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Biodegradation of phenol and sodium salicylate mixtures by suspended *Pseudomonas putida* CCRC 14365

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Abstract

The biodegradation of single phenol and sodium salicylate (SA) and their binary mixtures in water by free *Pseudomonas putida* (*P. putida*) CCRC 14365 was experimentally studied at 30 °C and pH 7. The initial concentration of the cells, adapted with either phenol or SA, was maintained at 0.025 g/L. Single substrate experiments were performed in the substrate level range 0.53–3.18 mM. The Haldane model has shown that phenol was biodegraded more quickly ($\mu_{max} = 0.245\ h^{-1}$) than SA ($0.137\ h^{-1}$) under the ranges studied, and SA had a more inhibitory effect on cell growth ($K_I = 5.21$ mM) than phenol (12.6 mM) at low substrate levels even by SA-adapted cells. Binary substrate experiments were carried out at two fixed total substrate levels of 1.06 and 3.18 mM, with a varying molar concentration ratio of 0.33–3.0. The presence of a small amount of phenol to SA could significantly enhance the biodegradation of SA, particularly when the phenol-adapted cells were employed. On the other hand, the addition of a small amount of SA to phenol would retard the biodegradation of phenol, especially at higher total substrate levels (3.18 mM).

Keywords: Biodegradation; Binary substrates; Phenol; Sodium salicylate; *Pseudomonas putida*

1. Introduction

A variety of toxic organic matters are produced in chemical and petroleum process industries, which have resulted in cumulative hazardous effects on the environment. These organic chemicals, especially for aromatic compounds, are rather resistant to natural biodegradation and persist in the environment. Phenolic compounds are the most common representatives of toxic organic pollutants. They are encountered in the effluents of such processes and operations as petroleum refineries, gas and coke oven industries, glass fiber units, pharmaceuticals, explosive manufacture, phenolic resin manufacture, plastic and varnish industries, textile units, and smelting and related metallurgical operations [1]. In addition to being potential carcinogens, phenols are either toxic (reduces enzyme activity) or lethal to fish at relatively low levels of 5–25 mg/L, depending on the temperature and the state of maturity for rainbow trout [2]. Moreover, toxic polychlorinated phenols can be formed when phenol–bearing water is chlorinated. Therefore, such effluents require proper treatment before being safely discharged to the water bodies.

Various treatment alternatives such as activated carbon adsorption, ion exchange, liquid–liquid extraction, and chemical oxidation have been reported [3]; however, they often suffer from serious drawbacks including high cost and the formation of hazardous by-products (secondary pollution) [4]. Among these methods available, biodegradation is environmental friendly and cost effective. Biological treatment of phenols has therefore been an increasingly important process in pollution control [5]. In contaminated groundwater and the effluents from industrial and municipal sources, organic matter mixtures are prevalent. The occurrence of pollutants in the mixture is an important issue, because microbial degradation of one component could be inhibited by other compounds in the mixture and different conditions may be required to treat different compounds. Biodegradation of a compound in a mixture could be strongly impacted by other components of the mixture [5,6]. To understand the mixture effects, one must consider the metabolic role of each compound playing for the biomass.

It was reported that the sodium salts of some carboxylic acids such as salicylate and glutamate not only support the growth of *Pseudomonas putida* (*P. puitda*) but also is responsible for

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Nomenclature

K_I	substrate-inhibition constant (mM)
K_S	substrate-affinity constant (mM)
SA	sodium salicylate
S_A, S_P	concentrations of SA and phenol, respectively (mM)
S_T	total substrate concentration (mM)
t	time (h)
t_{lag}	lag period for cell growth (h)
t_{100}	time required for complete removal of a given substrate (h)
x_A, x_P	mole fractions of SA and phenol, respectively
X	dried cell density (g/L)
X_{max}	maximum cell density (g/L)

Greek symbol

μ	specific cell growth rate (h^{-1})

inducing some specific enzymes for the degradation of non-growth substrates such as 4-chlorophenol and carbazole [7–9]. This special class of biological transformation is called the co-metabolism, which refers to the transformation of a non-growth substrate by cells growing on a growth substrate or by resting cells [9]. However, few studies have been done to examine the mixture effect when sodium salt of carboxylic acid and another growth substrate such as phenol are present.

The purpose of this work was to compare cell growth and biodegradation of single phenol and sodium salicylate (SA) at high initial substrate levels by suspended *P. putida*, adapted with either phenol or SA, in a batch mode, and to experimentally study the mutual inhibitory effect during the biodegradation of phenol and SA mixtures. The temperature and pH were maintained at 30 °C and 7, respectively, and the initial cell concentration was fixed at 0.025 g/L. *P. putida* strain was selected because it can effectively biodegrade both substrates, particularly for phenol [7,10–13]; and, pH 6.8–7.0 was reported to be optimal for the biodegradation of both substrates in the previous studies [1,7,13]. Single substrate experiments were performed in the level range of 0.53–3.18 mM. In the binary mixtures, the total substrate level was fixed at 1.06 or 3.18 mM, in which the mole fraction of one substrate was changed from 0.25 to 0.75.

