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PERFORMAMCE OF 2-CP BIODEGRADATION VIA HYDROGENOTROPHIC BIOREACTORS AND ITS MICROBIAL IDENTIFICATION

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ABSTRACT

This study utilized the hydrogenotrophic bioreactors to degrade 2-chlorophenol (2-CP). The hydrogenotrophic bioreactors had been acclimated with 2-CP under different reductive conditions for more than 18 months. Results show that under the condition with H_2, bacteria could use H_2 as electron donor for dechlorinating 2-CP with 95% removal efficiency. However, 2-CP dechlorination was inhibited in the presence of nitrate or sulfate because of the shifting of the role in bacteria's electron acceptor from 2-CP to nitrate or sulfate, which changed the role of 2-CP to a carbon source. Although the metabolic pathway of 2-CP varied in different reductive conditions, 2-CP removal efficiency revealed only minor differences. In this study, the cloning-denaturing gel gradient electrophoresis (DGGE) method was used to identify the microbial communities of the biofilms. Results show most identified bacteria belong to β-proteobacteria, and the most dominant was close to the genus *Ralstonia*. Other important bacteria genera found in the hydrogenotrophic bioreactors were similar to *MTBE-degrading bacterium* PM1, *Rhizobium*, *Zoogloea*, *Sporomusa*, *Comamonas*, *Acidovorax*, *Hydrogenophaga* and *sulfate-reducing bacterium* F1-7b. These bacteria were reported in literatures to have the ability to degrade several recalcitrant compounds.

INTRODUCTION

Chlorophenols (CPs) are the chemicals widely used in industry to produce insecticides, herbicides, fungicides, biocides and dyes, and thus they exist in the environment as one of the major causes for the pollution of air, waste, soil, and groundwater. The reductive dechlorination is actually the main metabolic pathway in the anaerobic biodegradation of CPs, in which the chlorinated substituents are replaced by hydrogen and produce less-chlorinated compounds [1]. Several external organics are often added to enhance the dechlorination of CPs [2-4]; unfortunately, the residual organics seem problematic when used in groundwater remediation. For this reason, H_2 can be used to replace the external organics as electron donor for the dechlorination of CPs. In fact, previous studies have demonstrated that H_2 could enhance the dechlorination of several chlorinated compounds [5-8], but the application of the hydrogenotrophic bioreactor for dechlorinating CPs still remains much to be explored.

The major difficulty in cultivating hydrogen bacteria is the low water solubility and the flammable property of hydrogen. Our group had developed a gas-permeable, silicone membrane bioreactor to cultivate hydrogen bacteria and showed good performance in nitrate removal [9-10]. Recently, this bioreactor was again applied to acclimating hydrogenotrophic biofilms for the dechlorination of 2-CP. Results showed that H_2 was utilized as electron donor for 2-CP dechlorination without the addition of external organics [11]. To further evaluate the application of this bioreactor in 2-CP removal, different bioreactors were conducted under different reductive conditions. Because the bioreactors were mixed cultures, the

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microbial communities responsible for 2-CP degradation were unknown. So, the cloning-Denaturing Gradient Gel Electrophoresis (DGGE) method was also used in this study to identify the microbial communities of the bioreactors. By analyzing the microbial communities of different bioreactors, we hope to understand better how the bioreactors work and make it a precursory movement in the application of the hydrogenotrophic bioreactor for degrading 2-CP.

