Journal of Hazardous Materials 168 (2009) 298–303

Contents lists available at ScienceDirect

Journal of Hazardous Materials

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

Aerobic degradation of nitrobenzene by immobilization of *Rhodotorula mucilaginosa* in polyurethane foam

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ARTICLE INFO

Article history:
Received 29 July 2008
Received in revised form 6 February 2009
Accepted 9 February 2009
Available online 20 February 2009

Keywords:
Nitrobenzene
Degradation
Kinetics
Immobilization
Rhodotorula mucilaginosa

ABSTRACT

Rhodotorula mucilaginosa Z1 capable of degrading nitrobenzene was immobilized in polyurethane foam. The nitrobenzene-degrading capacity of immobilized cells was compared to free cells in batches in shaken culture. Effects of pH and temperature on the nitrobenzene degradation showed that polyurethane-immobilized Z1 had higher tolerances toward acid, alkali, and heat than those of free cells. Kinetic studies revealed that higher concentrations of nitrobenzene were better tolerated and more quickly degraded by polyurethane-immobilized Z1 than by free cells. Moreover, the ability of polyurethane-immobilized Z1 to resist nitrobenzene shock load was enhanced. Experiments on the nitrobenzene degradation in different concentrations of NaCl and in the presence of phenol or aniline demonstrated that polyurethane-immobilized Z1 exhibited higher tolerance toward salinity and toxic chemicals than those of free cells. Immobilization therefore could be a promising method for treating nitrobenzene industrial wastewater. This is the first report on the degradation of nitrobenzene by a polyurethane-immobilized yeast strain.

1. Introduction

Immobilization technique has been receiving interest in the field of wastewater treatment. Compared to free cells, immobilized cells have the longer stability, higher activity and higher cell density. Thus, immobilized cell systems have the potential to degrade toxic chemicals more efficiently than free cell systems do [1]. Moreover immobilized cell systems can avoid the problem of separating cells from water, which is associated with free cell systems [2]. Polyurethanes are a class of polymers, which have been applied to medical field, automotive field [3], biosensor construction [4], etc. Polyurethanes are also used in the immobilization of microorganisms because of their ease of use, low cost, and low toxicity [2]. Many studies have described the degradation of toxic chemicals by polyurethane-immobilized cells such as naphthalene [2], pentachlorophenol [5], and oil [6].

Nitrobenzene-containing wastewater can be treated with aerobic biological method. Under aerobic condition, nitrobenzene was mineralized into CO_2 and H_2O. Many studies have shown the degradation of nitrobenzene by free cells. However, to date, the degradation of nitrobenzene by immobilized cells has not been reported.

In a previous study, we isolated a nitrobenzene-degrading yeast, *Rhodotorula mucilaginosa* Z1 [7]. The capacity of Z1 free cells to degrade nitrobenzene under aerobic condition has been investigated. In this paper, we do research on the degradation of nitrobenzene by polyurethane-immobilized Z1. The purpose of this paper is to demonstrate the application potential of immobilized cells to nitrobenzene industrial wastewater.

2. Materials and methods

2.1. Chemicals

All of the chemicals used in this study were of analytical grade. The water used was double de-ionized water. Polyurethane foam (patent No. ZL021417237, SIPO) was obtained from Landa Environmental & Biological Technology Co Ltd., Dalian, China. The foam was of elasticity, low wet bulk density (900 kg m^{-3}) and a large specific surface area (1.6–2.2 × 10^5 m^2 m^{-3}).

2.2. Growth of strain Z1 on nitrobenzene in the presence of polyurethane foam

The polyurethane foam was cut into approximately 1.5 cm cubes, washed twice with double de-ionized water, and autoclaved (20 min, 120 °C). One loop of Z1 stock culture on the agar plates was transferred aseptically to 100 mL sterilized MS medium [7] in a 250 mL flask with 200 mg L^{-1} nitrobenzene.

