Journal of Hazardous Materials 186 (2011) 978–983

Contents lists available at ScienceDirect

Journal of Hazardous Materials

journal homepage: www.elsevier.com/locate/jhazmat

The effects of nutrient amendment on biodegradation and cytochrome P450 activity of an *n*-alkane degrading strain of *Burkholderia* sp. GS3C

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ARTICLE INFO

Article history:
Received 24 April 2010
Received in revised form 29 October 2010
Accepted 23 November 2010
Available online 30 November 2010

Keywords:
Hexadecane
Biodegradation
Micronutrient
Amino acids

ABSTRACT

The promotion of hexadecane biodegradation activity by an *n*-alkane degrading strain of *Burkholderia cepacia* (GS3C) with yeast extract amendment was studied using various carbon, nitrogen, vitamin, and amino acid amendments. Cytochrome P450 monooxygenase enzymes play a very important role and are especially required to introduce oxygen in *n*-alkane degradation. These enzymes from GS3C were located and detected using amino acid amendments. It was shown that biodegradation activity was promoted with amino acids amendments. However, only specific amino acids (L-phenylalanine, L-glutamic acid, L-proline, L-lysine, L-valine and L-leucine) have biodegradation promoting ability for GS3C. Cell protein concentration and cytochrome P450 activity were promoted significantly with the addition of L-phenylalanine and yeast extract. Furthermore, a significant positive linear relationship between cytochrome P450 activity and biodegradation efficiency of GS3C was observed. The results indicate that amino acid is the primary factor of nutrient amendment in promoting hexadecane biodegradation by influencing cytochrome P450 activity in GS3C.

1. Introduction

Biodegradation of xenobiotics promoted by organic substrate amendment has been studied extensively [1–3]. A co-metabolic effect, which was first described by Leadbetter and Foster [4] was considered to be a reasonable explanation by various researchers [5–7]. Researchers also found that micronutrients, such as amino acids and vitamins were very important for biodegradation activity in some circumstances. For example, when vitamins and a particular mixture of two amino acids, tryptophan and phenylalanine, were provided in culture, it caused rapid degradation of 2,4,6-tribromophenol without the rapid cell growth seen by *Achromobacter piechaudii* strain TBPZ [8]. However, little research has been done to determine the exact reasons for the promotion of biodegradation activity by micronutrient amendment.

Monooxoygenase enzymes, such as cytochrome P450 are the foremost enzymes involved in the metabolism of xenobiotic compounds, including petroleum hydrocarbons [9]. The principal function of cytochrome P450 is monooxygenation of various substrates, which requires molecular oxygen and a supply of NADPH or NADH. Monooxygenation is considered to be the first step in aerobic degradation of alkanes by bacteria, yeasts and fungi [10,11]. Some prokaryotes that utilize hydrocarbons as their sole carbon and energy sources contain extremely high concentrations of special forms of cytochrome P450, such as P450cam, which specifically catabolizes hydrocarbons [10].

We recently isolated an *n*-alkane degrading strain of *Burkholderia cepacia* (GS3C) whose biodegradation activity was promoted significantly by yeast extract amendment. In this study, we found that amino acid amendments were the primary factor in promoting biodegradation by this strain. An explanation for the promotion, which was caused by enhanced cytochrome P450 activity due to the amino acid amendment, is also presented.

2. Methods

2.1. Bacteria strain and identification

The *n*-alkane-degrading strain GS3C was isolated from petroleum-contaminated soil in Guangzhou petrochemical industry and was identified by strain agar colony color, Gram staining

