Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2016.1149889

57 (2016) 25011–25017
November

Taylor & Francis
Taylor & Francis Group

Characterization and biodegradation potential of an aniline-degrading strain of *Pseudomonas* JA1 at low temperature

Yu Jiang, Yu Shang, Jun Zhou, Kai Yang*, Hongyu Wang*

School of Civil Engineering, Wuhan University, Wuhan 430072, China, emails: yjiangwhu10@163.com (Y. Jiang), yushangwhu@163.com (Y. Shang), zhoujun0913@hotmail.com (J. Zhou), yk220220@126.com (K. Yang), Tel. +86 027 61218623; Fax: +86 027 68775328; email: hyuwang@yeah.net (H. Wang)

Received 19 June 2015; Accepted 25 January 2016

ABSTRACT

Aniline is a common toxic compound in various industrial processes. It should be removed from effluents before their discharge into water bodies. A cold-tolerant bacterium JA1 capable of utilizing aniline as the sole source of carbon, nitrogen, and energy was isolated from activated sludge samples collected from a sewage treatment plant. The isolate was identified as *Pseudomonas* species by 16S rDNA sequence analysis. Strain JA1 was able to degrade less than 1600 mg l^{-1} aniline with high efficiency and grow well at pH varying from 5.0 to 9.0. The suitable temperature ranged from 10 to 35°C. Under optimal conditions (pH 7.0, 25°C), 800 mg l^{-1} of aniline was almost completely degraded within 24 h. Substrates utilization tests showed that the strain could grow well when sodium acetate, glucose, sodium citrate, benzene, aniline, phenol, nitrobenzene, and *m*-cresol acted as the sole source of carbon. The adaptation to wide range of aniline concentration, pH, temperature, and different substances made strain JA1 promising for aniline degradation from industrial wastewater.

Keywords: Aniline; Biodegradation; Low temperature; *Pseudomonas* sp.

1. Introduction

Aromatic compounds are widely distributed in nature via irresponsible discharge of industrial or agricultural wastewaters since most of these substrates are common intermediates in the manufacture of organic products [1–3]. One of typical aromatic amines is aniline, which is applied in the production of dyes, pharmacy, plastics, rubber, herbicides and many other industries [4–7]. Effluents from such processes are increasingly released into water body and pose a risk to life forms due to toxic properties of aniline. Therefore, the Ministry of Environmental Protection of China, the European Chemical Substances Information System, and US EPA have attached importance to its removal and classified it as one of the pollutants of major concern [8–11].

In order to remove pollutants efficiently with low cost, biological methods, especially aerobic biodegradation have been taken into account [12]. Though many organisms are able to degrade organic substances, aniline has had an inhibitory effect on cell growth and metabolism of most conventional bacteria [11]. However, it has been found that there are several micro-organisms naturally capable of synthesizing aromatic substances or developing catabolic pathways through gradual adaptation to environments exposed to this kind of pollutants. Previous studies have

*Corresponding authors.

reported aromatic biodegradation of bacteria and their potential applications for bioremediation. For instance, a strain *Bacillus* sp. was isolated to debrominate and mineralize 2,4,6-tribromophenol [13]. 2-chloro-4-nitroaniline could be used as the sole carbon, nitrogen, and energy source by *Rhodococcus* sp. strain MB-P1 under aerobic conditions [14]. Likewise, there are aniline-degrading bacteria, yeast, and fungi such as *Delftia*, *Erwinia*, and *Candida* [15–17]. *Pseudomonas* species as one of the most common strains applied to water treatment was documented to degrade phenol, 4-chlorophenol, aniline, methyl parathion, and many other recalcitrant compounds [18–21]. However, it is widely acknowledged that the change of environmental conditions may have a marked impact on microbial treatment [22–24]. Most aforementioned micro-organisms were mesophilic and when temperature dropped below 20˚C, their growth rates as well as degradation efficiencies of pollutants might decrease dramatically. Hence, it is significant for the isolated strain to maintain active at both moderate and low temperature.

This study isolated and identified a cold-adapted bacterium JA1 which could utilize aniline as the sole source of carbon, nitrogen, and energy. Effects of different initial aniline concentrations, pH values, temperatures, and carbon sources were evaluated to optimize the process parameters for aniline degradation. To our knowledge, there are limited information about aniline biodegradation of aerobic bacteria under both thermophilic and cryophylactic conditions. The adaptability to various substrates as well as a wide range of aniline concentration, temperature, and pH made the strain crucial for industrial wastewater treatment.

