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*Full Length Research Paper*

# Biodegradation and growth characteristics of a toluene-degrading strain

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**A toluene-degrading strain was isolated from active sludge in this study. Both growth characteristic and the performance to degrade toluene by the strain in batch culture mode were evaluated. Results showed that the isolated strain presented a good ability to remove toluene with the maximum removal efficiency of 93.8%. Growth and toluene degradation occurred at 20 to 50°C but the optimum was found to be 30°C for both. The optimal pH for growth and toluene degradation was 6.5. Lower toluene concentrations (1.19 to 2.45 mg/l) promoted faster growth rates than higher concentrations (3.28 to 6.17 mg/l) during the first 20 h; this could be probably due to the substrate inhibition effects. The removal efficiencies of toluene (90 to 95%) were almost the same within the concentrations ranges (1.19 to 6.17 mg/l). Kinetic analysis results indicated that the biodegradation of toluene followed first-order kinetics, and the removal rate constant (*k*) was 0.0385. Finally, the isolated strain was identified as *Pseudomonas* sp. using 16S rDNA sequencing.**

**Keywords:** Biodegradation, growth characteristic, toluene, *Pseudomonas.*

## INTRODUCTION

With the development of industry, the production of volatile organic compounds (VOCs) has been increased over the past two decades. This causes sometimes severe environmental problems. During the wide variety of VOCs, the aromatic VOCs, because of the non-degradation and high toxicity, are considered as the major environmental pollutants (Yadav et al., 1995).

Toluene is a common aromatic chemical, which is widely used in manufacturing process. It is a hydrophobic and carcinogenic compound. Even at low concentrations, toluene has been proven to damage human liver and kidney and paralyze the central nervous system (Murata et al., 1999). Large amounts of toluene released into the atmosphere every year would impair the air quality and are a threat to public health (Chan and You, 2010).

In response to the treatment of toluene and similar aromatic gaseous contaminates, the biological technology is becoming a cost-effective technology because of its high efficiency, low environmental risk and easy application. In the process of biological treatment, aromatic contaminates are removed through the biological activities of microorganisms. Bacteria and fungi are the two dominant microbial groups attributed to biological removal of aromatic contaminates. Bacteria present advantages of more rapid growth and higher removal efficiency than fungi (Okamoto et al., 2003; Sunday et al., 2007; Wang et al., 2003). There have been various investigations on aromatic compounds degradation by adaptive bacterial species (Lee et al., 1996; Connor et al., 1996).

In this study, a toluene-degrading strain was isolated from active sludge of wastewater treatment plant. Both growth characteristics and the performance to degrade toluene of a strain in batch culture were evaluated. The significant factors affecting toluene degradation were also studied. Finally, the strain was identified by 16S rDNA sequencing.

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**Table 1.** Experimental batches to evaluate factors affecting toluene degradation.

| Batch ID | Objective and series | Condition |
|---|---|---|
| Batch 1 | Effects of temperature (T= 20, 25 30, 35, 40, 45, 50°C) | pH= 6.5, $c_0$= 2.60 mg/l, reaction time 24 h, $OD_0$*= 0.26 |
| Batch 2 | Effects of pH (pH= 4, 5, 5.5, 6, 6.5, 7, 7.5, 8, 9) | T= 30°C, $c_0$= 2.60 mg/l, reaction time 24 h, $OD_0$*= 0.26 |
| Batch 3 | Effects of initial toluene concentration ($c_0$= 1.19, 2.45, 3.28, 4.26, 5.06, 6.17 mg/l) | pH= 6.5, T= 30°C, $OD_0$*= 0.05 |

*$OD_0$ means the initial bacteria biomass.