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doi:10.1016/j.jhazmat.2010.11.095

and morphology. Further characterization was performed using the API 20NE test (API, bio-mérieux, France) and 16S rRNA gene sequencing techniques [12,13]. The fragment of rRNA was amplified using a Gene Amp 2400 PCR System (PE, USA) under the following condition: 1 μL template DNA, 5.0 μL 10X PCR buffer (100 mM Tris/HCl (pH 8.3 at 20 °C), 15 mM $MgCl_2$, 500 mM KCl, and 0.1% (w/v) glutin), 0.5 μL Blend-Taq, 0.5 μL upstream primer 27F (AGAGT TTGAT CCTGG CTCAG), 0.5 μL downstream primer 1522R (AAGGA GGTGA TCCAG CCGCA), 5 μL dNTPs and distilled water with a total volume of 50 μL. The tubes were incubated at 94 °C for 3 min. After 3 min, the tubes were subjected to the following thermal cycling program: denaturation at 94 °C for 1 min, primer annealing at 54 °C for 1 min and chain extension at 72 °C for 2 min with an additional extension time of 7 min on the final cycle for a total of 30 cycles. The program BLAST was used to search 16S rRNA databases for similar sequences. The 16S rRNA gene sequence of strain CS3C was deposited into GenBank and named *Burkholderia* sp. GS3C (EU282110).

2.2. Culture conditions

Biodegradation batch experiments were performed in 100 mL flasks with 25 mL mineral salts medium (MSM) that contained 2500 mg/L hexadecane. The MSM for microbial culture was performed in media with an inorganic media base composed of the following [14] (in grams per liter): $MgSO_4{\cdot}7H_2O$, 0.2; $CaCl_2{\cdot}2H_2O$, 0.02; K_2HPO_4, 0.5; KH_2PO_4, 0.5; NH_4NO_3, 0.5; trace element solution, 10 mL/L. The trace elements of the MSM consisted of the following: (in milligrams per liter) $FeSO_4{\cdot}7H_2O$, 300; $MnSO_4{\cdot}H_2O$, 50; $CoCl_2{\cdot}6H_2O$, 106; $Na_2MoO_4{\cdot}2H_2O$, 34; $ZnSO_4{\cdot}7H_2O$, 40; $CuSO_4{\cdot}5H_2O$, 50. The pH of the MSM was adjusted to 7.5. The growth of GS3C was quantified with increasing cell density (colony forming units [cfu]/mL) during the experiment on a nutrient agar. A 5-day duration experiment was selected for this study because at 2500 mg/L hexadecane initial concentration enough hydrocarbon remained in cultures treated with and without yeast extract addition [15]. This condition allowed to evaluate the nutrient amendments effects and to support active biodegradation adequate to measure cytochrome P450 activity.

2.3. Nutrient amendment

Different nutrients were amended separately to the MSM to characterize the nutrient requirement of GS3C and to determine their ability to promote hexadecane degradation (Table 1). Yeast extract was added as a mixed nutrient, glucose and fructose were added separately as carbon sources, urea and ammonium nitrate were added separately as nitrogen sources, casamino acids, amino acids and mixed vitamin solution were added separately for micronutrients. The amino acids were divided into four groups based on Sørensen's classification [16]. The mixed vitamin solution was amended based on Balch's research [17].

2.4. Biodegradation of hexadecane by strain GS3C

Flasks that contained 25 mL of MSM and 2500 mg/L hexadecane were inoculated with 0.5 mL of cell culture, which was pre cultured for two days with hexadecane as the sole carbon source, and then incubated at 30 °C on a shaker preset to 150 rpm. Sterile control was incubated at the same time to determine abiotic loss. Three replicates were performed for each treatment except sterile control for two replicates. After 5 days of incubation three replicate flasks were taken from the shaker and the content of each flask was extracted twice with equal volume of hexane (25 mL). The combined extracts were pooled, dried over anhydrous Na_2SO_4, and then diluted with hexane to a suitable volume. The residual hexadecane was determined using a gas chromatograph (Agilent 6890N, USA) equipped with a flame-ionization detector and HP-5 type 30 m long capillary column (0.32 mm inside diameter with a 0.25 μm film thickness). During analysis, the column temperature was maintained isothermally at 80 °C for 1 min, and then increased to 280 °C at a rate of 15 min^{-1}. External hexadecane standards of 1–100 mg/L in hexane matrix were prepared and used to establish a calibration curve for quantification. Hexadecane recovery was 103.0 ± 1.8% after five days incubation based on two replicates.

