Promotive effect of pyridine on the biodegradation of isoquinoline by activated sludge under denitrifying conditions

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Abstract—Lab-scale batch experiments were conducted to investigate the promotive effect of pyridine on the biodegradability of isoquinoline under denitrifying conditions. The effect of pH and temperature on the biodegradation of isoquinoline in the presence of pyridine were also investigated. The biodegradation of isoquinoline was significantly influenced by pH and temperature. At pH of 7.5, both isoquinoline and pyridine were completely removed, whereas at pH of 6.5 and 8.0, isoquinoline was only removed by 84% and 86%, respectively. The high pH even inhibited the degradation of pyridine. While pyridine was completely degraded in the temperature range of 20-35 °C, isoquinoline was removed by 90% only in the temperature range of 28-30 °C. When the concentration ratio of isoquinoline to pyridine was in the range of 10:1 and 2:1, pyridine accelerated the degradation of isoquinoline; however, pyridine exhibited inhibition effect on the biodegradation of isoquinoline when the ratio reached 1:1.

Keywords- *promotive effect; isoquinoline; pyridine; activated sludge; denitrifying conditions*

I. INTRODUCTION

N-heterocyclic aromatic compounds such as quinoline, isoquinoline, pyridine etc. have received immense attention, because of their persistent, toxic, recalcitrant and carcinogenic nature [1, 2]. They are rated as priority pollutants by USEPA (United States Environmental Protection Agency) [3, 4]. These compounds could occur naturally in the environment; however, the majority of these compounds come from human activities. They are generated from manufacturing of dyes, explosives, pesticides and pharmaceuticals, also from mining industry, coal tar, oil shale processing operation, wood preserving facilities and chemical manufacturing plants [4-6]. Wastewater containing these compounds must be treated before being discharged into the environment, to prevent the damage to natural ecosystems and humankind. Comparing with chemical and physical methods, biological treatment has been accepted as one of the most feasible, eco-friendly and cost-effective options. A lot of researchers have investigated their biodegradation by using them as sole carbon and energy source [5, 7-12]. However, the tolerance of microorganisms towards the exhibited toxicity of these compounds is not unlimited. High concentrations of these compounds may change the activity of one or more enzymes, affect enzyme synthesis by interaction with genome or transcription process, alter cell's permeability and finally influence the total functional activity

of the cell [13]. In order to prevent the unfavorable impacts on cell metabolism when dealing with complex mix wastewater, researches must be done. In the wide majority of cases, conventional carbon sources at low concentrations stimulate microbial growth and thus more enzymes are available in order to degrade more recalcitrant compounds [14]. Another issue, that has to be pointed out is the importance of culture history on the effective biodegradation of priority pollutants, since it affects strongly the physiological state of the cells, meaning their macromolecular composition, which is the synthesis and reaction of enzyme systems [15].

Illuminating by above theories, in this paper two typical N-heterocyclic aromatic compounds, isoquinoline and pyridine were chosen. Pyridine only has single ring, resulting in the readily access by enzymes [16], whereas isoquinoline has nitrogenous heterocyclic and benzene ring. The increased molecular size made it more difficult for enzymes to access. Solubility also affects the degradability of compounds. The solubility of pyridine is much higher than isoquinoline, which may be another reason for the recalcitrant of isoquinoline. Previous research has confirmed that under denitrifying conditions, pyridine was easily degraded, whereas isoquinoline was more difficulty to be degraded [17]. In this study, pyridine was used as co-substrate, the promotive effect of pyridine on isoquinoline degradation under denitrifying conditions by activated sludge was investigated. Effects of pH and temperature on the biodegradation of isoquinoline in the presence of pyridine were also studied. The objective of the study is to provide useful information for the treatment of wastewater containing the mixture of N-heterocyclic aromatic compounds.

II. MATERIALS AND METHODS

A. Bioreactors

The bioreactor for acclimating activated sludge and degradation experiment was made of plexiglass, with diameter of 150 mm and height of 520 mm. The working volume was 9 L, the wastewater was filled at the height of 510 mm, resulting in 10 mm of head space above the water surface. In order to ensure anaerobic conditions, the top of the bioreactor was sealed by a plexiglass lid after filling. The dissolved oxygen remained in the 10 mm head space was consumed soon after the acclimation began, and couldn't been detected by the dissolved oxygen apparatus (DO5510HA, Taiwan). The reactor

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was run by the mode of SBR (Sequencing Batch Reactor). The wastewater was filled in the reactor, then the mixed liquid was stirred until isoquinoline and pyridine was completely degraded. The solution was then settled and the supernatant was drawn to the half of the working volume.

The bioreactor for the experiment investigating the effect of pH and temperature on the biodegradation was made of plexiglass,

with diameter of 80 mm and height of 210 mm. The working volume was 1 L. In order to ensure anaerobic conditions, the top of the bioreactor was also sealed by a plexiglass lid after filling. The bioreactor was conducted to investigate influencing factors during degradation like pH and temperature. The schematic figures of the two bioreactors were shown in Fig 1.