## MATERIALS AND METHODS

### Isolation of toluene-degrading bacteria

The toluene-degrading bacteria in this study were isolated from the actived sludge, designated as MJ001, which was collected from the excess sludge of an industrial wastewater treatment plant in Tianjin, China. For culture enrichment, 10 g actived sludge was added into 100 ml sterile water to get a uniform suspended liquid. Then 10 ml of suspension was added into 90 ml of inorganic nutrient medium with 1% (V/V) liquid toluene as carbon source. The inorganic nutrient medium contained 2 g/l $(NH_4)_2SO_4$, 1.3 g/l $Na_2HPO_4 \cdot 12H_2O$, 2 g/l $KH_2PO_4$, 0.2 g/l $MgSO_4 \cdot 7H_2O$. The mixed liquid suspension was cultivated for 2 days under shaking conditions (150 rpm, 30°C). The enriched consortium was transferred to the plate filled with toluene-added medium. After 2 days cultivation, the colonies on culture plate were selected and pure culture was added into the liquid medium. Then the cultivation procedure mentioned earlier was repeated twice. Finally, one microbial strain which could utilize toluene as the sole carbon source was acquired.

### Experimental procedure of bacterial culture on toluene

To test potential degradation of the strain on toluene, both the toluene concentration profile and microbial growth characteristics were examined. About 1 to 5 ml of enriched bacterial suspension was added into 100 ml liquid inorganic nutrient medium in 250 ml bottle closed with the shutoff stopper. A certain amount of toluene was injected into the medium. Then the bottles were incubated on a rotary shaker of 150 rpm under the conditions shown in Table 1. Both toluene concentration and bacteria biomass were analyzed at a certain interval (3 h). Meanwhile, control cultures, which only contain liquid toluene or microbial suspension (without toluene addition), were set up in order to determine the physical losses. All the samples were taken in duplicate.

### Identification of the toluene-degrading bacteria

In this study, 16s rDNA analysis were used to identify the isolated strain. The genomic DNA was extracted using the DNA extraction kit (QIAGEN GmbH, Hilden, Germany) and the 16s rDNA was amplified in PCR using the genomic DNA as template and bacterial universal primers: (5¢- AGAGTTTGATCC TGGCTCAG -3¢) and (5¢- AAGGAGGTGATC CAGCCGCA -3¢). The PCR reactions contained 1 μl template, 2 μl primers (each) and 25 μl PCR mixture (total volume: 50 μl). The reactions were preheated at 95°C for 5 min, followed by 30 cycles of 94°C for 3 min, 55°C for 30 s and 72°C for 90 s. The amplification products were purified using the PCR purification kit (QIAGEN GmbH, Hilden, Germany). The nucleotide sequence was established directly from the PCR product. Species identification was compared with GenBank database using basic local alignment search tool (BLAST) analysis.

### Analytical methods

The concentration of toluene was analyzed by a gas chromate-graph (Agilent 6890N, USA) with an FID detector and a capillary column (HP-5 30.0 m×320 μm×0.25 μm). Nitrogen was used as the carrier gas (1.0 ml/min), while pure hydrogen and air were supplied to the FID detector. The temperatures of the column oven, injector and detector were 80, 230 and 250°C, respectively. The capillary column pressure was set at 5.8 psi. Headspace (HSS 86.50) automatic injection instrument was used to determine the toluene in aqueous phase. Valve temperature of headspace was settled at 60°C with equilibrium time of 40 min and injection volume of 1 ml.

The bacterial biomass in culture bottle was measured in the aqueous phase by monitoring optical density (OD) at 600 nm using a protein nucleic acid analyzer (Bio-Rad, USA).

## RESULTS AND DISCUSSION

### Microbial growth characteristics

The isolated strain was first tested for its ability to grow on toluene under various cultivation conditions.

### Effect of temperature

Although, a number of different microorganisms have been tested for their growth ability on toluene (Xi et al., 2006; Genovese et al., 2008; Leng et al., 2010; Li et al., 2010), there has been little focus on microorganisms that can function at high temperature. Information is scarce regarding the effect of high temperatures on cell growth and toluene biodegradation, as experimental tempe-ratures in most studies have typically been lower than 40°C. Figure 1 shows the $OD_{600}$ values after 24 h cultiva-tion of the isolated strain under a wild temperature range

