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# An internal airlift loop bioreactor with *Burkholderia pickttii* immobilized onto ceramic honeycomb support for degradation of quinoline

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**Abstract**

A novel kind of bioreactor with a ceramic honeycomb support installed in a draught tube was developed and the ceramic honeycomb support could be used for immobilization of microbial cells. A strain of bacterium was isolated from activated sludge and identified as *Burkholderia pickttii* for degradation of quinoline and could grow on quinoline which served as a sole source of carbon, nitrogen and energy. Quinoline was degraded by the bioreactor in both batch and continuous flow operations when they were immobilized onto the ceramic honeycomb support. Experimental results indicated that quinoline could be degraded effectively by *B. pickttii* immobilized onto the ceramic support. Immobilized *B. pickttii* exhibited better stability in its metabolism and proliferation and degradation of quinoline in continuous flow operation conditions. More than 95% of quinoline could be removed for 4 h of hydraulic retention time (HRT). Quinoline was degraded through 2-hydroxy quinoline according to the analysis of gas chromatography–mass spectrum (GC–MS).


*Keywords:* Immobilized cells; Airlift bioreactors; Biodegradation; Wastewater treatment; Mixing; Quinoline

## 1. Introduction

Immobilized cell bioreactors prevent washout of biomass, as found in free cell flow bioreactors, and lend themselves to greater operational flexibility because flow rates and the kinetics of the bioreactors are controlled [1]. Immobilized cells have the potential to be much more resistant to fluctuation used in concentrations [2]. There have been many reports on the aerobic treatment of organic wastewater by bioreactors with immobilized cells including packed bed, fluidized bed or airlift reactors [3–9]. But there exists more resistance to flow in a packed bed of bioreactor, and gas–liquid transfer is usually uneven, especially as the bed would be blocked up partially with cell growth during the aerobic wastewater treatment. Partial anaerobic area was formed, and then influenced the result of wastewater treatment. The transfer coefficient from gas to liquid would be lowered, as the small air bubbles would easily form larger bubbles in fluidized bed bioreactors including airlift bioreactors as they float upwards. A novel kind of internal airlift loop (IAL) bioreactor with cells immobilized onto ceramic honeycomb support installed in a draught tube was developed to overcome all of these drawbacks.

The bioreactor was used for quinoline biodegradation. Quinoline belongs to a class of organic compounds called NHAs (*N*-heterocyclic aromatic compounds) which are ubiquitous environmental contaminants [10]. Quinoline and its derivatives are widely used in chemical processes, pharmaceutical industries and wood treatment [11]. Discharge of quinoline affects human health and causes environmental damage [12]. So far most of the research efforts on quinoline removal from the environment have been devoted to the isolation of different quinoline degraders and pathways of quinoline transformation by these strains [13,14].

## 2. Materials and methods

### 2.1. Bioreactor

The bioreactor was modified based on bubble column (BC) and IAL by installing a draught tube and ceramic honeycomb support in the draught tube respectively to make up an IAL with ceramic honeycomb support (IAL-CHS). The bioreactor was made of plexiglass, with internal

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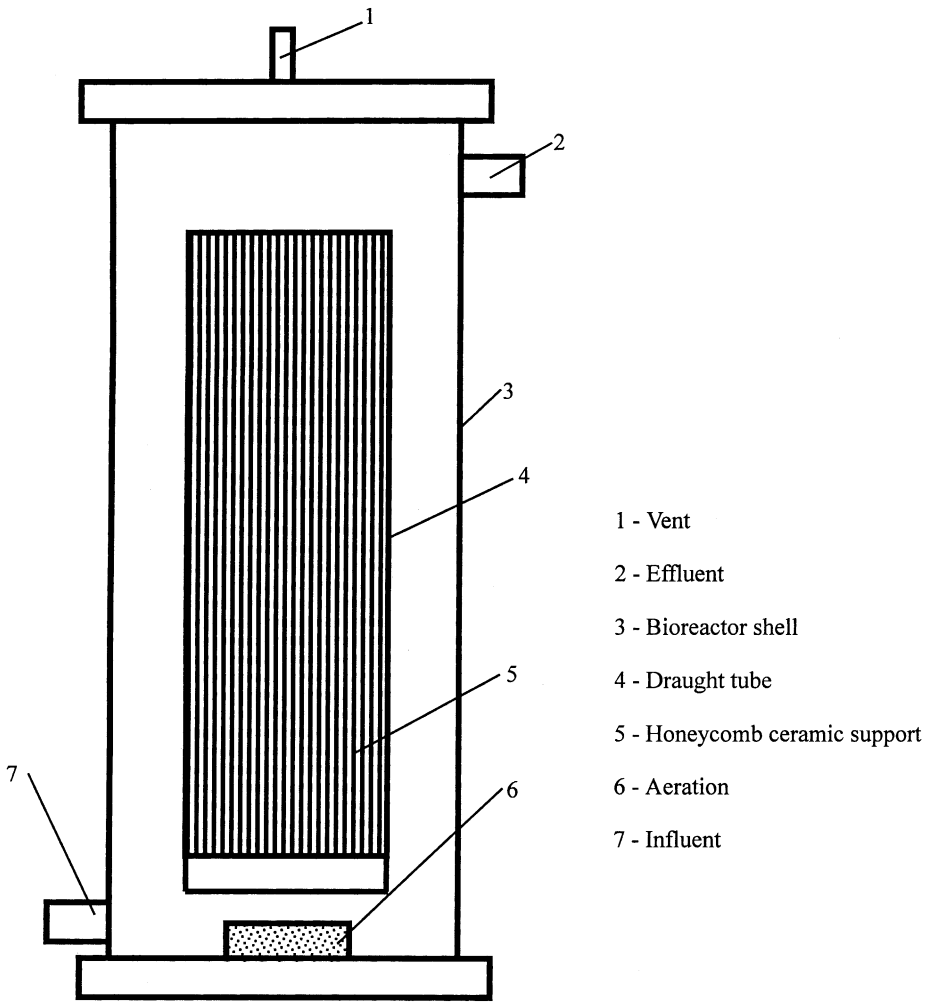