2. Materials and methods

2.1. Strain isolation and enrichment

The strain JA1 was isolated from sludge samples collected from a biological tank of Taiping sewage treatment plant in Harbin, Heilongjiang Province, China. The activated sludge was pretreated and acclimatized before spread on plates containing selective media in which aniline acted as the sole source of carbon, nitrogen, and energy. Cultivation was carried out in an incubator at 10˚C for 4 d. The strains were picked on the basis of their ability to grow at low temperature in the presence of aniline. One of them was named JA1, which was selected for further tests. After several times of streaking and multipoint inoculation, the strain was purified according to colony morphology and inoculated to a 500-mL conical flask with 200 mL of liquid selective media. The selective mineral medium composed of the following ingredients (g l^{-1}): Aniline 0.6, KH_2PO_4 2.0, K_2HPO_4 1.3, NaCl 5.0. The trace element solution was composed of the following ingredients (per liter): $FeCl_3{\cdot}6H_2O$ 0.01 g, H_3BO_3 0.15 g, $CuSO_4{\cdot}5H_2O$ 0.03 g, KI 0.18 g, $MnCl_2{\cdot}4H_2O$ 0.12 g, $Na_2MoO_4{\cdot}2H_2O$ 0.06 g, $ZnSO_4{\cdot}7H_2O$ 0.12 g, $CoCl_2{\cdot}6H2O$ 0.15 g, EDTA 10 g. The pH value of solution was adjusted to 7.0. Selective media were sterilized by autoclaving at 121˚C for 15 min before inoculation.

After cell density of seed culture dramatically increased, 10% (v/v) of mixed solution was transferred to beef extract peptone medium whose ingredient was (per liter): Beef extract 5.0 g, peptone 10.0 g, NaCl 5.0 g. Flask was incubated in a rotary shaker at 10˚C and 160 rpm. Subsequently, 10% (v/v) of culture was added into another 200 mL of selective medium. The inoculum transfers were done in exponential phase. At the end of alternate incubation, 200 mL of enriched culture was obtained and kept at 4˚C in a freezer for further characterization and aniline degradation studies.

2.2. Strain characterization and genetic identification

The strain was further characterized using biochemical and molecular methods. The cell size, shape, colony morphology, motility, and Gram stain reactions were observed using an optical microscope (CX31, Olympus Co., Ltd, Tokyo, Japan) and a scanning electron microscope (SEM, VEGA 3 LMU, TESCAN, Czech Republic). Genomic DNA of the bacterial strains was extracted from fresh bacterial culture using the Ezup Pillar bacterial genomic DNA Extraction Kit (Sangon Biotech Co., Ltd, Shanghai, China). The part of the 16S rRNA genes was amplified with a pair of forward (5′-CAGAGTTTGATCCTGGCT-3′) and reverse (5′-AGGAGGTGATCCAGCCGCA-3′) primers. PCR were conducted in 25 μL of PCR reaction system containing 25 ng of extracted DNA, 1 μL of 2.5 mM dNTPs, 2.5 μL of 10 × Buffer with Mg^{2+}, 0.2 μL of enzyme, 0.5 μL of 10 uM forward and reverse primers. Amplifications were carried out as follows: 4 min of denaturation at 94˚C, 30 cycles of 45 s at 94˚C, 45 s at 55˚C, and 1 min at 72˚C. An additional 10 min at 72˚C at the end of 30th cycle aimed at repair and extension. The amplified products were purified using agarose gel electrophoresis and the sequencing was carried out at Sangon Biotech Company (Shanghai, China). The nucleotide sequence was submitted to the GenBank databases for comparison and identification using the BLAST package software (http://blast.ncbi.nlm.nih.gov/Blast.cgi). GenBank accession number for the 16S rRNA gene sequences of strain JA1 is

KR006341. A maximum likelihood phylogenetic tree was generated by neighbor-joining methods [25] with MEGA 6.

2.3. Growth and aniline degradation

20 mL of seed culture kept in a freezer was added to a 500-mL conical flask with 200 mL of selective mineral medium followed by incubation in a rotary shaker at 10℃ at agitation speed of 160 rpm. When the optical density of cells was over 0.6, 10 mL of emulsion was transferred into 15 conical flasks with 200 mL of fresh selective mineral medium, respectively. Initial aniline concentrations were from 100 to 1,800 mg l^{-1} and all initial cell densities were controlled by adjusting OD_{600} to around 0.06. The organism was cultivated in rotary shaker for 7 d at 160 rpm and 10℃. 10 mL of samples from flasks were taken at intervals of 4 h to assay aniline concentration and cell density. All experiments were carried out in triplicate sets for checking the consistency of data.

