Isolation of a 2-picolinic acid-assimilating bacterium and its proposed degradation pathway

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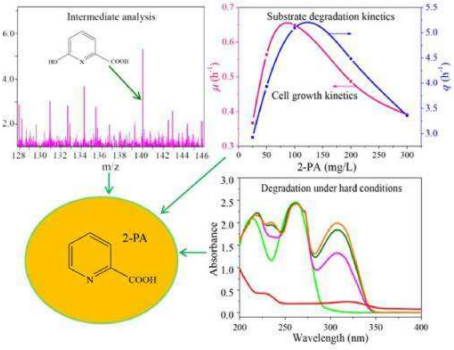
GRAPHICAL ABSTRACT



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ABSTRACT

Burkholderia sp. ZD1, aerobically utilizes 2-picolinic acid as a source of carbon, nitrogen and energy, was iso-lated. ZD1 completely degraded 2-picolinic acid when the initial concentrations ranged from 25 to 300 mg/L. Specific growth rate (μ) and specific consumption rate (q) increased continually in the concentration range of 25–100 mg/L, and then declined. Based on the Haldane model and Andrew's model, μ_{max} and q_{max} were cal-culated as 3.9 and 16.5 h^{-1}, respectively. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR-MS) was used to determine the main intermediates in the degradation pathway. Moreover, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was innovatively used to deduce the ring cleavage mechanism of N-heterocycle of 2-picolinic acid. To our knowledge, this is the first report on not only the utilization of 2-picolinic acid by a Burkholderia sp., but also applying FT-ICR-MS and ATR-FTIR for exploring the biodegradation pathway of organic compounds.

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1. Introduction

Pyridine and its derivatives are important representatives of N-heterocyclic aromatic compounds (Kaiser et al., 1996). Wastes con-taining large amounts of the pyridine ring are generated from mining industry, coal and shale oil processing, wood-preserving facilities, pharmaceutical, food, dye, and agriculture (Kaiser et al., 1996; Yao et al., 2013). It was reported that the concentration of pyridines in the wastewaters ranged from 20 to 300 mg/L, and pyridines were mainly from production of agricultural chemicals, drugs, dyestuffs and textile (Huang et al., 2017). Because of the heterocyclic structure, pyridine and its derivatives are more soluble in water than their homocyclic analogs, and thus, they can be transported more easily to the aquatic environ-ment and soil (Kaiser et al., 1996). Pyridine and its derivatives are classified as priority pollutants by the United States Environmental Protection Agency due to their carcinogenicity and toxicity (Yao et al., 2013). When men were treated orally with 1.8–2.5 mL of pyridine daily for up to two months, they experienced anorexia, nausea, vomiting, gastric, distress, headache, fatigue, faintness and depression (Buhler and Reed, 1990). Especially, two cases of liver and kidney damage and one death occurred. The probable oral lethal dose of pyridine was 0.5–5.0 g/kg and Lethal Dose Low (LDL_o) for man was 500 mg/kg p.o. (Buhler and Reed, 1990). For estuarine sediment microorganisms, median effect concentrations (EC_{50}) of pyridine and pyridine deriva-tives were in the range of 0.027–49.1 mmol/L (Liu et al., 1998).

2-Picolinic acid (2-PA) is an important pyridine derivative, which is widely used in the production of medicine, pesticide, daily chemicals, and feed additives in livestock and poultry industry (Li et al., 2005). Its hydrophilic nature (water solubility of 887 g/L at 20 °C) can do great damage to the environment (Tian and Li, 2005). Till now several stu-dies have shown that aerobic biodegradation is an effective method to remove 2-picolinic acid from water (Kaiser et al., 1996; Yao et al., 2013; Zheng et al., 2009a), in which the microorganisms mainly belong to the genera of Arthrobacter (Khasaeva et al., 2011; Siegmund et al., 1990) and Streptomyces (Zheng et al., 2009a). Currently, the knowledge gap exists on biodegradation of 2-picolinic acid with new strains. Particu-larly, information is not available on the kinetics of microbial growth and substrate utilization as well as metabolic pathway with the new strains under different conditions. Obtaining a strain belonging to a different genus and comparing it with previously reported strains (Khasaeva et al., 2011; Siegmund et al., 1990; Zheng et al., 2009a) are of scientific significance since it will be helpful for researchers to es-tablish a databank on how many kinds of microbes can utilize 2-pico-linic acid in nature.

To fill the knowledge gap, a new 2-picolinic acid-assimilating strain was isolated, and cell morphology and strain identification were carried out. The kinetics of cell growth and substrate consumption was studied systematically. The main intermediates in the degradation pathway were determined and the ring cleavage mechanism of N-heterocycle of 2-picolinic acid was deduced. The objectives of this paper were to quantitatively describe the capacity of the as-isolated strain on removal of 2-picolinic acid from water, explore how the as-isolated strain transfers 2-picolinic acid into carbon dioxide and water, and finally evaluate the implications of applying this as-isolated strain for 2-pico-linic acid-loaded wastewater treatment and site remediation.