MATERIALS AND METHODS

I. Bioreactor

The gas-permeable silicone membrane bioreactor was made of glass with an effective volume of 850 mL. The pillars in the reactor was wound around by a silicone tube (2.5 mm [i.d.] and 3.0 mm [o.d.] by 5.0 m long; Fuji system Co., Japan) , as shown in Fig.1. The 2-CP adsorption by the silicone tube had been tested and results showed less than 5% of adsorption effect. Anaerobic sludge from a swine wastewater treatment plant was immobilized on the tube wall to form a biofilm. The biofilm was immobilized by polyvinyl alcohol (PVA, Nacalai Tesque) and alginic acid (SIGMA), as described by Hsieh *et al*. [12]. A cylinder was used to supply pure H_2 (20 mL min^{-1}) to the lumen side of the silicone tube and the H_2 diffusion rate was 2.5 g m^{-2} d^{-1}[13]. The quantity of supplied H_2 was much greater than the need for 2-CP dechlorination. The solution in the bioreactor was stirred by a magnetic stirrer bar to form a complete mixing reactor. In addition, the bioreactors were kept in a water bath to ensure the temperature at 25 °C. The pH value of the bioreactor was manually controlled by adding 1 M HCl, or 1 M NaOH solution into the bioreactor. All the influent medium was de-oxygened by purging N_2 for 5 minutes to prevent the influence of O_2.

II. Operating Conditions of Bioreactors

Three hydrogenotrophic bioreactors were acclimated under dechlorinating (DC), denitrifying (DN) and sulfate-reducing (DS) conditions by continuously feeding the synthetic wastewater. The synthetic wastewater contained KH_2PO_4 (1.0 g L^{-1}), K_2HPO_4 (1.0 g L^{-1}), $NaHCO_3$ (1.0 g L^{-1}), $MgCl_2$ (20 mg L^{-1}), NH_4Cl (30 mg-N L^{-1}), trace elements (1 mg L^{-1}) and 2-chlorophenol. The trace elements solution was comprised of $CaCl_2 \cdot H_2O$ (7.3 g L^{-1}), $MnCl_2 \cdot 4H_2O$ (2.5 g L^{-1}), $CoCl_2 \cdot 6H_2O$ (0.5 g L^{-1}), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (0.5 g L^{-1}), $FeCl_2 \cdot 4H_2O$ (2.0 g L^{-1}), $ZnCl_2$ (1.0 g L^{-1}) and $CuCl_2$ (0.1 g L^{-1}). The other bioreactor (CON) was operated under the same condition of DC reactor but without H_2 supply for comparison with the DC reactor. In a normal continuous operation, the influent 2-CP was always kept at 25 mg/L with the hydraulic reten-

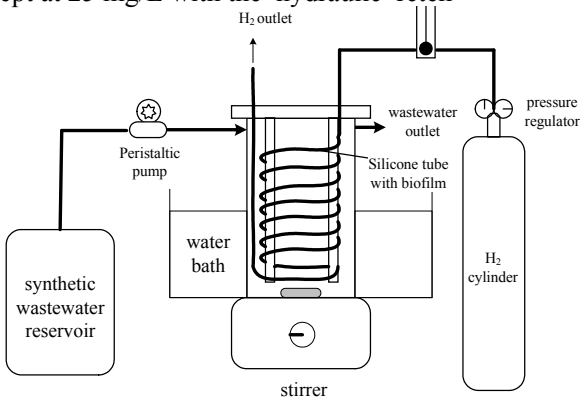

Fig. 1. Schematic diagram of the bioreactor.

tion time (HRT) of 15 h. Also, KNO_3 (50 mg-N L^{-1}) was added to the influent of DN reactor, and K_2SO_4 (200 mg L^{-1} as sulfate) was added to the influent of DS reactor, but the DC and CON reactors were acclimated in lack of nitrate and sulfate.

III. Analytical Methods

The 2-CP and phenol were determined by high-performance liquid chromatography (HPLC) with the mobile phase of a mixture of acetonitrile, distilled water and acetate in the proportion of 500/500/5.7 (v/v). The HPLC pump was controlled at the flow rate of 1.0 mL min^{-1}, and the UV detector was set at 254 nm. Nitrate, nitrite and sulfate were determined by the ion chromatography (Dionex DX-120) with the mobile phase of $NaHCO_3/Na_2CO_3$ (1.7 mM/1.8 mM) at a flow rate of 1.25 mL min^{-1}. The oxidation-reduction potential ($ORP_{Ag/AgCl}$) was determined by the ORP meter (ORIGIN model 720A, USA), and the pH value was by the pH meter (HTC-200). The hydrogen analyzer (Orbisphere Laboratories Model 3600) was used to measure the concentration of dissolved hydrogen and the hydrogen gas concentration in the silicone tube.

