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Bioaugmentation: a new strategy for removal of recalcitrant compounds in wastewater—a case study of quinoline

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Abstract: To demonstrate the feasibility of using bioaugmentation to enhance biodegradation of quinoline, four strains capable of using quinoline as sole source of carbon, nitrogen and energy were isolated from different environmental samples by enrichment technique. Screening for quinoline degrader with the highest quinoline-mineralizing rate was carried out in respirometer and one bacterium identified as *Burkholderia pickettii* W2 was chosen as inoculum in bioaugmentation tests. Quinoline biodegradation experiment results showed that this bacterium degraded quinoline very quickly. 100, 200 and 500 mg/L quinoline can be transformed completely within 1, 2 and 7 hours respectively. A bioaugmentation procedure was proposed and laboratory experiments confirmed that bioaugmentation was an effective way to improve the performance of traditional wastewater treatment facilities for quinoline removal. The effect of inoculum size on bioaugmentation was also investigated in this paper.

Key words: bioaugmentation; biodegradation; quinoline; inocula

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Introduction

In recent years xenobiotics have caught world's attention because they are poorly biodegradable and threaten public health if handle carelessly (Singleton, 1994). The immediate removal of xenobiotics is a real challenge for waste treatment facilities. When treating wastewater containing xenobiotics, most of these compounds pass through conventional wastewater treatment facilities unaltered. In addition, they also have adverse impact on the composition and activities of microorganism communities in activated sludge flocs, thus reducing the overall performance of these facilities(Buchtmann, 1997).

Bioaugmentation is an innovative way in dealing with this problem and some successes of applications have showed it is a promising technique(Limbergen, 1998). Through the addition of specialized indigenous or allochthonous microorganisms to waste treatment facilities, the removal of xenobiotics can be enhanced. Bioaugmentation has been used in bioremediation of polluted soil for decades, and recently it has also been applied to improve the treatability of wastewater containing xenobiotics(Babcock, 1992).

Quinoline belongs to a class of compounds called NHAs (N-heterocyclic aromatic compounds), which are ubiquitous environmental contaminants (Fetzner, 1998). Some studies showed that quinoline is a mutagen with the Ames assay and a carcinogen in mice and rats(Sutton, 1996). Quinoline has been produced and used in large quantities, and can not be destroyed easily in wastewater treatment facilities(Zhang, 1998). Several different strains capable of using quinoline for growth were isolated and identified (Brockman, 1989; Aslabie, 1990), but the bioaugmentation procedure using these strains to enhance the biodegradation of quinoline in wastewater has not yet been proposed so far.

The object of this study was to choose high-efficiency quinoline degrader and give a feasible bioaugmentation procedure.

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1 Materials and methods

1.1 Chemicals and media

All chemicals used in this study were reagent grade or better. Liquid mineral salts medium (MSM) with different quantity of quinoline was used for enrichment and degradation experiments. The composition of MSM is illustrated in Table 1. Solid quinoline-MSM (2% agar) was used for isolation and maintenance of microorganisms.

Table 1 The composition of MSM

Na_2HPO_4	KH_2PO_4	$MgSO_4 \cdot 7H_2O$	$FeSO_4 \cdot 7H_2O$	$CaCl_2$	$MnSO_4 \cdot 7H_2O$
4.26	2.65	0.2	0.01	0.02	0.002

1.2 Enrichment and isolation of microorganisms

Different environmental samples, such as sludge from coke-plant wastewater and domestic wastewater treatment facilities, were used for enrichment. After centrifugation, one gram of inoculum was introduced into 100 ml of MSM with 0.2 g/L quinoline. Cultures were maintained at 28℃ on a rotary shaker (180 r/min). After every five days of incubation, 5 ml of the enrichment cultures were transferred to 100 ml of fresh quinoline-MSM. Three to four transfers were made before pure cultures of microorganisms were recovered from the liquid enrichment medium by streak plating onto solid quinoline—MSM.

1.3 Screening and identification of microorganisms

Respirometer was used to screen high-efficiency quinoline degrading microorganism. The cumulative oxygen uptakes of each microorganism at different quinoline concentrations were recorded at intervals of 20—30 min over 24 hours. By comparing these data, a microorganism with highest quinoline-mineralizing rate was chosen for following experiments. The identification of microorganisms were conducted by biolog microstation system(Biolog Inc., USA).

1.4 Biodegradation of quinoline

The same inoculum size was used in every 250 ml Erlenmeyer flask containing 1000 ml of sterile MSM with different initial quinoline concentrations. The final cell density was about 1g dry weight per litter. The shaker condition was the same as in enrichment experiment.

1.5 Bioaugmentation experiment

Bioaugmentation experiment was carried out in 250 ml Erlenmeyer flasks, each of which contained 100 ml of domestic wastewater withdraw from the sewers of Tsinghua campus. The basic characteristics of this wastewater was: COD = 1100 mg/L, SS = 600 mg/L, NH_3-N = 25 mg/L. Different inoculum size was used to investigate its effect on bioaugmentation. A control with no inoculation was included. Every flask received the same quinoline concentration (300 mg/L).

