harbin 150090, People's Republic of China

ARTICLE INFO

Article history:
Received 6 September 2010
Received in revised form 19 December 2010
Accepted 20 December 2010
Available online 30 December 2010

Keywords:
Aniline
Mycelial pellet
Aspergillus niger Y3
Sequencing batch reactor (SBR)
Bioaugmentation

ABSTRACT

Mycelial pellet of *Aspergillus niger* Y3 was used as a biomass carrier to immobilize the aniline-degrading bacterium, *Acinetobacter calcoaceticus* JH-9 and the mix culture of the COD rapid degradation bacteria. In order to investigate its removal effect on aniline and COD, the combined mycelial pellets were applied in the SBR. Comparison of the performances was conducted between another SBR inoculated with sole strain JH-9 and the above SBR. The results showed that the stable degradations of aniline and COD were observed in both reactors. In the SBR with combined mycelial pellet, the biological removal efficiency was about 0.9 mg aniline/(L·d). It was much higher than that in the activated sludge reactor. Meanwhile, the performances of the sedimentation velocity, liquid–solid phase separation and the effluent quality were better in the SBR. According to SEM images and PCR–DGGE analysis, the species immobilized on the biomass carrier were more predominant in this system.

1. Introduction

In the sewage treatment system, bioaugmentation technology had its unique advantages: shortening the start-up time in the biological system, improving the removal efficiency and reducing the sludge production. Immobilized biological technology was an effective method to immobilize the bioaugmented bacteria onto the carrier in order to maintain the amount of bacteria inoculated. Meanwhile, the sequencing batch reactor (SBR) was applied in the wastewater treatment process, such as bacterial bioaugmentation using *Pseudomonas putida* ONBA-17 treating *o*-nitrobenzaldehyde synthetic wastewater (Yu et al., 2010), enhanced biological phosphorus removal process (Zengin et al., 2010), biological treatment of landfill leachate (Xu et al., 2010) and so on. It suggested that SBR is an effective reactor in the wastewater treatment, especially for the bioaugmentation process.

The choice of carrier is the key factor of immobilized biological technology. Mycelial pellet was a microorganism particle and formed naturally during the fermentation (Bayramoglu et al., 2009). Mycelial pellet could survive stably for more than two months in the flask experiment. And the mycelia pellets formed by *Aspergillus* sp. XF101 could be used as a biomass carrier to immobilize the H_2-producing bacteria, and the performance of this system was stable for over 15 days (Huang et al., 2005). Therefore, the continuous of system could be obtained by controlling the influent and operation conditions. It has been used to decolorize, remove the heavy metals and so on (Cho et al., 2006; Feng et al., 2004). The pellet had good biological activity and fast sedimentation velocity (Binupriya et al., 2008). Meanwhile, the immobilization technology using mycelial pellet as a biomass carrier could establish a micro-ecological environment for the mixed bacteria. Therefore, mycelia pellet could be widely used as the biomass carrier in wastewater treatment process in the future.

The mycelial pellet has been studied extensively in our laboratory. The feasibility and superiority of mycelial pellet as a biomass carrier has been determined. This method could solve the problems of conventional carrier, such as low efficiency mass transfer, less biomass, high cost and so on. And it could also improve the system efficiency and reduce the amount of excess sludge effectively (Zhao et al., 2007; Ma et al., 2008; Ma et al., 2009a,b). The optimal medium composition and culture conditions for the mycelial pellet of *Aspergillus niger* Y3 were determined. According to the characteristics of formation process, the formation and microbe immobilization mechanism of mycelial pellet were further proposed in details. In this study, the mycelial pellet was applied as a biomass carrier in SBR reactor, and the removal efficiency of the target contaminants were monitored. In addition, the dominant microorganisms in this system had been analyzed by SEM and PCR–DGGE.

* Corresponding author. Tel./fax: +86 451 86282107.
E-mail address: mafang@hit.edu.cn (F. Ma).