IV. Microbial Identification

The DNA of different biofilms was extracted by the Genomic DNA purification kit (Gentra, USA). The 16S rDNA of each DNA sample was amplified by the polymerase chain reaction (PCR) with the primer set, 11f (5'-GTTTGATCCTGGCTCAG-3') and 1492r (5'-TGCCTTGTTACGACTT-3') [13]. The PCR products were then used for cloning according to the manufacture's protocol (YT & A cloning kits, Yeastern Biotech, Taiwan). Fifty white colonies were picked and amplified by PCR for screening with the primer set, M13f (5'-TGTAAAACGACGGCCAGT-3') and M13r (5'-TCACACAGGAAACAGCTATGAC-

3'). The correct PCR products were further amplified by PCR with the DGGE primer set, GC341f (5'-CGCCCGCCGCGCGCGGGGGGCGGGGCGGGGG

Table 1. The performance of each reactor under continuous-operation (the 2-CP loading was the average ± standard deviation from three data).

Reactors	H_2 Supplied, g m^{-2} d^{-1}	2-CP loading, g m^{-2} d^{-1}	Nitrate loading, g-N m^{-2} d^{-1}	Sulfate loading, g m^{-2} d^{-1}	pH	ORP (mV)	2-CP decay rate, g m^{-2} d^{-1} (removal, %)	Nitrate decay rate , g-N m^{-2} d^{-1} (removal, %)	Sulfate decay rate, g m^{-2} d^{-1} (removal, %)
DC	2.5	0.72±0.01	0	0	6.2 - 6.4	-170 - -90	0.67(95)	0	0
DN	2.5	0.71±0.01	1.45±0.02	0	6.1 - 6.3	-40 - +50	0.67(95)	1.22(84)	0
DS	2.5	0.71±0.01	0	5.8±0.1	6.8 - 7.1	-280 - -210	0.66(94)	0	4.18(72)
CON	0	0.72±0.01	0	0	6.8 - 7.0	+105 - +135	0.72(100)	0	0

Note: The data of DC, DN and DS reactors were the average of three sample collected on day 118 - 122.
The data of CON reactor were the average of three sample collected on day 95 - 99.

CACGGGGGGCCTACGGGAGGCAGCAG-3') and 534r (5'-ATTACCGCGGCTGCTGG-3') [13], and were further screened with DGGE. The DGGE was run at 130V for 4 h with the 40%-70% denaturing gradient gel by using the Bio-rad Dcode system (USA), and then examined with silver stain. The same electrophoresis positions (operational taxonomic units, OTUs) in the DGGE gel were considered the same species. The clones of different species were sequenced with the primer 11f by using a DNA auto-sequencer (ABI 373 DNA sequencer), and then each sequence was compared with the GenBank in the NCBI by BLASTn.