2. Materials and methods

2.1. Microorganism, nutrient medium, and solutions

Here, *P. putida* CCRC 14365 was obtained from the Food Industry Research and Development Institute, Hsinchu, Taiwan. The stock cultures were stored at 4 °C. The nutrient medium contained 3 g/L of beef extract, 5 g/L of peptone, and mineral salt medium (MSM) at pH 7. The compositions of MSM were KH_2PO_4 (0.42 g/L), K_2HPO_4 (0.375 g/L), $(NH_4)_2SO_4$ (0.244 g/L), NaCl (0.015 g/L), $CaCl_2{\cdot}2H_2O$ (0.015 g/L), $MgSO_4{\cdot}7H_2O$ (0.05 g/L), and $FeCl_3{\cdot}6H_2O$ (0.054 g/L). A phosphate buffer (pH 7) was prepared by dissolving 8 g/L of NaCl, 0.2 g/L of KCl, 1.15 g/L of K_2HPO_4, and 0.2 g/L of KH_2PO_4 in deionized water (Millipore, Milli-Q). Prior to use, the MSM and phosphate buffer were sterilized in autoclave at 121 °C for 15 min. All inorganic chemicals were offered from Merck Co. as analytical reagent grade.

The single-substrate solution was prepared by dissolving phenol or SA (Merck Co.) in MSM to the desired concentrations (0.53–3.18 mM). In binary substrate systems, the total substrate level was fixed at 1.06 or 3.18 mM, in which the mole fraction of one substrate was varied. The pH was adjusted to 7 and the working volume was 100 mL in all experiments.

2.2. Free suspension cultivation

P. putida cells were activated at 30 °C in the nutrient medium, into which 1.06 mM of either phenol or SA was added for enzyme adaptation for 24 h. The activated cells in the late-exponential phase were harvested as inoculums. The cells collected after centrifugation (6000 rpm) for 10 min were re-suspended in phosphate buffer and re-centrifuged. After cleaning, the activated cells were inoculated into the culture medium (250 mL) in 500-mL Erlenmeyer flasks to give an initial cell concentration of around 1.3×10^8 cells/mL. This corresponds to an optical density at 600 nm (OD) of 0.064 or a dried cell weight of 0.025 g/L. After inoculation, the flask was capped with cotton plugs and placed in a shaker controlled at 120 rpm and 30 °C.

The number of colonies and dry cell weight were determined as follows. Serial dilutions of the culture were conducted and 0.1 mL of the diluted culture was spread on a plate. The plate was then incubated at 30 °C for 24 h and the number of colonies was counted. In a separate experiment, the cells in the cultures (40 mL) with different cell concentrations were harvested by centrifugation (6000 rpm) at 4 °C for 10 min several times and washed with deionized water. The cells were then dried at 60 °C over 24 h until a constant weight was obtained.

2.3. Biodegradation experiments

As the OD value of adapted cells reached 2.6–3.1, an aliquot of the culture was centrifuged at 6000 rpm and 4 °C for 10 min. To clean the biomass, it was re-suspended in phosphate buffer and centrifuged. The cells were then transferred and inoculated in a 250-mL conical flask, to which 100 mL of solution containing MSM and the substrate(s) was poured to yield an initial OD of 0.064. The cells were cultivated at 30 °C and 100 rpm. Samples were withdrawn at suitable time intervals, and the concentrations of cells, phenol, and SA were measured as described below.

2.4. Analysis of cells and substrates

The concentration of cells in the sample was analyzed by measuring OD at 600 nm using an UV/vis spectrophotometer (Jasco UV-550, Japan) with the culture medium as reference. The samples exceeding 0.8 OD were suitably diluted with the culture medium so that the Beer-Lambert law is applied.

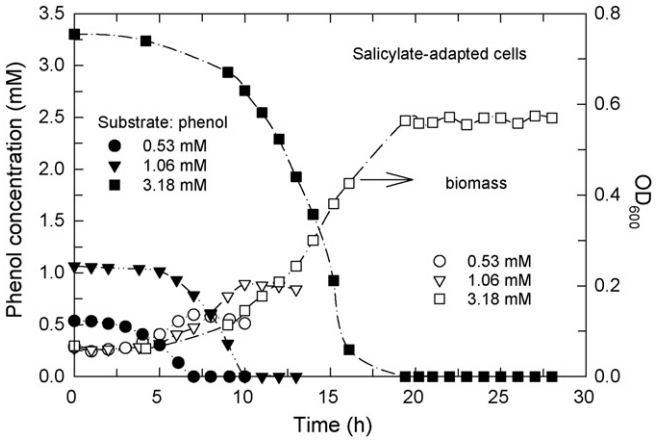

Fig. 1. Biodegradation of single phenol substrate by SA-adapted *P. putida* cells and their growth (1 OD = 0.39 g/L).