Fig. 1. Internal airlift loop bioreactor with ceramic honeycomb support (IAL-CHS).

diameter of 100 mm, height of 250 mm, draught tube diameter of 60 mm, and working volume of 1500 ml. The bioreactor was shown in Fig. 1. It can be regarded as fluidized bed bioreactor on the whole, but as packed bed bioreactor only from the draught tube.

### 2.2. Support

Ceramic honeycomb support was shown in Fig. 2, with a diameter of 59 mm, height of 150 mm, with a 3 mm × 3 mm of small hole in its section, and a number of micropores inside the ceramics. The hygroscopic rate of ceramics was 62.0%.

### 2.3. Strain

Activated sludge from aeration tank for coke-plant wastewater treatment was used for isolation of microorganisms. After centrifugation, 1 g of inoculum was introduced into 100 ml of mineral salts medium with 0.2 g/l quinoline. The mineral salts medium of 1000 ml consisted of 4.26 g of $Na_2HPO_4$, 2.65 g of $KH_2PO_4$, 0.2 g of $MgSO_4{\cdot}7H_2O$, 0.01 g of $FeSO_4{\cdot}7H_2O$, 0.02 g of $CaCl_2$ and 0.002 g of $MnSO_4{\cdot}7H_2O$. The culture was maintained at 28 °C in a shaker with 180 rpm. After every 5 days of incubation, 5 ml of the enrichment culture was transferred to 100 ml of fresh mineral salts medium with 0.2 g/l quinoline. Three to four transfers were made before pure culture of microorganisms was recovered from the liquid enrichment medium by streak plating onto solid quinoline–mineral salts medium. The strain of microorganism was identified as *B. pickttii* according to the report of Biolog Microstation System (Biolog, USA). *B. pickttii* is a Gram-negative, and a rod-shaped aerobe. It can grow on quinoline as the sole source of carbon, nitrogen and energy.

### 2.4. Wastewater

The wastewater was imitated by adding some quinoline into tap water, which consisted of $C_6H_4(CH)_3N$ mainly and some calcium and magnesium ions etc. coming from tap water.

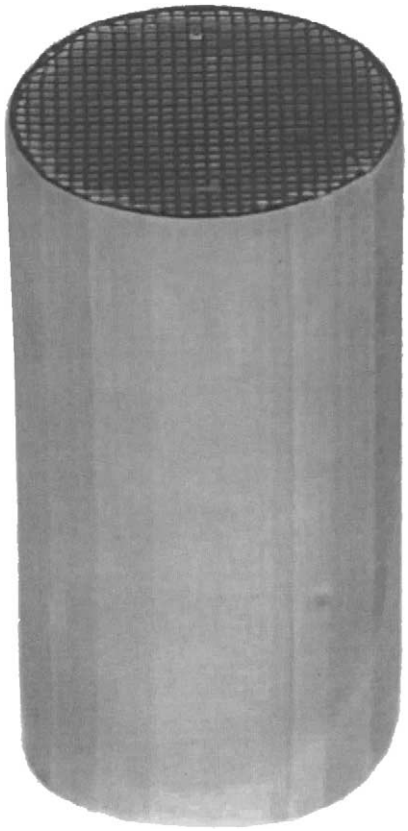

Fig. 2. Ceramic honeycomb support.

### 2.5. Culture medium

One liter of culture medium consisted of 10 g of tryptone, 5 g of glucose, 5 g of sodium chloride, 5 g of beef extract, and 2 g of yeast extract, and the pH of culture medium was adjusted between 7 and 7.2 by sodium hydroxide.