2.4. Effect of pH values and temperature on aniline biodegradation

Evaluation of acid and alkalinity tolerance of strain JA1 was carried out with optimal aniline concentration obtained from results of tests described in section 2.3. Seven 500-mL conical flasks containing 200 mL of selective mineral media were adjusted to different pH values varying from 4.0 to 10.0 at intervals of 1.0. To find optimal temperature ranges of the strain, another seven flasks were cultivated at temperatures ranging from 10 to 40℃. In each test, aniline concentrations as well as cell growth were investigated. All tests were done in triplicate.

2.5. Substrate utilization study

Several organics, namely sodium acetate, glucose, sodium citrate, alcohol, benzene, aniline, phenol, nitrobenzene, *m*-cresol, and nitrophenol were acted as sole source of carbon and energy. All the substrates were used at a concentration of 300 mg l^{-1}, in both solid and liquid media. The plates containing these 10 organics were incubated for 4 d. The substrates for carbon source utilization tests were sterilized before transferred into flasks. OD_{600} of flasks were tested every 4 h to determine cell growth rates.

2.6. Analytical methods

The cell density of the micro-organism was investigated by measuring the optical densities at 600 nm in a UV–visible spectrophotometer (nanbeijt, China). Samples collected at intervals of the tests were centrifuged for 10 min at 12,000 rpm to remove biomass. Aniline concentration was determined at 240 nm using high pressure liquid chromatography ((HPLC, Agilent 1,100 Series, Agilent, USA). The pH values of samples were measured by pH meter (PC-320).

3. Results and discussion

3.1. Isolation and characterization of the strain

Several colonies appeared on plates in the presence of 600 mg l^{-1} aniline after 4 d of incubation at 10℃. Five strains were separated by a multistep enrichment and screening technique according to morphologic distinction. One of them was named JA1 and was purified for further tests. The strain JA1 could utilize aniline as the sole source of carbon, nitrogen, and energy. The aerobic colonies were rod-shaped, gram negative, immotile, and capable of surviving in solid media supplemented with aniline ranging from 100 to 1,600 mg l^{-1}. The morphology of the strain under SEM was shown in Fig. 1. The strain was enriched in beef extract peptone medium followed by being transferred to the selective medium. Seed culture was placed in a shaking incubator. Aniline concentrations and OD_{600} were tested every 4 h. The time courses of residual

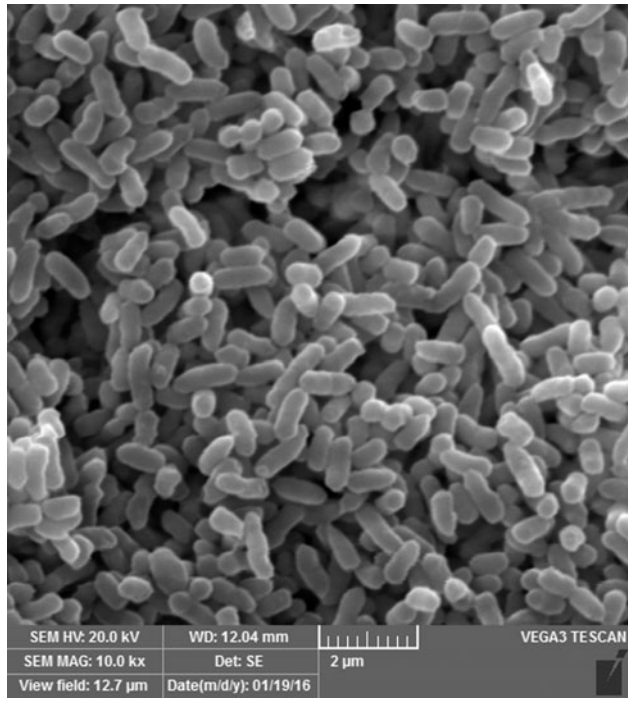

Fig. 1. Scanning electron micrographs of cells of strain JA1 with magnification of 10 k.