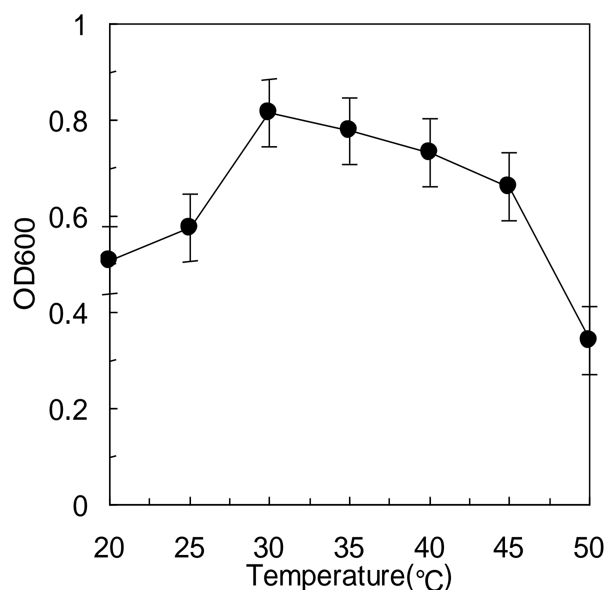


**Figure 1.** $OD_{600}$ values after 24 h cultivation under various temperatures (other conditions as following: initial toluene concentration $c_0$= 2.60 mg/l, initial OD600= 0.26, pH= 6.5).

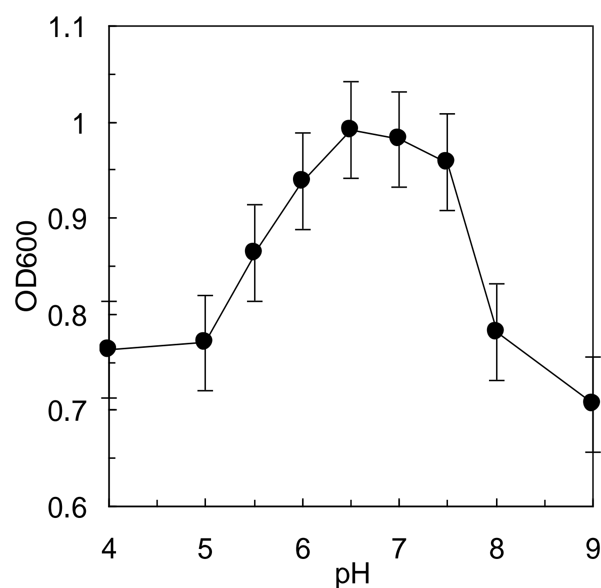


**Figure 2.** $OD_{600}$ values after 24 h cultivation under various pH conditions (other conditions as following: initial toluene concentration $c_0$= 2.60 mg/l, initial OD600= 0.26, temperature was maintained at 30°C).

vary from 20 to 50°C. The results showed that all the $OD_{600}$ values were higher than initial $OD_{600}$ value of 0.26 (the cultivation time is 0 h). And the optimum temperature for the isolated strain growth was around 30°C. The results suggested that the microbial strain could grow under all the tested temperatures even under 50°C ($OD_{600}$= 0.34). These results are especially helpful when considering biodegradation in contaminated areas with obvious annual temperature fluctuations.

Temperature influences microbial activity. Microbial species are adapted to their specific temperature optimum. If temperature declined, the metabolism of the microbial cell will slow down, reducing the growth rate. As reported by Devinny et al. (1999), most growth rates approximately double when the temperature is increased by 10°C. However, there must be limits since proteins are denatured beyond their optimal activity temperature. Moreover, other cell components such as structural lipids of the membranes could also be decomposed by high temperature (Devinny et al., 1999). Therefore, as the temperature increases, each microbial strain reaches a point where it can achieve the maximum growth.

## Effect of pH

Figure 2 compares the $OD_{600}$ values of the isolated strain under different initial pH conditions. As the initial pH increased from 4 to 6.5, the $OD_{600}$ values went up from 0.763 to 0.992. However, higher pH values beyond 6.5 (pH 6.5 to 9) resulted in a gradual decline of the microbial strain growth.

