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Phenol Biodegradation Kinetics in the Presence of Supplimentary Substrate

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ABSTRACT

Biodegradation of phenol in the presence of glucose as a supplementary substrate was investigated with mixed microbial consortium isolated from waste effluent of coke-steel factory. Batch experiments were carried out at room temperature and pH value of 7. Initial phenol and glucose concentrations were in the range of 25–1000 mg/l and 500–3000 mg/l, respectively. In a dual substrates system the concentration of supplementary source (glucose) was kept constant. It was obvious to find out that glucose as a simple carbon source was initially utilized in the presence of phenol. The rate of phenol degradation started once glucose concentration was significantly depleted. Phenol was known to be an inhibitory substrate, thus Haldane/Andrews kinetic model was applied to evaluate the growth kinetic parameters. The kinetic parameters, μ_m, K_s and K_i were 0.01 1/h, 27.04 and 127.55 mg/l, respectively. The specific growth rate of the culture in dual substrates system was expressed by SKIP model (R_2> 0.951). The values of interaction parameters showed uncompetitive partially inhibition at high phenol concentration.

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1. INTRODUCTION

Due to industrialization, mankind has been confronted with progressive environmental concerns, despite the improvements in quality of life. Rapid accumulation of recalcitrant chemicals in biosphere severely endangers environment and human health [1-2]. Among these chemicals, phenolic compounds are identified as potentially carcinogenic and toxic substances. They are known as aromatics in which a hydroxyl group is attached to a benzene ring [3-4]. Phenol is used as raw material to produce a variety of resins such as phenolic, epoxy, polyamide, etc [4]. Phenol and its derivatives may be released to environment by wastewater discharges from industries such as oil refinery, coke oven, coal conversion, synthesis of chemicals, steel mill, pulp and paper, textile and pharmaceutical factories [2, 5-7]. Concentration of phenol in these effluents varies in the range of 10–17,500 mg/l [5, 8]. The presence of phenol in water bodies leads to formation of noxious ploychlorinated phenols. These compounds create taste and odor in drinking water, even at low concentration of 2.0 µg/l [9-11]. Thus, phenol is regarded as high priority water pollutant [12]. It can internally affect liver, kidneys, heart, and vascular systems. Also, it is lethal to aquatic life in concentration in the range of 5–25 mg/l [13]. Due to high toxicity, phenols are prescribed as the most effective organic pollutants listed by U. S. EPA [1, 8]. WHO has identified permissible level of 1 µg/l phenol in drinking water [3]. Therefore, effluents containing phenolic compounds must be efficiently treated before being discharged to the surrounding environment.

Bioprocesses are proven to be efficient approaches for complete mineralization of organic contaminants [14-16] . Among various techniques for phenol removal, biodegradation has been highly appreciated as the most versatile promising approach. In compare to physicochemical methods, biological method has been preferred because of complete mineralization of phenol, economical aspects, and nontoxic by-products [3, 10, 13]. Biological treatment under anaerobic condition is more preferable than aerobic process due to economical aspects; oxygen requirements and high production of bio-sludge leads to high energy demand for performing aerobic process. In the other hand, biogas production which occurs during anaerobic process is a potential energy source in the industrial scale. However in terms of biomass generation, anaerobic process is slow [4, 17-21]. Several investigations on phenol biodegradation for the use of aerobic and pure culture were reported in the literature [22-26]. Limited research on anaerobic treatment of phenol was accomplished.

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Phenol is known as an inhibitory substrate for microbial population. Cell Growth may dramatically reduce as a result of increment in phenol concentration. The rate of cell growth reaches to a maximum level at a specific phenol concentration. In subsequence, any increase beyond this value diminishes the growth rate [6, 7, 9, 10, 20-24, 27-29]. Despite substrate inhibition, many types of microorganisms are capable of utilizing phenol as the sole carbon and energy source [2, 10]. Adopting a pure culture in phenol degradation may lead to production of toxic intermediates; while pure culture may not be able to degrade intermediate metabolites. Using mixed microbial consortium which possesses wide spectrum of metabolic properties may resolve such problem. Mixed culture with a variety of species has great potential to utilize intermediates and toxic end products [25]. Besides, environmental studies often use mixed culture for economical reasons in non sterile conditions.

Some strategies have been initiated to overcome substrate inhibition such as immobilizing the microorganisms, pre-acclimatizing the cells to high concentrations of phenol, and/or introducing a supplementary substrate to cells such as glucose [1-2]. Two earlier methods are discussed in a published paper [30]. Addition of simple alternate carbon/energy sources with intention may enhance biodegradation of pollutants. Biodegradation of hazardous substances in presence of nontoxic easily-biodegradable organic compounds such as simple sugars are of the major interests considering the interactions in competitive utilization of organic wastes. Simultaneous degradation of toxic organic effluents blended with supplementary nutrients may enhance the rate of utilization of toxic wastes [26].

The main purpose of the present research is to define biodegradation of phenol under anaerobic condition in the presence of glucose as supplementary substrate. A mixed culture was used to carry out batch experiments with a wide range of phenol concentration. The consortia of microorganisms were isolated from coke oven effluent. Growth kinetics was determined for complete understanding of the culture tolerance and capacities of phenol and glucose biodegradation as single and dual substrates. This investigation led to development of biokinetic model to predict phenol degradation.