Table 1
Composition of different nutrient amendments.

Amendment	Component	Concentration
Yeast extract	Yeast extract	600 mg/L
Fructose	Fructose	600 mg/L
Glucose	Glucose	600 mg/L
Urea	Urea	600 mg/L
Ammonium nitrate	Ammonium nitrate	600 mg/L
Casamino acids	Casamino acids	600 mg/L
Amino acid mixture 1 (G1)	L-Glutamic acid, L-proline, L-lysine, L-valine and L-leucine	20 mg/L of each single amino acid
Amino acid mixture 2 (G2)	L-Phenylalanine, L-isoleucine, L-aspartic acid, L-alanine and L-threonine	20 mg/L of each single amino acid
Amino acid mixture 3 (G3)	L-Arginine, L-serine, L-histidine, L-glycine and L-methionine	20 mg/L of each single amino acid
Amino acid mixture 4 (G4)	L-Cysteine, L-tryptophan, L-tyrosine, L-cystine, L-glutamine, L-asparagine	20 mg/L of each single amino acid
Mixed vitamin (MV)	Thiamine, riboflavin, niacinamide, pyridoxine, cyanocobalamin, biotin, folacin, niacin and p-aminobenzoic acid	Concentration of each vitamin can be found in [16]

2.5. Preparation of microsomes

After 5 days of incubation, the whole culture broth of four independent replicates was centrifuged at 5000 r/min for 10 min at 4 °C. Hexadecane solidified at this temperature and was removed from the top of the culture broth. Additionally, the supernatant (Sup-1) was collected and pooled. The cell pellets of the four replicates were also pooled to a single sample for protein and cytochrome P450 activity measurements. Then the combined cell pellets was suspended in a cold solution containing 20% sucrose and 10 mmol/L Tris–HCl (pH 7.5). Periplasmic fractions (labeled Sup-2) were collected using the method described by Koshland and Botstein [18]. The cells were centrifuged in an Eppendorf centrifuge tube for 2 min, and then the pellets were placed on ice. All subsequent steps were carried out at 4 °C. The pellets were re-suspended in 3.75 mL of a cold solution containing 20% sucrose and 10 mmol/L Tris–HCl (pH 7.5). EDTA was added (75 μL of 0.5 mol/L (pH 8)), and incubation on ice was continued for 10 min. Cells were centrifuged in an Eppendorf centrifuge tube for 5 min while cold. The supernatant fluid was quickly removed and the pellets were rapidly re-suspended by vigorous agitation in 2.5 mL cold distilled water. The mixture was incubated for 10 min on ice and then centrifuged again for 5 min. The supernatant was removed and saved as the periplasmic fraction (Sup-2). The remaining pellets were suspended again and disrupted with ultrasonication for 10 min. The mixture was centrifuged at 8000 r/min for 10 min at 4 °C and the supernatant (Sup-3) was collected. The supernatant was then precipitated by