The physiological pH effect on microbial activity is similar to the effect of temperature. All species have optimal pH conditions, some are more tolerant to a wider pH range, but some are prefer to a lower pH range. Some species are good at high pH and some at low pH. In this study, a moderate pH range (pH= 6.5) was found to be optimal to support growth for the isolated specie. However, under certain conditions, the microbial growth was even observed in the presence of toluene down to pH 4, which is quite unusual and indicates the ability of the present strain to remove toluene under acidic conditions. Other researchers also reported the micro-organism under acid conditions. Veiga et al. (1999) observed higher microbial activities in the presence of alkylbenzenes down to pH 2.5 with mixed cultures.

## Effect of initial toluene concentration

Figure 3 shows the effects of initial toluene concentration on growth of the isolated strain. It was observed that the $OD_{600}$ value remained at around 0.05 in the control test ($c_0$= 0 mg/l) as the microbial strain did not grow without substrate. During the first 20 h, the microbial strain under the lower initial toluene concentrations ($c_0$= 1.19 and 2.45 mg/l) grew faster than under the higher initial toluene concentrations ($c_0$= 3.28 to 6.17 mg/l). This suggests that perhaps the high-concentration of substrate inhibited the growth of the strain.

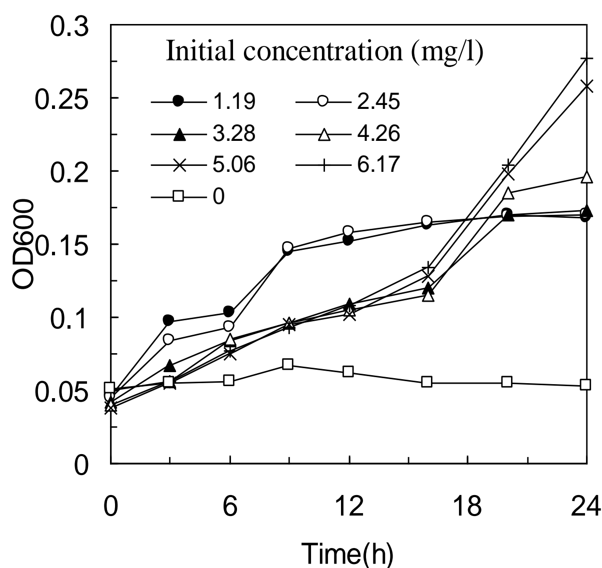


**Figure 3.** $OD_{600}$ values during cultivation process under various initial toluene concentrations (other conditions as following: initial OD600= 0.05, pH= 6.5, temperature was maintained at 30°C).

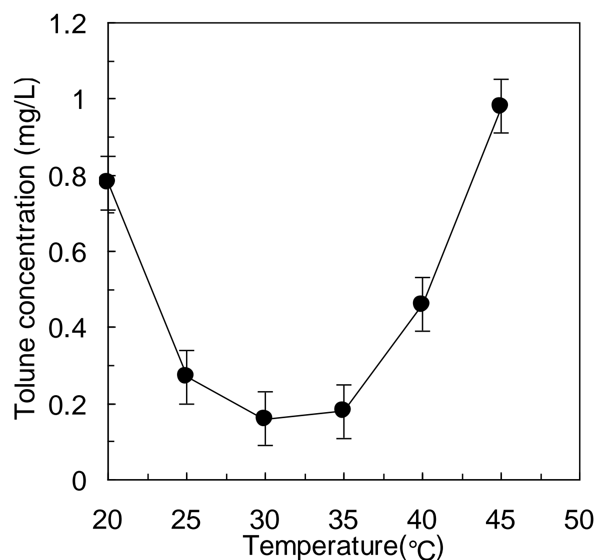


**Figure 4.** Toluene concentrations after 24 h degradation under various temperatures (other conditions as following: initial toluene concentration $c_0$= 2.60 mg/l, initial $OD_{600}$= 0.26, pH= 6.5).

After the 20th h, the $OD_{600}$ value in the higher toluene concentrations increased rapidly (represented by the slope of $OD_{600}$ curves). This is probably caused by the inhibition effects of elimination due to the toluene concentrations reduction during the cultivation process. Finally, the microbial strain under the higher toluene concentrations yielded higher biomass than those under the lower concentrations. Similar results were also reported by Zilli et al. (2005) and Duldhardt et al. (2007). The author found that the microbial activity could be inhibited by excess benzene concentrations.