2. Materials and methods

2.1. Chemicals

2-Picolinic acid (purity 99%), 6-hydroxypicolinic acid (purity 99%), 2,5-dihydroxypyridine and α-oxoglutaric acid (purity 99%) were ob-tained from J & K Scientific Ltd. (Beijing, China). All these substrates were chromatographic grade. Other chemicals with analytical grade were purchased from Tianjin Kermel Chemical Reagent Co., Ltd., China. Ultrapure water was produced from a UPT-II system (Ulupure Science and Technology Co., Ltd., China).

2.2. Enrichment, isolation and identification of strain ZD1

The activated sludge used in this work was collected from a was-tewater treatment plant (located in Xi'an, China) that treated waste-water contaminated with 2-picolinic acid. A certain volume (10 mL) of the activated sludge were aseptically added to 90 mL of the sterilized MS medium (Zheng et al., 2007) in a 250-mL flask. The MS medium contained (per liter): $Na_2HPO_4 \cdot 12H_2O$, 7 g; KH_2PO_4, 1 g; $CaCl_2 \cdot 2H_2O$, 10 mg; $FeCl_3$, 2 mg; and $MgSO_4 \cdot 7H_2O$, 20 mg. The pH of the MS medium was adjusted to 7.0. The 250-mL flask was supplemented with 2-picolinic acid to a final concentration of 100 mg/L and incubated under the aerobic condition in a rotary shaker (ZHWY-2102C, Zhicheng Analytical Instrument Manufacturing Co., Ltd., Shanghai, China) at 180 rpm and 30 °C. When the culture became obviously turbid, 10 mL of the culture was transferred to 90 mL fresh MS medium in a new 250-mL flask with 100 mg/L 2-picolinic acid. This operation was repeated until the degradation of 2-picolinic acid came to a stable level, and then the culture was diluted with sterilized NaH_2PO_4-K_2HPO_4 buffer (pH = 7.0) and spread onto agar (1.5%) plates with MS medium and 100 mg/L 2-picolinic acid. The agar plates were incubated at 30 °C for 2–3 d. There were three colonies appearing on the agar plates and stain ZD1 was selected for the further study. The 16S rDNA sequence of strain ZD1 was amplified by PCR with primers 27F (5′-AGTTTGATCMTGGC-TCAG-3′) and 1492R (5′-GGTTACCTTGTTA CGACTT-3′).

2.3. Growth kinetics and 2-picolinic acid degradation kinetics

One loop of strain ZD1 on the agar plates was transferred aseptically to 100 mL sterilized MS medium in a 250-mL flask with 100 mg/L 2-picolinic acid. After 12 h of cultivation, 10 mL of the culture was aseptically inoculated into 90 mL sterilized MS medium in a 250-mL flask. The flask was supplemented with 2-picolinic acid at different initial concentrations ranged from 25 to 300 mg/L. These cultures were incubated on a rotary shaker (30 °C, 180 rpm). All cultivations were repeated three times. Data were analyzed statistically by Origin 7.0 (Origin lab, USA) and the error bars depicted the 95% confidence in-tervals. A flask with the same amount of the autoclaved cells (20 min, 120 °C) was used as abiotic degradation. Samples were withdrawn periodically for the analysis of 2-picolinic acid concentration, total or-ganic carbon (TOC), growth of strain ZD1, and dehydrogenase activity (DHA). Prior to the analysis of 2-picolinic acid concentration, TOC and DHA, samples were centrifuged under 15 °C at 12,000 rpm for 20 min to collect the supernatant, which then was filtered through a 0.22 μm-pore-size membrane (G-Biosciences Geno Technology Inc., USA) to re-move the biomass. Degradation of 2-picolinic acid under different pH, temperatures and salinities were also investigated.

2.4. Preparation of the resting cells

One loop of Strain ZD1 stock culture on the agar plate was trans-ferred aseptically to 100 mL sterilized MS medium in a 250-mL flask with 200 mg/L 2-picolinic acid served as a sole source of carbon, ni-trogen and energy. The culture was incubated on a rotary shaker at 30 °C and 180 rpm. The culture in the late exponential phase was aseptically centrifuged at 12,000 rpm for 20 min and under 4 °C to collect cells. The cells were washed twice with sterilized ultrapure water (pH = 7.0 and electrical resistivity of 18 mΩ cm). The resting cells (100 mg, wet cell weight) were suspended in 10 mL of sterilized phosphate buffer (Na_2HPO_4-KH_2PO_4, pH = 7.0) and then incubated with 50 mg/L of 2-picolinic acid at 30 °C and 180 rpm. Samples were taken regularly from the cultures and centrifuged at 12,000 rpm for 20 min and under 4 °C to collect supernatant. The supernatant was filtered through a 0.22 μm-pore-size membrane. After removal of the biomass, some supernatant was directly used for the analysis of total

organic carbon (TOC) and ammonia concentrations, whereas other supernatant was concentrated by freeze-drying (Boyikang Lab Instrument Co., Ltd., Beijing, China) at −60 °C, vacuum degree 12 Pa for 48 h. The concentrated samples were analyzed by FT-ICR-MS and ATR-FTIR. The same setup was used for the experiments of 6-hydroxy picolinic acid, 2,5-dihydroxypyridine, and oxoglutaric acid degradation by the resting cells of ZD1.