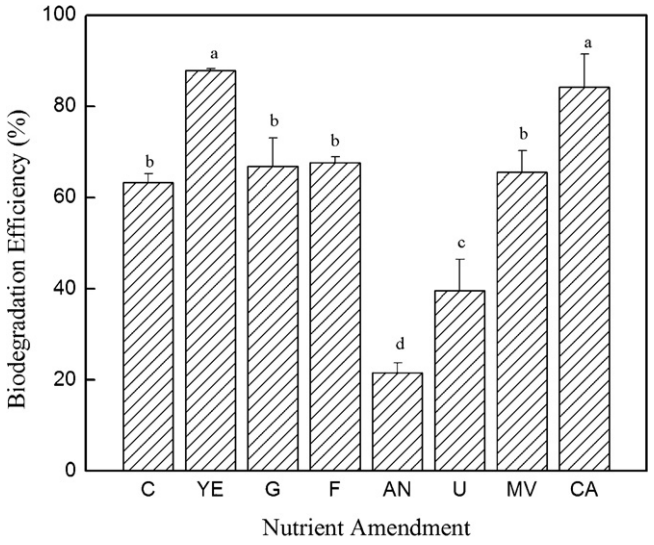

Fig. 1. Biodegradation activity of hexadecane by strain GS3C with yeast extract (YE), glucose (G), fructose (F), ammonium nitrate (AN), urea (U), mixed vitamins (MV) casamino acids (CA) and no amendment (C). All of the amendment concentrations were 600 mg/L except vitamin solution (2.8 mg/L of total concentration). Values represent mean ± S.D. ($n = 3$). Different letters indicate significant differences at $p < 0.05$.

ultracentrifugation at 105,000 × *g* for 90 min in a Hitachi model 85P-72 ultracentrifuge. The microsomes were separated and suspended in 50 mM Tris–HCl buffer (pH 8.0) for cytochrome P450 measurement. The protein concentration of each fraction was measured using the method described by Bradford [19].

2.6. Assay of cytochrome P450

Cytochrome P450 activities were measured after 5 days culture at 2500 mg/L hexadecane initial concentration as strain GS3C was still keeping active biodegradation activities under this condition. The amount of cytochrome P450 was determined using a modified method from Omura and Sato [20]. Several milligrams of sodium dithionite were added to the microsome samples, which were equally divided into two optically matched cuvettes. The contents of the cuvette were exposed to carbon monoxide (CO) for 2 min by gently bubbling the gas through the cuvette. Immediately afterwards, the UV spectrum was obtained and compared to the spectrum of a non-CO-exposed cuvette using a UV spectrophotometer (UV-2450, Shimadzu). The presence of cytochrome P450 was confirmed by the characteristic absorption peak around 450 nm. Cytochrome P450 activity was quantified using a difference extinction coefficient of 0.091 M^{-1} cm^{-1}.

3. Results and discussion

3.1. Effect of different nutrient amendments on biodegradation

Our previous research indicated that biodegradation activity was promoted significantly with yeast extract amendment at day 5 and day 7 [15]. In this study, we first investigated biodegradation activity of strain GS3C with different nutrient amendments harvested after 5 days of culturing. Yeast extract amendment is most effective for promoting biodegradation; almost 88% of the hexadecane was degraded with yeast extract amendment compared to 63.3% without nutrient amendment (Fig. 1). Yeast extract is made by yeast cells and is typically rich in carbohydrate (4–13%), nitrogen (8–12%) and microbial growth stimulants, such as amino acids (3.0–5.2%) and vitamins. Therefore, yeast extract is often used in media for the cultivation of microorganisms. To investigate the function of specific ingredients in the yeast extract used for biodegradation of strain GS3C, different kinds of carbon sources, nitrogen sources, casamino acids and mixed vitamin solutions were amended separately to the MSM. As shown in Fig. 1, biodegradation with glucose or fructose amendments was similar to control samples (without any extra nutrient addition) even the amendment concentration (600 mg/L) obviously higher than carbohydrate concentration in yeast extract. Mixed vitamin also did not promote biodegradation activity. Biodegradation was even inhibited by the addition of extra nitrogen sources, especially with extra ammonium nitrate, which was a component of MSM used in all the treatment. This was also observed by Nejidat et al. [21]. The inhibition of biodegradation by adding nitrogen sources could be caused by the formation of nitrite, a toxic byproduct of metabolism. Or GS3C is nitrate sensitive, and the amended concentration of ammonium nitrate (600 mg/L) was more than the tolerance limit. Only casamino acids promoted biodegradation as much as yeast extract amendment. It was speculated that amino acids are the primary fac-