## Biodegrading of toluene

### Effect of temperature

The degradation efficiency of toluene by the isolated strain after 24 h was examined under various temperatures, as shown in Figure 4. The toluene degradation was investigated at 30 and 35°C at concentrations of 0.16 and 0.18 mg/l. It was found that the degradation at 30 and 35°C was close to each other. Under the other tested temperatures, it was observed that toluene was also removed but the degradation efficiency was much lower compared with 30 and 35°C. For example, when the temperatures were set to 20 and 45°C, the toluene concentrations declined to 0.78 (removal efficiencies of 70%) and 0.98 mg/l (removal efficiencies of 62.3%), respectively. These results indicated that the moderate temperature of 30°C promoted the degradation of toluene compared with other temperatures.

Yadav and Reddy, (1993) observed substantially greater degradation of toluene by *Pseudomonas chrysosporium* at 25 than 37°C, which indicated that the optimum growth temperature of the microorganism at 37°C appeared not to be the optimum temperature for pollutants degradation. Our data showed the same optimum temperature of 30°C for both microbial growth and pollutants degradation. The reason for the different optimum temperatures mentioned earlier was mainly due to the distinct enzyme systems.

### Effect of pH

Figure 5 compares the removal performance of toluene with respect to various pH values. When the pH increased from 4 to 6.5, the residual toluene concentration decreased from 0.66 mg/l (removal efficiency of 74.6%) to 0.16 mg/l (removal efficiency of 93.8%) after 24 h. Further increase of pH values beyond pH= 6.5 resulted in a gradual increase of residual toluene concentrations indicating a lower toluene degradation efficiency. When the pH went up to pH= 9, the residual toluene concentration was as high as 1.22 mg/l (removal efficiency of 53.1%).

Each species of microorganisms is most successful over a certain range of pH and will be inhibited or killed if conditions are more outside this range. Lu et al. (2002) showed the BTEX removal efficiencies as a function of pH of the nutrient feed. It was seen that toluene removal efficiencies increased as the pH of the nutrient feed

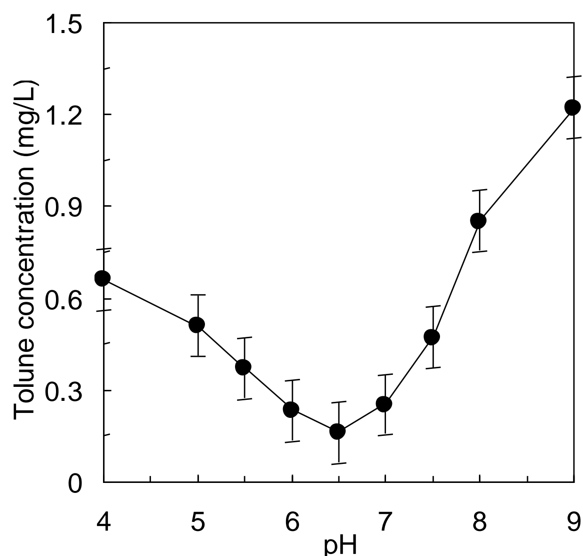


**Figure 5.** Toluene concentrations after 24 h degradation under various pH conditions (other conditions as following: initial toluene concentration $c_0$= 2.60 mg/l, initial $OD_{600}$= 0.26, temperature was maintained at 30°C).