### 2.6. Cells of immobilization

*B. pickttii* was first inoculated into culture medium in a shake flask to proliferate under 28 °C and 180 rpm, then the enrichment solution of the cells was added into the bioreactor directly and the cells were immobilized onto ceramic honeycomb support by adsorption. The adsorbed *B. pickttii* was cultured in the bioreactor for 48 h, here adsorbed in the micropore of ceramics firmly.

### 2.7. RTD of the bioreactor

The hydraulics of the bioreactor is usually an important parameter of the bioreactor. So the hydraulics of the bioreactor was investigated by the tracer element to determine their resident time distribution (RTD), and compared with IAL and BC, as it was modified based on the IAL and BC. During the experiment, when pure water flowed through these bioreactors at steady flow rate, 5 ml of solution including potassium dihydrogen phosphate was injected into the bioreactor instantaneously. Then effluent samples were taken at a certain interval to monitor the concentration of potassium ion by spectrophotometer of model 180-80, with a wavelength of 766.5 nm, and flame air–acetylene under oxygenation.

### 2.8. Biodegradation and detection of quinoline

The wastewater including quinoline was biologically treated in both batch and continuous flow operations. The reaction pathway for quinoline degradation was mainly that quinoline was translated into final products through 2-hydroxy quinoline by *B. pickttii*. For quinoline quantification, high performance liquid chromatography (HPLC) system (Hewlett-Packard model 5050 with an UV detector) was used. A sample of 20 μl was injected after centrifugation and filtration. Separation was carried out in a $C^{18}$ reverse-phase column, 250 mm × 4.6 mm, 5 μm (Hewlett-Packard Zorbax SV-C18, USA). The elution solvent, which consisted of a mixture of methanol and water (60:40, v/v), was introduced into the column at a flow rate of 1 ml/min. Quinoline was detected at 275 nm.

## 3. Results and discussion

### 3.1. Configuration of immobilized B. pickttii

The configuration of *B. pickttii* immobilized in micropore of ceramic support was shown in Fig. 3. The micropore in ceramics is an excellent microenvironment for *B. pickttii* to metabolize, proliferate and degrade quinoline as it has better absorptivity to both cells and quinoline. The debris-like deposits may be those of calcium and magnesium due to better adsorbability of microporous ceramics according to the authors' opinion.

### 3.2. The hydrodynamics of the bioreactor

The concentration of potassium ion vs time was drafted in Fig. 4. The concentration of potassium ion achieved the top in split second, then declined then declines as exponential model, which showed that the bioreactor belonged to complete mixed model, which could be described by a combined mathematical model [15–17], i.e. distributive density function $E(t)$ could be expressed as

$$E(t) = \frac{(1-\lambda)^2}{\eta} \exp\left[-\frac{q(1-\lambda)}{\eta V}t\right]$$

Here $\lambda$ is the bypass ratio of liquid flowing through the bioreactor, $\eta$ the ratio of full mixed zone, $q$ the liquid flux and $V$ the volume of the bioreactor. For the three kinds of bioreactor, their $\lambda$ and $\eta$ were shown in Fig. 5. IAL-CHS bioreactor had improvement over IAL and BC; according to Fig. 5 its bypass ratio was much less than that of IAL

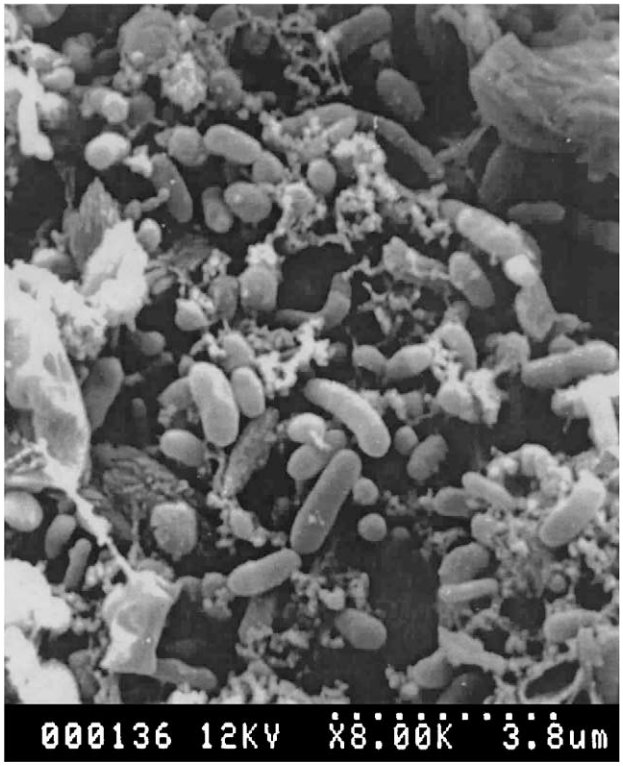


Fig. 3. Photomicrographs of *B. pickttii* immobilized in micropore of ceramics.

and BC bioreactor and full mixed zone ratio was greater than that of IAL and BC. In addition, ceramic honeycomb support had a function to cut bubbles into smaller ones, and prompt oxygen transfer.