2.5. Analysis

Residual 2-picolinic acid concentration was determined at 265 nm by HPLC (S500, Sykam, Germany) using a Pinnacle II C18 (5 μm, 250 mm × 4.6 mm) (Restek Corporation, Bellefonte, PA, USA). The mobile phase was a mixture of water: methanol (v/v, 90: 10) containing 0.2% of formic acid. The flow rate was 1 mL/min and the column temperature was 30 °C. FT-ICR-MS analyses were performed using a 9.4 T Solari X FT-ICR mass spectrometer from Bruker Daltonics (USA). The FT-IR spectra were measured on a Bruker Tensor 27FT-IR spec-trophotometer (Germany) in Attenuated Total Reflection (ATR, MIRacle, PIKE Co., USA) mode. The resolution of the FT-IR was 4.0 cm^{-1}. The scans number accumulated was 20. The TOC contents were determined using a multi N/C2100 analyzer (Analytik Jena AG, Germany) (Zheng et al., 2009b). Ammonia concentration was measured by a standard method (Zheng et al., 2009c). DHA was analyzed by TTC (2,3,5-triphenyltetramlium chloride) (Tluczkiewicz et al., 2016).

3. Results and discussion

3.1. Isolation and characterization

A strain able to utilize 2-picolinic acid as a sole source of carbon, nitrogen and energy under aerobic condition was isolated. SEM pho-tography shows that the morphology of this strain was baculiform with a size of 1–2 μm. After incubation on the agar culture medium for 3 d at 30 °C, the colony was milk white, rounded protuberance, translucent, and smooth with regular edge. A phylogenetic tree was constructed, and it was found that this strain was related to Burkholderia sp. The homology between this strain and Burkholderia sp. C10 (2010) reached 99%. Thus it was identified as Burkholderia sp. and named as ZD1. The gene bank accession number for strain ZD1 is KP900019. Burkholderia sp. is widely found in soil (Compant et al., 2008), in activated sludge (Wang et al., 2010), and on human skin (Vandamme et al., 2007). Several studies have shown that Burkholderia sp. is capable of degrading various organic pollutants under aerobic conditions including phenol (Dobslaw and Engesser, 2015), phenanthrene (Jia et al., 2009), qui-nolone (Quan et al., 2000). However, reports on the utilization 2-pi-colinic acid by Burkholderia sp. are still very few.

3.2. Degradation and growth kinetics

The cell growth of ZD1, the consumption of 2-picolinic acid and TOC removal results were shown in Fig. 1. After 12 h, 2-picolinic acid was degraded completely. However, the TOC removal rate was only 15%, which indicated that 2-picolinic acid was transformed into other substances. After 24 h, the TOC removal rate reached 99%, suggesting that these substances were finally mineralized into carbon dioxide and water. DHA reached a maximum at 24 h.

Fig. 2a–e shows the cell growth of strain ZD1 and 2-picolinic acid degradation when the initial concentration of 2-picolinic acid in MS medium ranged from 25 to 300 mg/L. The degradation rate corre-sponding to initial concentrations of 25, 50, 100, 200 and 300 mg/L was 97.9%, 99.0%, 98.0%, 99.0%, and 95.6%, respectively. It is noted here that no abiotic degradation happened. Estimated values of the specific growth rate (μ) based on linear and logistic models (Ghosh et al., 2014) were listed in Table 1. Here, μ was determined through linear regression. Fig. 2f demonstrates that the logistic model pr

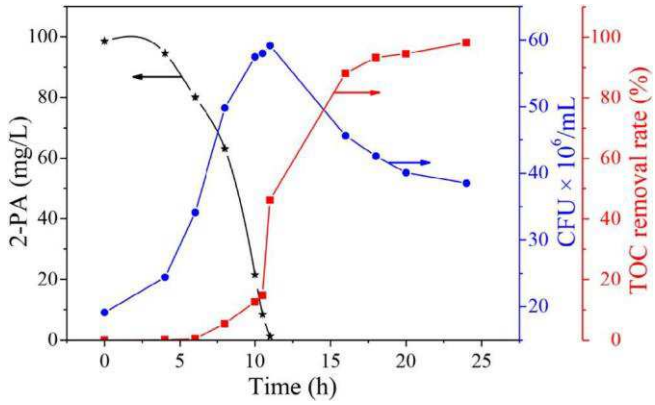

Fig. 1. Curves described the cell growth of Burkholderia sp. ZD1, degradation of 2-pico-linic acid (2-PA) and TOC removal rate (initial concentration of 2-picolinic acid in MS medium was 100 mg/L, temperature of 30 °C, pH of 7.0, 0% salinity (w/v, NaCl)). Data were analyzed statistically and the error bars depicted the 95% confidence interval. Each data was repeated three times.

a good fit for the entire growth profile (including the lag and log phase growth data) up to N_{max}. The linear model could only fit the log phase data. Table 1 shows that the values of μ based on logistic model were higher than those calculated from the linear model at any 2-picolinic acid concentrations. In order to obtain the maximum specific growth rate (μ_{max}), the Haldane model was used (Fig. 3). The value of R^2 was 0.987, which demonstrated that the experimental data were well cor-related by the Haldane model. By using a non-linear regression analysis, the kinetic parameters were as followed: μ_{max} = 3.9 h^{-1}, K_S = 221.6 mg/L, and K_i = 35.5 mg/L.

