ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 12 (2): 125-127, 2013
ISSN 1682-296X / DOI: 10.3923/biotech.2013.125.127

Effect of Inhibitory Factors on Biodegradation of Phenol by an Isolated *Pseudomonas* Strain

Ben-Jun Zhou
School of Resources and Environmental Engineering, Hefei University of Technology, 230009, Hefei, China

Abstract: The effect of various factors such as inoculum size, glucose concentrations, cell density, agitation rate and salinity concentrations on phenol degradation by *Pseudomonas* JF122 during incubation was evaluated respectively. Tests results indicated the phenol-degrading rates were enhanced when glucose concentrations were in rang of 50-100 mg L^{-1}, however, biodegradation of phenol were inhibited when glucose higher than 100 mg L^{-1}. The speed of 150-200 rpm were efficient agitation rates for phenol degradation, the efficient cell concentrations for phenol degradation was 0.2 (OD_{600}), NaCl concentrations of 200-600 mg L^{-1} were found to be the optimum saline concentration for biodegradation of phenol and the efficient inoculum size (V/V%) for phenol degradation by *P.* JF122 was 1%.

Key words: Phenol degradation, agitation rates, inoculum size, saline concentrations

INTRODUCTION

Phenol is a widespread pollutant found in many industrial effluents such as wastewaters from coal processing plants, oil refineries, petrochemical and phenol resin industry plant, etc. (Annadurai *et al*., 2002; Ahmed *et al*., 1995; Bandyopadhyay *et al*., 1998). Phenol is currently removed by many treatment techniques such as chemical oxidation, coagulation, solvent extraction, ion-exchange, activated carbon adsorption, ultrafiltration and conventional biological methods generally using activated sludges or anaerobic cultures (Watanabe *et al*., 1996; Jianmin *et al*., 1993; Singleton, 1994; Ra *et al*., 2008). However, these techniques have proven to be costly and inefficient (Kotresha and Vidyasagar, 2008; Shourian *et al*., 2009). Thus, biodegradation of phenol has been considered as an alternative, since phenol is aerobically biodegraded to CO_2 and H_2O by microorganisms (Bai *et al*., 2007).

The application potential of phenol degrading microorganisms are affected by various environmental factors, such as temperature, pH, phenol concentration in polluted environments. Phenol-degrading strain *Pseudomonas* sp. JF122 was isolated in our previous study, Thus, the aim of this study was to investigate the effect of various factors, such as inoculum size, glucose concentrations, cell density, agitation rate and salinity concentrations on phenol degradation by *P.* JF122, so as to ensure the strain can be efficiently applied for phenol polluted environments clean-up.

MATERIALS AND METHODS

Phenol biodegradation: The *P.* JF122 strain was cultivated aerobically 30°C with 150 mL mineral medium containing 300 mg phenol for 24 h at 170 rpm shaking speed, which was used as an inoculum. The composition of mineral medium was 1.0 g L^{-1} $(NH_4)_2SO_4$, 0.5 g L^{-1} K_2HPO_4, 0.5 g L^{-1} NaH_2PO_4, 0.2 g L^{-1} $CaCl_2$, 0.2 g L^{-1} NaCl, 0.2 g L^{-1} $MgSO_4 \cdot 7H_2O$ and 0.01 g L^{-1} $Fe_2(SO_4)_3 \cdot 7H_2O$. The degradation experiments were carried out under static incubation condition at 30°C with mineral medium containing 600 mg L^{-1} phenol, the effect of inoculum size, glucose concentrations, cell density, agitation rate and salinity concentrations on phenol degradation during incubation was evaluated respectively.

Analysis methods: The 1 mL samples from batch assays were collected at regular time and analyzed for phenol concentration using the 4-aminoantipyrine method. The effect of various factors on the phenol degradation by *P.* JF122 were measured by calculating the phenol degradation rate, which was calculated based on the following equation:

$$\text{Phenol degradation rate} = \frac{C0\text{-}C}{C0} \times 100\%$$

where, C_0 is initial phenol concentration (mg L^{-1}) and C is residual concentration of phenol (mg L^{-1}).