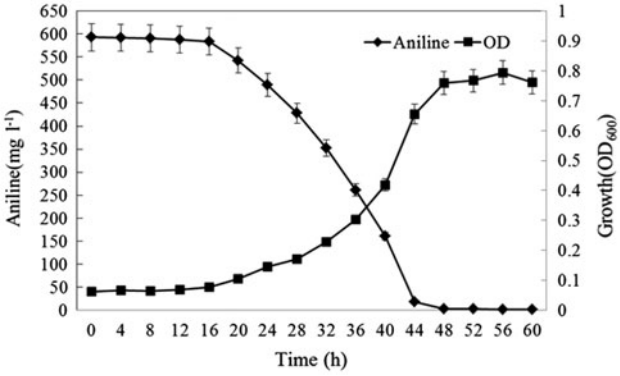

Fig. 2. Time course of growth and aniline degradation by strain JA1 in selective medium (600 mg l^{-1} aniline).

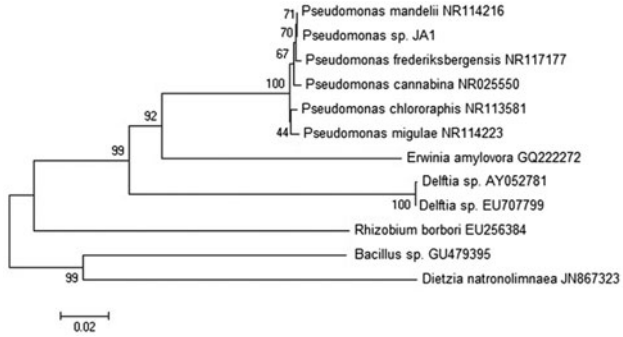

Fig. 3. Phylogenetic tree of strain JA1 based on 1435 unambiguous nucleotides of the 16S rRNA sequence.

aniline concentration in medium and cell growth were shown in Fig. 2. The lag phase was 16 h and there was a slight decline in aniline concentration during this period. In logarithmic phase of cell growth, aniline biodegradation rate increased sharply. Similar trend was found in curve of OD_{600}. More than 99% of 600 mg l^{-1} aniline was degraded in 48 h, with OD_{600} increasing to 0.76, indicating that strain JA1 grew substantially well in the flask. OD_{600} peaked at 0.79 in about 56 h after inoculation, just before the stationary phase. OD_{600} decreased moderately after 60 h (data not shown), due to the fact that the substrate became a limiting factor in metabolic activity of JA1 strain. Trends of the two curves illustrated that aniline biodegradation was closely related to bacterial reproduction.

3.2. Identification of strain JA1

The partial sequence of 1,435 bp 16S rRNA gene fragment of strain JA1 was obtained by PCR amplification and submitted for sequencing. The result has been deposited in the GenBank database. The isolate JA1 was identified as *Pseudomonas* sp. based on 16S rRNA gene sequences. Phylogenetic relationship of strain JA1 with several other bacteria was shown in Fig. 3. 16S rRNA of the strain had 99% sequence similarity with *Pseudomonas mandelii*, *Pseudomonas frederiksbergensis*, and *Pseudomonas cannabina*. As can be seen, the homology between strain JA1 and two other *Pseudomonas* sp. was 98%, indicating that these strains formed a cluster.

3.3. Utilization of aniline

Utilization of aniline by strain JA1 at low temperature was evaluated by investigating substrate removal efficiencies and cell densities in selective media containing different initial concentrations of aniline ranging from 100 to 1,800 mg l^{-1} at 10°C. As shown in Fig. 4, strain JA1 was able to metabolize substrates in both low and relative high concentrations of aniline. It was found that the isolate was capable of removing aniline completely within 5 d when initial aniline concentration was below 1,600 mg l^{-1} (data not shown). The highest average degradation rate appeared in culture supplied with 800 mg l^{-1} of aniline and removal rate was 15.46 mg l^{-1} h^{-1}, indicating strong biodegradation ability of the strain (Fig. 5). Wang et al. reported a *Pseudomonas* strain PN1001 isolated from activated sludge of petrochemical wastewater treatment process which could degrade 81% of 130 mg l^{-1} aniline in 24 h [10]. In contrast with strain PN1001, JA1 had higher efficiencies and tolerance in aniline biodegradation. The adaptability of a wide range of aniline concentration at low temperature made the strain suitable for industrial wastewater treatment on account of notable concentration

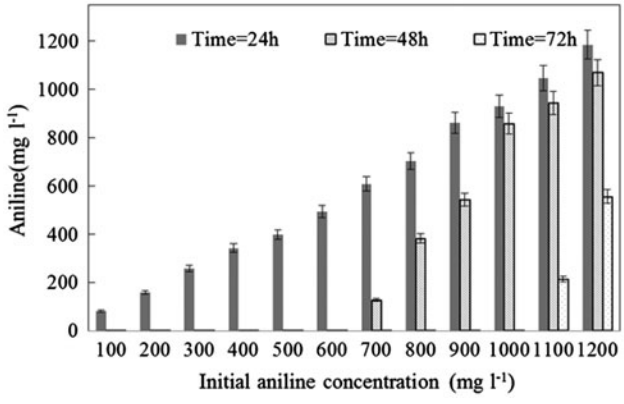

Fig. 4. The effect of different initial aniline concentrations on aniline removal.