The samples were subject to filtration through a Millipore filter (0.2 μm) before the analysis of phenol and SA by HPLC on a Phenomenex C18 column (particle size, 5 μm). A mixture of methanol (49%, v/v), acetic acid (1%, v/v), and water was used as the mobile phase, and the flow rate was 1.0 mL/min. An aliquot of 10 μL of the sample was injected and analyzed using an UV detector (Jasco 975, Japan). The wavelengths for phenol and SA were set at 280 and 240 nm, respectively. Each experiment was duplicated under identical conditions. The reproducibility of the concentration measurements is mostly within 5%.

3. Results and discussion

3.1. Biodegradation of single substrates

Figs. 1 and 2 show the growth of SA-adapted *P. putida* cells and the biodegradation of single phenol and SA, respectively. To discuss the effects of cell adaptation and/or the presence of second substrate on the overall degradation more definitely in single and binary substrate systems, three quantities are compared here. They are the lag time for cell growth (t_{lag}), the maximum cell density (X_{max}), and the time required for complete degradation

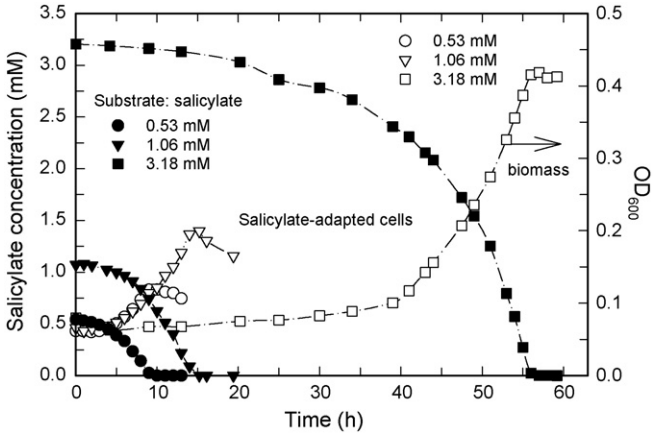

Fig. 2. Biodegradation of single SA substrate by SA-adapted *P. putida* cells and their growth (1 OD = 0.39 g/L).

of a given substrate (t_{100}). It is found that the t_{100} values with 0.53, 1.06, and 3.18 mM of single phenol are 7, 10, and 20 h, respectively. Their t_{lag} values are 3.0, 4.6, and 7.0 h, and the X_{max} values are 0.055, 0.082, and 0.222 g/L. On the other hand, the t_{100} values with 0.53, 1.06, and 3.18 mM of single SA are 9, 15, and 56 h, respectively. Their t_{lag} values are 3.7, 3.8, and 38 h, and the X_{max} values are 0.047, 0.078, and 0.164 g/L. The t_{100} values for phenol and SA are comparable at a low substrate level; however, the t_{100} value for SA becomes longer than for phenol at a higher substrate level (>1.06 mM). Exact comparisons of the degradation performance with literature results are difficult because the cell density and medium compositions are more or less different. Without considering these factors, it was reported that 1.25 mM of SA can be completely biodegraded within 13 h by *P. putida* ATCC 17484 [7].

In general, the specific growth rate of cell, μ (h^{-1}), in a batch reactor is calculated as [14]

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{d\ln X}{dt} \quad (1)$$

where X is the cell concentration in g/L. The μ value is determined based on the exponential phase of growth curve. The following Haldane model is tried here because of its wide applicability and mathematical simplicity for representing cell growth kinetics on single inhibitory substrates [1]

$$\mu = \frac{\mu_{max} S}{K_S + S + (S^2/K_I)} \quad (2)$$

where S is the substrate concentration (mM) and μ_{max} is the maximum growth rate (h^{-1}). K_S is the substrate affinity constant (mM) and K_I is the substrate inhibition constant (mM). A larger K_I value indicates that the culture is less sensitive to substrate inhibition [1].

According to the results of Figs. 1 and 2, the Haldane model can be expressed as Eqs. (3) and (4) for single phenol and SA substrates, respectively (correlation coefficient $R^2 > 0.9737$).

$$\mu_P = \frac{0.245 S_P}{0.129 + S_P + (S_P^2/12.6)} \quad (3)$$

$$\mu_A = \frac{0.137 S_A}{0.111 + S_A + (S_A^2/5.21)} \quad (4)$$

Here, the kinetic parameters are obtained by a non-linear least-squares regression method using the Sigmaplot software (SPSS Inc., Surrey, UK). Evidently, cell growth is inhibited by both substrates under the conditions studied. It is expected that phenol is degraded by *P. putida* more rapidly than SA, even in binary substrate system, because the maximum growth rate (μ_{max}) on phenol is larger. Moreover, the magnitude of K_S value indicates that the biomass has a slightly stronger affinity to phenol in comparison with SA although the cell was adapted with SA. On the other hand, SA has a more seriously inhibitory effect on cell growth than phenol at low substrate levels.