Ten milliliters of the culture in the late exponential phase was aseptically inoculated into 90 mL sterilized MS medium in a 250 mL flask with foam cubes. The best condition was 0.5 g of foam cubes in 90 mL of MS medium. The 250 mL flask was supplemented with 200 mg L^{-1} nitrobenzene. The cultures were incubated on a rotary

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doi:10.1016/j.jhazmat.2009.02.029

shaker (180 rpm) at 30 °C. A flask with same amount of the autoclaved cells (10 min, 60 °C) was used as control. Samples were withdrawn periodically for the analysis of nitrobenzene concentration, total organic content (TOC), and dry cell mass. As far as the dry cell mass is concerned, the suspension was decanted and the foam cubes with immobilized cells were collected. Dry weights of the decanted suspension and the microbes-laden foam cubes were determined as described by Du et al. [8]. The average, predetermined dry weight of foam cube was then subtracted.

The degradations of nitrobenzene by polyurethane-immobilized cells and free cells at different pH (3.0–12.0, 30 °C, 180 rpm) and temperature (10 °C-50 °C, pH 7.0, 180 rpm) were compared. The initial concentration of nitrobenzene was 200 mg L^{-1}. After 30 h of incubation, samples in immobilized cells system were withdrawn for the analysis of nitrobenzene concentrations. With the free cells systems, samples were withdrawn after 60 h.

If not noted otherwise, each experiment was repeated five times. Data were analyzed statistically and the error bars depict 95% confidence intervals.

2.3. Kinetics of nitrobenzene degradation by polyurethane-immobilized Z1

Ten milliliters of the culture in the late exponential phase was aseptically inoculated into a 250 mL flask with 90 mL sterilized MS medium and 0.5 g foam cubes. The 250 mL flask was supplemented with nitrobenzene at different initial concentrations. The cultures were incubated on a rotary shaker (180 rpm) at 30 °C. Samples were withdrawn periodically for the analysis of nitrobenzene concentration. A flask with same amount of the autoclaved cells (10 min, 60 °C) was used as control.

2.4. Degradation of nitrobenzene in different salinities or in the presence of other toxicants by polyurethane-immobilized Z1

Ten milliliters of the culture in the late exponential phase was aseptically inoculated into each 250 mL flask with 90 mL sterilized MS medium and 0.5 g foam cubes. Each 250 mL flask was supplement with 200 mg L^{-1} nitrobenzene and 2–7% (quality concentration) NaCl. The cultures were incubated on a rotary shaker (180 rpm) at 30 °C. Samples were withdrawn periodically for the analysis of nitrobenzene concentration. Flask with the same amount of the autoclaved cells (10 min, 60 °C) was used as control.

The same setup was also used for testing the capacity of polyurethane-immobilized Z1 to degrade nitrobenzene in the presence of aniline or phenol. Phenol concentrations ranged from 50 to 200 mg L^{-1} and aniline from 25 to 100 mg L^{-1}.

2.5. Analytical methods

Nitrobenzene concentrations were measured with a V-560 UV–vis spectrophotometer (JASCO, Japan) using N-(1-Naphthyl) ethylenediamine dihydrochloride as per standard procedure [9]. Prior to a total organic carbon (TOC) content analysis, samples were centrifuged (15 °C) at 12,000 rpm for 20 min to remove the biomass. The TOC contents were determined using a multiN/C2100 analyzer (Analytik Jena AG, Germany) [10].

3. Results and discussion

3.1. Growth of strain Z1 on nitrobenzene in the presence of polyurethane foam

Fig. 1 shows the profiles of nitrobenzene consumption and cell growth in the presence of polyurethane foam. The initial concentration of nitrobenzene was 200 mg L^{-1} and the equilibrium adsorption of nitrobenzene on the polyurethane foam was about 20 mg g^{-1}. The equilibrium adsorption was negligible and not used to correct the values of nitrobenzene concentration. As shown in Fig. 1, polyurethane-immobilized Z1 completely degraded 200 mg L^{-1} nitrobenzene within 30 h and the maximum dry cell mass was 193.08 mg L^{-1}. Over 99% of TOC was removed. The previous study showed that the time for complete degradation of 200 mg L^{-1} nitrobenzene by Z1 free cells was 60 h and the maximum dry cell mass was only 96.51 mg L^{-1} [7]. Since the polyurethane foam protected Z1 cells, it was good for remaining the stable pH value inside Z1 cells, avoiding acidification of Z1 cells, resisting shock load, and so on [11,12]. Thus, higher biomass amounts were produced. Due to the high local cell density in polyurethane foam, faster degradation of nitrobenzene was achieved by immobilized cells.