1.6 Analytical method

For quinoline quantification, a HPLC system (Hewlett-Packard model 5050 with an UV detector) was used. Samples were prepared by centrifugation and filtration, 20 μl of which was injected. Separation was carried out in a C^{18} reverse-phase column, 250 × 4.6 mm, 5 μm(Hewlett-Packard Zorbax SB-C^{18}, USA). The elution solvent, which consisted of a mixture of methanol and water (60 : 40, v/v), was introduced to the column at a flow rate of 1 ml/min. Quinoline was detected at 275 nm.

2 Results and discussion

2.1 Screening and identification of high-efficiency quinoline degrader

From enrichment cultures of different environmental samples, four different bacteria, named *W2*, *G2*, *GM* and *WY*, were isolated. All of these four strains can use quinoline as sole carbon,

nitrogen and energy source. Screening the high-efficiency quinoline degrading bacterium with the highest quinoline-mineralizing rate among these four strains was conducted by respirometer. Results are shown in Fig. 1.

It can be seen from Fig. 1 that at different quinoline concentrations, strain *W2* was always the fastest one to achieve the maximum cumulative oxygen uptake, i. e. strain *W2* had the highest mineralizing rate at a specific time compared with the other three strains. Therefore, strain *W2* was chosen as inocula for further biodegradation and bioaugmentation experiments.

Strain *W2* was identified as *Burkholderia pickettii W2* according to the report of biolog microstation system (ID = 0.733). It was a gram negative, rod-shaped, aerobe. Colonies were mucoid and light orange when grown on solid quinoline-MSM used in this study.

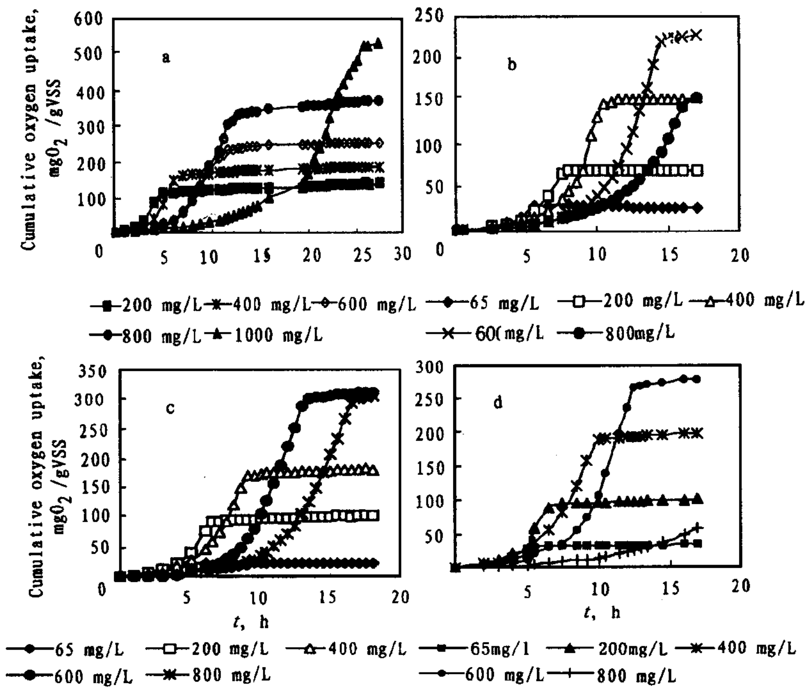

Fig. 1 Oxygen uptakes of different strains
a. W2; b. G2; c. GM; d. WY

2.2 Biodegradation of quinoline

Four different initial quinoline concentrations were used in this experiment. The time course of quinoline degradation by *Burkholderia pickettii W2* is illustrated in Fig. 2.

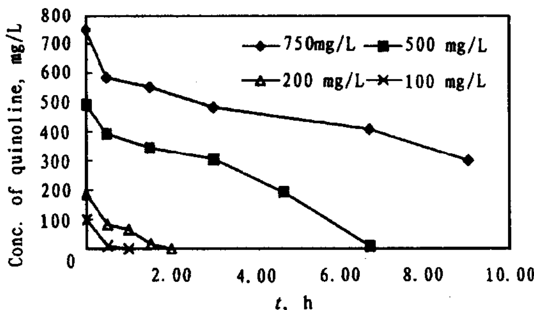

Fig. 2 Quinoline degradation by *Burkholderia pickettii W2*

Fig. 2 demonstrated that *Burkholderia pickettii W2* degraded quinoline as soon as it was inoculated without lag time. This was desirable for application in bioaugmentation. Compared with other quinoline degrading microorganisms isolated by other researchers (Aslabie, 1990), this one not only degraded quinoline much faster, but also transformed a much higher quinoline concentration. For instance, it can degrade 100, 200 mg/L and 500 mg/L quinoline completely within 1, 2 and 7 hours respectively, and transform 750 mg/L