Literature survey on phenol degradation [1,13,15] shows that the K_I values are within 0.57 and 9.6 mM, which were obtained by *P. putida* DMS 548 and *P. putida* DSM 50222, respectively. The slightly larger K_I value obtained here (12.6 mM) indicates

SA-adapted *P. putida* cells have a higher resistance of substrate inhibition. The K_S value (0.129 mM) obtained here falls within the literature ranges of 0.01–0.197 mM. In the case of SA degradation, the Haldane's parameters of μ_{max}, K_S, and K_I were reported to be 0.52 h^{-1}, 0.226, and 7.51 mM, respectively, by *P. putida* ATCC 17484 at 30 °C and pH 6.8 [7]. Compared to the use of phenol-adapted *P. putida* CCRC 14365 for phenol degradation, we obtained μ_{max}, K_S, and K_I to be 0.33 h^{-1}, 0.148, and 7.12 mM, respectively, at 30 °C and pH 7 [13]. Therefore, *P. putida* adapted with SA is superior to that with phenol due to the weaker substrate inhibitory effect for SA-adapted cells.

Using phenol-adapted cells, as we can see in Table 1, the t_{100} values with 1.06 and 3.18 mM of single phenol ($x_P = 1$) are 8.5 and 16 h, respectively (the t_{lag} values are 4.0 and 7.0 h, and the X_{max} values are 0.086 and 0.195 g/L). However, the t_{100} values with 1.06 and 3.18 mM of single SA ($x_P = 0$) are 65 and 103 h, respectively (the t_{lag} values are 55 and 92 h, the X_{max} values are 0.082 and 0.168 g/L). It is evident that the cells adapted with phenol do not largely improve the degradation of phenol itself; conversely, the results of t_{lag} and t_{100} reveal that cell growth and SA degradation is significantly retarded. Thus, the cells adapted with SA are favored for this subject.

3.2. Biodegradation of binary substrates when phenol is in excess

Figs. 3 and 4 show the growth of phenol-adapted cells and the degradation of binary substrates when phenol is in excess. The total substrate level (S_T) is fixed at 1.06 and 3.18 mM, respectively. Similarly, the results of using SA-adapted cells are illustrated in Figs. 5 and 6. When phenol is in excess, the lag times with phenol- and SA-adapted cells are not significantly affected (within 4–6 h) by substrate compositions at low substrate levels (S_T = 1.06 mM). At S_T = 3.18 mM, however, the

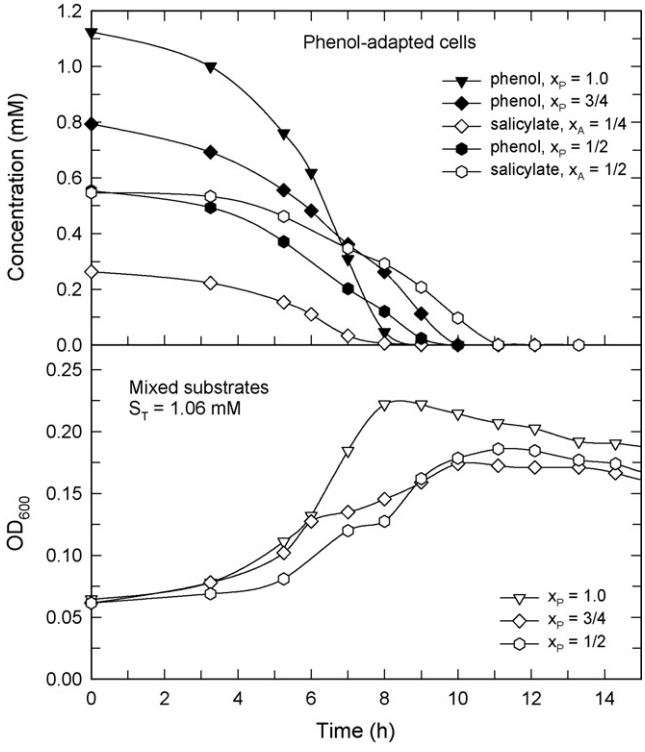

Fig. 4. Biodegradation of binary substrates by phenol-adapted *P. putida* cells and their growth when phenol is in excess (S_T = 3.18 mM, 1 OD = 0.39 g/L).

Fig. 3. Biodegradation of binary substrates by phenol-adapted *P. putida* cells and their growth when phenol is in excess (S_T = 1.06 mM, 1 OD = 0.39 g/L).