Based on the degradation data shown in Fig. 2a–e, the specific substrate consumption rate (q) was calculated. Fig. 3 shows the re-lationship between q and the initial concentrations of 2-picolinic acid. It was observed that the values of q increased continually at the initial substrate concentration of 25–100 mg/L, and then declined at 100–300 mg/L. As seen in Table 1, the values of logistic model based μ also reached the peak at 100 mg/L and then decreased. Therefore, it can be concluded when the initial concentration of 2-picolinic acid in MS medium was higher than 100 mg/L, the inhibitory effect occurred. Based on Fig. 3, the maximum specific consumption rate (q_{max}) was calculated to be 16.5 h^{-1} with R^2 of 0.900, suggesting a good fit. So far there are only a few reports on the aerobic degradation of 2-picolinic acid by microbes (Kaiser et al., 1996; Yao et al., 2013; Zheng et al., 2009a). In these limited reports, the values of μ_{max} and q_{max} were not given. Therefore, it is hard to compare the growth kinetics of strain ZD1 and its degradation capacity with other 2-picolinic acid assimilating strains. We have reported a nitrobenzene-assimilating strain which was identified as R. Mucilaginosa (Zheng et al., 2008). R. mucilaginosa uti-lized nitrobenzene as a sole source of carbon, nitrogen and energy under aerobic conditions. The value of q_{max} was calculated to be 1.5 h^{-1} based on the Andrew's model. After immobilized R. mucilagi-nosa on a kind of polyurethane foam, its degrading capacity was en-hanced largely in which the value of q_{max} equaled to 6.0 h^{-1} (Zheng et al., 2009b). As mentioned above, it is hardly to compare the growth activity of strain ZD1 and its degradation ability with other 2-picolinic acid assimilating strains since for other 2-picolinic acid assimilating strains the data of μ, μ_{max}, q and q_{max} are still absent. However, based on the values of μ, q and q_{max} released by our previous study (Zheng et al., 2009b, 2008), it might be concluded that the growth activity of strain ZD1 and its degrading capacity is really high and within the expecta-tion.