RESULTS AND DISCUSSION

Effect of glucose concentration on phenol degradation: The effect of glucose concentrations on phenol degradation by *P.* JF122 was investigated by varying glucose concentrations from 50-400 mg L^{-1} in present of 600 mg L^{-1} phenol. As shown in Fig. 1, the phenol-degrading rates were enhanced when glucose concentrations were in rang of 50-100 mg L^{-1}, The degradation rates were higher than 95%. Further increase glucose concentration the phenol-degrading rates decreased greatly. The reason may be glucose is the preferable carbon sources for *P.* JF122, which interferes with phenol utilisation. Similar observation were reported by Leitao *et al.* (2007).

Effect of agitation rates on phenol degradation: The effect of agitation rates on phenol degradation by *P.* JF122 was investigated by varying shaking speeds from 50-250 rpm. As shown in Fig. 2, the Phenol-degrading rate increased when agitation rates increased from 50-150 rpm, the degradation rates were found to be higher than 93% at speed of 150-200 rpm. However, at 250 rpm, the Phenol-degrading rate decreased greatly and was less than 40%, thus, the speed of 150-200 rpm were efficient agitation rates for phenol degradation by *P.* JF122.

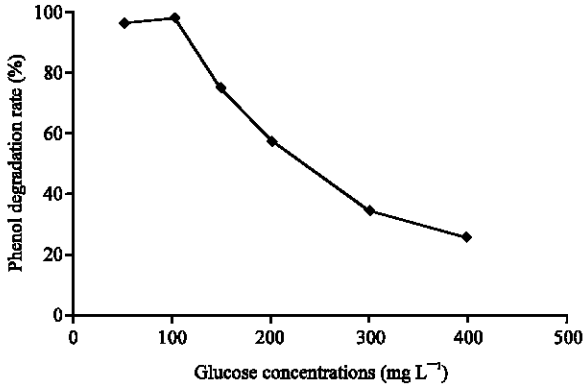

Fig. 1: Effect of glucose concentrations on phenol degradation by JF122

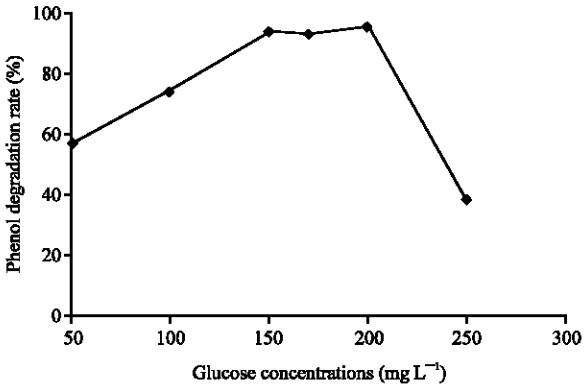

Fig. 2: Effect of agitation rates on phenol degradation by JF122

Effect of cell concentrations on phenol degradation: The effect of cell concentrations on phenol degradation by *P.* JF122 was investigated by inoculating different amount of cell concentrations (OD_{600}), varying from 0.05-0.3. As shown in Fig. 3, the best phenol-degrading rate occurred at 0.2 (OD_{600}), the degradation rates were found to be similar at 0.2-0.3 range. However, at 0.05-0.15 range, the Phenol-degrading rate was less than 58%, thus, the efficient cell concentrations for phenol degradation by *P.* JF122 was 0.2.

Effect of inoculum size on phenol degradation: The effect of inoculum size on phenol degradation by *P.* JF122 was investigated by varying inoculum size (V/V%) from 0 to 2%. As shown in Fig. 4, the phenol-degrading rates were improved with increased inoculum sizes. When the inoculum size varying from 0-1%, the degradation rates were increased from 0-92%. Further increase inoculum size the Phenol-degrading rates kept almost constant. The reason may be the batch experiments were carried out in present of 600 mg L^{-1} phenol, which means the growth of cell was accounted for the limit carbon sources. So it can be inferred that the efficient inoculum size for 600 mg L^{-1} phenol degradation by *P.* JF122 was 1%.