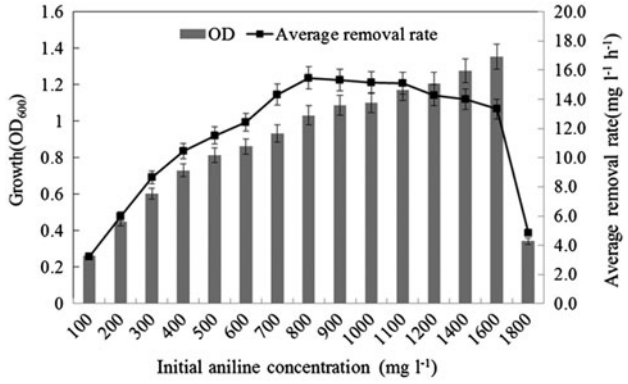

Fig. 5. The cell growth and average removal rate in media containing different initial concentrations of aniline.

variation in industrial effluents. Biodegradation with initial aniline concentration from 100 to 700 mg l^{-1} showed that although lower initial concentration of aniline needed shorter time to be removed, the average degradation rate increased when there was more initial carbon source since the lack of substance would limit cell growth in log period. A similar phenomenon that low concentrations of substrate to some extent retarded bacterial growth was also described by Haddadi and Shavandi [26]. The average removal rate of strain JA1 dropped in media with up to 900 mg l^{-1} of aniline. When initial concentration of aniline was over 1,600 mg l^{-1}, the OD_{600} of strain JA1 significantly decreased, illustrating that bacterial growth was remarkably inhibited beyond this concentration. Growth retardation of many other kinds of bacteria for wastewater treatment was common in media with high aniline concentration. For instance, O'Neill et al. who have studied the biodegradation of aniline by bacterial consortia showed that the rate of bacterial growth decreased at elevated concentrations at neutral pH [4]. Li et al. isolated strain HSA 6 capable of degrading aniline and found that the degradation rate dropped as the concentration of aniline increased and rose when the culture time prolonged [16].

3.4. Effect of pH and temperature on aniline biodegradation

The pH tolerance of strain JA1 was determined by comparing cell density and aniline degradation at different pH values. Results were shown in Fig. 6(a). Strain JA1 was able to survive at pH values varying from 5.0 to 9.0. In view of the time spent reducing pollutants, the suitable pH range for wastewater treatment was from 6.0 to 8.0. At this pH range, 800 mg l^{-1} of aniline could be completely degraded within 4 d and the ideal pH was found to be 7.0, at which 99% of aniline was removed in 52 h (data not shown). In contrast with biodegradation at pH of 8.0, the removal rate at pH of 6.0 was higher. However, the degradation rate noticeably dropped when pH value of media was adjusted to 5.0 or 9.0. Only 22.5% of aniline was degraded within 72 h at pH of 9.0, suggesting that although biodegradation ability of strain JA1 maintained in acid and alkaline culture, the lag-phase prior to logarithmic growth was extended. Adaptability of micro-organisms to adverse environment was also documented by Wang et al. In their research, an aniline-degrading strain *Candida tropicalis* was able to degrade about 15% of 400 mg l^{-1} aniline in 24 h at pH of 5.0 or 9.0. However, aniline concentration dropped to nearly 0 mg l^{-1} in the same period when pH of culture was 7.0 [22].

Biodegradation of the isolate at a range of temperature (10–40°C) was described in Fig. 6(b). The suitable temperature for strain JA1 ranged from 10 to 30°C and 800 mg l^{-1} of initial aniline could be completely degraded within 60 h (data not shown). The highest average removal rate of aniline appeared at 25°C. At