3.3. Degradation of 2-picolinic acid under different conditions

The degradation of 2-picolinic acid by strain ZD1 under different

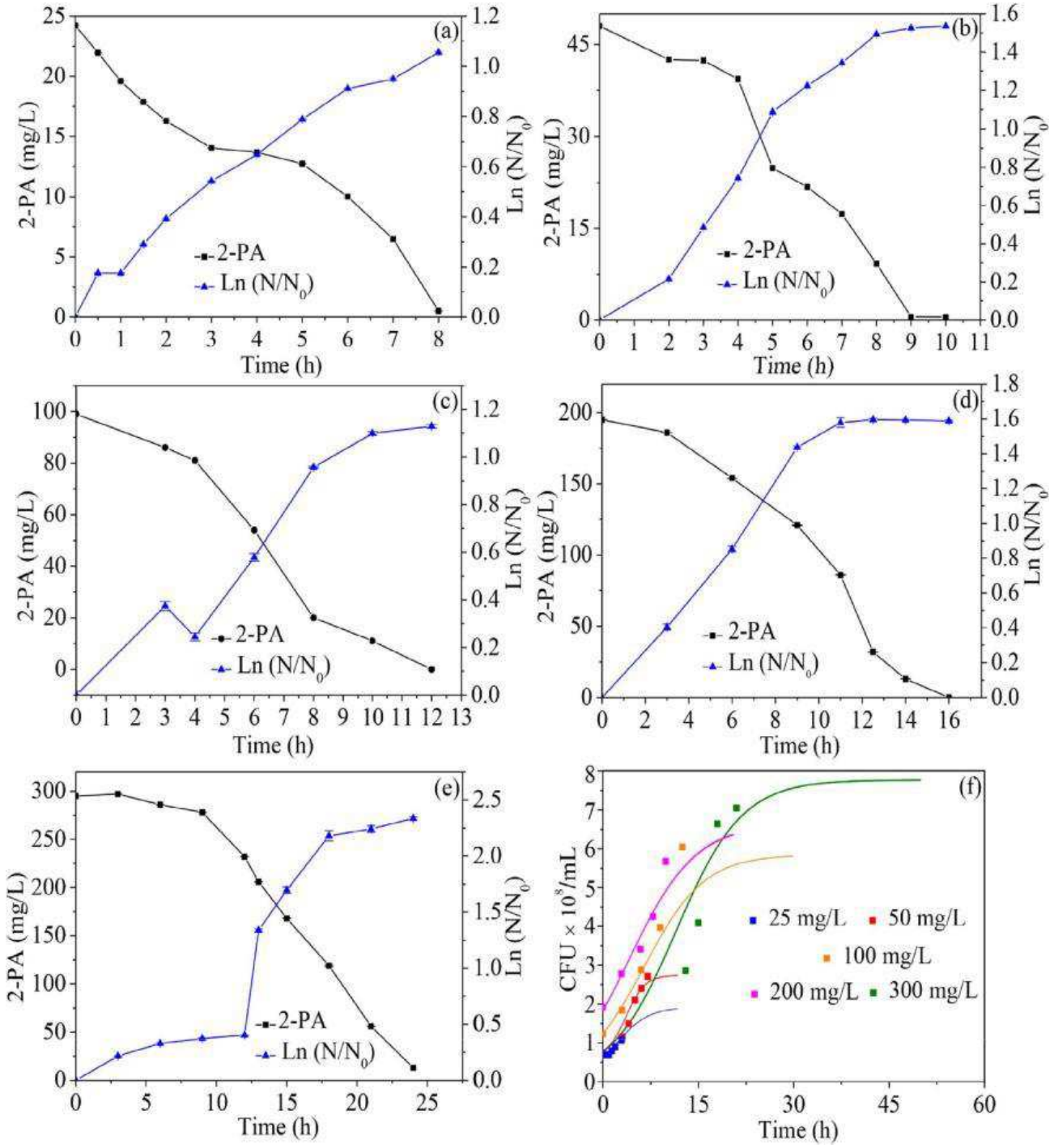

Fig. 2. Degradation of 2-piconlinic acid (2-PA) and the growth of strain ZD1. Initial concentration (in mg/L) of 2-PA in MS medium was (a) 25, (b) 50, (c) 100, (d) 200, and (e) 300, respectively (data were analyzed statistically and the error bars depicted the 95% confidence interval); (f) growth of strain ZD1 in MS medium with different concentration of 2-picolinic acid as a sole source of carbon, nitrogen and energy. Each data was the mean value of triplicates (n = 3).

pH, temperatures and salinities was investigated in order to evaluate the bioactivity of strain ZD1 under hard conditions, as shown in Fig. 4. Strain ZD1 exhibited a good activity at a relatively wide pH and tem-perature range. However, the tolerance of strain ZD1 towards salinity was relatively low, and strain ZD1 can survive at salinity less than 1% (w/v, NaCl). Interestingly, even under low temperature (4 °C) and strong acidic conditions (pH = 3.0), strain ZD1 also kept active. Ul-traviolet spectra show that after 4 d, 2-picolinic acid (A_{265}) degraded slightly and simultaneously a new compound (A_{310}) emerged. When the reaction time was prolonged to 8 d, the peak intensity of 2-picolinic acid almost kept unchanged compared to it shown in 4 d, however, the peak intensity at A_{310} was a little bit higher than it shown in 4 d. With the increase in reaction time, the peak intensity at A_{310} began to ob-viously decline after 12 d. After 15 d all the peaks disappeared and the TOC removal was more than 98% (not shown). These results demon-strated that 2-picolinic acid was finally mineralized and the new com-pound (A_{310}) was an intermediate in the degradation pathway. Based on the values of μ_{max} and q_{max} as well as the degradation capacity under severe conditions, it can be concluded that strain ZD1 has a significant potential to biologically treat 2-picolinic acid-loaded wastewater and may be used in remediation 2-picolinic acid contaminated sites. It is noted here that the substance (A_{310}) was identified as 6-hydroxy

Table 1
Kinetic parameters for 2-picolinic acid degradation by strain ZD1.

C_e (mg/L)	Initial N_0 (CFU/mL)	N_{max} (CFU/mL)	Logistic model		Linear model		Yield (CFU/mg)*
			μ (h^{-1})	R^2	μ (h^{-1})	R^2	
25	7.05E+07	1.95E+08	0.367	0.993	0.147	0.985	1.38E+10
50	7.05E+07	3.28E+08	0.564	0.986	0.193	0.928	1.29E+10
100	1.91E+08	5.61E+08	0.649	0.959	0.147	0.970	1.23E+10
200	1.22E+08	6.24E+08	0.487	0.955	0.153	0.983	1.22E+10
300	7.50E+07	7.75E+08	0.391	0.975	0.201	0.988	1.19E+10

* Yield (CFU/mg) equals to CFU/mL divided by VSS (mg/mL).

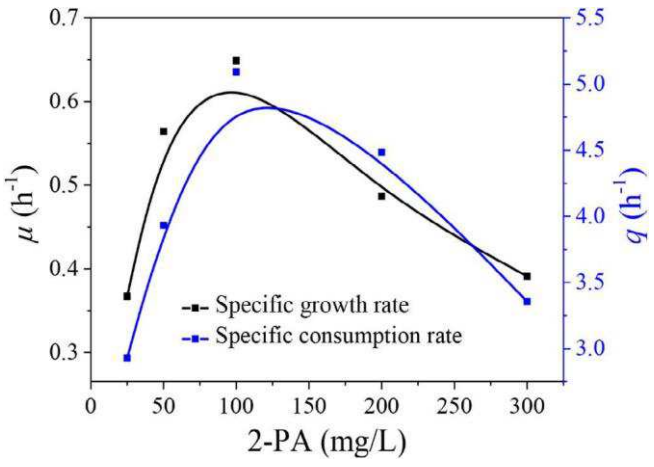

Fig. 3. Relationships between specific growth rate/specific consumption rate and initial 2-picolinic acid (2-PA) concentrations. Each data was the mean value of triplicates (n = 3).

picolinic acid by FT-ICR-MS. Many studies have shown that during the aerobic microbial breakdown of heterocyclic substrates, the most common mode of initial attach on the aromatic ring is hydroxylation of carbon adjacent to the heteroatom (Kaiser et al., 1996; Yao et al., 2013).

