Journal of Hazardous Materials 164 (2009) 646–650

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

The potential of the acetonitrile biodegradation by *Mesorhizobium* sp. F28

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ARTICLE INFO

Article history:
Received 3 April 2008
Received in revised form 8 August 2008
Accepted 14 August 2008
Available online 22 August 2008

Keywords:
Acetonitrile
Amidase
Biodegradation
Mesorhizobium sp.
Nitrile hydratase

ABSTRACT

Mesorhizobium sp. F28 was used in the NHase/amidase enzyme system to convert acetonitrile into acetamide and acetic acid, and the cells grew with the production of acetic acid. The NHase activity of the strain F28 was 78 U mg^{-1} dcw, observed in the conversion of 19.5 mM acetonitrile at 0.2 h. As the initial pH value was between 6.5 and 8.3, 18.3 mM acetonitrile completely converted into acetamide within 2 h and the accumulation of acetamide subsequently converted into acetic acid and ammonia within 46 h. When 20.3 mM acetamide was added in the medium, the conversion rate of acetonitrile was 80% at 2 h and the conversion rate of the accumulative acetamide was slightly affected. The concentrations of acetic acid and ammonia were respectively 6.01 and 6.68 mM at 46 h. The addition of acetic acid decreased the activities of the NHase and amidase. The conversion rate of acetonitrile was 94% at 9.5 h and traces of acetic acid (0.25 mM) and ammonia (0.29 mM) were produced. The effects of product-inhibition indicated that the appropriate operation of bioreactor would be beneficial for *Mesorizobium* sp. F28 to degrade acetonitrile continuously.

1. Introduction

Acetonitrile is widely used in the industries as perfumes, rubber products, pesticides, or used to make pharmaceuticals. It is also applied as a mobile phase for HPLC analyses, or as a solvent to extract fatty acids from animal and vegetable oils [1,2]. The efficient process for acetonitrile removal should be designed to avoid the risks of waste discharging into the natural environment since acetonitrile is a toxic chemical that may cause severe health effects [3]. The microbial process is of interest to use in the detoxification of acetonitrile-containing waste. The packed-bed biofilm reactor equipped with an external aeration chamber for acetonitrile removal under aerobic conditions has been designed to investigate the treatment of acetonitrile HPLC waste [4]. The biodegradation of acetonitrile into ammonia and acetic acid has been developed, and the conversion efficiencies were 92–100% as the organic load was 2 g acetonitrile l^{-1} d^{-1} [1]. The biodegradation of acetonitrile of the adapted mixed culture in the batch reactor shows a two-step pathway with the generation of acetamide followed by acetic acid and ammonia [5]. For complete removal of acetonitrile in the bioprocess, microorganisms which can efficiently degrade acetonitrile are of considerable importance, and the proper operation must be determined. The strains, *Nocardia rhodochrous* LL100-21 [6], *Pseudomonas putida* [3], *Chromobacterium* sp., *Pseudomonas aeruginosa* [7], *Geotrichum* sp. JR1 [8], and *Rhodococcus* sp. RHA1 [9] can use acetonitrile as the sole carbon and nitrogen source. *Mesorhizibium* sp. F28, which was isolated from the nitrile-polluted wastewater, contains the nitrile hydratase (NHase, EC 4.2.1.84)/amidase (EC 3.5.1.4) enzyme system to efficiently convert acrylonitrile [10]. In this research, the biodegradation of acetonitrile by *Mesorhizibium* sp. F28 was investigated. Moreover, the optimum pH and the product-inhibitory effects for acetonitrile removal had been examined to realize the suitable operational process.

2. Materials and methods

2.1. Organisms, media and culture conditions

Mesorhizobium sp. F28 (GenBank accession number EU350515) was isolated from a wastewater treatment system in a polyacrylonitrile (PAN) fibre factory in Taiwan. The bacterium was maintained by transferring colonies at approximately monthly intervals on R2A agar [11] stored at 4 °C. For the preparation of resting cells, single colonies from R2A agar plate stock cultures were subcultured at 30 °C and shaken at 120 rpm in R2A liquid medium. The preculture was inoculated into R2A liquid medium containing 0.01 g l^{-1} $CoCl_2 \cdot H_2O$. The medium incubated at 30 °C

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doi:10.1016/j.jhazmat.2008.08.039

for 24 h under aeration and harvested by centrifugation (6000 × *g* for 12 min). The harvested cells were washed with the phosphate buffered medium (PBM, pH 7.5) [12,13], and recentrifugated under the same conditions. The cell pellets were used as resting cells.

2.2. Acetonitrile biodegradation

To observe acetonitrile degradation by *Mesorhizobium* sp. F28, a series of batch experiments were conducted in 120 ml serum bottles. Each serum bottle containing cells suspended in 40 ml phosphate buffer medium (PBM, pH 7.5) to a cell concentration of 10^7 cell ml $^{-1}$, and then various concentrations of acetonitrile (2.32, 4.20, 9.34, 12.1, 18.3, and 19.5 mM) were added to the medium. After sealing with teflon/silicon stoppers, the reactors were shaken at 120 rpm in the dark at 30 °C. The concentrations of acetonitrile, acetamide, acetic acid and ammonia were analyzed at regular intervals. The value of pH and O.D.$_{600}$ were also observed in the experiment. One unit (U) of NHase activity was defined as the amount of resting cells that catalyses 1 μmol of acetonitrile per min under the specified conditions.