0960-8524/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.biortech.2010.12.079

2. Methods

2.1. Strains and media

A. niger Y3 used in this study was isolated in our laboratory, and it could flocculate into mycelial pellet under the submerged shaking culture condition. The aniline-degrading bacterium, *Acinetobacter calcoaceticus* JH-9 (XP81659) (Shan et al., 2007) and the mix culture of COD rapid degradation microorganisms were isolated and enriched from secondary settling sludge of Jilin Chemical wastewater treatment plant. The COD rapid degradation microorganisms contained *Aeromonas* sp. F19, *Bacillus* sp. F18, *Acinetobacter* sp. F22, and *Pseudomonas* sp. W2 and *Bacillus* sp. W5 (Su et al., 2006; Wang et al., 2008).

The medium for culturing the mycelial pellet contained (g/L): 10 glucose, 1.0 $KH_2PO_4{\cdot}3H2O$, 1.0 NH_4Cl, and 0.5 $MgSO_4{\cdot}7H_2O$. And the medium for culturing and conserving the spores was Czapek, which contained (g/L): 30 sucrose, 20 agar, 0.5 $MgSO_4{\cdot}7H_2O$, 2.0 $NaNO_3$, 1.0 $K_2HPO_4{\cdot}3H_2O$, 0.5 KCl, and 0.01 $FeSO_4{\cdot}7H_2O$. Both media were autoclaved at 112 °C for 20 min. The medium for strain JH-9 was nutrient broth medium, which contained (g/L): 3.0 beef extract, 10 peptone, 5.0 NaCl, pH 7.0–7.2. The solid medium contained 20 g/L agar. Both media were autoclaved at 121 °C for 20 min (Cox and Thomas, 1992; Mclntyre et al., 2001; Casas López et al., 2005; Kim and Kim, 2004).

2.2. Combined mycelial pellet culture

2.2.1. Preparation of spore suspension

In order to quantify the spores conveniently, spore suspension was used for inoculation in the experiment (Van Suijdam et al., 1982; Hamanaka et al., 2001; Saraswathy and Hallberg, 2005; Van Suijdam et al., 1980). Preparation of the spore suspension was as follows: the distilled water was added onto the solid medium covered with spores under sterile condition, and then the suspension was poured onto a sterile flask after gently shaking. Spore suspension with high concentration was obtained after several repetitions of the above operations. Distilled water was added to dilute the spore suspension to obtain the appropriate concentration, which was measured by visible spectrophotometer under the wavelength of 620 nm.

$$\text{Volume of spore suspension for inoculation} = \frac{0.25 \times 0.1\% \times \text{volume of liquid medium}}{\text{absorbance of spore suspension (A)}}$$

2.2.2. Combined mycelial pellet culture

The culture of strain JH-9 and the mix culture of the COD rapid degradation bacteria were centrifuged for 5 min at 6000 r/min, and the pellets were washed twice using phosphate buffer. Finally, the cells were re-suspended into distilled water, and then the suspensions were obtained.

Spore suspension and the strain JH-9 suspension were inoculated in the medium for mycelium pellet at the same time. After 48 h culture at 30 °C and 140 r/min, the suspension of the COD rapid degradation bacteria was inoculated in order to degrade the intermediates rapidly formed by the aniline biodegradation. Finally, the combined mycelial pellet was formed after 12 h for adsorption (Fomina and Gadd, 2002; Yang and Liau, 1998).

2.3. Reactors and activated sludge

Two laboratory-scale SBRs were operated under the same conditions. The column type reactors have the working volume of 2 L, the total height of 35 cm, and the internal diameter of 10 cm. They were used to cultivate the combined mycelial pellet and aerobic activated sludge, respectively (Adav et al., 2008; Ni et al., 2009; Kreuk et al., 2005). The reactors were operated at 30 °C, in successive cycles of 12 h, including 5 min of feeding, 600 min of reaction, 20 min of sludge settling, 5 min of effluent discharge, 90 min of idling, and a constant airflow of 2 L/h. The volumetric exchange ratio was 60%, and the volume loads of the two reactors were same. The solid retention times were 60 days, and the effective hydraulic retention times were 20 h.