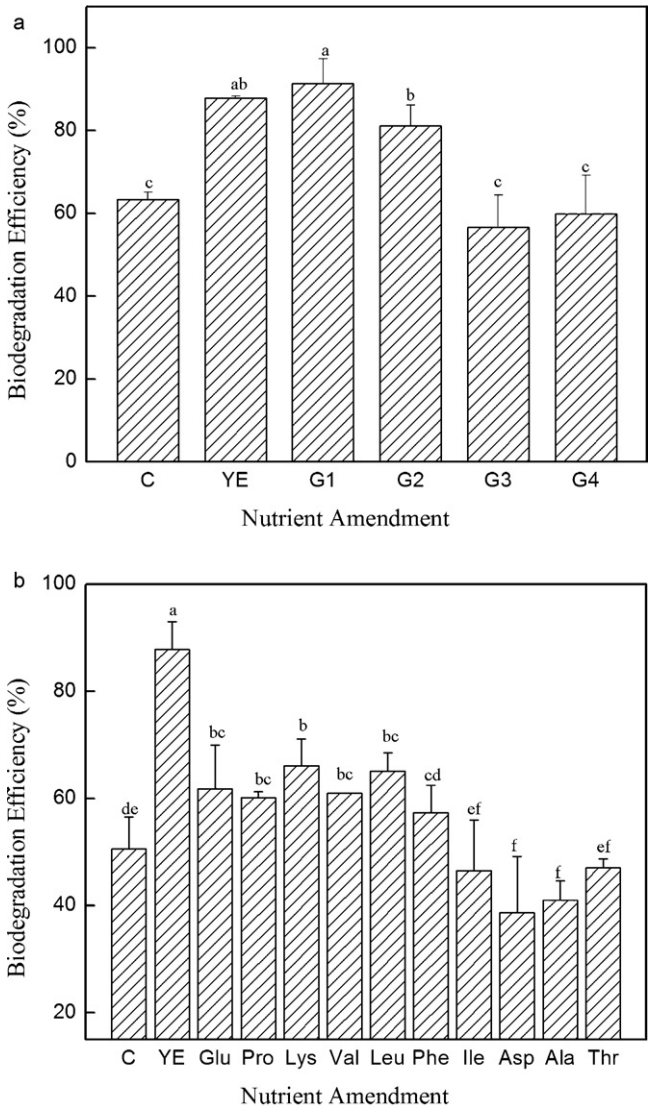

Fig. 2. Biodegradation activity of hexadecane by strain GS3C with (a) mixed amino acids and (b) separated amino acids amendment compared with yeast extract (YE) and no amendment (C). Concentration of each amino acid in each group or separated amendment was 20 mg/mL, concentration of yeast extract (YE) was 600 mg/L. Values represent mean ± S.D. ($n = 3$). Different letters indicate significant differences at $p < 0.05$.

Table 2
Distribution of protein and cytochrome P450 in different fractions of strain GS3C.

Sample	Volume (mL)	Protein concentration (mg/mL)	Protein mass (mg)	Protein proportion (%)	Microsome concentration (mg/mL)	Cytochrome P450 (nmol/mg Pro)	Cytochrome P450 proportion (%)
Sup-1	100	0.02	2.00	11.7%	–	–	0
Sup-2	5	0.05	0.25	1.4%	–	–	0
Sup-3	5	2.98	14.90	86.9%	0.7	0.173	100

tor in yeast extract that promote biodegradation because casamino acids are obtained from acid hydrolysis of casein and are a mixture of amino acids.

Despite the fact that pure and mixed cultures can use xenobiotic substrates as their sole carbon source, it is possible that substrate inhibition occurs during biodegradation [22]. Amendment of growth substrates and nutrients to overcome this inhibition has been suggested and tested extensively. It has been reported that the presence of additional carbon sources is necessary to promote biodegradation activity. One approach is to employ a co-metabolic effect, in which metabolic byproducts of a co-substrate could be used to stimulate the biodegradation of the desired substrate. Schukat et al. found that glucose proved to be the most effective nutrient addition to co-metabolic degradation of 2- and 3-chloroaniline [6]. The enzyme responsible for aniline catabolism was detected when various organic compounds were tested for their ability to stimulate degradation of monochloroanilines. Additionally, different co-substrates may be required for different bacteria. Lee and Lee found that adding phenol as a growth co-substrate to a 4-chlorophenol degrading culture was better than adding glucose as phenol enhanced 4-chlorophenol transformation [7]. Namkoonga et al. found that degradation rates of total petroleum hydrocarbons and *n*-alkanes were promoted greatest at the ratio of 1:0.5 of contaminated soil to organic amendments (sewage sludge) for bioremediation of diesel-contaminated soil with composting [23]. However, the co-metabolic effect of glucose and fructose are not responsible for promoting biodegradation in this study because stimulation was not observed when they were added as co-substrates.