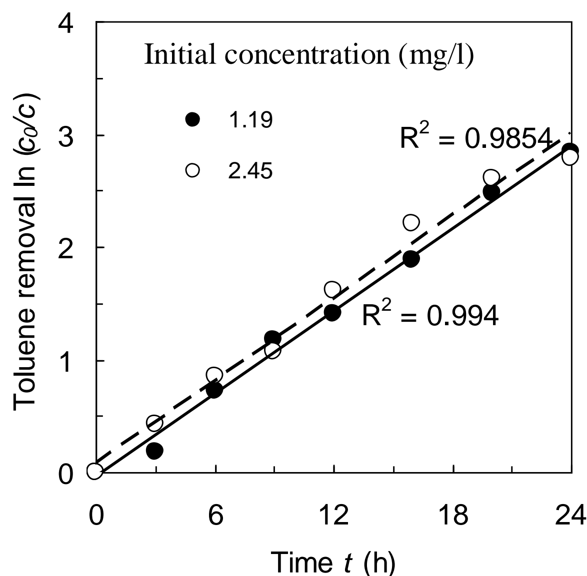


**Figure 7.** Relationship between toluene removal ln($c_0$/$c$) and reaction time $t$.

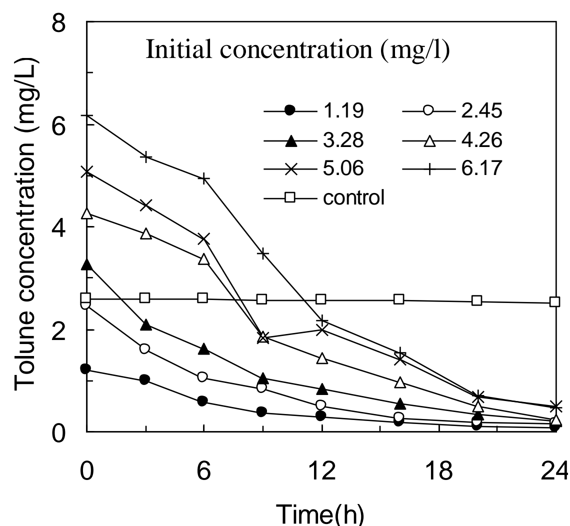


**Figure 6.** Toluene concentration profile during degradation process under various initial toluene concentrations (other conditions as following: initial $OD_{600}$= 0.05, pH= 6.5, temperature was maintained at 30°C).

increased in the pH range of 5 to 8. However, an opposite trend was observed for pH between 8 and 8.5. This indicated that most bacteria preferred a weak basic environment. In this study, our results indicate that the moderate pH of 6.5 is the optimum pH for toluene degradation. This is consistent with results for micro-organism growth (Figure 2).

## Effect of initial toluene concentration

Figure 6 shows the effects of initial concentration on degradation of toluene by the isolated strain. It was observed that only 3% of toluene was lost during the 24 h degradation, suggesting that the physical loss in this case could be ignored.

The results showed that the strain was able to grow on toluene as its sole carbon source. After 24 h, the residual toluene concentrations in the all tested initial concentrations declined below 0.5 mg/l (Figure 6). The removal efficiency of 94.1% of toluene was observed in the low initial concentration of 1.19 mg/l. The removal efficiencies were almost the same with the increase of initial concentrations. Values of removal efficiencies were 93.3 and 92.5% when the initial concentrations were raised to 3.28 and 6.17 mg/l, respectively.

## Kinetic analysis

The experimental data of toluene degradation were further used for kinetic analysis. Figure 7 shows the relationship between the toluene removal ln($c_0$/$c$) and reaction time ($t$) ($c_0$= 1.19 and 2.45 mg/l). The linear fitting curves describe the toluene removal with time. Similar results have also been required under other initial concentrations. These results suggested that the

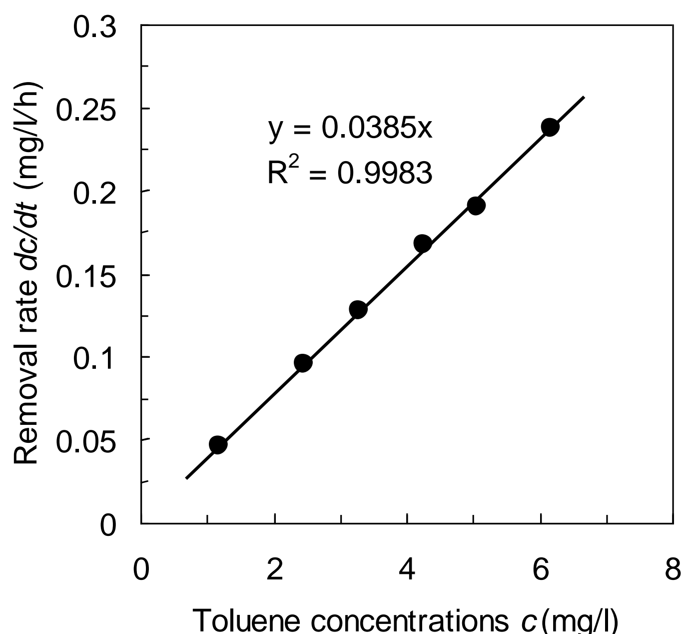


**Figure 8.** Relationship between toluene removal rate and concentrations.

biodegradation reaction of toluene followed first-order kinetics, defined as the following equations (1 and 2).