2. MATERIALS AND METHODS

2. 1. Microorganism and Growth Medium A mixed microbial consortium was employed for anaerobic biodegradation of phenol. Microbial population was originated from the industrial effluent of coke oven, Isfahan, Iran. The culture was cultivated and enriched in 100 ml of a growth medium contained (in mg/l): glucose, 2000; yeast extract, 1450; and mineral salt medium (MSM). The MSM was composed of (in mg/l): K_2HPO_4, 522.54; KH_2PO_4, 408.27; and NH_4Cl, 50; NaCl, 200; KCl, 200; $CaCl_2.2H_2O$, 150; $MgCl_2$, 100; $MgSO_4.7H_2O$, 5; $FeSO_4.7H_2O$, 10; $CoCl_2.2H_2O$, 0.2; $NiCl_2.2H_2O$, 0.2; $ZnCl_2$, 0.2; $CuCl_2.2H_2O$, 0.2; $MnCl_2.4H_2O$, 0.2; $NaMoO_4.2H_2O$, 0.5; H_3BO_3, 0.2 (pH= 7.0). The media was primarily sterilized in an autoclave at 121 ºC and 15 psi for 20 min. No acclimation period was implemented in order to observe the effect of glucose as co-substrate on phenol biodegradation.

For preparing single substrate solution, phenol and glucose were dissolved in MSM to obtain the defined range of 25–1000 mg/l and 500–3000 mg/l, respectively. For dual substrates experiments, concentration of glucose was fixed at 1800 mg/l and for phenol varied in the range of 25 to 1000 mg/l. A concentration of 1450 mg/l yeast extract was applied to single and dual substrates solutions. The initial pH was adjusted to 7. The experiments were conducted at room temperature (23 ± 2 ºC).

2. 2. Batch Biodegradation Assay Batch experiments on phenol biodegradation were performed in 250 ml Erlenmeyer flasks containing 100 ml of growth medium. Freshly grown seed culture was used as inoculums. The inoculums size was 9% (v/v) which was harvested at exponential phase of the growth. The experiments were conducted under anaerobic condition by placing the flasks in vacuum desiccators. During 30 days of experiments, samples were collected at proper time intervals, centrifuged, and analyzed for pH, biomass, residual phenol and glucose concentrations. Supernatants of the drawn samples were used for analysis.

2. 3. Analytical Procedures Biomass concentration was determined in colorimetric method by measuring absorbance at 600nm using a spectrophotometer (UNICO, 2100, USA). Standard curve was developed for defining biomass concentration. For measuring the residual phenol concentration, samples were centrifuged in a micro centrifuge (HERMLE Z 233 M-2, Germany) at 10000 g for 5 min. Phenol concentration was detected by direct photometric method. The method is based on rapid condensation of 4-amino antipyrene followed by oxidation with alkaline potassium ferricyanide resulting red coloration which can be measured by spectrophotometer. The developed procedure was in accordance with standard method [31]. Samples were centrifuged at 7000 rpm for 7 min by micro centrifuge for determination of carbohydrate concentration. The sugar was measured by chemical reagent, 3,5-dinitrosalicylic acid (DNS) via colorimetric method [32]. The pH of medium was

detected by a pH meter (HANNA pH212, Germany).

3. KINETICS CONCEPT

3. 1. Single Substrate System For determination of microbial tolerance in degradation and operation of treatment units, the growth kinetics has been investigated on biodegradation of different refractory materials such as phenol and VOCs [10, 33-34]. Cell growth kinetic model at the exponential phase is defined by Malthus law. In this model, the maintenance term has been neglected [35-36]:

$$dX/dt = \mu X \quad (1)$$

where, μ stands for specific growth rate (1/h). The cell concentration (X) may be defined on the basis of either cell dry weight (mg/l) or optical density. The Monod model is the simple equation that relates the specific growth rate to a non-inhibitory growth-limiting substrate [10, 37]:

$$\mu = \mu_m S/K_s + S \quad (2)$$

where, μ_m and K_s represent maximum specific growth rate (1/h) and half-saturation substrate constant (mg/l), respectively. The substrate concentration (S) is in mg/l and K_s is described by substrate concentration in which the specific growth rate (μ) reached to the half of its maximum value (μ_m). This parameter exhibits cell affinity to the targeted substrate [13]. Glucose effect on cell growth as non-inhibitory compound may be prescribed using Monod rate model. In order to predict dynamic behavior of the culture grown on phenol, Haldane or Andrew's model was applied:

$$\mu = \mu_m S/(K_s + S + S^2/K_i) \quad (3)$$

where, K_i is substrate inhibition coefficient (mg/l) which demonstrates cell sensitivity to substrate inhibition. For very large inhibition coefficient (K_i) Haldane model reduced to Monod equation [13]. When K_i is large, the microorganisms are less sensitive to substrate inhibition [11]. Haldane model is often used as a simple mathematical equation which incorporates substrate inhibition while growth is in progress [10].