### 3.3. Quinoline degradation rate in batch test

Quinoline was degraded by immobilized *B. pickttii* in the first batch test; the degradation rate of quinoline was slow

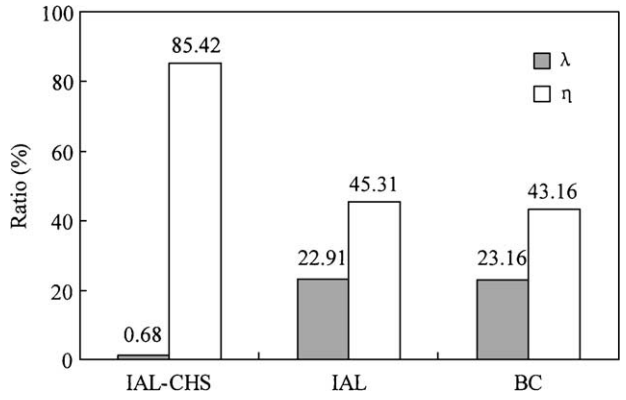


Fig. 5. Bypass ratio and full mixture zone ratio for the three kinds of bioreactor.

in the first instance, and the rate quickened subsequently in the second instance, as the immobilized *B. pickttii* had acclimated to new microenvironment soon and the cells proliferated in ceramic media, which was shown in Fig. 6. The result showed that *B. pickttii* immobilized onto ceramics had an acclimation period for degradation of quinoline and the acclimation elapsed was less than 20 h. It took 12 h for immobilized *B. pickttii* to degrade incompletely before acclimation, but only 8 h after acclimation. The shoulder appeared in the degradation curve in Fig. 6, which indicated that quinoline could be hardly degraded in the first 0.5 h before immobilized *B. pickttii* acclimated into the microenvironment, but quinoline could be degraded rapidly in the first 0.5 h after acclimation. The degradation rates for different concentrations of quinoline were shown in Fig. 7, which indicated that higher the concentration of quinoline, the longer it would take to degrade completely. There are several reasons why reaction rates might slowdown after some time in a batch test: buildup of an inhibitory intermediate or product, depletion of oxygen, or saturation of a physical/chemical

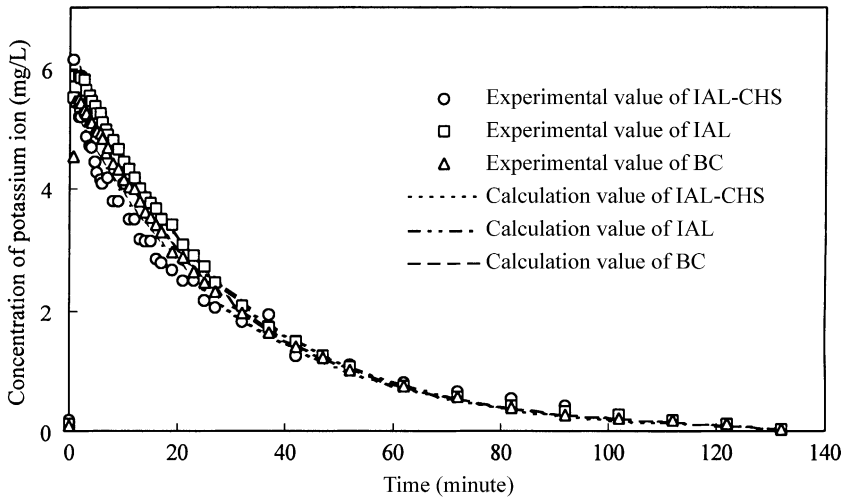


Fig. 4. Change of concentration of potassium ion in effluent vs time for three kinds of bioreactor.

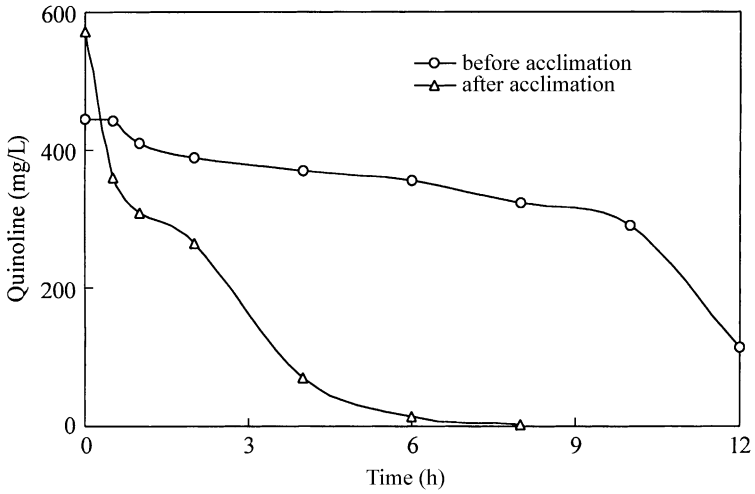


Fig. 6. Quinoline biodegradation rate in batch test before acclimation and after acclimation, respectively.