The influent of two reactors was as follows (g/L): 0.5 glucose, 0.025 NH_4Cl, 0.005 KH_2PO_4, 0.005 K_2HPO_4, 0.042 $CaCl_2$, 0.06 $MgSO_4{\cdot}7H_2O$, 0.13 $NaHCO_3$, the trace element solution (1 mL/L), a certain proportion of condensed aniline solution, pH 7.0–7.2. And the trace element solution contained (g/L): 0.3 $FeSO_4$, 0.038 $CuSO_4{\cdot}5H_2O$, 0.115 $ZnSO_4{\cdot}7H_2O$, 0.169 $MnSO_4$, 0.116 H_3BO_3, 0.024 $CoCl{\cdot}6H_2O$, 0.017 $Na_2Mo{\cdot}2H_2O$.

Combined mycelial pellet (refers to 1.5) was inoculated into reactor 1, and the initial mixed liquor suspended solids (MLSS) concentration was about 2364 mg/L. The aniline-degrading bacterium, strain JH-9 were added in reactor 2 with the aerobic activated sludge obtained from the secondary settling tank of Harbin Taiping municipal wastewater treatment plant. The initial MLSS concentration was about 3677 mg/L. And the removal efficiency of the pollutants was expressed as follows:

$$\text{Removal efficiency of the pollutant} = \frac{\text{removal of some pollutant in one cycle (mg/L)}}{\text{MLSS} \times \text{times of one cycle (d)}}$$

2.4. DNA extraction of the sludge samples

Genomic DNA was extracted using a modified technique for extracting DNA from the sludge samples and the combined mycelial pellet (Neilan, 1995).

2.5. Amplification of the bacterial 16S rDNA gene sequences

Polymerase Chain Reaction (PCR) was performed to amplify a variable V3 region of the bacterial 16S rDNA gene with the GC-clamp forward primer GC338f (5′-CGC CCG CCG CGC GCG GCG GGC GGG GCG GGG GCA CGG GGG GAC TCC TAC GGG AGG CAG CAG-3′) and the reverse primer 518r (5′-ATT ACC GCG GCT GCT GG-3′) (Ma et al., 2008). Amplification was done using a PCR thermal cycler Dice (BioRad Co., Ltd., USA) in a reaction mixture: 20 μL containing 2.0 μL 10 × PCR buffer, 2 μL dNTP mixture (2.0 mmol/L), 0.3 μL rTaq polymerase, 1.0 μL of each primer (0.01 mmol/L) and DNA template, and 12.7 μL sterile deionized water. The PCR was run with an initial denaturation at 95 °C for 5 min, and 30 cycles of denaturation at 94 °C for 1 min, annealing at 52 °C for 30 s, and extension at 72 °C for 2 min, with a final extension at 72 °C for 20 min. And the amplified fragments of PCR products were about 200 bp.

2.6. PCR–DGGE analysis

The PCR-amplified DNA products were separated by Denaturing Gradient Gel Electrophoresis (DGGE) using a BioRad Dcode system (BioRad Co., Ltd., USA). The mixtures of 6 μL PCR sample and 6 μL 10 × buffer were applied onto 6–12% (w/v) polyacrylamide gels in a running TE buffer. The gels were prepared with a denaturing gradient from 40% to 60% of urea and formamide (7 mol/L urea and 40% formamide as 100% denaturants). Electrophoresis was conducted at a constant voltage of 130 V for 7 h at 60 °C. After electrophoresis, the gels were silver stained and photographed by a gel imaging instrument. The gel images scanned were transferred to