3. 2. Dual Substrates System In an actual biological system each substrate may lead to an intermediate compound; as substrates are utilized end products are formed. In a media with multi-substrates, a number of intermediates are present. The consecutive biological reactions should lead to final end product. Applying pseudo-steady state hypothesis to intermediates, a generalized Monod model can be obtained for a dual substrates system composed of mixed substrates S_1 and S_2. Yoon and his coworkers derived the following model for cell growth utilizing dual substrates [38]:

$$\mu = \frac{\mu_{m1} S_1}{K_{s1} + S_1 + a_{21} S_2} + \frac{\mu_{m2} S_2}{K_{s2} + S_2 + a_{12} S_1} \quad (4)$$

The model implies that two substrates interact with each others. The coefficient of a_{ij} indicates that the substrate i inhibits utilization of substrate j by microorganisms. Large value of a_{ij} demonstrates stronger inhibition [38-39]. The proposed equation is commonly known as "sum kinetics with interaction parameters" (SKIP) model [40, 41]. Hereby, modification of the above model contributes evaluation of the interactions exist between glucose (S_1) and phenol (S_2) on culture growth and substrate uptake [11, 26]:

$$\mu = \frac{\mu_{m1} S_1}{K_{s1} + S_1 + S_1^2/K_{i1} + K'_{31} S_2 + K_{31} S_1 S_2} + \frac{\mu_{m2} S_2}{K_{s2} + S_2 + S_2^2/K_{i2} + K'_{32} S_1 + K_{32} S_1 S_2} \quad (5)$$

No inhibition term (S^2/K_i) is involved in the proposed model given in Eq. (4). The objective of this model in Eq. (5) is defined by interaction parameters between glucose and phenol under inhibition effect caused by the presence of phenol. If the values of K'_{31}, K'_{32}, K_{31} and K_{32} are equal to zero, substrates do not interact with each other and the expressions in Eq. (5) simplifies to Haldane equations. Analogy in enzymatic reactions reveals that the following four possible cases may exist for dual substrates [26]:

- Competitive cross-inhibition, when $K'_{31} \neq 0$, $K'_{32} \neq 0$, $K_{31} = K_{32} = 0$.
- Uncompetitive cross-inhibition, when $K'_{31} = 0$, $K'_{32} = 0$, $K_{31} \neq 0$, $K_{32} \neq 0$.
- Competitive partially inhibition, when $K_{31} = 0$, $K_{32} = 0$, and either (a) $K'_{31} = 0$, $K'_{32} \neq 0$ or (b) $K'_{31} \neq 0$, $K'_{32} = 0$.
- Uncompetitive partially inhibition, when $K'_{31} = 0$, $K'_{32} = 0$, and either (a) $K_{31} = 0$, $K_{32} \neq 0$ or (b) $K_{31} \neq 0$, $K_{32} = 0$.

Considering these cases, SKIP model can be solved for understanding how phenol and glucose interact in a dual substrates system.

4. RESULTS AND DISCUSSION

4. 1. Culture Growth and Biodegradation of Single Substrate Figures 1 and 2 show concentration and cell growth profiles based on phenol and glucose as

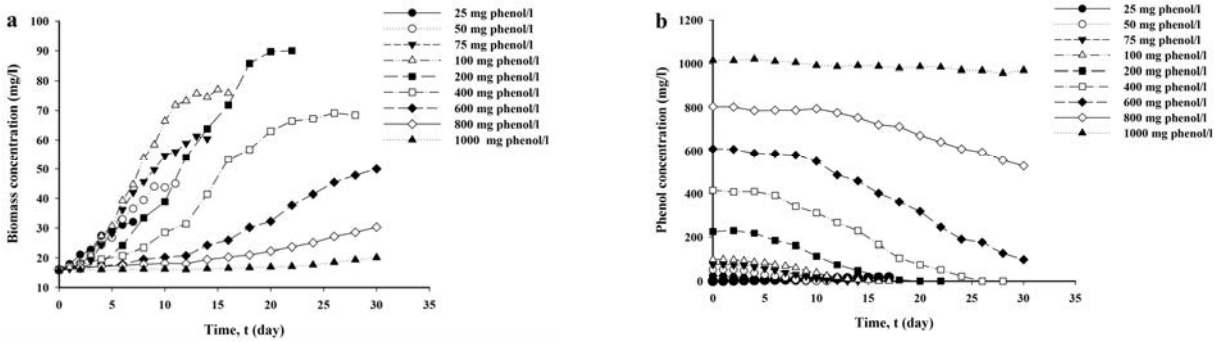

Figure 1. Cell growth (a) and single phenol degradation (b) with defined initial phenol concentrations

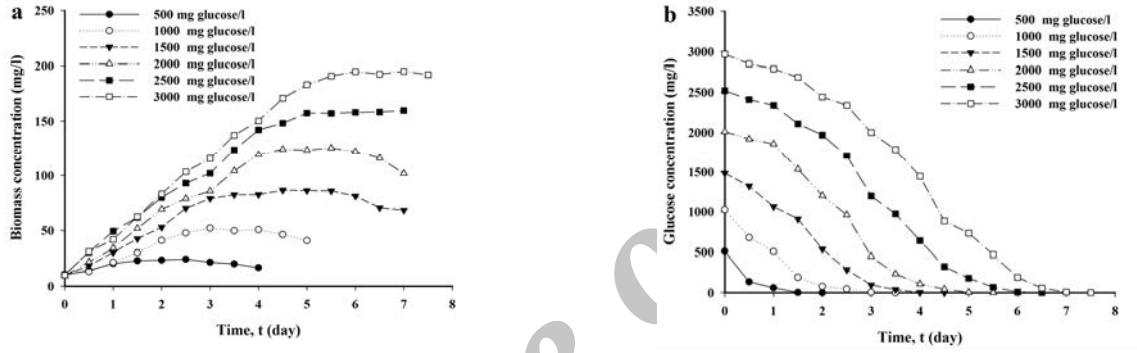

Figure 2. Cell growth (a) and single glucose utilization (b) with defined initial glucose concentrations

single substrates, respectively. Generally, glucose is easily utilized while phenol is highly toxic and recalcitrant compound; therefore, the rate of glucose utilization in compare to phenol is quite high. Phenol as a recalcitrant compound resists to biodegradation showing a prolonging degradation period. However, it is biodegradable in general.