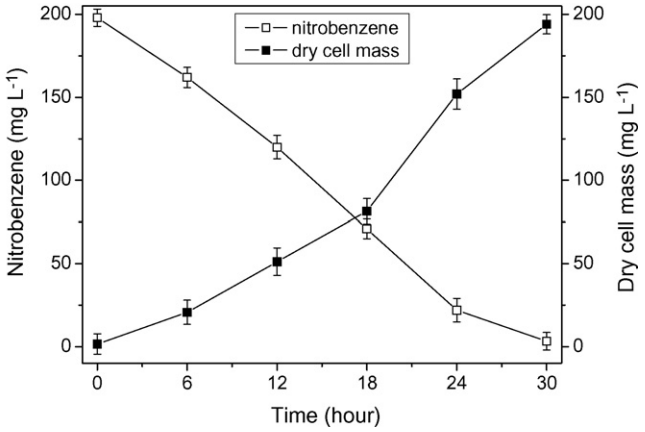

Fig. 1. Growth of strain Z1 with nitrobenzene as a sole source of carbon, nitrogen, and energy in the presence of polyurethane foam. The initial concentration of nitrobenzene was 200 mg L^{-1}. Nitrobenzene (-□-), dry cell mass (-■-).

As shown in Fig. 2, the optimal pH and temperature for nitrobenzene degradation by polyurethane-immobilized Z1 were the same as free cells. The optimal pH and temperature were pH 7.0 and 30 °C, respectively. However, at other pH values and temperatures, the degrading rates of nitrobenzene by polyurethane-immobilized Z1 were higher than those by free cells. These results indicate that polyurethane-immobilized cells exhibit higher tolerances toward acid, alkali and heat than those of free cells. Immobilization can provide a kind of membrane stabilization and increase cell permeability, thus cells are protected and higher degrading rates are realized [2].

3.2. Nitrobenzene degradation kinetics by polyurethane-immobilized Z1

The consumption rate of organic compounds by microbes can be expressed by the equation as followed [1]:

$$q = \frac{q_{\max} S}{k + S} \quad (1)$$

where $q_{\max}$ is the maximum specific consumption rate, S is the concentration of substrate, and k is the half-saturation constant.

If S«k, Eq. (1) can be reduced to:

$$q = \frac{q_{\max} S}{k} \quad (2)$$

Eq. (2) is the typical first-order model. Assuming $k_1 = (q_{\max}/k)$ and integrating Eq. (2), the relationship of substrate concentration to time can be expressed as followed:

$$\ln S = a + k_1 t \quad (3)$$

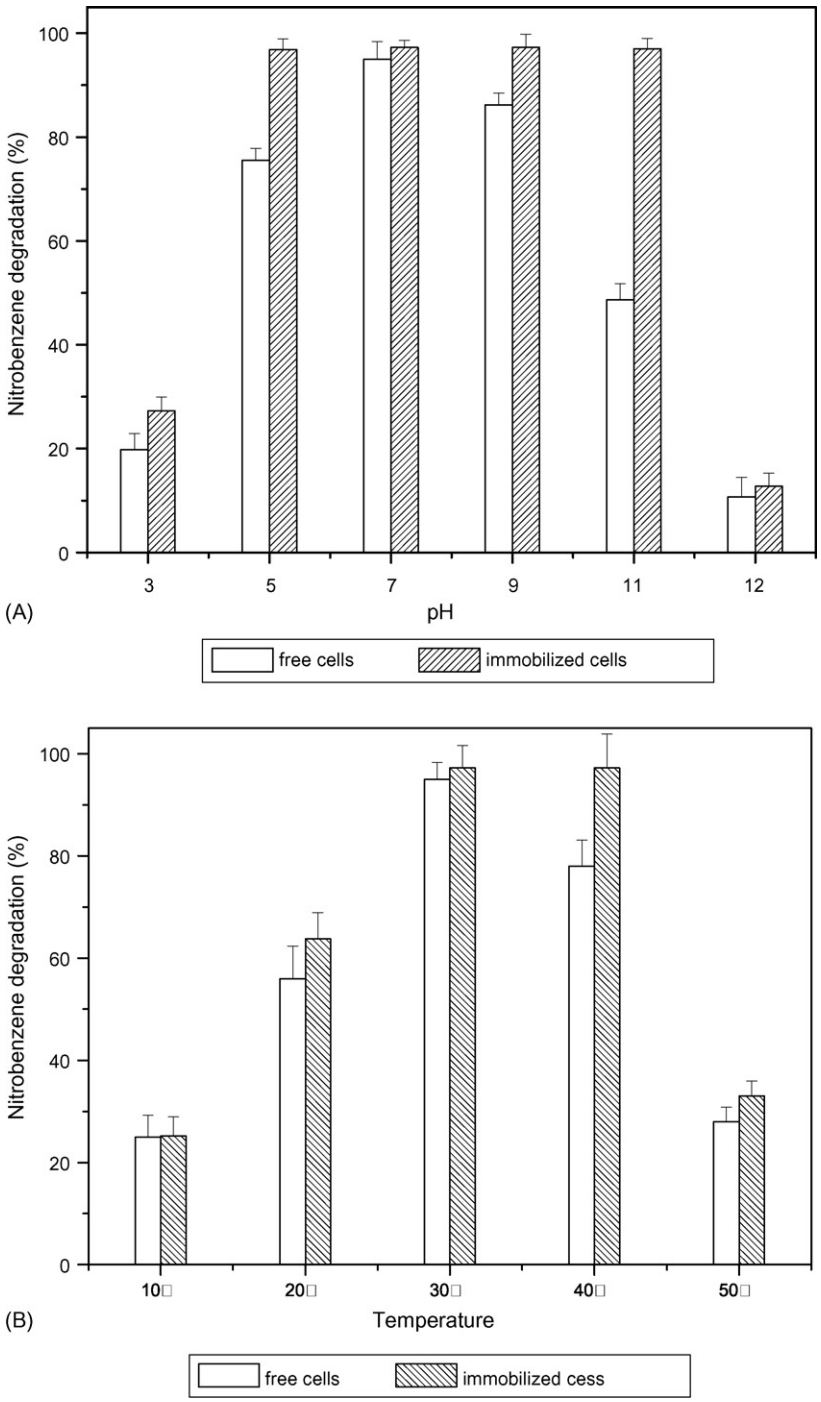

Fig. 2. Effects of pH (A) and temperature (B) on the nitrobenzene degradation by polyurethane-immobilized Z1 and free cells. The initial concentration of nitrobenzene was 200 mg L^{-1}.

If $S \gg k$, Eq. (1) can be reduced to:

$$q = q_{max} \quad (4)$$

Eq. (4) is the zero-order model and the rate constant $k_0 = q_{max}$. Thus, the relationship of substrate concentration to time is:

$$S = b + k_0 t \quad (5)$$

When the initial concentrations of nitrobenzene ranged from 16.63 mg L^{-1} to 112.18 mg L^{-1}, the kinetics of nitrobenzene degradation by polyurethane-immobilized Z1 was well expressed by the first-order reaction model (Fig. 3). The values of q increased with the increase in initial nitrobenzene concentration. At a concentration range of 133.23–233.39 mg L^{-1}, the kinetics of nitrobenzene degradation by polyurethane-immobilized Z1 was well expressed by the zero-order reaction model (Fig. 4). The kinetic results were summarized in Table 1. The previous study showed that the degradation of nitrobenzene by Z1 free cells is inhibited when the initial concentrations of nitrobenzene are above 90 mg L^{-1} [7]. However, polyurethane-immobilized Z1 can degrade nitrobenzene at a constant rate in the concentration range of 133.23–233.39 mg L^{-1}. These results demonstrate that the ability of polyurethane-immobilized Z1 to resist nitrobenzene shock load was enhanced. At higher concentration of 269.34 mg L^{-1}, the degradation of nitrobenzene by polyurethane-immobilized Z1 was inhibited. Thus, the