Another approach to promote biodegradation is to amend special micronutrients that are not adequately synthesized by microbial metabolism. As seen in Maymó-Gatell's research [24], dechlorination of tetrachloroethene (PCE) to give vinyl chloride (VC) followed by subsequent transformations to give nontoxic products, such as ethene and ethane was performed using strain 195. When yeast extract was added to culture, 288 μmol/L of VC was detected, while only 35 μmol/L were detected without nutrient addition. Dechlorination was stimulated greatly with the addition of casamino acids, which resulted in 1600 μmol/L of VC formed. Research has also shown that only providing vitamins and a defined mixture of amino acids together cause rapid degradation of 2,4,6-tribromophenol [8]. These studies showed that biodegradation might be controlled by micronutrients. In the present study, it was demonstrated that the degrading strain GS3C was sensitive to the promotion of biodegradation by amino acids addition based on following experiments.

3.2. Effect of different amino acids amendment on biodegradation

Based on the results above, it was assumed that if the degrading strain GS3C was cultured with individual amino acid compound amendment instead of yeast extract or casamino acid amendment, the biodegradation activity would be promoted similarly. To confirm this assumption, amino acids were amended in the MSM for further study. In this experiment, amino acids were divided into four groups, and each group was added to the MSM separately. The results showed that both mixture 1 (G1) and mixture 2 (G2) amendment produced significant biodegradation promotion (Fig. 2a) and without significant difference compared with yeast extract addition. However, no significant difference of promotion was found with either mixture 3 (G3) or mixture 4 (G4) amendment compared to the blank control sample (without extra nutrient addition). To examine the effect of single amino acids, each amino acid in mixture 1 (G1) and mixture 2 (G2) was amended separately into a batch culture. The results show that all of the amino acids in mixture 1 (G1), and only L-phenylalanine in mixture 2 (G2) promote biodegradation at various levels. However, no single amino acid can promote biodegradation to the extent of the mixture of amino acids (Fig. 2b).

The observation that specific amino acids were needed to stimulate hexadecane biodegradation was shown by the selective promotion of biodegradation by G1 and G2 (amino acids addition) instead of G3 and G4. Although G3 and G4 did not promote biodegradation in comparison with the control experiment, both amendments could serve as low concentration nitrogen sources. This fact together with the results derived from the experiments with ammonium nitrate and urea amendments, suggest that the availability of higher concentration of nitrogen sources was not responsible for promoting biodegradation in this study. Likely the nitrogen source was not the limiting factor for promoting the *n*-hexadecane biodegradation efficiency. The G1 concentration of 20 mg/mL was much lower than the concentration of yeast extract amendment (600 mg/mL); however, biodegradation using G1 amendment was better than yeast extract (Fig. 2a). This shows that amino acids were especially needed by GS3C and that amino acids were the active ingredients in yeast extract that caused enhanced biodegradation. Furthermore, Fig. 2b shows that only specific amino acids (L-phenylalanine, L-glutamic acid, L-proline, L-lysine, L-valine and L-leucine) supply the micronutrients needed by specific microorganisms. As seen in Sørensen's research [16], L-methionine was essential for *Sphingomonas* sp. SRS2 to rapidly metabolize isoproturon to CO_2. Additionally, initial mineralization was decreased when L-methionine and glycine were excluded from the mixture of amino acids. Ronen et al. found that only a specific mixture of two amino acids, tryptophan and phenylalanine, caused rapid degradation of 2,4,6-tribromophenol without rapid microbial growth [8]. In this study, it was demonstrated that some specific amino acids (L-phenylalanine, L-glutamic acid, L-proline, L-lysine, L-valine and L-leucine) are needed for hexadecane metabolism by strain GS3C.