As the figures depict, the time required for complete degradation of phenol was incomparable to that of glucose. The culture required at least 8 days for complete degradation of low phenol concentration (25 mg/l). The obtained results showed high biodegradability of glucose in compare to phenol. Rapid assimilation of glucose indicates non-inhibitory effect of substrate on cell growth. However, initial phenol concentrations of 25 to 400 mg/l were successfully degraded. Removal efficiencies of phenol with initial concentrations of 600, 800, and 1000 mg/l were 83.7, 34.3, and 4.4%, respectively. At low concentrations of phenol, biomass concentration profiles indicated no lag phase. As the concentration of phenol increased, long lag phase was imposed evidently. Lag phase was prolonged to 4 to 6 days as phenol concentration increased from 200 to 400 mg/l. At phenol concentrations of 600 and 800 mg/l due to substrate inhibition, the biodegradations of phenol were prolonged and delayed to 8 and 10 days. No growth was observed while phenol concentration reached to 1000 mg/l which was absolutely due to intensive inhibition imposed by high phenol concentration.

4. 2. Growth Kinetics on Single Substrate

Figures 3a and 3b represent specific growth rates for phenol and glucose as single substrates, respectively. It is apparent that cell growth was inhibited by phenol as sole carbon source; while growth on glucose consumption showed no substrate inhibition on its utilization.

Monod and Haldane models were fitted to the experimental data. Table 1 lists the values of bio-kinetic parameters that were obtained using a non-linear least-squares regression method (SigmaPlot 11.0). The simulated values shown in Figure 3 were correlated by experimental data with square regression coefficients (R^2) of more than 0.9. Both Monod and Haldane models predict glucose degradation trend satisfactorily. Correlation coefficients (R^2) obtained by Monod and Haldane models on glucose utilization were 0.982 and 0.984, respectively. The value of K_i for glucose degradation

was very large (14299.151 mg/l) which was due to non-inhibitory effect of glucose; as numerical evident, the Haldane equation simplifies to the Monod model for very large values of K_i [13].

For phenol degradation, Haldane biokinetic parameters, μ_m, K_s and K_i were 0.01 1/h, 27.04 mg/l and 127.55 mg/l, respectively. It was observed that the maximum specific growth rate (μ_m) was achieved at phenol concentration of 100 mg/l. By increasing phenol concentration more than 100 mg/l, cell growth was retained due to substrate inhibition. Suboptimal growth

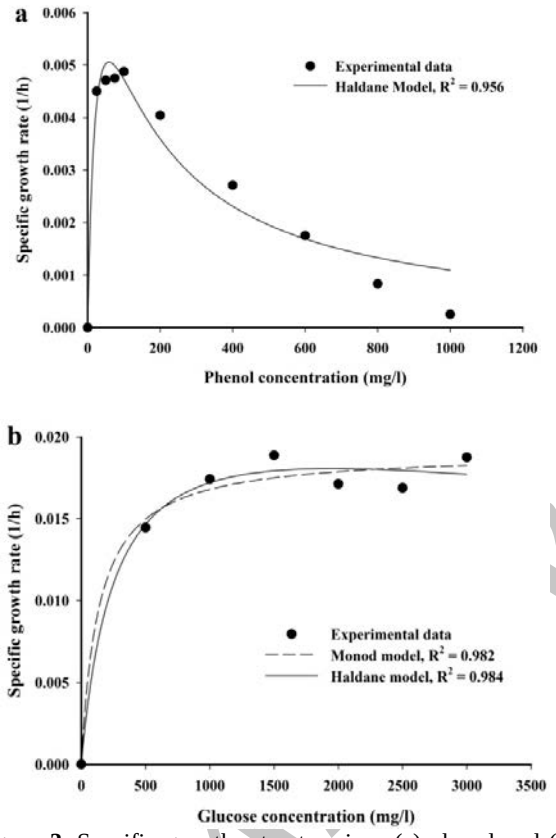

Figure 3. Specific growth rate at various (a) phenol and (b) glucose concentrations

TABLE 1. Bio-kinetic parameters obtained in single substrate systems

Substrate	Model	μ_m (1/h)	K_s (mg/l)	K_i (mg/l)	R^2
Phenol	Haldane	0.01	27.04	127.55	0.956
Glucose	Monod	0.019	138.24	–	0.982
	Haldane	0.023	267.55	14299.151	0.984