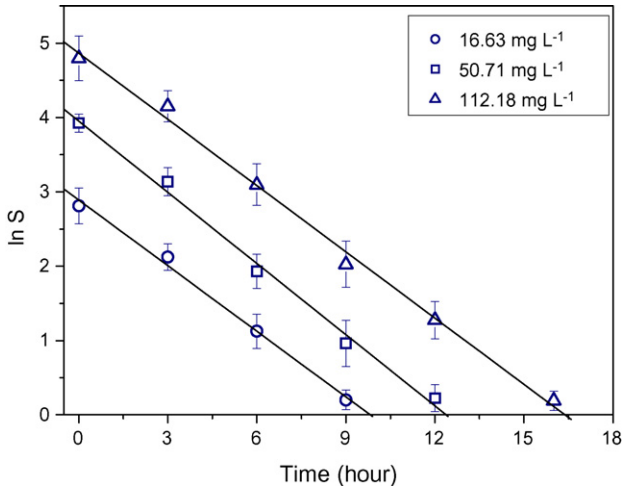

Fig. 3. Degradation of nitrobenzene at different initial concentrations by polyurethane-immobilized Z1. Initial concentration of nitrobenzene: 16.63 mg L^{-1} (-○-), 50.71 mg L^{-1} (-□-), and 112.18 mg L^{-1} (-□-). The first-order reaction model was used to express the nitrobenzene degradation.

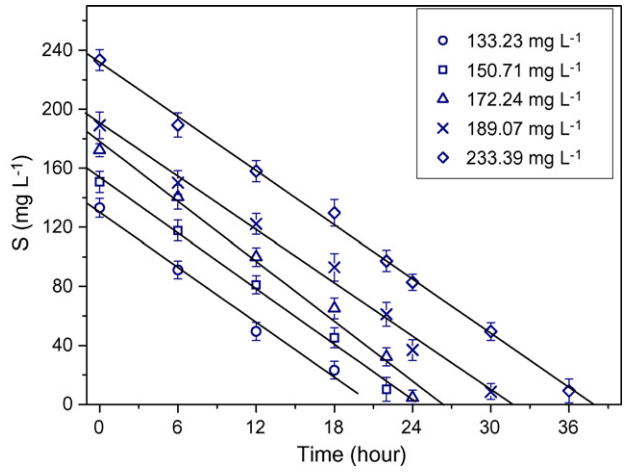

Fig. 4. Degradation of nitrobenzene at different initial concentrations by polyurethane-immobilized Z1. Initial concentration of nitrobenzene: 133.23 mg L^{-1} (-○-), 150.71 mg L^{-1} (-□-), and 172.24 mg L^{-1} (-□-), 189.07 mg L^{-1} (-×-), and 233.39 mg L^{-1} (-□-). The zero-order reaction model was used to express the nitrobenzene degradation.

Andrews equation [13] was used to express the dependence of q on nitrobenzene concentration (Fig. 5):

$$q = \frac{q_{max} S}{S + K_s + (S^2/K_i)} \quad (6)$$

where q_{max} is the maximum specific substrate consumption rate, K_i is the inhibition constant, K_s is the half-rate constant, and S is the substrate concentration.

Table 1
The kinetic equations of nitrobenzene degradation by immobilized polyurethane-immobilized Z1.