2.3. The optimum pH for acetonitrile biodegradation

The same process of the batch experiments was used except that the pH value of the PBM. Various pH values of phosphate buffer medium (pH 4.2, 5.5, 6.5, 7.5, 8.3), which were adjusted with NaOH/HCl, were adjusted to investigate the optimum pH for acetonitrile biodegradation. Acetonitrile (18.3 mM) was added to observe its biodegradation by *Mesorhizobium* sp. F28.

2.4. The effect of product-inhibiton on the acetonitrile biotransformation

To investigate the effect of acetamide and acetic acid on the acetonitrile biotransformation by *Mesorhizobium* sp. F28, the same process of the batch experiment was conducted. Each serum bottle (40 ml PBM, pH 7.5), which contained cell suspension (10^7 cell ml^{-1}) and acetonitrile (15.4 mM), was supplemented with acetamide (12.4, 16.4, and 20.3 mM) or acetic acid (12.8, 16.5, and 21.6 mM). The reactors were shaken at 120 rpm in the dark at 30 °C to observe acetonitrile biodegradation.

2.5. Analytical methods and chemicals

Samples were collected directly from the reactors using a syringe and then filtered (Millex GV-Filter, 0.22 μm pore size, Millipore). Acetonitrile, acetamide, and acetic acid were determined by a gas chromatograph (GC) equipped with a flame ionization detector. GC was performed with a HP 6890 system equipped with an Agilent DB-WAXETR column (1.0 μm × 30 m, 0.35 mm I.D.; USA). The injector and detector temperatures were both set at 250 °C. The column temperature was 100 °C for the initial 10 min after injection, followed by a temperature ramp at 6 °C min^{-1} up to 180 °C. Nitrogen gas was supplied as the carrier gas and the flow rate was 3.0 ml min^{-1}. The ammonia concentration was measured using the indophenol blue method [14]. The pH and O.D.$_{600}$ were measured respectively using a pH meter and Spectrophotometer (HITACHI U2800). All chemicals were from commercial sources and of analytical grade.

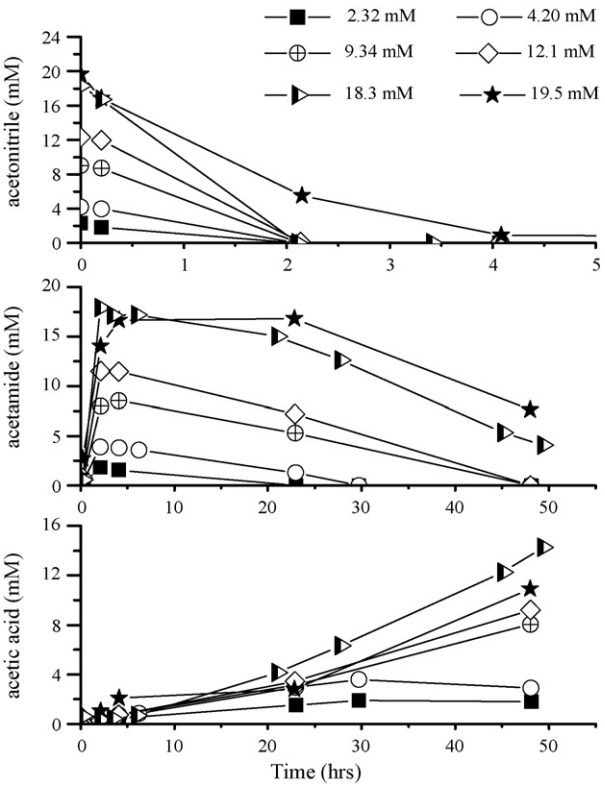

Fig. 1. The biodegradation of acetonitrile by *Mesorhizobium* sp. F28. Various concentrations of acetonitrile (2.32, 4.20, 9.34, 12.1, 18.3, and 19.5 mM) were added to the phosphate buffer medium (PBM, pH 7.5). The reaction was carried out at 30 °C with 120 rpm shaking.

3. Results and discussion

3.1. The biodegradation of acetonitrile by Mesorhizobium sp. F28

Fig. 1 shows the results of acetonitrile in different initial concentrations (2.32, 4.20, 9.34, 12.1, 18.3, and 19.5 mM) being converted into acetamide and acetic acid by the strain F28. Complete acetonitrile removal was achieved within 2 h when the initial concentration of acetonitrile was lower than 18.3 mM. The accumulation of acetamide gradually converted into acetic acid and ammonia by the amidase of *Mesorhizobium* sp. F28. As the acetonitrile concentration reached to 19.5 mM, this strain completely converted acetonitrile into acetamide and acetic acid within 4 h. The maximum NHase activity (78 U mg^{-1} dcw) was observed in the conversion of 19.5 mM acetonitrile at 0.2 h. Though the high concentrations of acetonitrile (2.32, 4.20, 9.34, 12.1, 18.3, and 19.5 mM) were added, the high accumulation of acetamide (1.83, 3.90, 8.54, 11.5, 17.9, and 16.8 mM) still efficiently converted into acetic acid and ammonia. At the end of the reaction, the acetic acid concentrations were 1.81, 3.60, 8.05, 9.20, 14.2, and 10.9 mM, respectively. The ammonia concentrations were 2.54, 4.50, 7.60, 8.58, 11.4, and 8.83 mM, respectively. During the experiment period, the O.D.$_{600}$ values increased obviously as the acetic acid was produced (data not shown).

The results of the acetonitrile biodegradation by *Pseudomonas putida* indicated that the pH increased rapidly from 7.0 to 8.9 with the high accumulation of ammonia (60.0 mM) and low production of acetic acid which was initially produced to 3.80 mM while disappeared after 36 h of incubation [3]. As *Pseudomonas aeruginosa* converted acetonitrile into acetic acid and ammonia, the pH increased from 7.0 to 8.9 since the ammonia concentration