RESULTS AND DISCUSSION

I. Performance of the Bioreactors

The hydrogenotrophic bioreactors (DC, DN and DS) had been acclimated with 2-CP for more than 18 months, and the CON reactor had been acclimated for about 1 year under the condition in lack of H_2. The DC reactor showed good 2-CP dechlorinating efficiency. 2-CP was dechlorinated to produce phenol by using H_2 as electron donor. However, dechlorination was inhibited in the presence of nitrate or sulfate because these two compounds could take the place of 2-CP as electron acceptors. Although 2-CP dechlorination was inhibited by nitrate or sulfate, 2-CP still could be degraded by other pathways, such as being utilized as carbon or energy source. Moreover, 2-CP could also be degraded in lack of H_2, where 2-CP was also utilized as carbon or energy source but not as electron acceptor. Under the continuous operating condition, all the reactors demonstrated good 2-CP removal efficiency, as shown in Table 1. 2-CP removal efficiency was about 94% in all hydrogenotrophic bioreactors under the condition of influent 2-CP of 23.5-24.9 mg L^{-1} and HRT of 15 h (influent 2-CP loading-rate was 0.71-0.72 g m^{-2} d^{-1}) and was 100% in the CON bioreactor. Dechlorination occurred in the DC reactor, but not in the DN, DS and CON reactors. Instead, denitrification and sulfate-reduction were the main reactions in DN and DS reactors, respectively, and fermentation might occur in the CON reactor. The main reactions of the DN, DS and DC reactors were as below:

$$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O \text{ (denitrification)}$$

$$SO_4^{2-} + 4H_2 + H^+ \rightarrow HS^- + 4H_2O \text{ (sulfate-reduction)}$$

$$2\text{-CP} + H_2 \rightarrow \text{phenol} + H^+ + Cl^- \text{ (dechlorination)}$$

The nitrate removal efficiency of DN reactor reaching 84% as the influent nitrate loading-rate was 1.45 g-N m^{-2} d^{-1}. While the sulfate-reduction efficiency of DS reactor was 72% at the influent sulfate loading-rate of 5.8 g m^{-2} d^{-1}.

Different environmental conditions would cause varied 2-CP metabolic pathways in different reactors, and these varied 2-CP metabolic pathways could be clearly seen from the ORP range in Table 1. Generally, the ORP range was below +50 mV in the hydrogenotrophic bioreactors, but it was found to increase up to +100 mV in the CON reactor. The lower ORP range reflected that the hydrogenotrophic bioreactors proceeded with reductive reactions such as dechlorination, denitrification, and sulfate-reduction by using H_2 as electron donor [11]. On the other hand, 2-CP dechlorination did not occur for lack of H_2 in the CON reactor because of the higher ORP value.

II. Effects of Influent 2-CP, Nitrate and Sulfate Loading-rate

The hydrogenotrophic bioreactors were operated under different influent loading-rate, such as 2-CP, nitrate, and sulfate loading-rate, to evaluate these loading-rate effects on 2-CP removal. The bioreactors were operated continuously by changing the influent

2-CP (10, 25, 40, 50 mg/L), nitrate (25, 50, 100 mg-N/L), and sulfate (50, 100, 200 mg/L) concentrations with fixed HRT of 15 h. The bioreactors were kept at each concentration for 2-3 weeks to reach a stable

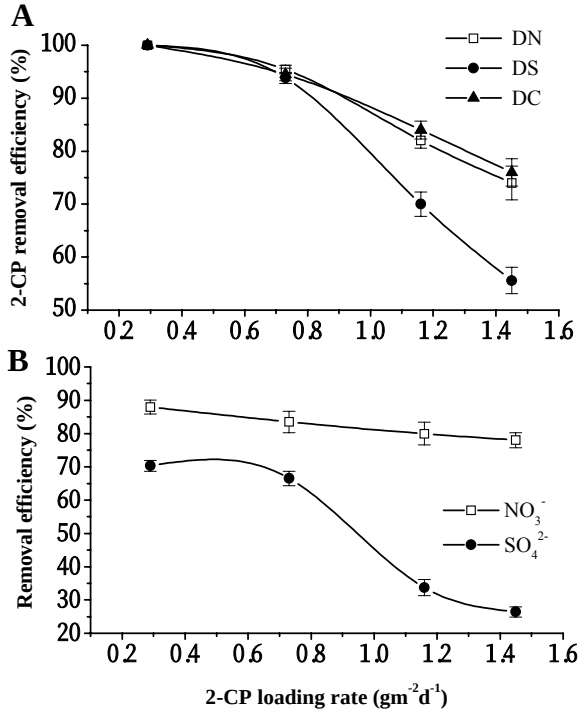

Fig. 2. The effects of 2-CP loading rate on the 2-CP removal (A) and nitrate, sulfate reduction (B) (Error bars represent the standard deviation)