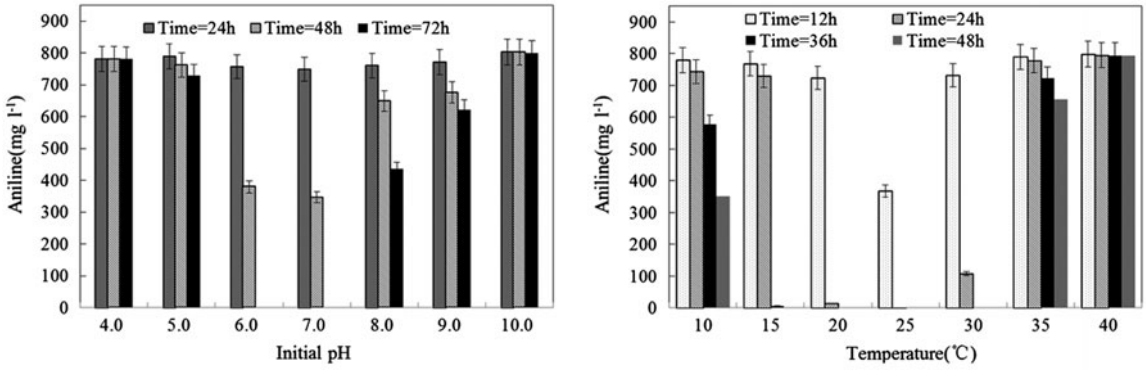

Fig. 6. The effect of different pH values (a) and temperature (b) on aniline removal (800 mg l^{-1} aniline).

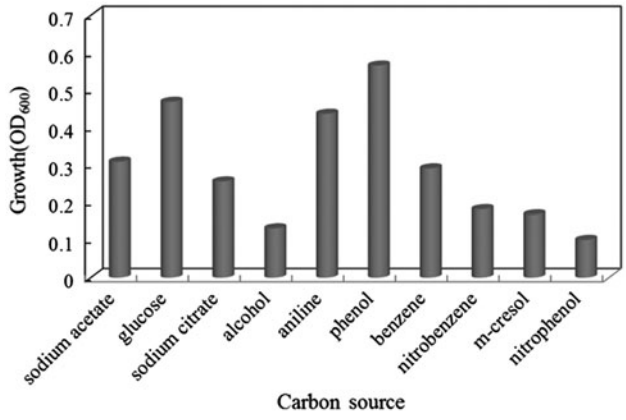

Fig. 7. The cell growth of strain JA1 in media containing different carbon sources.

the optimal temperature, nearly 100% of aniline was degraded within 24 h. Similar result could be found in a study of *Delftia* sp. XYJ6 degrading aniline at different temperature. The suitable temperature for the isolate ranged from 25 to 35˚C [27]. Compared to strain XYJ6, the cold-adapted JA1 was able to grow at a wider range of temperature. In contrast with cell metabolism at temperature beyond 30˚C, bacterial growth was better at relatively low temperature (15 and 10˚C). Aniline concentration dropped to about 720 mg l^{-1} in 36 h at 35˚C, while aniline was almost completely removed at 15˚C in the same period. There was no sign that aniline would be removed at 40˚C, indicating that cell growth of strain JA1 was severely inhibited under this condition. This was consistent with the findings of Zhang et al., who reported cold-adaptive aniline-degrading bacteria *Rhizobium borbori* sp. nov., of which the optimum temperature for cell growth was 28˚C and no growth was observed when temperature was above 37˚C [28]. According to results of temperature tests, strain JA1 belonged to psychrotrophs which was capable of surviving in both moderate and cold conditions [29].

3.5. Utilization of different substrates

The utilization of different carbon sources by strain JA1 was shown in Fig. 7. Cell growth was observed in the presence of sodium acetate, glucose, sodium citrate, alcohol, benzene, aniline, phenol, nitrobenzene, *m*-cresol, and nitrophenol. Strain JA1 showed the best growth in phenol-rich culture, followed by growing in media containing glucose. 99% of 300 mg l^{-1} initial phenol was degraded within 2 d. Another study has discussed a laboratory isolate *Pseudomonas* sp. CP4 which was able to degrade aromatics such as phenol and cresols [30]. Aromatic compounds biodegradation potential of *Pseudomonas* strain was also studied by Al-Zuhair and El-Naas, who immobilized *Pseudomonas putida* in PVA gel particles to remove high concentration of phenol [31]. It was found from Fig. 7 that bacterial growth of strain JA1 was noticeably inhibited in nitrophenol, which was consistent with the cell growth inhibition of *Halomonas* sp. reported by Haddadi and Shavandi [26]. Most aniline-containing industrial effluents are complex and rich in numerous aromatic substances. Hence, the characteristics of strain JA1 capable of utilizing different kinds of carbon sources especially some aromatics are crucial to industrial wastewater treatment.