3.4. Degradation pathway of 2-picolinic acid by strain ZD1

Electrospray ionization coupled to Fourier transform ion cyclotron resonance mass spectrometry (ESI FT-ICR-MS) enables the identifica-tion of molecular formula with extremely high mass resolution powers (> 300,000) and mass accuracy (< 1 mg/L) (Han et al., 2016; Herzsprung et al., 2016; Le Vot et al., 2016; Wozniak et al., 2008). Since each detected mass peak can be analyzed for possible elemental com-position based on predefined chemical constraints, ESI FT-ICR-MS is regarded as a viable method to directly ascertain molecular composi-tion of each constituent in a complicated matrix without any prior ex-traction or separation steps (Han et al., 2016; Herzsprung et al., 2016). Till now, ESI FT-ICR-MS has been successfully applied to various fields, including 1) environmental science, e.g., characterization of organic matter at the detailed molecular level in natural waters (Altieri et al., 2009b; Herzsprung et al., 2016; Lv et al., 2016; Wozniak et al., 2008) and refinery wastewater (Fang et al., 2017), atmospheric dissolved organic nitrogen in precipitation (Altieri et al., 2009a), and organo-sulfates in atmospheric samples) (Schmitt-Kopplin et al., 2010); 2) medicine industry (e.g., investigation of the characteristic fragment ions of phillyrin) (Lin et al., 2016); 3) biology (e.g., identification of peptide de novo amino acid sequencing for a seven-protein mixture) (Guan et al., 2017), and characterization of the covalent interactions between cisplatin and Cox17-a key copper chaperone protein (Li et al., 2016); and 4) energy industry (e.g., analysis of oxygen compounds in the total organic acid content of crude oils) (Rojas-Ruiz and Orrego-Ruiz, 2016).

Fourier transform infrared spectroscopy (FTIR) has been used for decades to investigate the structure and bonding of chemicals. It has many desirable features such as simple sample preparation, few sam-ples requirement, delivering reliable and fast results and being non-destructive (Banas et al., 2017; Özgenc et al., 2017). Currently, with the development of IR reflectance technique such as attenuated total re-flectance (ATR), FTIR is capable of directly measuring substances in solid (Banas et al., 2017) or liquid state (Zhang et al., 2017). Therefore, the application of ATR-FTIR has been extended to many fields such as monitoring the solution concentration during a cooling crystallization process (Zhang et al., 2017), distinguishing 150 tablets with or without lactose (Banas et al., 2017), determining changes in the chemical structure of heat-treated woods (Özgenc et al., 2017), and exploring phosphate adsorption mechanisms at the ferrihydrite-water interface (Arai and Sparks, 2001).

Based on the characteristics of ESI FT-ICR-MS and ATR-FTIR, we hypothesized that these technologies can be applied to a new field, that is, deduction of microbial metabolism of aromatic ring compounds under aerobic conditions. The justifications are as follows: 1) ESI is regarded as a “soft” ionization technique as it produces minimal frag-mentation of the analytes, and thus, allows for the detection of intact molecules (Wozniak et al., 2008). Moreover, FT-ICR allows for direct injection; thus more concise and accurate raw data of intermediates in the biodegradation pathway can be obtained as compared to liquid or gas chromatographic mass spectrometry, in which sample pretreatment is needed (Han et al., 2016). 2) During the biodegradation process of aromatic ring compounds under aerobic conditions, the polar groups such as hydroxyl and carboxyl are added to the aromatic ring under the catalysis of dioxygenases or monooxygenases, suggesting that the po-larity of intermediates is enhanced compared to parent compounds. Since FTIR is sensitive to polar groups, it may provide useful and sup-plementary information on the identification of intermediates once ESI FT-ICR-MS cannot capture any signals. It is noted here that the biode-gradation of aromatic ring compounds occurs in aqueous solution; thus, it is suitable to use infrared technique in ATR mode instead of tradi-tional transmission mode. To test our hypothesis, we combined ESI FT-ICR-MS and ATR-FTIR to determine the main intermediates in the de-gradation pathway and to deduce the ring cleavage mechanism of N-heterocycle of 2-picolinic acid.

3.4.1. Hydroxylation of 2-picolinic acid to form 6-hydroxy picolinic acid

The mass spectra of 2-picolinic acid, in which the greatest peak magnitude located at m/z 146.02130 corresponded to $[M + Na]^+$, and the peak magnitude centered at m/z 124.03934 was related to $[M + H]^+$. 2-Picolinic acid underwent hydroxylation, and then 6-hydroxy picolinic acid was produced (m/z at 162.01616 represented $[M + Na]^+$). Many studies have shown that during the aerobic microbial breakdown of heterocyclic substrates, the most common mode of initial attach on the aromatic ring is hydroxylation of carbon adjacent to the heteroatom (Kaiser et al., 1996; Yao et al., 2013; Zheng et al., 2009a).