condition. Experimental results showed that when 2-CP loading-rate was below 0.72 g m^{-2} d^{-1}, 2-CP removal efficiency could remain over 90% (Fig. 2). When 2-CP loading-rate increased higher than 0.72 g m^{-2} d^{-1}, the 2-CP removal efficiency rapidly dropped, especially in the DS reactor. The decrease of 2-CP removal efficiency might be caused by the inhibition of the microbial activity under the higher 2-CP concentration. Because the denitrifying and sulfate-reducing efficiency also decreased as the 2-CP loading-rate increased.

The 2-CP loading-rate could affect the denitrification in the DN reactor and the sulfate-reduction in the DS reactor. Under the conditions of influent nitrate of 50 mg-N L^{-1} for DN reactor and influent sulfate of 200 mg L^{-1} for DS reactor, the nitrate and sulfate removal efficiency decreased with the increasing 2-CP loading-rate (Fig. 2). When the 2-CP loading-rate increased from 0.29 to 1.45 g m^{-2} d^{-1}, the nitrate and sulfate removal efficiency decreased from 88% to 78% and from 70% to 26%, respectively. The results also showed that the sulfate reducing bacteria might be more sensitive to the 2-CP loading-rate, thus there was a marked decline in the removal efficiency of the sulfate and 2-CP as the 2-CP loading-rate increased.

To elucidate how denitrification and sulfate-reduction affected 2-CP degradation, different nitrate and sulfate loading-rate were proceeded. Results showed that under the condition of fixed influent 2-CP of 25 mg L^{-1}, both nitrate and sulfate loading-rate had

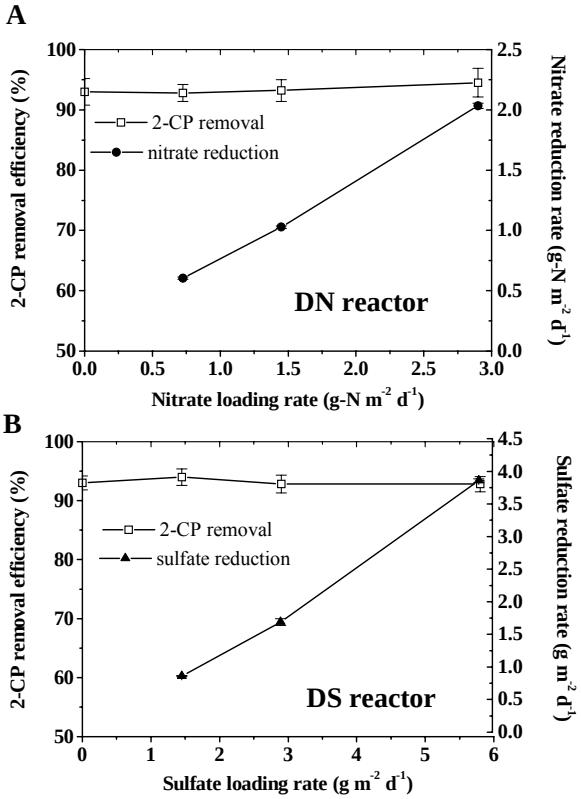

Fig. 3. The effects of nitrate (A) and sulfate (B) loading rate on the 2-CP removal, nitrate and sulfate reduction rate (Error bars represent the standard deviation).