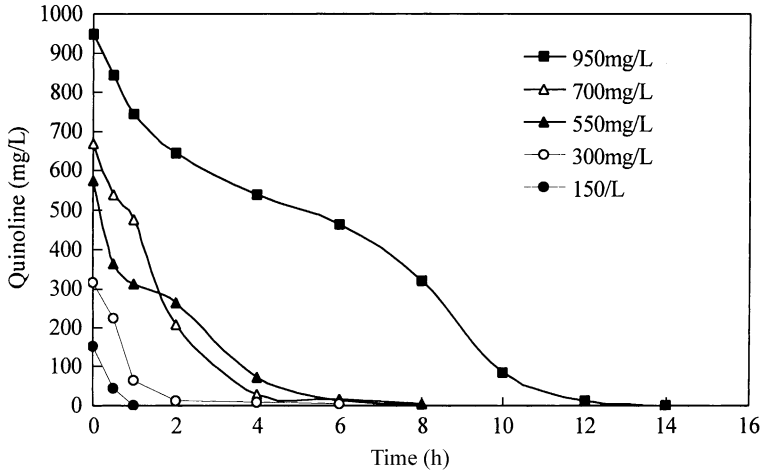


Fig. 7. Quinoline biodegradation rate in batch test for different initial concentrations.

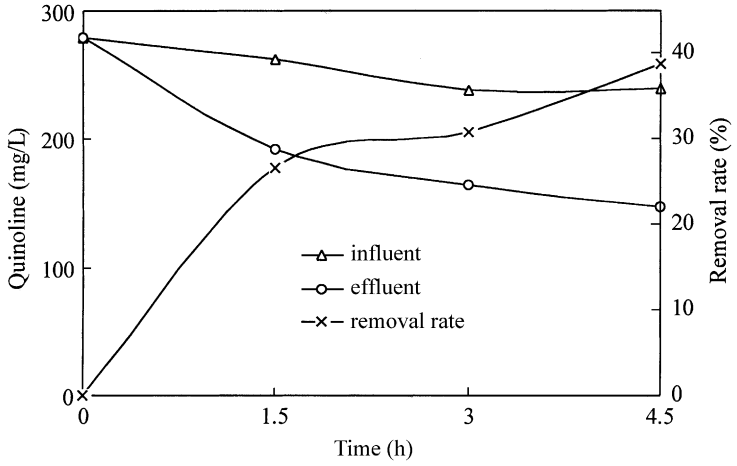


Fig. 8. Quinoline biodegradation and removal rate in continuous test for 1.5 h of hydraulic retention time.

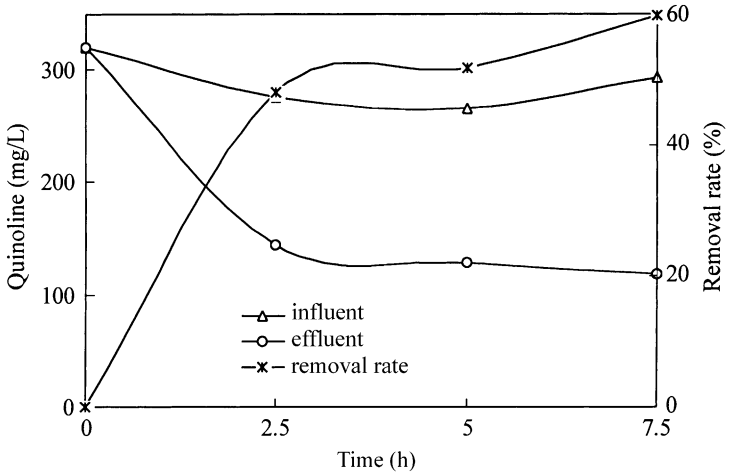


Fig. 9. Quinoline biodegradation and removal rate in continuous test for 2.5 h of hydraulic retention time.

mechanism, such as adsorption according to the author's opinion.

### 3.4. Quinoline biodegradation in continuous flow test

The quinoline was degraded in continuous flow test for different concentrations of quinoline with the hydraulic retention time (HRT) of 1.5, 2.5 and 4 h, respectively. The concentrations of influent, effluent and quinoline removal rate were shown respectively in Figs. 8–10. For the influent concentration from 260 to 320 mg/l, the quinoline removal rates were 40% when HRT was 1.5 h, 60% when the HRT was 2.5 h, and 95% when the HRT was 4 h.

When Fig. 8 was compared with Figs. 9 and 10, the effluent concentration and quinoline removal rate did not become stable when the HRT was 1.5 h but attained stability when the HRT was 2.5 and 4 h. HRT should be 4 h at least for achieving good removal rate of quinoline according to Fig. 10.