happened at concentrations below this value because of substrate limitation. On the other hand, high concentrations of phenol may result in damage of some cells which cause cell leakage and improper activities. In addition, partial cell death may enhance inhibition at high phenol concentrations. Thus, very high concentrations of phenol may lead to negative values for the growth rate [29]. The maximum specific growth rate, μ_m, for phenol and glucose as single substrate were 0.01 and 0.021 1/h, respectively. The calculated μ_m for glucose shows that the value is two times higher than phenol; it indicated that the culture could grow considerably on glucose. In single phenol system, the growth of the culture is dramatically restricted. The range of values of K_s for phenol (27.04 mg/l) and glucose (138–267 mg/l) described that the mixed culture was able to grow on low concentration of substrates. A comparison of phenol degradation reported in the literature with the results of the present work is summarized in Table 2. The values of K_s in the present study are in the range of the data reported in literature. The value of K_s (27.04 mg/l) shows that the culture has tendency to uptake phenol as substrate. Though, the values of μ_m and K_i were low; considering the fact that the culture experienced no acclimation period on phenol. Maximum specific growth rate (μ_m) is significantly lower than the data obtained by aerobic biodegradation process. Maximum specific growth rate of 0.051–0.656 1/h has been reported for phenol degradation under aerobic condition [9, 23, 29, 39, 42, 43]. That may be due to higher biomass yield in presence of oxygen as electron acceptor. However, high biomass yield may not be the reason of aerobic process's privilege. Concerning disposal of biological sludge, it can be rather a disadvantage. The value of μ_m in this study is in the range of reported data for anaerobic digestion in literature (Table 2). As the data in Table 2 shows, the value of K_i for aerobic degradation of phenol varies between 121–1470 mg/l, and it is in the range of 363–1027 mg/l for anaerobic digestion of phenol. The obtained value of 127.55 mg/l demonstrates low inhibition of phenol on culture growth. It may be due to the presence of phenol in coke oven effluent, thus, pre-adaption of organisms to this compound [13].

4. 3. Biodegradation of Phenol/Glucose Mixture

Phenol biodegradation, glucose consumption, and cell growth were monitored at various phenol concentrations ranging from 25 to 1000 mg/l in the presence of glucose at a constant concentration of 1800 mg/l. Tay and his coworkers [38] have observed that glucose concentration of 500 to 4000 mg/l promoted phenol biodegradation with phenol feed concentration of 420–2100 mg/l. However, glucose supplemental effect on phenol biodegradation was more significant in the range of 500–2000 mg/l glucose [47].

Figures 4a and 4b depict phenol biodegradation at initial concentrations of 25–1000 mg/l. Phenol degradation was slightly retarded at initial stage of the experiment where the rate of biodegradation was

TABLE 2. Growth kinetic parameters cited in literature for phenol biodegradation

Microbes	Growth Condition	Model	Phenol Conc.(mg/l)	μ_m (1/h)	K_s (mg/l)	K_i (mg/l)	Ref.
Corynebacterium sp. DJ1	Aerobic	Haldane	500–2500	0.656	33.1	1470	[23]
Mixed culture	Aerobic	Haldane	23.5–659	0.3095	74.65	648.13	[29]
Mixed culture	Aerobic	Haldane	0– 800	0.308	44.92	525.0	[9]
Mixed culture	Aerobic	Haldane	0–900	0.260	25.4	173.0	[42]
Mixed culture	Aerobic	Haldane	40	0.258	3.9	121.7	[43]
P. putida F1 ATCC 700007	Aerobic	Haldane	50	0.051	18	430.0	[39]
P. putida	Anaerobic	Haldane	300-1000	0.031	63.9	450	[34]
Mixed culture	Anaerobic	Haldane	–	0.027	0.03	363.0	[44]
Mixed culture	Anaerobic	Modified Haldane	250–1250	0.003	700	966	[45]
Mixed culture	Anaerobic	Modified Haldane	–	0.0002	93± 22	1027 ± 37	[46]
Mixed culture	Anaerobic	Haldane	25-1000	0.01	27.04	127.55	present study

constant, nearly zero. The lag phase for phenol uptake was prolonged as the concentration was increased. A lag phase of 4–8 days was resulted for degradation of high phenol concentrations (400–800 mg/l). It seems that the organisms initially have no tendency to uptake phenol in the presence of glucose. As a consequence, the period of phenol degradation was delayed. Glucose is consumed rapidly at initial stage of degradation (Figure 5). However, glucose consumption was influenced by the presence of phenol with inhibitory characteristic. A prolonging lag phase was observed in glucose consumption as well. Once concentration of glucose considerably dropped, phenol concentration gradually decreased. The same behavior was observed in the course of aerobic degradation of phenol using pure culture of *Bacillus brevis*, while dextrose was present as co-substrate [10]. Comparatively the removal efficiency of phenol was decreased as dextrose availed [10]. Though, complete degradation of phenol took place in shorter period of time rather than the single substrate system. The employed mixed culture was able to degrade phenol up to a maximum concentration of 600 mg/l within 30 days. It was observed that the existence of glucose has positively affected on phenol biodegradation process. In the presence of glucose, short lag phase was observed for phenol concentrations of 200 mg/l. Owing to phenol toxicity, further increase in initial phenol concentration may lead to long period for complete degradation of phenol and consumption of glucose. The lag phase of phenol at concentrations of 200, 400, 600, and 800 mg/l were 2, 4, 6, 10 days, respectively. The culture efficiency to degrade phenol with initial concentration of 800 mg/l was enhanced to about 48%. A strong inhibition was imposed by phenol concentration of 1000 mg/l at which the lowest degradation rate of phenol was obtained (8.9%). Inhibitory effect of phenol clearly observed on glucose assimilation while phenol concentration increased. Glucose is completely consumed in the presence of phenol with concentration of up to 600 mg/l. By further increase in concentration of phenol to 800 mg/l and 1000 mg/l, the rate of glucose consumption may be reduced.