no significant influence on 2-CP removal efficiency (Fig. 3). When the nitrate loading-rate increased from 0 to 2.9 g-N m^{-2} d^{-1}, the nitrate reduction rate increased from 0 to 2.1 g-N m^{-2} d^{-1}. However, 2-CP removal efficiency showed almost no difference. Similarly, as the sulfate loading-rate increased from 0 to 5.8 g m^{-2} d^{-1}, the sulfate reduction rate was increased from 0 to 3.8 g m^{-2} d^{-1}, and 2-CP removal efficiency always maintained at around 92 - 95 %. The results showed that the increased nitrate and sulfate reduction rate had no effects on 2-CP removal efficiency. This result indicated that 2-CP was not involved in the denitrification and sulfate-reduction. The denitrification and sulfate-reduction were controlled by the H_2, and thus these reactions did not occur in lack of H_2.

III. Microbial Identification

To clarify how the microorganism affected 2-CP degradation in the four bioreactors, Cells of the biofilm were collected for microbial identification

after the acclimation of these bioreactors. The 16S rDNA of the samples was extracted and followed the cloning-DGGE method for microbial identification. Results showed that most identified species of these bioreactors belong to β-proteobacteria, about 68%, 52%, 60% and 68% in the DC, DN, DS and CON reactors, respectively (Table 2). More specifically, eight DNA sequences were identified in the DC reactor, and among them, the DC1 (20% in abundance) was similar to *Ralstonia sp*. 50 with similarity of 98%. Other dominant species identified in the DC reactor were similar to *MTBE-degrading bacterium* PM1, *Comamonas sp*. MBIC3885, *Sporomusa aerovorans*, and *Zoogloea sp*. DhA-35 (Table 2). From the phylogenetic tree (Fig. 4), we can make sure that the DC1 belongs to genus *Ralstonia*, and the DC3, DC6, DC15, DC21 belong to genera *MTBE-degrading bacterium* PM1, *Zoogloea*, *Comamonas* and *Acidovorax*, respectively. As for the DN reactor, there were ten identified species, and the most dominant species DN2, probably the same species with DC1, was also similar to *Ralstonia sp*. 50 (Table 2). The other species in the DN reactor were similar to *MTBE-degrading bacterium* PM1, *Clostridium sp*. Rpec1, *Acidovorax avenae* and *Bacterium* BA128.

Table 2. The microbial identification of the bioreactors by clone-DGGE method.

Reactors	Clone No.	Percentage (%)[a]	Best matched organism (accession no.)	Similarity (%)	Sequence length compared (bp)[b]
DC	DC1	20	*Ralstonia sp.* 50 (AY177368)	98	1093 (45-1135)
	DC2	4	*Flexibacter sancti* (AB078068)	92	665 (187-851)
	DC3	16	*MTBE-degrading bacterium* PM1 (AF176594)	99	751 (60-809)
	DC5	8	*Rhizobium sp.* Aei-4 (AF511493)	91	1105 (31-1135)
	DC6	12	*Zoogloea sp.* DhA-35 (AJ011506)	96	1124 (44-1163)
	DC9	16	*Sporomusa aerovorans* TMA03 (AJ506191)	95	1044 (70-1111)
	DC15	16	*Comamonas sp.* MBIC3885 (AB008429)	98	1002 (34-1035)
	DC21	4	*Acidovorax avenae* (AF508114)	97	993 (22-1114)
DN	DN2	16	*Ralstonia sp.* 50 (AY177368)	96	989 (134-1122)
	DN3	8	*MTBE-degrading bacterium* PM1 (AF176594)	99	1036 (92-1127)
	DN7	8	*Clostridium sp.* Rpec1 (Y15985)	97	551 (200-750)
	DN12	12	*Acidovorax avenae* (AF508114)	99	687 (141-827)
	DN13	4	*Comamonas terrigena (CTE430343)*	97	935 (67-1001)
	DN16	8	*Bacterium* BA128 (AF364862)	97	659 (117-775)
	DN18	4	*Comamonas sp.* MBIC3885 (AB008429)	99	940 (1-940)
	DN19	4	*Bacterium* Ellin371 (AF498753)	90	931 (23-953)
	DN22	4	*Flavobacterium ferrugineum* (M62798)	91	1002 (15-1014)
	DN23	4	*Helicobacter bilis* (U51873)	89	778 (33-810)
DS	DS1	8	*β-proteobacterium* 5Z-C1 (AJ224618)	96	947 (1-947)
	DS2	20	*Sulfate-reducing bacterium* F1-7b (AJ012594)	96	985 (1-985)
	DS4	16	*Ralstonia sp.* 50 (AY177368)	98	994 (34-1127)
	DS7	12	*Hydrogenophaga flava* (AF078771)	98	834 (45-878)
	DS13	12	*Comamonas sp.* TK41 (AJ550282)	98	935 (44-978)
	DS18	12	*MTBE-degrading bacterium* PM1 (AF176594)	99	981 (78-1158)
CON	CON1	8	*Flexibacter sp.* CF1 (AF361187)	92	895 (6-900)
	CON2	68	*Ralstonia sp.* 50 (AY177368)	99	923 (12-934)
	CON6	8	*β-proteobacterium* 5Z-C1 (AJ224618)	96	885 (114-998)