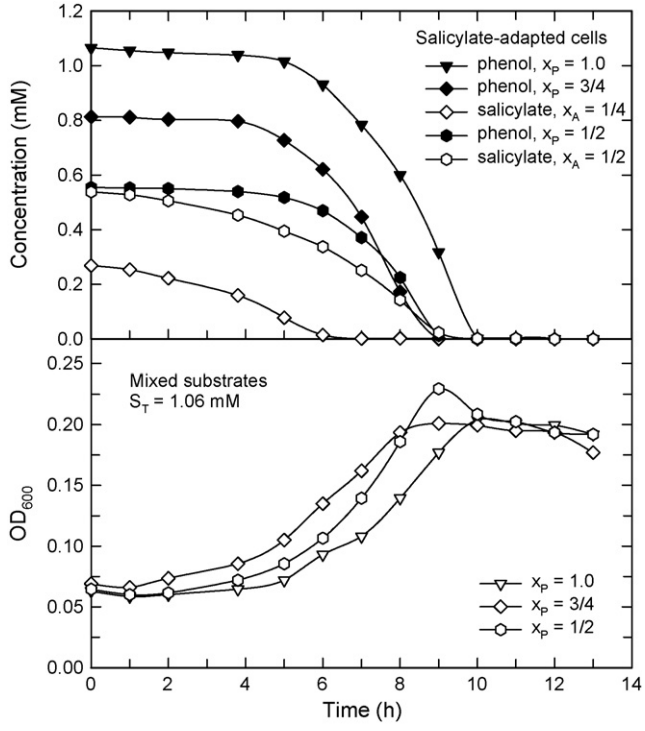

Fig. 5. Biodegradation of binary substrates by SA-adapted *P. putida* cells and their growth when phenol is in excess (S_T = 1.06 mM, 1 OD = 0.39 g/L).

Table 1
The lag time (t_{lag}) and maximum cell density (X_{max}) for cell growth, and the time required for complete degradation of phenol and SA mixtures (t_{100}) by *P. putida* at 30 °C and pH 7

S_T (mM)	x_P	Cell conditions	t_{lag} (h)	X_{max} (g/L)	t_{100} (h)	
					Phenol	*SA*
1.06	1	Phenol-adapted	4.0	0.086	8.5	–
	0.75		4.2	0.066	10	8
	0.5		4.6	0.070	10	11
	0.25		5.0	0.070	7	13
	0		55	0.082	–	65
1.06	1	SA-adapted	4.6	0.082	10	–
	0.75		4.2	0.078	9	7
	0.5		4.0	0.090	9	10
	0.25		3.9	0.082	10	11
	0		3.8	0.078	–	15
3.18	1	Phenol-adapted	7	0.195	16	–
	0.75		9	0.187	19	15
	0.5		22	0.191	28	30
	0.25		28	0.187	31	43
	0		92	0.168	–	103
3.18	1	SA-adapted	8	0.222	20	–
	0.75		9	0.176	20	13
	0.5		14	0.176	22	23
	0.25		20	0.160	28	33
	0		38	0.164	–	56

lag time becomes longer when the mole fraction of phenol (x_P) decreases, particularly by phenol-adapted cells. The t_{lag} value increases from 7 to 22 h with phenol-adapted cells and from 8 to 14 h with SA-adapted cells when x_P deceases from 1 to 0.5.

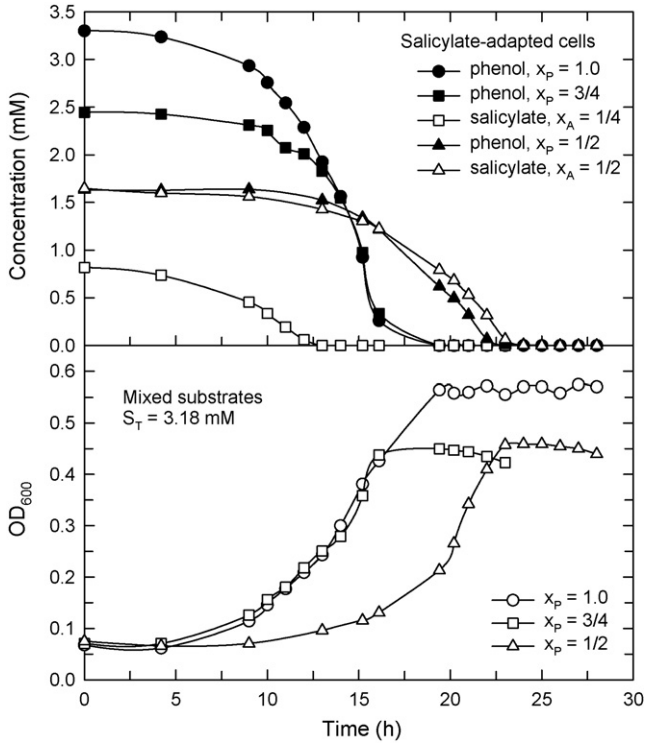

Fig. 6. Biodegradation of binary substrates by SA-adapted *P. putida* cells and their growth when phenol is in excess (S_T = 3.18 mM, 1 OD = 0.39 g/L).

That is, the cells adapted with SA reduce the lag time of cell growth when SA is present.

The addition of SA to phenol, and *vice versa*, does not considerably influence the X_{max} value, regardless of using phenol- or SA-adapted cells. They are within 0.07–0.09 and 0.164–0.195 g/L at a total substrate level of 1.06 and 3.18 mM, respectively. Also, the t_{100} value for phenol remains nearly unchanged when a smaller amount of SA is present by phenol- and SA-adapted cells at S_T = 1.06 mM. This is also the case by SA-adapted cells even at S_T = 3.18 mM; however, the t_{100} values for phenol significantly increase from 16 to 28 h by phenol-adapted cells when x_P deceases from 1 to 0.5. On the other hand, the increment of t_{100} value for SA by SA-adapted cells is smaller than by phenol-adapted cells. These findings indicate that the presence of a small amount of SA retards the degradation of phenol (competitive inhibition), and such a negative effect is reduced when the cells are adapted with SA. It is noticed that the substrate with a fewer amount in the mixture, SA in this case ($x_A < 0.5$), is completely degraded more rapidly.