4. Conclusions

The cold-adapted strain JA1 identified as *Pseudomonas* sp. was isolated from selective culture with aniline as the sole source of carbon, nitrogen, and energy. Strain JA1 was able to withstand relatively wide range of aniline concentration (0–1,600 mg l^{-1}), pH (5.0–9.0), and temperature (10–35˚C). The optimal initial aniline concentration, pH value, and temperature for aniline biodegradation was 800 mg l^{-1}, 7.0, and 25˚C, respectively. 800 mg l^{-1} of aniline was almost completely degraded within 24 h under this condition. Besides aniline, strain JA1 could efficiently utilize sodium acetate, glucose, sodium citrate, benzene, phenol, nitrobenzene, *m*-cresol as the sole source of carbon and energy. The tolerance of the bacteria to vast fluctuations in aniline concentration, temperature, and different aromatic compounds is of great importance to industrial wastewater treatment.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (NSFC) (No. 51378400), the National Science and Technology Pillar Program (2014BAL04B04), the Natural Science Foundation of Hubei Province, China (No. 2013CFB289).

References

[1] N. Boon, J. Goris, P. De Vos, W. Verstraete, E.M. Top, Genetic diversity among 3-chloroaniline-and aniline-degrading strains of the Comamonadaceae, Appl. Environ. Microbiol. 67 (2000) 1107–1115.
[2] D.E.C. Mazzeo, C.E. Levy, D.D.F. de Angelis, M.A. Marin-Morales, BTEX biodegradation by bacteria from effluents of petroleum refinery, Sci. Total Environ. 408 (2010) 4334–4340.