[a] Percentage is calculated from the operational taxonomic units (OTUs)

[b] Escherichia coli numbering in parentheses

The major species of the DS reactor, however, showed great difference from the other reactors. The DS2, similar to *sulfate-reducing bacterium* F1-7b with similarity of 96%, was the most dominant in the DS reactor (Table 2). The species *Ralstonia sp*. 50 also appeared in the DS reactor with a large proportion, indicating that this species could also adapt to the sulfate-reducing environment. The DS7, similar to *Hydrogenophaga flava* with similarity of 98%, was a rare species only found in the DS reactor. The remaining species found in the DS reactor were also found in the other reactors (Table 2). As for the CON reactor, the microbial community here was not so complicated. Only three species, *Ralstonia sp*.50, *Flexibacte sp*. CF1 and *β-proteobacterium* 5Z-C1 were identified, and *Ralstonia sp*.50 accounted for 68% in amount.

The 2-CP degrading pathway varied with different environmental conditions, and the microbial data could help us understand more about how it worked in different bioreactors. From the microbial identification, it was suggested that the *Ralstonia* might be the major 2-CP degrading bacteria and easily adapted to various environmental conditions to degrade 2-CP via

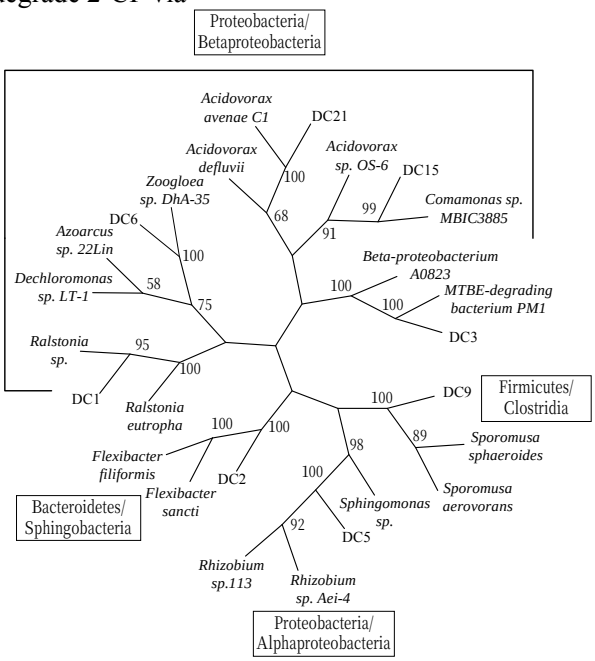

Fig. 4. The phylogenetic tree of the DC reactor.

various metabolic pathways. Several studies pointed out that *Ralstonia* could degrade phenol, 4-CP, 2,4,5-TCP and 2,4,6-TCP under the aerobic condition [14-16], and could reductively dechlorinate 4-CP under the anaerobic condition [17]. Moreover, under the anaerobic condition, *Ralstonia* could reduce the 3-nitrophenol and 2-chloro-5-nitrophenol [18]. *Ralstonia* could even utilize H_2 as electron donor for denitrification [19]. That accounted for the reason why the DC and DS reactors could also present good denitrification efficiency [11]. It was also the same reason that *Ralstonia* could sustain dominant in the DN reactor and DC reactor (Table 2).