The effect of other compounds in a mixture of homologous carbon and energy substrates on biodegradation of an organic compound is commonly negative [8,16,17]. Reasons for decreased degradation include competitive inhibition and toxicity as the case of BTEX mixtures [18], and the formation of toxic intermediates by the non-specific enzymes as the case of chlorinated phenolic mixtures [19,20]. For instance, Klecka and Maier [16] have indicated that 2,4,5-trichlorophenol or phenol reduces the degradation rate of pentachlorophenol by an enrichment culture, and that the substrate interaction is not purely competitive in nature. Yu and Loh [8] examined the degradation of SA (1.25 mM) and *p*-cresol (0.09–1.11 mM) mixtures by *P.*

putida ATCC 17484 at 30 °C and pH 6.8, and observed that the specific growth rate on mixed *p*-cresol and SA is higher compared to the corresponding conditions without SA. That is, the presence of SA enhances cell growth. But, the negative effect of substrate interaction is more manifested in cell mass yield; the maximum cell density obtained from SA and *p*-cresol mixture is much lower than the sum of the maximum cell density on each substrate alone at the corresponding levels. Moreover, the degradation of *p*-cresol was not affected significantly by the presence of SA. This is understood because *p*-cresol is a better growth substrate than SA, resulting in faster growth [7,17]. However, Yu and Loh [8] have found that with 0.93 mM of *p*-cresol the degradation of SA (1.25 mM) is retarded by 25 h compared to the absence of *p*-cresol. Such different trends from those obtained in this work, as we can see in Section 3.3, are likely because the total substrate levels are varying in their study.

3.3. Biodegradation of binary substrates when SA is in excess

Figs. 7 and 8 show the growth of phenol-adapted cells and the degradation of binary substrates when SA is in excess. The results of using SA-adapted cells are also shown in Figs. 9 and 10. When SA is in excess, the t_{lag} values with phenol- and SA-adapted cells are nearly not altered (within 4–6 h) by substrate compositions at $S_T = 1.06$ mM, except the case of $x_P = 0$ by phenol-adapted cells. However, the lag time notably reduces when phenol is added to SA at $S_T = 3.18$ mM particularly by phenol-adapted cells. The t_{lag} value decreases from 92 to 28 h

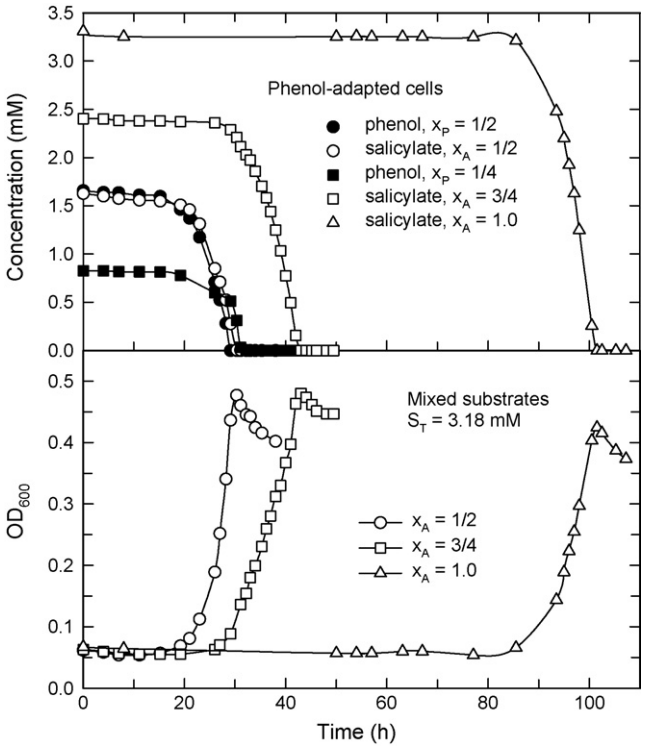

Fig. 8. Biodegradation of binary substrates by phenol-adapted *P. putida* cells and their growth when SA is in excess ($S_T = 3.18$ mM, 1 OD = 0.39 g/L).