S (nitrobenzene, mg L^{-1})	Kinetic equation	Rate constant (mg L^{-1} h^{-1})	Correlation coefficient (R^2)
16.63	$\ln S = -0.2942t + 2.8897$	0.2942	0.9946
50.72	$\ln S = -0.3192t + 3.9519$	0.3192	0.9939
112.18	$\ln S = -0.2971t + 4.8671$	0.2971	0.9954
133.23	$S = -6.1725t + 130.08$	6.1725	0.9896
150.71	$S = -6.2769t + 153.86$	6.2769	0.9946
172.24	$S = -6.7604t + 178.10$	6.7604	0.9872
189.07	$S = -6.0191t + 190.79$	6.0191	0.9900
233.39	$S = -6.1133t + 231.75$	6.1133	0.9970

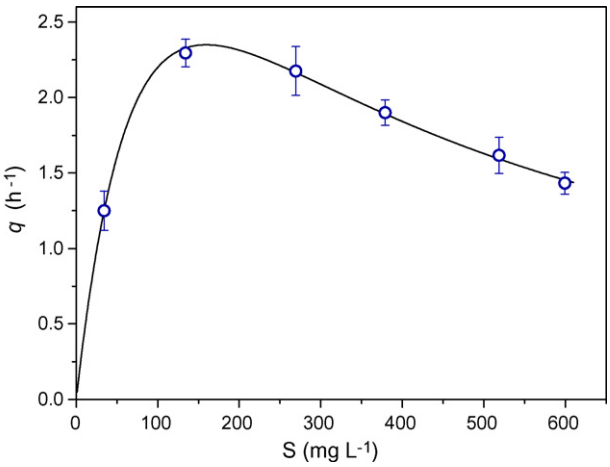

Fig. 5. Comparison between kinetic predictions and experimental data of specific substrate consumption rate by polyurethane-immobilized Z1. The initial concentrations of nitrobenzene ranged from 0 to 600 mg L^{-1}.

The value of R^2 is 0.9973, which demonstrates that the experimental data are well correlated by Andrews equation. By using a non-linear regression analysis, the kinetic parameters are as follows: $q_{max} = 6.03\ h^{-1}$, $K_s = 125.00$ mg L^{-1}, and $K_i = 203.96$ mg L^{-1}. The kinetic parameters of nitrobenzene degradation by free cells and polyurethane-immobilized Z1 were listed in Table 2. These results indicate that higher concentrations of nitrobenzene are better tolerated and more quickly degraded by polyurethane-immobilized Z1 than by free cells.

3.3. Degradation of nitrobenzene in different salinities by polyurethane-immobilized Z1

Nitrobenzene industrial wastewater usually contains inorganic salts and multi-organic compounds. The existence of these compounds can influence the performance of biological process. It has been generally accepted that conventional microbes could not be used to treat wastewater containing salts (mainly NaCl) over 3% [14]. The previous study showed that Z1 free cells can degrade 200 mg L^{-1} nitrobenzene effectively in 5% NaCl. In this paper, the nitrobenzene degradation by polyurethane-immobilized Z1 in different salinities was tested (Fig. 6). The degradation of nitrobenzene by polyurethane-immobilized Z1 in 2% NaCl and zero percent NaCl presented no distinction. At the higher concentration of NaCl (3%), the nitrobenzene degradation was less effective within 30 h. However, over a longer period, 54 h, polyurethane-immobilized Z1 degraded 200 mg L^{-1} nitrobenzene efficiently. After 54 h, the degrading rate of nitrobenzene was 98%. Even the concentration of NaCl reached to 5%, polyurethane-immobilized Z1 degraded, up to 81%, nitrobenzene with 200 mg L^{-1} after 72 h. At 7% NaCl, the degrading rate of nitrobenzene was decreased sharply.

The degradation of 200 mg L^{-1} nitrobenzene in different salinities by Z1 free cells and polyurethane-immobilized Z1 were listed in Table 3. These results indicate that the salinity tolerance of polyurethane-immobilized Z1 is higher than that of free cells.

Table 2
The kinetic parameters of nitrobenzene degradation by free cells and polyurethane-immobilized Z1.