$$-\frac{dc}{dt} = kc \quad (1)$$

$$ln(c_0/c) = kt \quad (2)$$

In order to test the toluene removal rate constant (*k*), the average removal rate of toluene (d*c*/d*t*) under various initial concentrations (*c*) were calculated. The relationship between toluene removal rates and concentrations were analyzed as shown in Figure 8. The results showed that the removal rates of toluene increased in a linear relationship with increasing concentrations (coefficient of determination, $R^2$= 0.9983). The maximum removal rate of 0.24 mg/l/h of toluene were achieved by the strain (Figure 8). From the slope fitting curve, a rate constant (*k*) of 0.0385 was obtained.

## Identification results of the isolated strain

The isolated strain in this study, designated MJ001, was an aerobic Gram-negative motile rod, with size of 0.5 to 0.7μm in width and 1.5 to 2.5μm in length and having a flagellum. Taxonomical identification of the isolated strain was performed by amplification and sequencing the 16s rRNA genes and comparing them to the database of known 16s rRNA sequences. Alignment of the 16s rRNA gene sequences of MJ001 with sequences obtained by doing a Blast searching revealed 100% similarity to *Pseudomonas* sp. (Figure 9). In this study, this strain was designated as *Pseudomonas* sp. strain MJ001.

*Pseudomonas* sp. has been used to degrade aromatic solvents in previous studies. Lee (2008) has reported around 80% degradation of toluene by *Pseudomonas putida* BZ918. And *P. putida* F1 removed more than 90% of toluene (about 1 mg/l) in nearly 25 h in the presence of an alternative substrate succinate (Rueegg et al., 2007). In this study, the isolated strain, *Pseudomonas* sp. strain MJ001, almost completely degraded toluene of 6.17 mg/l in 24 h. Therefore, the ability to remove toluene would differentiate this strain as specific, even within the same species.

## Conclusions

The following conclusions were drawn from the results presented in this study: (1) an isolated strain from the active sludge presented a good ability to remove toluene with the maximum removal efficiency of 93.8% under the conditions as illustrated in this study; (2) the temperature of 30°C was better than the other temperature within the range of 20 to 50°C for both microbial growth and toluene degradation. Moreover, the microbial strain could grow under all the tested temperatures even under 50°C.

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**Figure 9.** 16s rDNA sequence of MJ001.

These results are especially helpful when considering biodegradation in contaminated areas with obvious annual temperature fluctuations; (3) a moderate pH of 6.5 promoted the growth and the removal of toluene. Under certain conditions, the microbial growth was even observed in the presence of toluene down to pH 4, which is quite unusual and indicates the ability of the present strain to remove toluene under acidic conditions; (4) low toluene concentrations (1.19 to 2.45 mg/l) presented greater growth rates than those under the high initial toluene concentrations (3.28 to 6.17 mg/l) during the first 20 h, probably due to the substrate inhibition effects; (5) kinetic analysis results indicated that the biodegradation of toluene followed first-order kinetics. The removal rate constant ($k$) was 0.0385; (6) finally, the isolated strain was identified as *Pseudomonas* sp. using 16s rDNA sequencing.

## ACKNOWLEDGEMENT

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**REFERENCES**

Chan WC, You HY (2010). The influence of nonionic surfactant Brij 30 on biodegradation of toluene in a biofilter. Afr. J. Biotechnol. 9(36): 5914-5921.

Connor K, Duetz W, Wind B, Dobson ADW (1996). The effect of nutrient limitation of styrene metabolism in *Pseudomonas putida* CA-3. Appl. Environ. Microbiol. 62: 3594-3599.