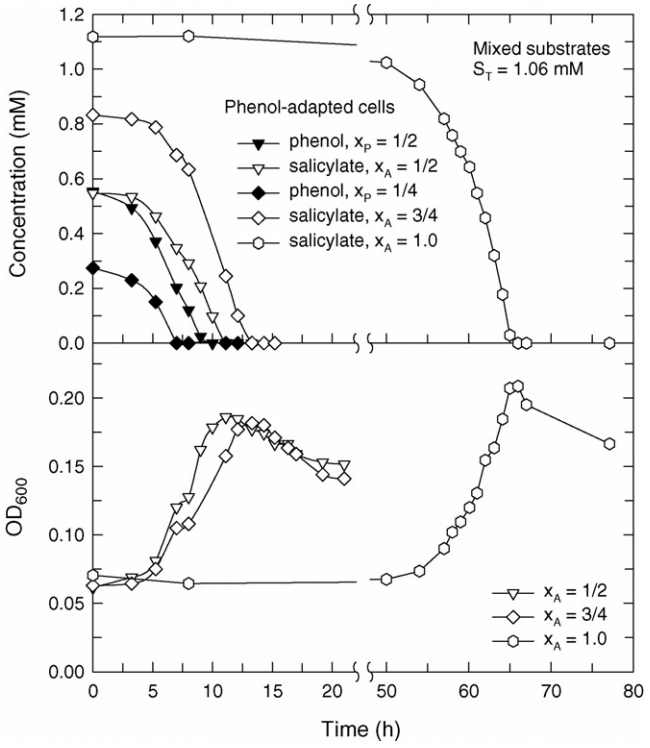

Fig. 7. Biodegradation of binary substrates by phenol-adapted *P. putida* cells and their growth when SA is in excess ($S_T = 1.06$ mM, 1 OD = 0.39 g/L).

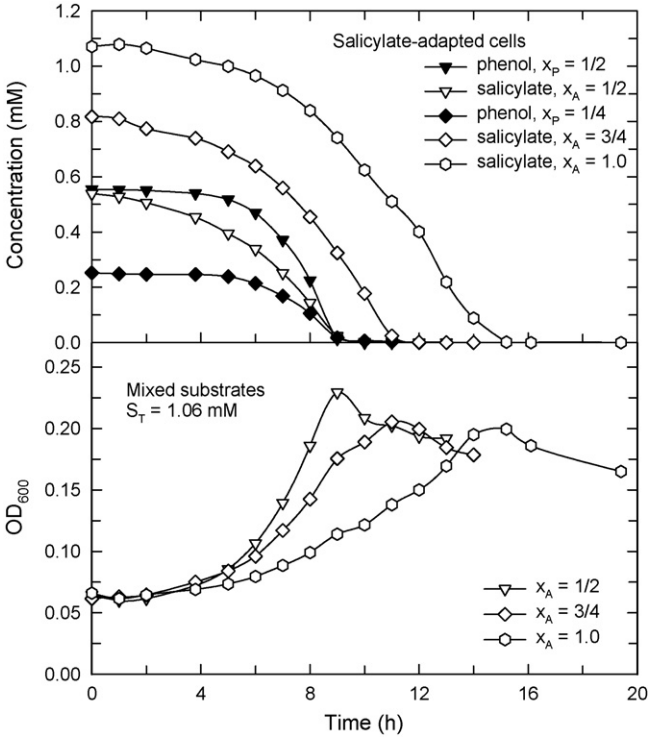

Fig. 9. Biodegradation of binary substrates by SA-adapted *P. putida* cells and their growth when SA is in excess ($S_T = 1.06$ mM, 1 OD = 0.39 g/L).

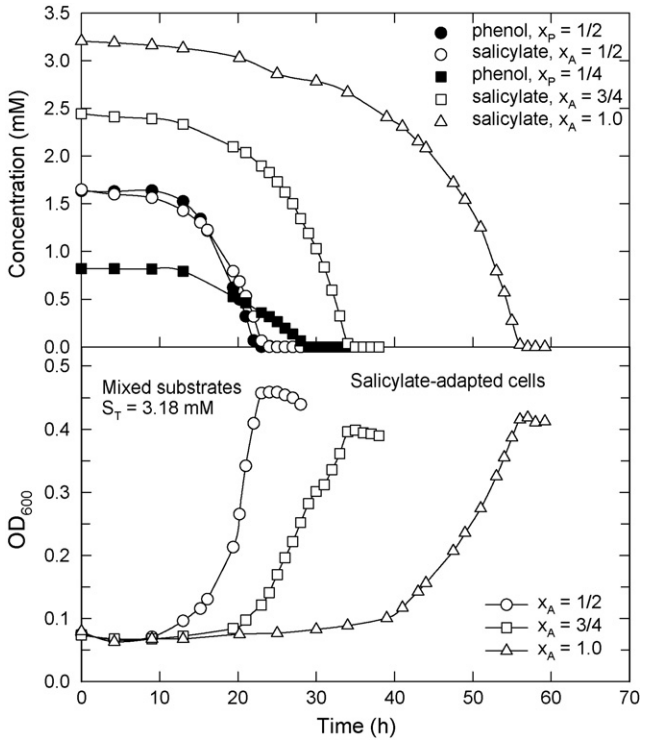

Fig. 10. Biodegradation of binary substrates by SA-adapted *P. putida* cells and their growth when SA is in excess (S_T = 3.18 mM, 1 OD = 0.39 g/L).

by phenol-adapted cells and from 38 to 20 h by SA-adapted cells when x_A deceases from 1 to 0.75.