Phenol degradation and glucose consumption rates were determined in order to evaluate the effect of initial phenol concentration (Figure 6a and 6b). In a single substrate system with phenol concentration of 600 mg/l, the maximum biodegradation rate of phenol was 0.71 mg/l/h. In a dual substrates system, maximum phenol biodegradation rate was 0.84 mg/l/h at initial phenol concentration of 600 mg/l. Inhibitory effect of phenol led to lower degradation rate at concentration below and above 600 mg/l [9]. It was found that the presence of glucose has enhanced biodegradation rate in a certain phenol concentration (200-800 mg/l). However at very low concentrations of phenol (25 – 100 mg/l) and very high concentrations of phenol (1000 mg/l), no significant differences were observed in phenol degradation with and without glucose. Hence, optimum concentration of glucose and phenol should be determined for maximum phenol degradation. Glucose consumption rate decreased as the initial concentration of phenol increased.

Variation of pH was monitored for dual substrates experiments. Figure 7 shows the pH pattern with respect to incubation time. Initial pH was adjusted to 7.0 ± 0.2.

The pH of medium decreased to a mean value of 5.48 in the initial stage of experiment (1 day). Subsequently the value of pH gradually increased to about 6.97. This may be due to glucose consumption and formation of intermediate organic acid products. The populations of methanogenic bacteria may not be dominant at initial stages of anaerobic degradation for utilizing excessive volatile fatty acids (VFAs). Hence, VFAs are not consumed as fast as those are produced. Thus, the pH of the medium rapidly dropped due to accumulation of intermediate compounds. In the next stage, the intermediate VFAs and organic compounds were further consumed and stable end products such as methane and carbon dioxide were formed. Decreasing in concentration of glucose caused the culture to be faced to phenol as other source of carbon which was happened to be an inhibitory substrate. Because of decrease in glucose concentration and relatively low consumption rate of phenol, a balance system may be established between the produced and consumed VFAs. This may led to pH increment in medium and latterly its stabilization [48].

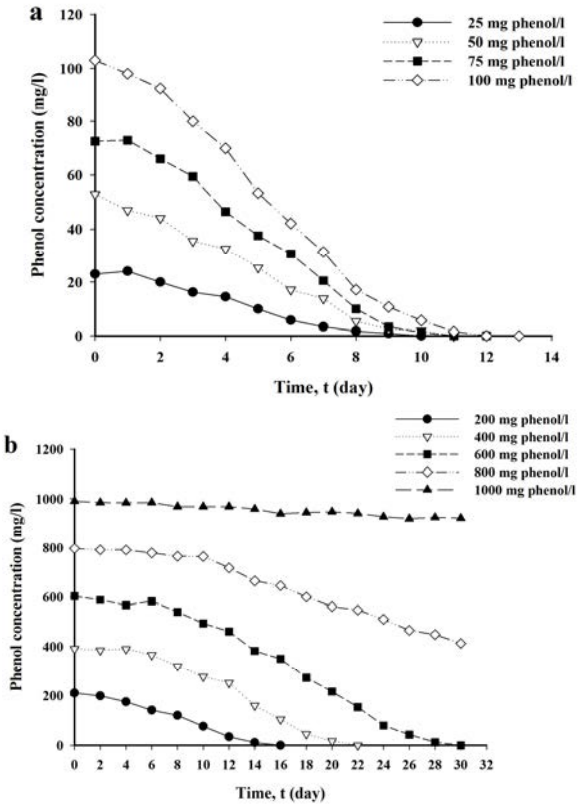

Figure 4. Phenol residual concentration with respect to incubation time for (a) low and (b) high initial phenol concentrations in presence of 1800 mg/ l glucose in a dual substrate system

This assumption can be investigated experimentally in future studies by measuring VFAs concentration and alkalinity of the media. At initial stage of the experiment, for phenol concentration of 1000 mg/l, the value of pH decreased to 5.8. It confirmed the assumption that the biomass was less grown on glucose while high concentrations of phenol were predominant in the media.

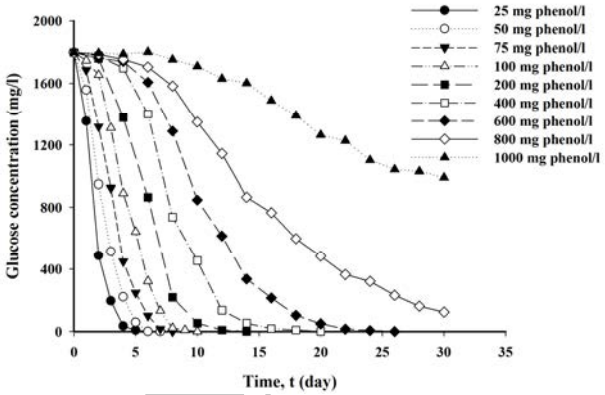

Figure 5. Glucose concentration profiles for different initial phenol concentrations