3.3. Location of cytochrome P450

Monooxygenase enzymes, such as cytochrome P450 are the foremost enzymes involved in the metabolism of xenobiotic compounds (petroleum hydrocarbons) [9]. These enzymes are thought to be involved in the first step of aerobic degradation of alkanes by bacteria, yeasts and fungi [10,11]. A number of researchers have shown that the catabolic enzymes of various microorganisms may be distributed in different parts of cells [25–28]. There are also reports about the location of cytochrome P450 in microsomes [29] and cell membrane fractions [30]. In this study, proteins and cytochrome P450 that are located in different part of cells (inside the cells, at periplasmic and out of cells) were separated using sonication and centrifugation methods. The results (Table 2) showed

Table 3
Biodegradation activity, cell protein concentration, cell density and cytochrome P450 activity of degrading strain GS3C with nutrient amendment.

Nutrient amendment[a]	Biodegradation efficiency (%)	Protein concentration (mg/mL)	Cell density (10^9 cfu/mL)	Cytochrome P450 activity (nmol/mg Pro)
Control	63.3 ± 1.9[e]	2.98	4.3 ± 0.9	0.173
P1	78.9 ± 2.5[c]	6.82	3.0 ± 0.8	0.286
P2	77.4 ± 4.0[c]	6.72	8.1 ± 1.0	0.289
P3	84.4 ± 2.7[b]	6.72	6.6 ± 1.0	0.304
Glu	67.6 ± 1.3[d]	3.61	4.7 ± 1.0	0.182
YE	87.9 ± 0.4[a]	7.52	5.6 ± 0.4	0.340

Values of biodegradation efficiency and cell density represent mean ± S.D. ($n = 3$). Different letters in columns indicate significant differences at $p < 0.05$.
[a] Control, no nutrient amendment; P1, L-phenylalanine, 20 mg/L; P2, L-phenylalanine, 40 mg/L; P3, L-phenylalanine, 60 mg/L; Glu, glucose, 600 mg/L; YE, yeast extract, 600 mg/L.

that most of protein (86.9%) was found in the intracellular fraction (Sup-3) and only 11.7% and 1.4% were found in the extracellular fraction (Sup-1) and the periplasmic fraction (Sup-2), respectively. It was also important to find out the location of catabolic enzymes before further study. The results of the enzyme location study showed that all of the cytochrome P450 was located in the intracellular fraction (Table 2). No cytochrome P450 was detected in the extracellular fraction (Sup-1) or the periplasmic fraction (Sup-2). This indicated that hexadecane-degrading enzymatic activities of strain GS3C occur in the intracellular fraction. Thus, hexadecane in the batch medium must penetrate extracellular/intracellular gradients with the help of a transporter or is driven by diffusion due to substrate concentration before degradation by cytochrome P450 [31].

3.4. Activity of cytochrome P450

Biodegradation efficiency, cell protein concentration, cell density and cytochrome P450 activity of strain GS3C were measured after 5 days of culturing. The results are presented in Table 3. Compared with the control treatment (no extra nutrient addition), promotion of biodegradation activity and cell protein concentration were observed in all of the treatments. It also seems that increased biodegradation can be obtained with additional L-phenylalanine amendment. Therefore, an increase in cell protein concentration due to amino acid amendment (yeast extract and L-phenylalanine) resulted in enhanced biodegradation. However, no obvious correlation between cell density and biodegradation activity was found in this study. The possible reason is that not all of bacteria formed separately colony when it was diluted and spread on agar plate to count colonies number. It is possible that the ratio of single stayed and bonded bacteria is not stable at different nutrient conditions in this study.