3.4.2. Transformation of 6-hydroxy picolinic acid to 2,5-dihydroxypyridine

Since 6-hydroxy picolinic acid was ascertained to be one of the in-termediates in the biodegradation pathway, it was used as a substrate to identify the consequent degrading product. A strong peak at m/z of 162.01616 ($[M + Na]^+$) referring to 6-hydroxy picolinic acid when the reaction time was 0 h. The peak at 163.01952 was isotopic peak of 6-hydroxy picolinic acid. After 8 h, 2,5-dihydroxypyridine with m/z at 134.02110 ($[M + Na]^+$) was produced, and simultaneously the peak intensity of 6-hydroxy picolinic acid became weaker. It can be con-cluded that 6-hydroxy picolinic acid was transformed to 2,5-

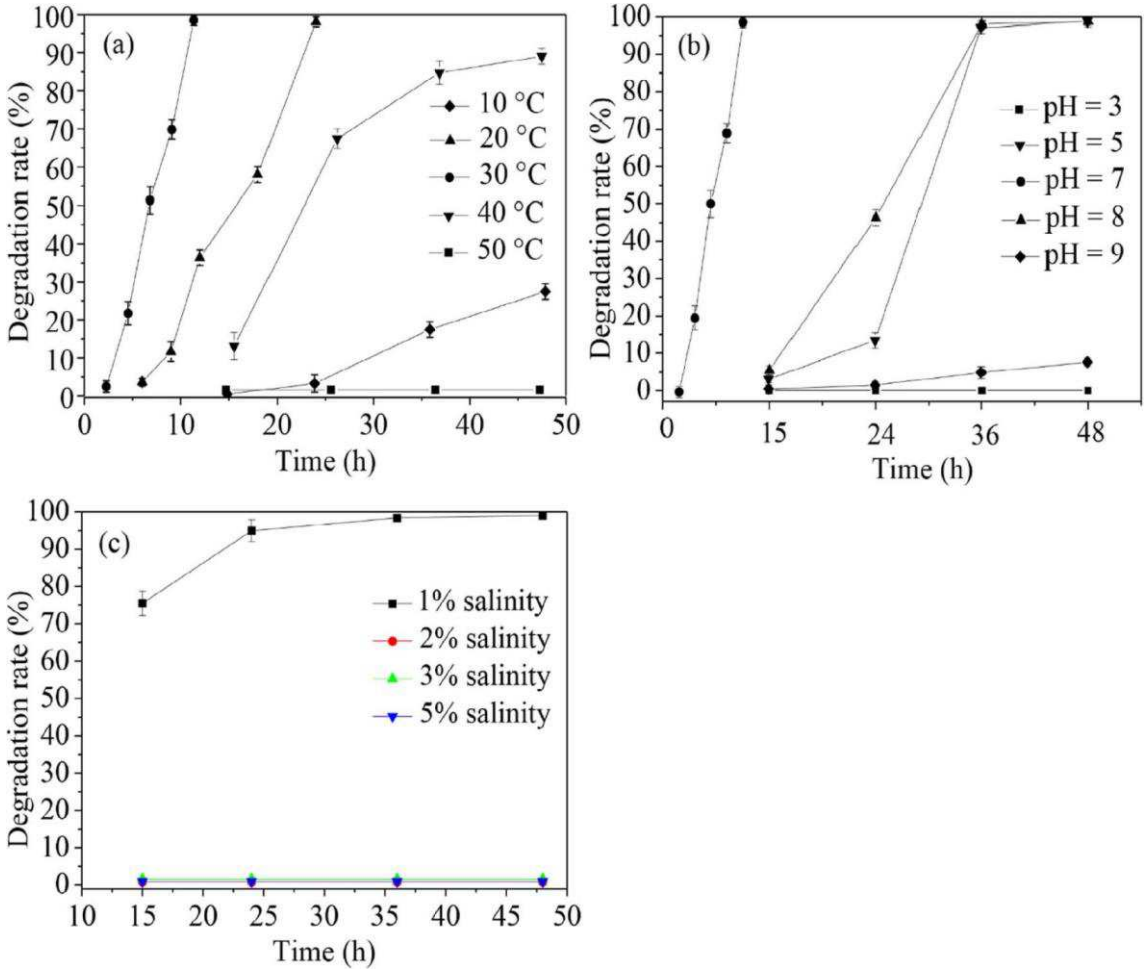

Fig. 4. Degradation of 2-picolinc acid by strain ZD1 under (a) different temperatures, (b) different pH, and (c) different salinities (w/v, NaCl). Data were analyzed statistically and the error bars depicted the 95% confidence interval. Each data was repeated three times.

dihydroxypyridine, which is consistent with the previous reports (Kaiser et al., 1996; Yao et al., 2013).

3.4.3. Production of oxoglutaric acid from 2,5-dihydroxypyridine

Since 2,5-dihydroxypyridine was ascertained to be one of the intermediates in the biodegradation pathway, it was used as a substrate to identify the consequent degrading product. 2,5-Dihydroxypyridine with two strong signals at m/z of 223.07322 and 245.05553 respectively corresponding to the $[2M + H]^+$ and $[2M + Na]^+$ adducts were clearly detected when the reaction time was 0 h. After 7 h, the peaks of 2,5-dihydroxypyridine disappeared, but new peaks located at m/z of 147.02875 and 169.01078 emerged. The two peaks respectively at-tributed to the $[M + H]^+$ and $[M + Na]^+$ adducts of oxoglutaric acid. These results suggested that 2,5-dihydroxypyridine was transformed into oxoglutaric acid, which accords with the previous reports (Kaiser et al., 1996; Yao et al., 2013).