Figure 1. The bioreactors used in the experiment. (a) bioreactor for acclimating activated sludge and degradation experiment, (b) bioreactor for studying the effect of pH and temperature.

B. Chemicals

Isoquinoline and pyridine were obtained from Sigma chemical co. (St. Louis, MO, USA) and was used to prepare the media. Synthetic wastewater was prepared using tap water and supplemented with isoquinoline and pyridine at different ratios. Sodium nitrate was supplemented at the desired ratio of chemical oxygen demand (COD) to NO_3^- -N. Phosphorus (KH_2PO_4), calcium (CaCl_2), and magnesium (MgSO_4) were used at ratio of COD: P: Ca: Mg= 100: 1: 0.18: 0.18. The COD was the calculated oxygen demand based on isoquinoline or pyridine as the carbon sources, and organic nitrogen was oxidized to nitrate. The COD was calculated according to the following equations

where 1.0g of isoquinoline is equivalent to 2.98g of COD, and 1.0g of pyridine is equivalent to 3.03g of COD.

C. Analyses

Samples used for isoquinoline and pyridine quantification were taken periodically from the bioreactors, and filtered through a $0.45\mu\text{m}$ cellulose ester membrane. Samples were extracted with dichloromethane three times and then dried by anhydrous sodium sulphate. The concentration of isoquinoline and pyridine of each prepared sample was determined with a gas chromatography (Agilent 6890) equipped with flame ionization detector and $30.0\text{m} \times 320\mu\text{m} \times 0.25\mu\text{m}$ (insider diameter) stainless-steel column (HP-5). Injector and detector temperatures were kept at 250°C and 280°C , respectively. The column temperature was stayed at 100°C for 1min, then increase at the rate of $10^\circ\text{C}/\text{min}$ to 160°C , and kept on 2min. Nitrogen was used as the carrier gas.

Measurement of nitrite, ammonia nitrogen and COD (chemical oxygen demand) were according to standard methods [18]. Because isoquinoline was absorbs strongly at

220 and 275 nm, nitrate was measured by rapid spectrophotometric determination using phenol [19].

III. RESULTS AND DISCUSSION

A. Effects of initial pH

In the experiments investigating the effect of pH on the biodegradation of isoquinoline, the initial concentrations of isoquinoline and pyridine were both 100 mg/L. Previous studies indicated that the optimal C/N (the concentration ratio of COD to NO_3^- -N) ratios for both isoquinoline and pyridine degradation under denitrifying conditions were 8 to 10 when isoquinoline and pyridine were used as the sole carbon sources, respectively. Therefore, the C/N ratio was set at around 10 in

this experiment. The initial pH of the wastewater was controlled at 6.5, 7.0, 7.5 and 8.0 by phosphate buffer solution, except one sample was not adjusted pH. The reaction time was controlled at 10 hours. The results were shown in Table I. pH played an important role in the degradation of isoquinoline and pyridine. Pyridine was completely degraded when pH was in the range of 6.5-7.5, whereas when pH at 8.0, its removal efficiency was only 55%, indicating the inhibition effect of high pH on the biodegradation of N-heterocyclic compounds. The optimal pH for the biodegradation of isoquinoline was 7.5. Around this pH value more than 95% isoquinoline was removed, whereas at pH of 6.5 and 8.0, the removal efficiencies of isoquinoline were only 84% and 86%, respectively.

TABLE I. EFFECTS OF INITIAL pH ON THE DEGRADATION WHEN CONCENTRATION RATIO OF ISOQUINOLINE TO PYRIDINE WAS 1:1

Influent (mg/L)				Effluent (mg/L)				RE-Iso (%)
Isoq	Pyr	NO_3^- -N	pH	Iso	Pyr	NO_3^- -N	pH	
100	100	60.11	7.39*	4.96	n.d.	37.18	8.25	95.05
			6.5	15.59	n.d.	37.51	7.69	84.41
			7.0	9.35	n.d.	37.38	7.44	90.65
			7.5	n.d.	n.d.	34.89	7.00	100
			8.0	13.35	45.04	48.05	8.01	86.65

Notes: Isoq: concentration of isoquinoline; Pyr: concentration of pyridine; RE-Isoq: removal efficiency of isoquinoline; *: the sample whose pH was not adjusted; n.d.: not detected.

B. Effects of temperature

In the experiments investigating the effect of temperature on the biodegradation of isoquinoline, the initial concentrations of isoquinoline and pyridine were both 100 mg/L, and the C/N ratio was 10. The initial pH in the bioreactors was controlled at 7.5, and the reaction time was controlled at 24h. The reactors were run at the temperature of 20, 25, 28, 30 and 35 °C, respectively, by thermostatic magnetic agitator (85-2, China). The results were shown in Table II. The temperature played an

important role in the biodegradation of isoquinoline and pyridine. At the five temperatures, all the pyridine was degraded in 24h. When the temperature was 20 °C, isoquinoline was only removed by 50%. In the range of 25-35 °C, isoquinoline was well degraded. The optimal temperature range was 28-30 °C, in which more than 90% isoquinoline was removed.