Kinetic parameter	Free cells	Polyurethane-immobilized Z1
q_{max} (h^{-1})	1.50	6.03
K_s (mg L^{-1})	31.31	125.00
K_i (mg L^{-1})	101.34	203.96

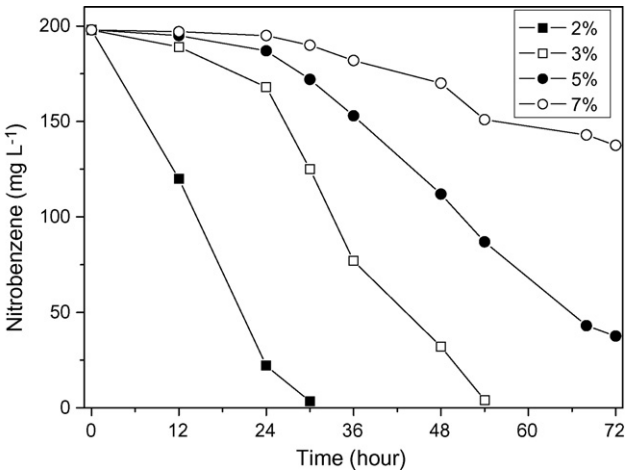

Fig. 6. Degradation of nitrobenzene in different quality concentrations of NaCl by polyurethane-immobilized Z1. The initial concentration of nitrobenzene was 200 mg L^{-1}. Quality concentration of NaCl: 2% (-■-), 3% (-□-), 5% (-●-), and 7% (-○-). Each experiment was repeated five times and the means of these data were presented.

Table 3
The nitrobenzene degradation in different salinities by free cells and polyurethane-immobilized Z1.

	NaCl (%)	Nitrobenzene degradation (%)	Time (h)
Z1 free cells	2	98.23	60
	3	95.66	100
	5	70.13	120
	7	33.71	120
Polyurethane-immobilized Z1	2	98.96	30
	3	98.05	54
	5	81.18	72
	7	37.72	72

3.4. Degradation of nitrobenzene in the presence of phenol or aniline by polyurethane-immobilized Z1

The degradation of nitrobenzene in the presence of aniline or phenol by Z1 free cells has been tested, since these toxicants are present along with nitrobenzene in industrial wastewaters [7]. Z1 free cells degrade 200 mg L^{-1} nitrobenzene in the presence of 50 mg L^{-1} aniline without inhibition. Similar to free cells, polyurethane-immobilized Z1 completely degraded 200 mg L^{-1} nitrobenzene in the presence of 50 mg L^{-1} aniline within 30 h (Fig. 7). At 75 mg L^{-1} of aniline, the degrading rate of nitrobenzene decreased sharply.

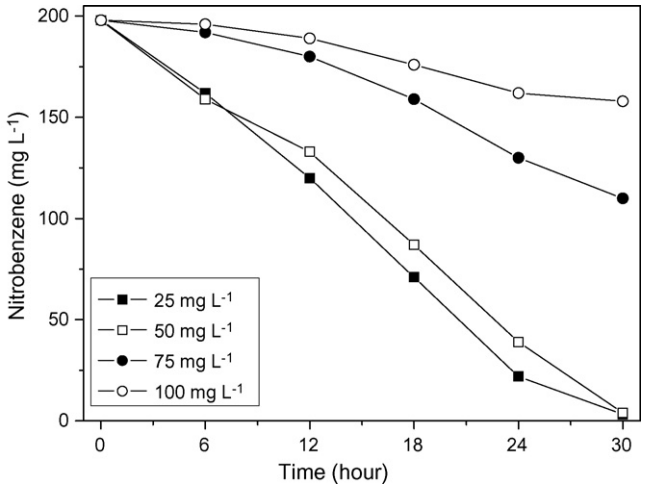

Fig. 7. Degradation of nitrobenzene in the presence of aniline by polyurethane-immobilized Z1. The initial concentration of nitrobenzene was 200 mg L^{-1}. Initial concentration of aniline: 25 mg L^{-1} (-■-), 50 mg L^{-1} (-□-), 75 mg L^{-1} (-●-), and 100 mg L^{-1} (-○-). Each experiment was repeated five times and the means of these data were presented.