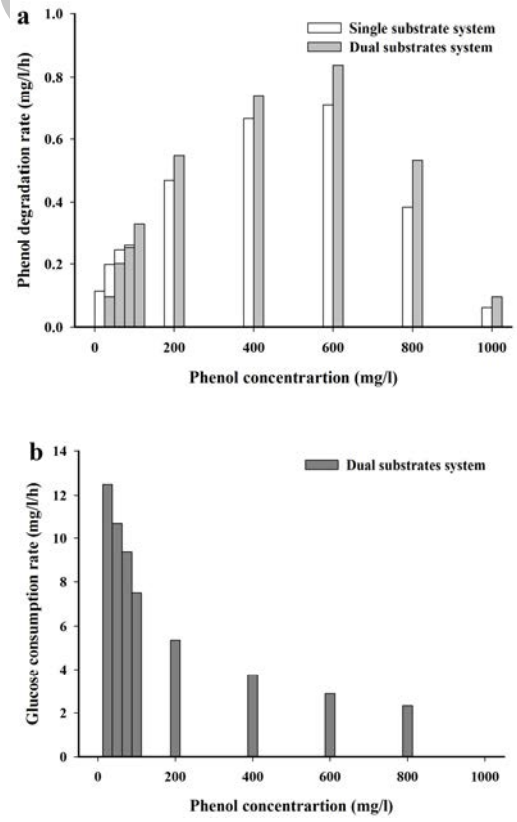

Figure 6. Effect of initial phenol concentrations (25– 1000 mg/l) on phenol biodegradation rate (a) and glucose (1800 mg/l) consumption rate (b)

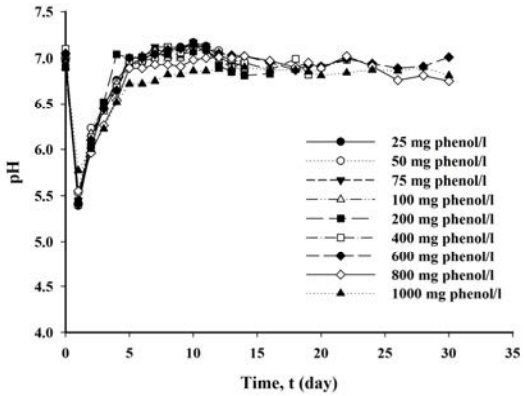

Figure 7. pH variation as a function of incubation time with respect to various initial phenol concentrations in dual substrates system

4. 4. Culture Growth on Dual Substrates System Figure 8 presents biomass dry weight corresponding to low and high phenol concentrations. In this system, biomass concentration was yielded by utilization of glucose and phenol as dual substrates. It seems that cell growth was related to consumption of glucose at initial days of degradation process since a significant growth primarily occurred. Though, a short-term lag phase (about 2 days) was observed for phenol concentration of 200 mg/l. The lag phase was severely prolonged by increasing an increment in phenol concentration. Moreover, cell growth rate subsequently diminished which may be correlated to duration of phenol uptake. During exponential phase, microorganisms attained maximum growth (203 mg/l) at phenol concentration of 200 mg/l within 30 days. At phenol concentration beyond this value, biomass activity was reduced as a result of substrate inhibition. Biodegradation interval of phenol in aerobic process was considerably less than the anaerobic process [9]. This may be due to relatively slower cell growth during anaerobic degradation than aerobic process [4, 18-21].

4. 5. Determination of Interaction Parameters in Dual Substrates System The specific growth rates (μ) for dual substrates media were calculated using Eq. (1) on the basis of cell concentration with respect to time. For determination of interaction parameters, Eq. (5) was solved using a non-linear least-squares regression method by SigmaPlot 11.0. Bio-kinetic parameters, μ_m, K_s and K_i were the same as those of single substrate systems [38, 39]. Interaction parameters obtained by solving SKIP model are summarized in Table 3.

As mentioned earlier, interaction constants indicate the inhibition effect of single substrate on degradation rate of other available substrates for the organisms. For large interaction parameters, strong inhibition is imposed [38, 39]. For fitting and solving Eq. (5), there were two defined conditions: (1) $K_{31} = K_{32} = 0$, (2) $K'_{31} = K'_{32} = 0$. As mentioned before, these two conditions stand for competitive and uncompetitive inhibition, respectively. Uncompetitive inhibition condition was the best fit as it resulted in the highest correlation coefficient ($R^2 = 0.977$). Dissimilar structure of glucose and phenol may lead to an uncompetitive inhibition. However, the values of R^2 were more than 0.9 for both conditions with no significant differences. For uncompetitive inhibition K_{31} and K_{32} were 0.95 and 0.0005 l/mg, respectively; it depicts inhibition effect of phenol on glucose utilization. When the values of K'_{31} and K'_{32} reached to zero, the value of K_{32} (0.0005 l/mg) was also zero; which expresses uncompetitive partial inhibition of phenol on glucose.

Considering competitive inhibition for correlating SKIP model to experimental data, the values of K'_{31} = 95 and K'_{32} = 1.5 were determined. If large values of interaction parameters, K'_3 and K_3 were obtained, extreme case of diauxic growth may happen [26, 38]. In this study, biomass concentration patterns absolutely illustrated the