TABLE II. EFFECTS OF TEMPERATURE ON THE DEGRADATION OF ISOQUINOLINE AND PYRIDINE

T(°C)	Effluent (mg/L)					RE-Iso (%)
	Isoq	Pyr	NO_3^- -N	NO_2^- -N	NH_3 -N	
20	49.66	n.d.	37.68	35.47	19.59	50.34
25	13.66	n.d.	23.53	20.47	22.90	86.34
28	3.32	n.d.	4.54	3.98	31.33	96.68
30	6.84	n.d.	9.51	23.94	25.63	93.16
35	12.34	n.d.	6.11	14.60	36.23	87.66

Notes: Isoq: concentration of isoquinoline; Pyr: concentration of pyridine; RE-Isoq: removal efficiency of isoquinoline; n.d.: not detected.

C. Promotive effect of pyridine on the biodegradation of isoquinoline

Different concentration ratio of isoquinoline to pyridine was chosen to proceed the experiments, as 10:1, 6:1, 4:1, 2:1 and 1:1. Fig 2 shows that an increase of pyridine concentration in the wastewater led to faster isoquinoline degradation. This indicates that pyridine can promote the degradation of isoquinoline.

Figure 2. Degradation of isoquinoline and pyridine at different concentration ratios of isoquinoline to pyridine. (a) 10:1, (b) 6:1, (c) 4:1, (d) 2:1 and (e) 1:1.

TABLE III. KINETIC EQUATIONS AND THE SPECIFIC DEGRADATION RATE OF ISOQUINOLINE IN THE PRESENCE AND ABSENCE OF PYRIDINE

Isoq/Pyr	Time range	Equation	K (10 ⁻³ /h)	R ²	Time range	Equation	K [*] (10 ⁻³ /h)	R ²
10:1	0~1.5h	y=-5.279t+96.881	1.313	0.9756	1.5~28h	y=-3.252t+90.847	0.820	0.9960
6:1	0~2.5h	y=-10.321t+106.02	2.356	0.9698	2.5~28h	y=-3.1023t+87.137	0.708	0.9975
4:1	0~4.0h	y=-6.4579t+100.16	1.497	0.9984	4.0~28h	y=-3.273t+88.472	0.759	0.9951
2:1	0~7.0h	y=-5.1206t+103.3	1.247	0.9967	7.0~33h	y=-2.6527t+87.864	0.646	0.9960
1:1	0~10h	y=-2.97t+96.582	0.751	0.9490	10~32h	y=-3.0572t+94.729	0.773	0.9860

Notes: Isoq/Pyr: concentration ratio of isoquinoline to pyridine; K: specific degradation rate of isoquinoline in the presence of pyridine; K^{*}: specific degradation rate of isoquinoline in the absence of pyridine.

Pyridine always completely degraded prior to isoquinoline, and isoquinoline degraded much slower. The degradation of pyridine conformed to zero model and its specific degradation rate increased along with its concentration. The reason was pyridine could be easily degraded, when degradation with isoquinoline, it used as preferential carbon source easily degraded by microorganism, moreover, with its concentration increasing the advantage was more markedly. The degradation rate of isoquinoline appeared different in the presence and absence of pyridine, and it was shown in Table III.

Except for the ratio 1:1, at all the other four ratios, the specific degradation rate of isoquinoline in the presence of pyridine was greater than that in the absence of pyridine. The reason was the existence of pyridine could accelerate the degradation of isoquinoline. However, when the concentration of pyridine arrived at high concentration of 100mg/L, it exhibited the inhibition for isoquinoline degradation. The reason was that the existence of pyridine induced to synthesize the critical enzyme for N-heterocyclic aromatic compounds degradation, and the enzyme also could be utilized by

isoquinoline. However, if the concentration of pyridine was too high, pyridine may compete with isoquinoline on the critical enzyme, and degradation of isoquinoline was inhibited as pyridine could utilize the enzyme more easily. So, pyridine could accelerate the degradation of isoquinoline under their concentration ratio between 10:1-2:1, but much higher concentration of pyridine inhibited the degradation of isoquinoline.

IV. CONCLUSIONS

Experiments were conducted to investigate the promotive effect of pyridine on the biodegradability of isoquinoline under denitrifying conditions. The effect of pH and temperature on the biodegradation of isoquinoline in the presence of pyridine were also investigated. pH and temperature significantly influenced the biodegradation of isoquinoline. At pH of 7.5, both isoquinoline and pyridine were completely removed, whereas at pH of 6.5 and 8.0, isoquinoline was only removed by 84% and 86%, respectively. The high pH even inhibited the degradation of pyridine. Pyridine was completely degraded in

the temperature range of 20-35 °C, while isoquinoline was removed by 90% only in the temperature range of 28-30 °C. When the concentration ratio of isoquinoline to pyridine was in the range of 10:1 and 2:1, pyridine accelerated the degradation of isoquinoline; however, pyridine exhibited inhibition effect on the biodegradation of isoquinoline when the ratio reached 1:1.

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