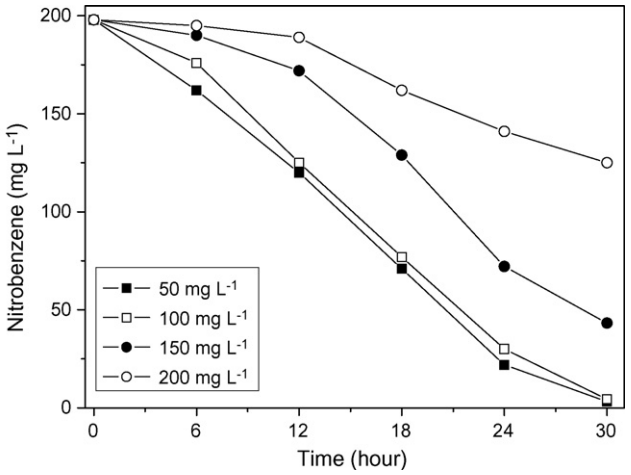

Fig. 8. Degradation of nitrobenzene in the presence of phenol by polyurethane-immobilized Z1. The initial concentration of nitrobenzene was 200 mg L^{-1}. Initial concentration of phenol: 50 mg L^{-1} (-■-), 100 mg L^{-1} (-□-), 150 mg L^{-1} (-●-), and 200 mg L^{-1} (-○-). Each experiment was repeated five times and the means of these data were presented.

Table 4
The nitrobenzene degradation in the presence of aniline by free cells and polyurethane-immobilized Z1.

	Aniline (mg L^{-1})	Nitrobenzene degradation (%)	Time (h)
Z1 free cells	25	60.20	30
	50	44.90	30
	75	21.43	30
	100	9.95	30
Polyurethane-immobilized Z1	25	98.36	30
	50	98.57	30
	75	44.13	30
	100	20.02	30

Z1 free cells degrade 200 mg L^{-1} nitrobenzene in the presence of 100 mg L^{-1} phenol without inhibition. However, at higher concentration of 150 mg L^{-1} phenol, the degrading rate of nitrobenzene by free cells decreases sharply. In this paper, phenol concentrations of up to 100 mg L^{-1} also did not inhibit the nitrobenzene degradation by polyurethane-immobilized Z1 (Fig. 8). Even the concentration of phenol reached to 150 mg L^{-1}, polyurethane-immobilized Z1 degraded, up to 78%, nitrobenzene with 200 mg L^{-1} after 30 h. At higher concentration of 200 mg L^{-1}, the inhibition effect of phenol

Table 5
The nitrobenzene degradation in the presence of phenol by free cells and polyurethane-immobilized Z1.

	Phenol (mg L^{-1})	Nitrobenzene degradation (%)	Time (h)
Z1 free cells	50	60.43	30
	100	61.28	30
	150	41.62	30
	200	10.92	30
Polyurethane-immobilized Z1	50	97.81	30
	100	98.67	30
	150	78.13	30
	200	36.73	30

on nitrobenzene degradation became predominant. During the degradation period, the concentrations of aniline and phenol kept invariable.

The degradations of 200 mg L^{-1} nitrobenzene in the presence of phenol or aniline by Z1 free cells and polyurethane-immobilized Z1 were listed in Tables 4 and 5, respectively. These results demonstrate that the tolerance of polyurethane-immobilized Z1 toward toxic chemicals is higher than that of free cells.

4. Conclusions

R. mucilaginosa Z1 is able to utilize nitrobenzene as a sole source of carbon, nitrogen, and energy under aerobic condition. In the previous study, we have investigated the capacity of Z1 free cells to degrade nitrobenzene. In this paper, we further do research on the capacity of polyurethane-immobilized Z1 to degrade nitrobenzene. Based on the kinetic study and the nitrobenzene degradations under different conditions, it is found that more efficient degradation of nitrobenzene is achieved by polyurethane-immobilized Z1 than by free cells. Polyurethane-immobilized Z1 has higher tolerances toward acid, alkali, heat, salinity, and toxic chemicals than those of free cells. Compared to free cells, the ability of polyurethane-immobilized Z1 to resist nitrobenzene shock load is enhanced. Moreover, the immobilization of microbes in polyurethane is versatile and cost-effective. Immobilization technology has a higher potential for being applied to the treatment of nitrobenzene wastewater.

Acknowledgements

The authors are thankful to Dr. Qidong Zhao in Dalian University of Technology and associated professor Yusuo Yang in Dalian University for helpful discussions.

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