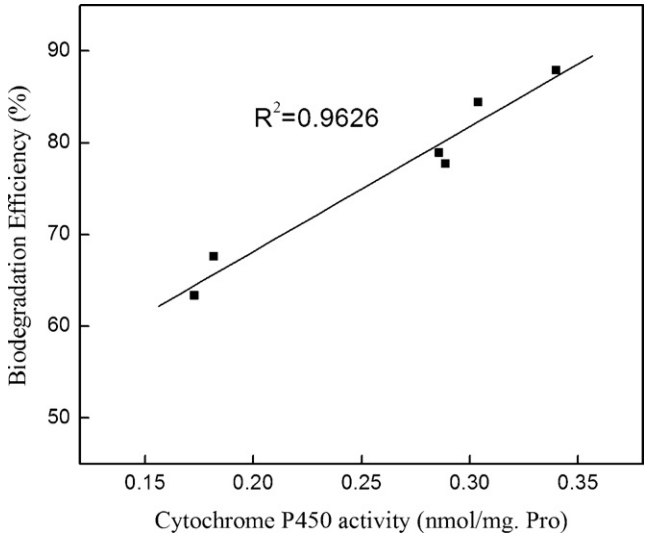

Fig. 3. Relationship between cytochrome P450 activity and hexadecane biodegradation activity by strain GS3C.

Although researchers have found that amendment with amino acids and vitamins can promote biodegradation activity [8,16,24], little research has been done on the exact reason of this observation. Cytochrome P450 is considered as a monooxygenase enzyme involved in the metabolism of endogenous and xenobiotic compounds. To obtain more information on promoting degradation using yeast extract and amino acid amendment, cytochrome P450 was studied to explain the mechanism of promotion due to their involvement in the first step of aerobic degradation of alkanes by bacteria, yeasts and fungi [10,32]. These enzymes, which introduce oxygen atoms derived from molecular oxygen into substrate, play an important role in the biodegradation of xenobiotics. After the first oxygenase step, the resulting carboxylic acids enter the β-oxidation cycle [33]. Depending on the chain length of the alkane substrate, different oxygenases are required to introduce oxygen into the substrate, and subsequently, cytochrome P450 is required for hexadecane degradation [32]. In this study, a significant positive correlation ($R^2 = 0.963$) was observed between cytochrome P450 activity and biodegradation promotion (Fig. 3). Based on the alkane metabolism mechanism presented above, our results demonstrate that amino acid is the primary factor of nutrient amendment in promoting hexadecane biodegradation by influencing cytochrome P450 activity in GS3C. Also, the data presented in Fig. 2 and Table 3 indicate that even low concentrations of amino acid amendment (20 mg/L) can promote cytochrome P450 activity and biodegradation significantly. It is concluded from the results presented above that the terminal oxidation, which is important for alkane biodegradation, was promoted by amino acid amendment. The results from this study also indicate that the amendment of micronutrients to stimulate the activity of a degrading strain is also a possible strategy for bioremediation.

4. Conclusions

Significant promotion of biodegradation of strain *B. cepacia* GS3C was observed with yeast extract amendment. The results indicated that strain GS3C was sensitive to amino acids for promoting biodegradation. However, only specific amino acids (all those of L-glutamic acid, L-proline, L-lysine, L-valine and L-leucine in mixture 1, and L-phenylalanine in mixture 2) were effective promoters. Further studies showed that cytochrome P450 activity was promoted significantly with L-phenylalanine and yeast extract amendment. Furthermore, a significant positive linear relationship between cytochrome P450 activity and biodegradation activity was found. This indicated amino acid is the primary factor of nutrient

amendment in promoting hexadecane biodegradation by influencing cytochrome P450 activity in GS3C.

Acknowledgements

This work was supported by a grant from the National Natural Science Foundation of China (40730741 and 41073088), the Guangdong Provincial Natural Science Foundation (No. 9351064101000001), the Guangdong Provincial Science and Technology Planning Project (2009B050900005), the Fundamental Research Funds for the Central Universities (Nos. 2009ZM0034 and 2009ZM0119), and the Open Foundation of the State Key Laboratory of Environmental Geochemistry.

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