### 3.5. GC–MS analysis for quinoline biodegradation

It was shown that quinoline was first degraded into 2-hydroxy quinoline before it was converted into final product by the analysis of gas chromatography–mass spectrum (GC–MS). Fig. 11 shows the map of HPLC analysis of original quinoline solution, and Figs. 12–14 respectively showed the maps of HPLC for quinoline biodegraded in 4, 6 and 8 h of batch test. Peak value in 4 min was represented for 2-hydroxy quinoline and peak value in 6 min was represented for quinoline. Peak value in 6 min decreased gradually, and peak value in 4 min increased first, and then decreased gradually with quinoline biodegradation, which indicated that 2-hydroxy quinoline was also biodegraded ultimately. The experimental results indicated that quinoline was indeed degraded by *B. pickttii* immobilized onto ceramic honeycomb support, but not volatilized.

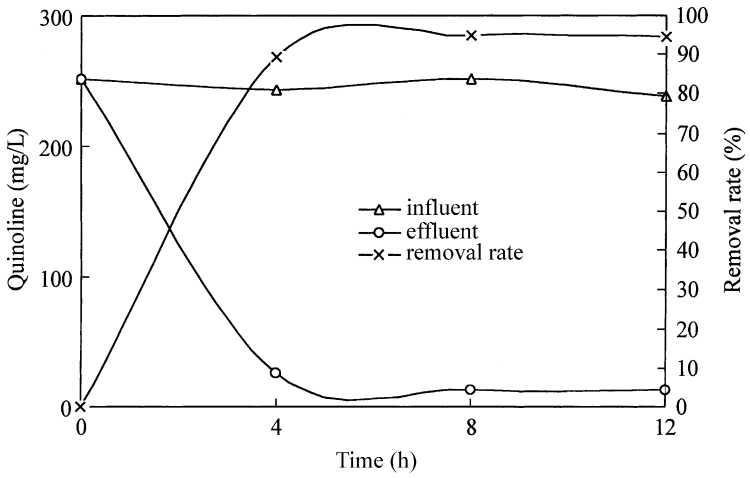


Fig. 10. Quinoline biodegradation and removal ratio in continuous test for 4 h of hydraulic retention time.

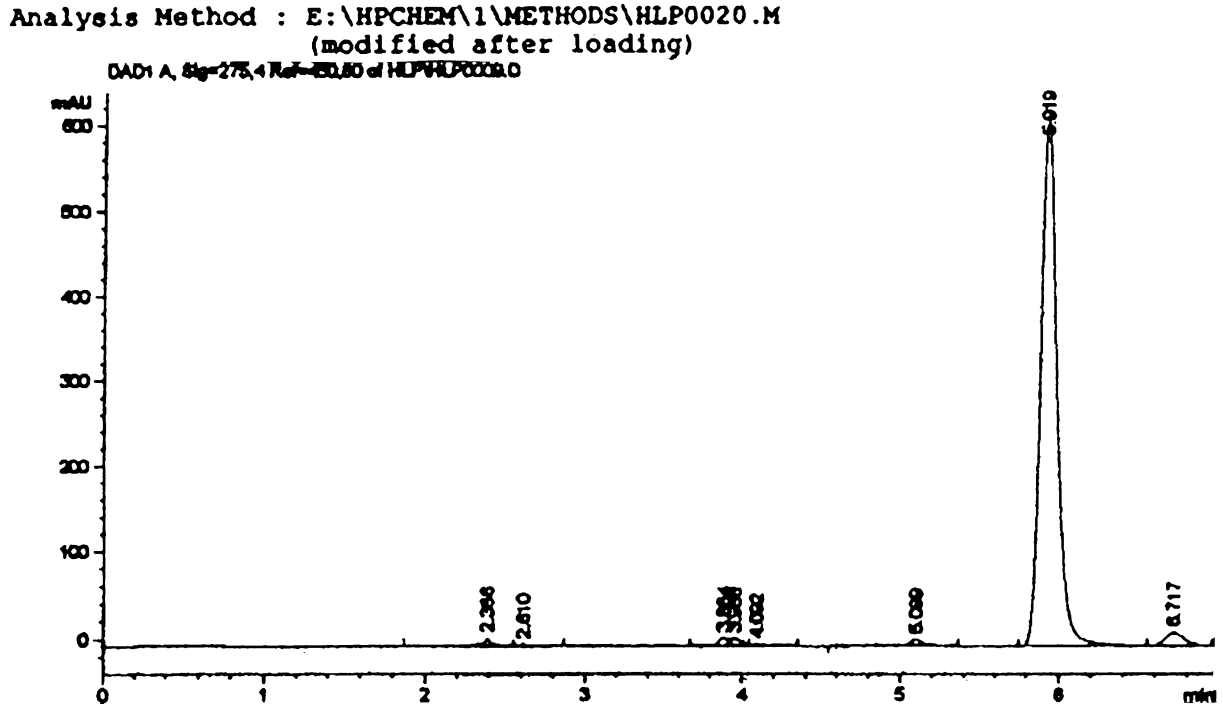


Fig. 11. HPLC analysis of initial quinoline to be biodegraded.