When a small amount of phenol is added, the t_{100} value for phenol remains nearly unchanged by phenol- or SA-adapted cells at S_T = 1.06 mM; however, the t_{100} value for SA remarkably reduces from 65 to 13 h by phenol-adapted cells and from 15 to 11 h by SA-adapted cells as x_A decreases from 1 to 0.75. Such positive effect becomes more apparent at S_T = 3.18 mM especially by phenol-adapted cells; e.g., the t_{100} value for SA largely decreases from 103 to 43 h by phenol-adapted cells and from 56 to 33 h by SA-adapted cells as x_A deceases from 1 to 0.75. These results indicate that the presence of a small amount of phenol significantly enhances the degradation of SA although the degradation of phenol is retarded (uncompetitive inhibition) in contrast to single substrate.

Wang and Loh [9] have studied the degradation of mixed phenol and sodium glutamate (SG) by *P. putida* ATCC 49451 at 30 °C. They found that the effect of SG on phenol degradation is more significant at lower SG levels (phenol/SG at 0.53/0.12, 0.53/0.30, 0.53/0.59 mM), compared to the level ratio of phenol/SG at 0.53/2.96 and 1.06/5.92 mM. Despite the fact that the presence of SG reduces specific phenol degradation rate by 46% even at high SG levels, phenol was completely consumed in all the experiments. The effect of SG, over a level range of 0–23.7 mM, on the overall phenol degradation kinetics (S/S_0 versus t) in the initial level of 2.12, 4.25, 6.38, and 8.51 mM was not significant. Although the specific degradation rate of phenol is lower in the presence of SG, more cells are generated with simultaneous utilization of phenol and SG.

It is reminded that the substrate with a fewer amount in the mixture, phenol in this case ($x_P < 0.5$), is completely degraded more rapidly. Wang and Loh [9] have also found that only less than 40% of the initially added SG is consumed when phenol is depleted for the experiments containing phenol/SG at 0.53/2.96 or 1.06/5.92 mM. The same trends were also observed in the degradation of binary phenol–toluene and phenol–benzene systems by *P. putida* F1 [18].

The positive effect of second compound in a mixture on the degradation of an organic matter has been reported in the case of increased growth at low substrate levels [21]. For instance, Meyer et al. [6] studied the interactions between benzene and other aromatic hydrocarbons, and found that benzene degradation is enhanced in the presence of toluene or *p*-xylene. Reardon et al. [18] have indicated that the presence of toluene and benzene enhance the degradation of phenol by *P. putida* F1, while phenol does not considerably affect the degradation of toluene and benzene and, there is no significant interaction between benzene and toluene. This is likely because the cell growth on toluene is slightly faster than on benzene, and toluene and benzene are much better substrates than is phenol [18,22,23]; e.g., the μ_{max} values for single toluene, benzene, and phenol are 0.86, 0.73, and 0.11 h^{-1}, respectively, in the low level range (<0.57 mM) [18]. Abu Hamed et al. [22] has also reported that toluene and benzene enhance the degradation of phenol, and the effect of toluene on the degradation of phenol by *P. putida* F1 ATCC 700007 is more positive than the effect of benzene. However, phenol inhibits the degradation of benzene and toluene [23]. In a word, the *P. putida* cells adapted with SA, a comparatively hard-to-degrade substrate, favor the overall biodegradation of binary phenol-SA substrates because the substrate inhibitory effect becomes weaker.

4. Conclusions

The mixture effects on the biodegradation of phenol and sodium salicylate (SA) by *P. putida* CCRC 14365, adapted with phenol or SA, have been examined at 30 °C and pH 7. Growth kinetics showed that phenol was a better substrate for *P. putida* than SA. The addition of SA to phenol, and *vice versa*, did not notably alter the maximum cell density by phenol- or SA-adapted cells under the ranges studied. Substrate with a fewer amount in the mixture would be completely consumed more rapidly. The presence of small amount of SA retarded phenol degradation (competitive inhibition) particularly at high total substrate levels; however, such a negative effect could be reduced if the cells were adapted with SA. At a total substrate level of 3.18 mM, for example, the time needed for complete removal of phenol increased from 16 to 28 h by phenol-adapted cells but from 20 to 22 h by SA-adapted cells when mole fraction of SA increased from 0 to 0.5.

On the other hand, the addition of small amount of phenol to SA considerably enhanced SA degradation particularly by phenol-adapted cells although the degradation of phenol itself was also retarded (uncompetitive inhibition). At a total substrate level of 3.18 mM, the time required for complete removal of SA largely decreased from 103 to 43 h by phenol-adapted cells

and from 56 to 33 h by SA-adapted cells when mole fraction of phenol increased form 0 to 0.5. The present results proved that cells adapted with comparatively hard-to-degrade substrate (SA, in this case) facilitated the overall biodegradation efficiency of mixed homologous carbon and energy substrates.

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