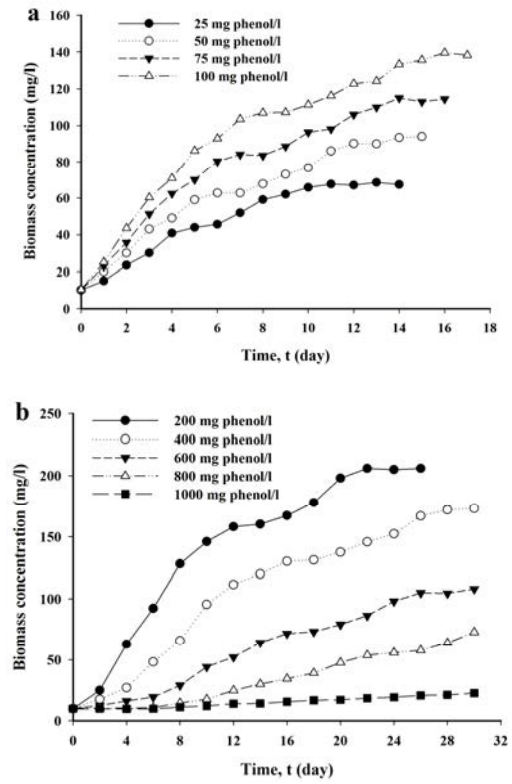

Figure 8. Biomass concentration profiles for (a) low and (b) high initial phenol concentrations in presence of 1800 mg/ l glucose in dual substrates system

TABLE 3. Interaction parameters obtained by SKIP model

Case	Parameter	K'_{31}	K'_{32}	K_{31} (l/mg)	K_{32} (l/mg)	R^2
Competitive	Value	95	1.5	0	0	0.951
Uncompetitive	Value	0	0	0.95	0.0005	0.977

diauxic growth (see Figure 8). The obtained values of K'_{31} and K'_{32} demonstrated that phenol intensively hinders glucose consumption; whereas the effect of the glucose presence on phenol degradation was negligible. Wang, et al. [26] studied kinetics of phenol biodegradation using a pre-adapted culture in the presence of supplemental glucose. In recent study, it was reported that assuming competitive cross-inhibition condition in dual substrates system showed a very poor prediction. In uncompetitive cross-inhibition, very good results were obtained [26]. In the present study, it is assumed that if the seed culture was grown on a mixture of glucose and phenol as carbon and energy sources, uncompetitive cross-inhibition condition might happen; as it was reported by Wang and his coworkers [26].

5. CONCLUSIONS

In this study anaerobic phenol biodegradation in the presence of glucose was successfully achieved. Mixed culture was able to degrade phenol as sole carbon/energy source up to concentration 400 mg/l. Haldane bio-kinetics parameters for phenol degradation (μ_m, K_s, K_i) were 0.01 1/h, 27.04 and 127.55 mg/l, respectively. Low values of μ_m and K_i revealed relatively high substrate inhibition due to no pre-acclimation. Both Monod and Haldane models satisfactorily predicted glucose utilization trend. The value of K_i for glucose degradation was very large (14299.151 mg/l) which confirmed the assumption that when K_i is a very large value, the Haldane equation simplifies to Monod model. In the presence of glucose, phenol biodegradation capacity of the culture was enhanced; maximum biodegradation rate of phenol at 600 mg/l increased to 0.84 mg/l/h. Also, phenol removal efficiency slightly improved at high phenol concentrations. In initial stage of experiment conducted for dual substrates media, considerable increase in biomass concentration and decrease in pH were observed. The pH of medium initially decreased to 5.5 which gradually increased and stabilized at 6.9. Rapid consumption of glucose resulted in accumulation of intermediate VFAs at the beginning of experiments which may lead to pH drop. Growth kinetics of the culture in dual substrates system was well predicted by SKIP model (R^2>0.951). The values of K_{31} = 0.95 l/mg, K_{32} = 0.0005 l/mg, and K'_{31} = K'_{32} = 0 (R^2 = 0.977) showed uncompetitive partially inhibitory condition. The interaction parameters represent that phenol intensively hinders glucose consumption; whereas the effect of glucose on phenol degradation was negligible. This problem may be resolved by accomplishment of an acclimation period on mixture of phenol and glucose. The large value of either K_{31} or K'_{31} demonstrates extreme case of diauxic growth.

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Phenol Biodegradation Kinetics in the Presence of Supplimentary Substrate

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چکیده

تجزیه زیستی فنل در حضور گلوکز به عنوان رشدمایه مکمل توسط کشت میکروبی مختلط که از پساب کارخانه فولاد کشت گردیده بود مورد بررسی قرار گرفت. آزمایشهای ناپیوسته در دمای اتاق و pH ۷ انجام شد. غلظت های اولیه فنل و گلوکز به ترتیب در محدوده ۱۰۰۰– ۲۵ میلی گرم در لیتر و ۳۰۰۰–۵۰۰ میلی گرم در لیتر تغییر می‌کرد. در سیستم دو رشدمایه، غلظت منبع غذایی مکمل (گلوکز) ثابت نگه داشته شد. واضح است که ابتداً گلوکز به عنوان یک منبع غذایی ساده در محیط دو رشدمایه مصرف شد. نرخ تجزیه زیستی فنل زمانی آغاز شد که غلظت گلوکز به طور چشمگیری کاهش یافت. از آنجائیکه فنل به عنوان یک رشدمایه بازدارنده شناخته می شود، مدل سینتیکی اندروز/هیلدن برای بررسی پارامترهای رشد سینتیکی به کار گرفته شد. پارامترهای سینتیکی رشد μm، Ks و Ki به ترتیب برابر با 1/h ۰/۰۱، ۲۷/۰٤ و ۱۲۷/۵۵ میلی گرم در لیتر به دست آمدند. نرخ رشد ویژه در سیستم دو رشدمایه توسط مدل SKIP (R2 > 0.951) بیان گردید. مقادیر پارامترهای اندرکنش نشان دهنده بازدارندگی نسبتا غیر رقابتی در غلظت های بالای فنل بود.

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