Devinny JS, Deshusses MA, Webster TS (1999). Biofiltration for air pollution control. Lewis press, Boca Raton, Florida, USA.

Duldhardt I, Nijenhuis I, Schauer F, Heipieper HJ (2007). Anaerobically grown *Thauera aromatica, desulfococcus multivorans, geobacter sulfurreducens* are more sensitive towards organic solvents than aerobic bacteria. Appl. Microbiol. Biotechnol. 77(3): 705-711.

Genovese M, Denaro R, Cappello S, Di Marco G, La Spada G, Giuliano L, Genovese L, Yakimov MM (2008). Bioremediation of benzene, toluene, ethylbenzene, xylenes-contaminated soil: a biopile pilot experiment. J. Appl. Microbiol. 105(5): 1694-1702.

Lee K, Gibson DT (1996). Stereospecific dehydroxylation of the styrene vinyl group by purified naphthalene dioxygenase from *Pseudomonas* sp. strain NCIB 9816-4. J. Bacteriol. 178: 3353-3356.

Lee ES, Park JY, Yeom SH, Yoo YJ (2008). Effects of nitrogen sources on toluene degradation by *Pseudomonas putida* BZ918. Kor. J. Chem. Eng. 25 (1): 139-143.

Leng L, Zhang J, Li SP (2010). Isolation, Identification and Characteristics of a Toluene-degrading Bacterium JB-1. Chinese J. Appl. Environ. Biol. 16(1): 118-121.

Li Y, Li J, Wang C, Wang PF (2010). Growth kinetics and phenol biodegradation of *psychrotrophic Pseudomonas putida* LY1, Bioresour. Technol. 101 (17): 6740-6744.

Lu C, Lin MR, Chu C (2002). Effects of pH, moisture, and flow pattern on trickle-bed air biofilter performance for BTEX removal. Adv. Environ. Res. 6: 99-106.

Murata M, Tsujikawa M, Kawanishi S (1999). Oxidative DNA damage by minor metabolites of toluene may lead to carcinogenesis and reproductive dysfunction. Biochem. Biophys. Res. Commun. 261: 478-483.

Okamoto K, Izawa M, Yanase H (2003). Isolation and application of a styrene-degradation strain of *Pseudomonas putida* to biofiltration. J. Biosci. Bioeng. 95: 633-636.

Ruegg I, Hafner T, Bucheli-Witschel M, Egli T (2007). Dynamics of benzene and toluene degradation in *Pseudomonas putida* F1 in the presence of the alternative substrate succinate. Eng. Life Sci. 7(4): 331-342.

Sunday AA, Flynn WP, Matthew OI, Olukayode OA, Clay F, Nathan G (2007). Aerobic degradation of di- and trichlorobenzenes by two bacteria isolated from polluted tropical soils. Chemosphere, 6(10): 1939-1946.

Veiga MC, Fraga M, Amor L, Kennes C (1999). Biofilter performance and characterization of a biocatalyst degrading alkylbenzene gases. Biodegradation, 10(3): 169-176.

Wang C, Xi JY, Hu HY, Wen XH (2008). Biodegradation of gaseous chlorobenzene by white rot fungi. Biomed. Environ. Sci. 21(6): 474-478.

Xi JY, Hu HY, Qian Y (2006). Effect of operating conditions on long-term performance of a biofilter treating gaseous toluene: biomass accumulation and stable-run time estimation. Biochem. Eng. J. 31: 165-172.

Yadav JS, Reddy CA (1993). Degradation of benzene, toluene, ethylbenzene, and xylenes (BTEX) by the lignin-degrading basidiomycete *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 59(3): 756-762.

Yadav JS, Wallace RE, Reddy CA (1995). Mineralization of mono- and diCBs and simultaneous degradation of chloro- and methyl-substituted benzenes by the white rot fungus *Phanerochaete chrysosporium*. Appl. Environ. Microbiol. 61(2): 677-680.

Zilli MC, Guarino D, Daffonchio S, Borin AC (2005). Laboratory-scale experiments with a powdered compost biofilter treating benzene-polluted air. Process Biochem. 40(6): 2035-2043.