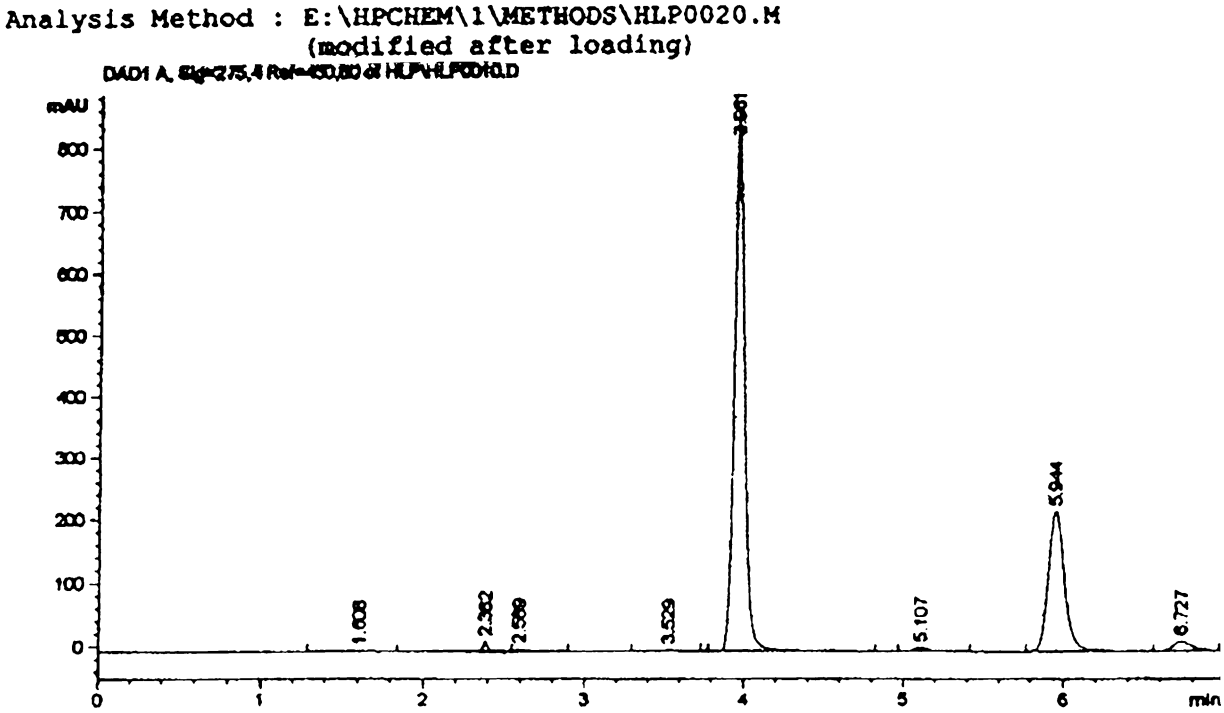


Fig. 12. HPLC analysis of quinoline biodegradation in 4 h.