The mass error of the intermediates analysis by FT-ICR-MS was found lower than 2 mg/L and allowed a credible conversion of ex-perimental mass peaks into compound formulas with high confidence. It is noted here that during the degradation process ammonia was de-tected (not shown), which means that 2,5-dihydroxypyridine under-went ring cleavage and N atom was released from the N-heterocycle.

3.4.4. Ring cleavage of 2,5-dihydroxypyridine

Till now there are few reports on how the ring of 2,5-dihydrox-ypyridine was opened to form oxoglutaric acid (Kaiser et al., 1996; Yao et al., 2013). The possible reason is that it is hard for mass spectra to detect the ring cleavage product since sometimes it is difficult to find out the optimal ionization conditions. In order to explore the ring cleavage mechanism of 2,5-dihydroxypyridine, we adopted the method as follows: first, we proposed that there may exist four ways for the ring open of 2,5-dihydroxypyridine based on Kaiser et al. (1996), who summarized the metabolic pathways of N-heterocyclic compounds in-cluding pyridine, quinolone, acridine and their derivatives under aerobic conditions. The heterocycle was opened by dioxygenases at the position between two carbon atoms (one C atom is located at ortho position to N atom while the other at meta position) or between N atom and its adjacent C atom (Kaiser et al., 1996). As for pathways I and IV, the ring cleavage of 2,5-dihydroxypyridine was assumed to happen between C_5 and C_6 or between C_2 and C_3, respectively. After the release of formamide (eNH_2CHO) (Kaiser et al., 1996) the products for the two pathways would contain four carbon atoms only, which is obviously contradictory to the results given by FT-ICR-MS since oxoglutaric acid contains five carbon atoms. As for pathways II and III, the ring cleavage of 2,5-dihydroxypyridine was assumed to happen between N atom and its adjacent C atom, that is, C_6 for pathway II and C_2 for pathway III, respectively. After the release of eNH_2 and hydrogenation (Kaiser et al., 1996) the products for pathways II and III would be oxoglutaric acid, which is in agreement with the results provided by FT-ICR-MS.

Next, we used the technology of Attenuated Total Reflectance FTIR to determine whether pathway II or III is correct. The band that appears at 1623 and 1436 cm^{-1} was attributed to pyridine ring skeleton stretching vibration. CeO stretching vibration was recognized by two sorption bands at 1159 and 1049 cm^{-1} as well as the band at

2312 cm^{-1} was assigned to CO_2. After 6 h of degradation, the peaks of 2,5-dihydroxypyridine disappeared, and a new band at 1648 cm^{-1} appeared, which was attributed to amide stretching vibration. It was found that only the product of 2,5-dihydroxypyridine ring cleavage based on way II possesses amide group.

Since oxoglutaric acid was ascertained to be one of the inter-mediates in the biodegradation pathway, it was used as a substrate to identify the consequent degrading product. Unfortunately, FT-ICR-MS did not provide any information. However, TOC analysis showed that oxoglutaric acid was finally mineralized into CO_2 and H_2O. Based on the results provided by FT-ICR-MS, ATR-FTIR and TOC, the proposed biodegradation pathway of 2-picolinic acid by strain ZD1 was pre-sented. 2-Picolinic acid firstly underwent hydroxylation to form 6-hy-droxy picolinic acid. Then 6-hydroxy picolinic acid was transformed into 2,5-dihydroxypyridine. Under the catalysis of dioxygenase, the ring of 2,5-dihydroxypyridine was opened in which the cleavage happened between N atom and C_6. After the release of eNH_2 and hydrogenation, oxoglutaric acid was produced and finally mineralized into carbon di-oxide and water.

Herein, the main intermediates were qualitatively analyzed, and the biodegradation process of 2-picolinic acid was described at molecular level. Based on the qualitative information, quantitative analysis will be done later by monitoring the concentration of each intermediate. Pure enzyme responsible for each biotransformation reaction in the de-gradation pathway will be extracted. Kinetics of enzyme-catalyzed re-actions will be established and the rate-limiting step in the degradation pathway will be found out. These future works might contribute to the construction of genic engineering strain and the field of biocatalysis. Moreover, both μ_{max} and q_{max} were calculated, which will be helpful for ascertaining organic and hydraulic shock loadings and as well as design of the corresponding wastewater treatment processes in the future.

4. Conclusions

This paper reported the aerobic degradation of 2-picolinic acid by Burkholderia sp. ZD1. Growth and degradation kinetics were studied. ZD1 exhibited a good activity under relative wide pH and temperature ranges. FT-ICR-MS and ATR-FTIR were used to deduce the biode-gradation pathway. To our knowledge, this is the first time to use FT-ICR-MS and ATR-FTIR in such a field. This work not only clarified the metabolic mechanism of 2-picolinic acid but also may provide a new method for exploring the biodegradation pathway of organic com-pounds.

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