[3] Y. Hou, W. Dong, F. Wang, J. Li, W. Shen, Y. Li, Z. Cui, Degradation of acetochlor by a bacterial consortium of *Rhodococcus* sp. T3-1, *Delftia* sp. T3-6 and *Sphingobium* sp.MEA3-1, Lett. Appl. Microbiol. 59 (2014) 35–42.
[4] F.J. O'Neill, K.C.A. Bromley-Challenor, R.J. Greenwood, J.S. Knapp, Bacterial growth on aniline: Implications for the biotreatment of industrial wastewater, Water Res. 34 (2000) 4397–4409.
[5] G. Emtiazi, M. Satarii, F. Mazaherion, The utilization of aniline, chlorinated aniline, and aniline blue as the only source of nitrogen by fungi in water, Water Res. 35 (2001) 1219–1224.
[6] E. Matsumura, S. Ooi, S. Murakami, S. Takenaka, K. Aoki, Constitutive synthesis, purification, and characterization of catechol 1,2-dioxygenase from the aniline-assimilating bacterium *Rhodococcus* sp. AN-22, J. Biosci. Bioeng. 98 (2004) 71–76.
[7] M. Martins, F. Rodrigues-Lima, J. Dairou, A. Lamouri, F. Malagnac, P. Silar, J. Dupret, An acetyltransferase conferring tolerance to toxic aromatic amine chemicals: Molecular and functional studies, J. Biol. Chem. 284 (2009) 18726–18733.
[8] C.D. Lyons, S.E. Katz, R. Bartha, Persistence and mutagenic potential of herbicide-derived aniline residues in pond water, Bull. Environ. Contam. Toxicol. 35 (1985) 696–703.
[9] M.F. Khan, X. Wu, B.S. Kaphalia, P.J. Boor, G.A.S. Ansari, Nitrotyrosine formation in splenic toxicity of aniline, Toxicology 194 (2003) 95–102.
[10] L. Wang, S. Barrington, J. Kim, Biodegradation of pentyl amine and aniline from petrochemical wastewater, J. Environ. Manage. 83 (2007) 191–197.
[11] M. Sihtmäe, M. Mortimer, A. Kahru, I. Blinova, Toxicity of five anilines to crustaceans, protozoa and bacteria, J. Serb. Chem. Soc. 75 (2010) 1291–1302.
[12] D. Zhao, C. Liu, Y. Zhang, Q. Liu, Biodegradation of nitrobenzene by aerobic granular sludge in a sequencing batch reactor (SBR), Desalination 281 (2011) 17–22.
[13] L. Zu, G. Li, T. An, P. Wong, Biodegradation kinetics and mechanism of 2,4,6-tribromophenol by *Bacillus* sp. GZT: A phenomenon of xenobiotic methylation during debromination, Bioresour. Technol. 110 (2012) 153–159.
[14] F. Khan, D. Pal, S. Vikram, S.S. Cameotra, Metabolism of 2-chloro-4-nitroaniline via novel aerobic degradation pathway by *Rhodococcus* sp. Strain MB-P1, PLoS One 8 (2013) e62178.
[15] Q. Liang, M. Takeo, M. Chen, W. Zhang, Y. Xu, M. Lin, Chromosome-encoded gene cluster for the metabolic pathway that converts aniline to TCA-cycle intermediates in *Delftia tsuruhatensis* AD9, Microbiology 151 (2005) 3435–3446.
[16] J. Li, Z. Jin, B. Yu, Isolation and characterization of aniline degradation slightly halophilic bacterium, *Erwinia* sp. Strain HSA 6, Microbiol. Res. 165 (2010) 418–426.
[17] K. Mucha, E. Kwapisz, U. Kucharska, A. Okruszeki, Mechanism of aniline degradation by yeast strain *Candida methanosorbosa* BP-6, Pol. J. Microbiol. 59 (2010) 311–315.
[18] S. Bathe, Conjugal transfer of plasmid pNB2 to activated sludge bacteria leads to 3-chloroaniline degradation in enrichment cultures, Lett. Appl. Microbiol. 38 (2004) 527–531.
[19] L. Wang, X. Chi, J. Zhang, D. Sun, N. Zhou, Bioaugmentation of a methyl parathion contaminated soil with *Pseudomonas* sp. strain WBC-3, Int. Biodeterior. Biodegrad. 87 (2014) 116–121.
[20] W.S. El-Sayed, M.K. Ibrahim, S.A. Ouf, Molecular characterization of the alpha subunit of multicomponent phenol hydroxylase from 4-chlorophenol-degrading *Pseudomonas* sp. strain PT3, J. Microbiol. 52 (2014) 13–19.
[21] N. Ahmad, I. Ahmed, A. Shahzad, N. Khalid, K. Ahad, G.M. Ali, F. Mehboob, Molecular identification and characterization of *Pseudomonas* sp. NCCP-407 for phenol degradation isolated from industrial waste, J. Korean Soc. Appl. Biol. Chem. 57 (2014) 341–346.
[22] D. Wang, G. Zheng, S. Wang, D. Zhang, L. Zhou, Biodegradation of aniline by *Candida tropicalis* AN1 isolated from aerobic granular sludge, J. Environ. Sci. 23 (2011) 2063–2068.
[23] J.P. Bassin, R. Kleerebezem, M. Dezotti, M.C.M. van Loosdrecht, Simultaneous nitrogen and phosphate removal in aerobic granular sludge reactors operated at different temperatures, Water Res. 46 (2012) 3805–3816.
[24] A. Kumar, B. Bhunia, D. Dasgupta, T. Mandal, A. Dey, S.D.P. Bhattacharya, Optimization of culture condition for growth and phenol degradation by Alcaligenes faecalis JF339228 using Taguchi Methodology, Desalin. Water Treat. 51 (2013) 3153–3163.
[25] N. Saitou, M. Nei, The neighbor-joining method: A new method for reconstructing phylogenetic trees, Mol. Biol. Evol. 4 (1987) 406–425.
[26] A. Haddadi, M. Shavandi, Biodegradation of phenol in hypersaline conditions by *Halomonas* sp. strain PH2-2 isolated from saline soil, Int. Biodeterior. Biodegrad. 85 (2013) 29–34.
[27] C. Xiao, J. Ning, H. Yan, X. Sun, J. Hu, Biodegradation of aniline by a newly isolated *Delftia* sp. XYJ6, Chinese, J. Chem. Eng. 17 (2009) 500–505.
[28] G.X. Zhang, S.Z. Ren, M.Y. Xu, G.Q. Zeng, H.D. Luo, J.L. Chen, Z.Y. Tan, G.P.Sun, *Rhizobium borbori* sp. nov., aniline-degrading bacteria isolated from activated sludge, Int. J. Syst. Evol. Microbiol. 61 (2011) 816–822.
[29] L.G. Whyte, C.W. Greer, W.E. Inniss, Assessment of the biodegradation potential of psychrotrophic microorganisms, Can. J. Microbiol. 42 (1996) 99–106.
[30] P.Y. Aneez Ahamad, A.A. Mohammad Kunhi, Enhanced degradation of phenol by Pseudomonas sp. CP4 entrapped in agar and calcium alginate beads in batch and continuous processes, Biodegradation 22 (2011) 253–265.
[31] S. Al-Zuhair, M. El-Naas, Immobilization of *Pseudomonas putida* in PVA gel particles for the biodegradation of phenol at high concentrations, Biochem. Eng. J. 56 (2011) 46–50.