Effect of saline concentrations on phenol degradation: In order to investigate the effect of saline concentrations on phenol degradation by *P.* JF122, the concentrations of NaCl in 150 mL mineral medium were altered to 0, 200, 400,

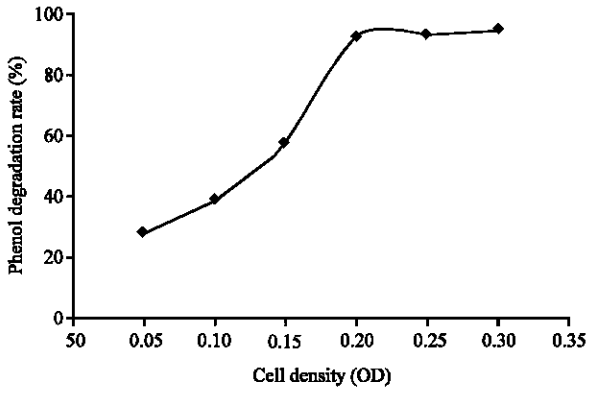

Fig. 3: Effect of cell concentrations on phenol degradation by JF122

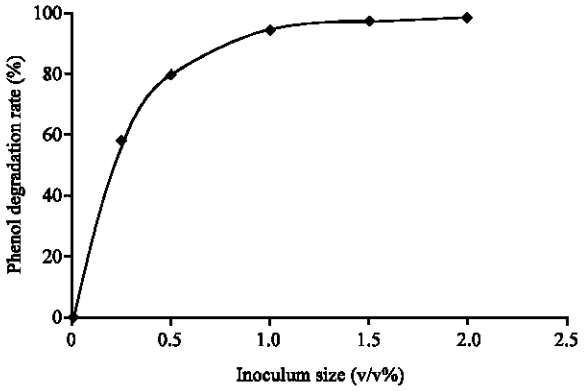

Fig. 4: Effect of inoculum size on phenol degradation by JF122

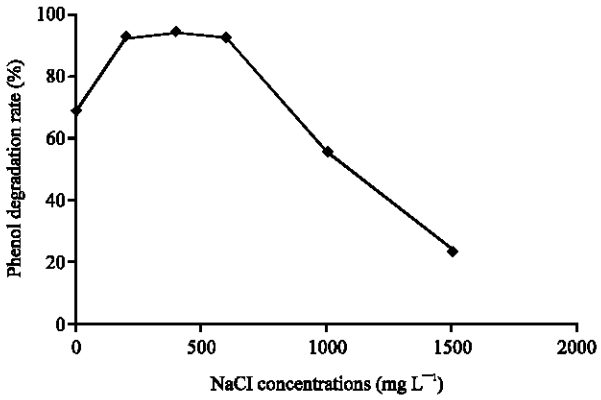

Fig. 5: Effect of NaCl concentrations on phenol degradation by JF122

600, 1000 and 1500 mg L^{-1}. As shown in Fig. 5, there was no obvious acceleration in phenol-degrading rates when NaCl concentrations were in range of 200-600 mg L^{-1}. When NaCl concentrations varying from 600-1500 mg L^{-1}, The degradation rates were decreased from 92-24%. On the other hand, the degradation rate was more than 68% in present of 0 mg L^{-1} NaCl. These result indicated that phenol degradation by *P.* JF122 was affected by saline concentrations, among the six NaCl concentrations test, 200-600 mg L^{-1} NaCl was found to be the optimum concentration for biodegradation of phenol.

CONCLUSION

The biodegradation of phenol by *P.* JF122 was affected by various factors. In the present study, the effect of inoculum size, glucose concentrations, cell density, agitation rate and salinity concentrations on phenol degradation during incubation was evaluated, respectively, Test results showed that the Phenol-degrading rates were enhanced when glucose concentrations were in rang of 50-100 mg L^{-1}, the speed of 150-200 rpm were efficient agitation rates for phenol degradation, the efficient cell concentrations for phenol degradation was 0.2 (OD_{600}), 200-600 mg L^{-1} NaCl were found to be the optimum concentration for biodegradation of phenol and the efficient inoculum size (V/V%) for phenol degradation by *P.* JF122 was 1%.

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