The other genera found in the hydrogenotrophic bioreactors were *Sporomusa*, *MTBE-degrading bacterium* PM1, *Comamonas*, *Hydrogenophaga*, *Acidovorax* and *Clostridium* (Table 2). These bacteria were reported in literatures to possess the ability to degrade some aromatics or other recalcitrant compounds, but none of these bacteria had been reported to have CPs degrading ability. *Sporomusa*, only found in the DC reactor, was reported to have the ability for PCE dechlorination [20], and could utilize H_2 and CO_2 as the energy and carbon source [21]. *Hydrogenophaga* could also grow by using H_2 as the energy source [22]. The diverse microbial communities also demonstrated that the metabolic pathway of 2-CP varied with the environmental conditions.

CONCLUSIONS

The experimental results showed that 2-CP dechlorination occurred in the hydrogenotrophic bioreactor. 2-CP was dechlorinated to produce phenol by using H_2 as electron donor. However, nitrate and sulfate would inhibited 2-CP dechlorination by taking the place of 2-CP as electron acceptor. 2-CP dechlorination did not occur in lack of H_2, but it could be degraded by other pathways. This study investigates the performance of 2-CP biodegradation under different conditions and their microbial communities. In the DC, DN and DS reactors, the dominant reactions were 2-CP dechlorination, denitrification and sulfate-reduction, respectively. 2-CP was utilized as carbon or energy source in DN, DS and CON reactors. From the microbial identification, most bacteria belonged to β-proteobacteria , in which, *Ralstonia* might be the major genus for degrading 2-CP. This bacterium could grow in aerobic and anaerobic environment and degrade CPs in different pathways. Other important bacterial genera found in the hydrogenotrophic bioreactors were similar to *MTBE-degrading bacterium* PM1, *Rhizobium*, *Zoogloea*, *Sporomusa*, *Comamonas*, *Acidovorax*, *Hydrogenophaga* and *sulfate-reducing bacterium* F1-7b.

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氫氣自營性生物反應槽對 2-氯酚之降解及其菌種鑑定

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關鍵詞：氯酚、脫氯反應、 氫氣自營性、生物反應槽

摘 要

本研究利用氫氣自營性生物反應槽進行 2-氯酚(2-CP)之降解，反應槽於不同還原條件下連續進流 2-CP 馴養超過 18 個月，結果顯示在供氫條件下，微生物可利用氫氣為電子供給者進行 2-CP 之脫氯反應，而其 2-CP 去除效率達 95%。然而在硝酸鹽或硫酸鹽存在的條件下，2-CP 之脫氯反應則被抑制，主要是因為微生物的電子接受者由 2-CP 轉變為硝酸鹽或硫酸鹽，而 2-CP 則變成碳源被分解。本研究也利用 clone-DGGE 方法進行反應槽之菌種鑑定，結果顯示大部分菌種屬於 Proteobacteria/β-proteobacteria，最主要的 2-CP 分解菌應為 *Ralstonia* 菌屬，而反應槽出現的其他菌種較近似於 *MTBE-degrading bacterium* PM1, *Rhizobium*, *Zoogloea*, *Sporomusa*, *Comamonas*, *Acidovorax*, *Hydrogenophaga* 及 *sulfate-reducing bacterium* F1-7b，文獻中這些菌種大多具有分解某些難分解有機物的能力。