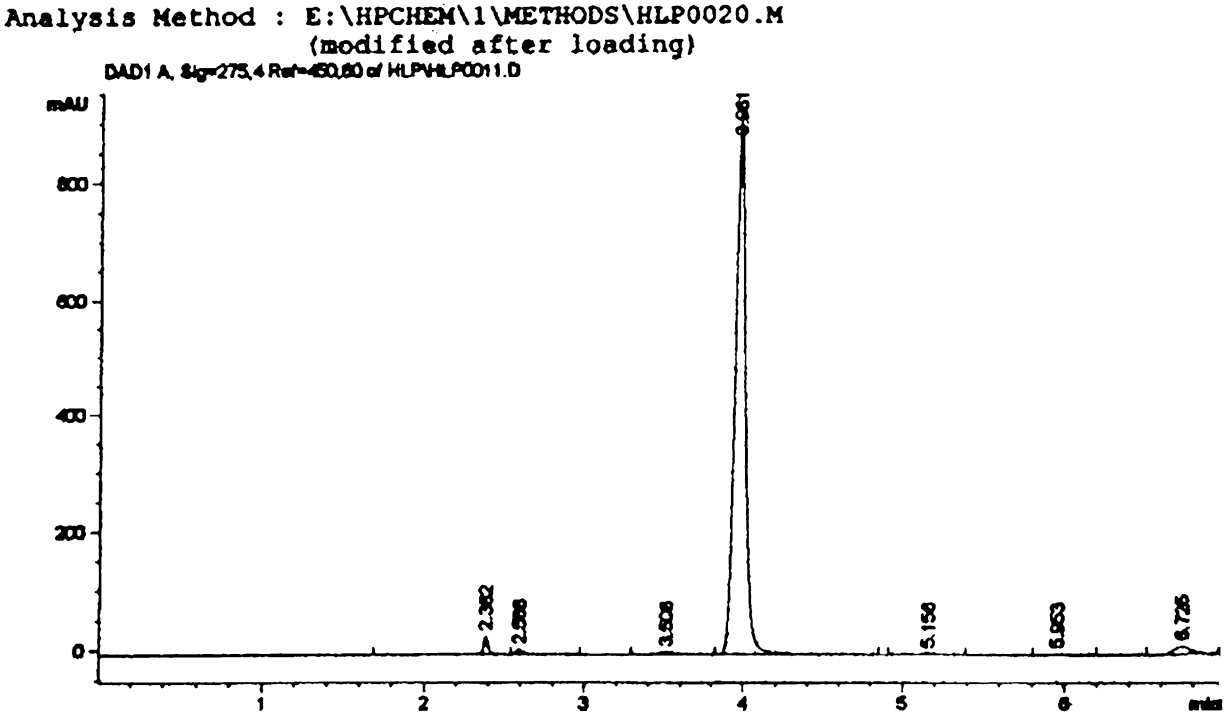


Fig. 13. HPLC analysis of quinoline biodegradation in 6 h.

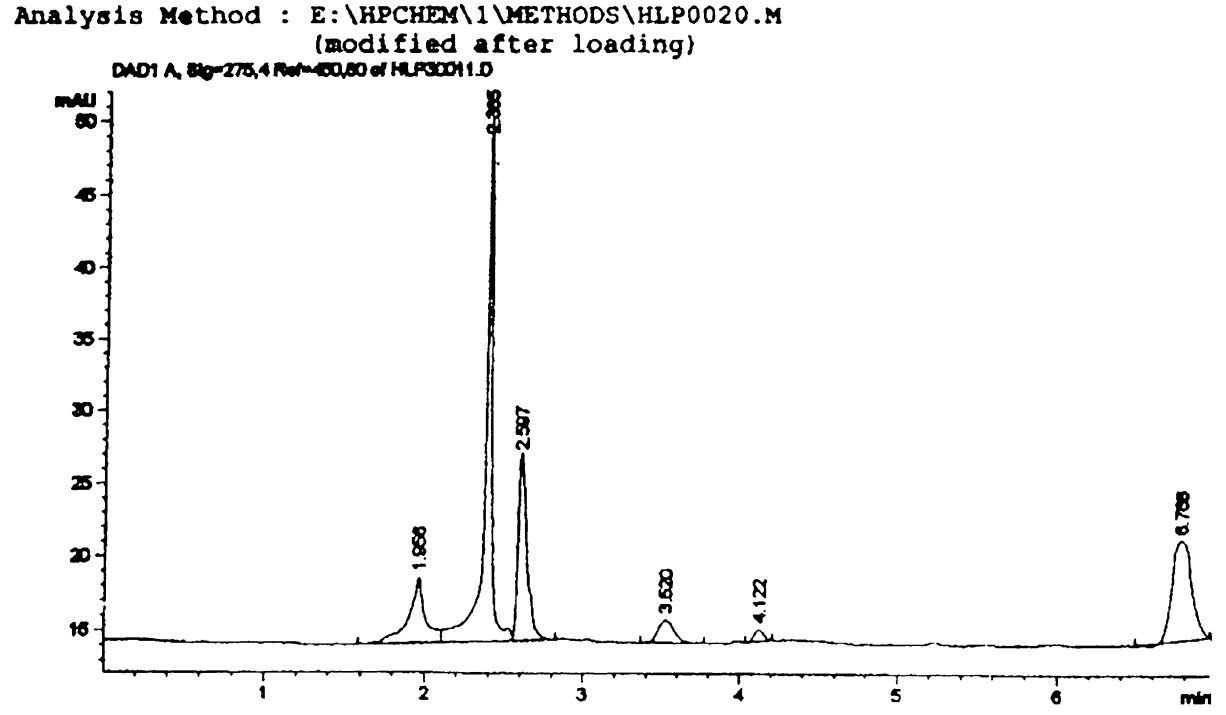


Fig. 14. HPLC analysis of quinoline biodegradation in 8 h.

## 4. Conclusion

IAL-CHS bioreactor could decrease the bypass ratio by installing a ceramic honeycomb support and increase the ratio of full mixture zone. *B. pickttii* could be easily immobilized into the micropore within ceramics, as ceramics' better absorptivity to cells, which supplied a better microenvironment for *B. pickttii* to metabolize, proliferate and degrade quinoline steadily. Immobilized *B. pickttii* exhibited better stability in continuous flow test. Quinoline

could be removed more than 95% for 4 h of HRT. Quinoline was degraded first into 2-hydroxy quinoline and then degraded further according to the analysis of gas chromatography–mass spectrum (GC–MS).

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