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KINETICS OF PHENOL BIODEGRADATION BY *PROVIDENCIA RETTGERI*

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ABSTRACT

Providencia rettgeri, Providencia stuartii and *Enterobacter cloaceae* had been isolated from phenolic wastewater. The isolates exhibited differences in their phenolic tolerance and biodegradation activity. Out of 16 isolates, 9,4 and 3 of them tolerated phenol to 0.5, 1.0 and 1.5% w/v phenol, respectively. *Providencia species* exhibited a potent biodegradability than *Enterobacter cloaceae* aganist phenol. The retaining of this character was not manifested by most of these isolates. Three isolates of *Providencia rettgeri* represent the most active phenol biodegradable isolates, were capable to utilize and degrade phenol up to concentrations 600, 800 and 1000 µg/ml and 84, 88.8 and 100% of phenol was degraded, respectively, after 5 days. The kinetic parameters of phenol biodegradation indicated that the maximum specific growth rate is inversely proportional with the bioconversion and indicates a high affinity of the organism for the substrate. However, higher concentration (> 1.5 mg/ml) were shown to be inhibitory for metabolism and growth. The rate of phenol biodegradation increased with tolerated moderate concentration of the substrate, time of incubation (3-5 days) and optimal temperature (at 35°). Addition of glucose or yeast extract, although enhanced the growth rate, yet it retarded the biodegradation. These kinetic parameters can be used to define the optimal conditions for the removal of phenol in biological treatment systems.

INTRODUCTION

Prediction of the presistence and concentration of toxic chemicals in natural environment and the possible exposure of humans or other creatures to such compounds, require information about the kinetics of their biodegradation. Waste water containing phenols have traditionally been treated by using physical or chemical processes, moreover, aerobic or anaerobic biological processes[1-3] have been used for such treatment. Some phenolic compounds, including phenol, have been shown to be biodegraded to methane and carbon dioxide in batch anaerobic cultures[4-7]. Phenolic compounds may be removed from the environment by complete or partial biodegradation or by bioconversion. Moreover, mineralization of the phenol ring was shown in previous studies[4].

A number of mesophilic microorganisms have been reported to degrade phenol at low concentrations, including *Arthorbacter species* [8], *Pseudomonas species*[9], *Azotobacter species* [10] *Alcaligenes species*[11], *Bacillus stearothermophilus*[12], anaerobic bacterial population[13], *Streptomyces setonii*[14] and the yeasts *Trichosporon cutaneum* and *Candida tropicalis*[15,16]. Many studies of the biodegradation of phenolic compounds and its kinetics in aquatic environments in lake water or other surface fresh waters have been conducted[17-19].

A number of models have been formulated to characterize the kinetics of biodegradation by pure cultures of bacteria[20-22].

In the study of Schmidt and his associates[23] models were described for the kinetics of biodegradtion of organic substrates by bacteria that grow logisitically, logarithmatically or linearly while metabolizing the organic substrate of interest.

Because of their presence in the effluents of oil refineries, petrochemical plants and other industrial processes, phenols are hazardous pollutants and their biodegradation has been intensively studied[24-26].

The present study focused on trying to isolate phenol degradable bacteria from industrial effluent, studying the biodegradabilities of phenol by these isolates and the factors affecting the rate of such biodegradation.

MATERIALS AND METHODS

Chemicals and Reagents: Phenol was obtained as white pure crystals from Jenssen, Belgium. All of the other chemicals and reagents were analytical grade.

Media: Salt liquid medium (SLM) was used according to Grurujeyalakshmi and Oreiel[12]. It consists of (per litre of bidist. water): K_2HPO_4 0.5 g; NH_4Cl 1.0 gm $MgSO_4.7H_2O$ 0.02 g; yeast extract 0.2 g, Casamino acid 0.1 g and trace elements 1.0 ml. Phenol was added as a sole carbon and energy source and the pH was adjusted to 6.9 by NaOH.

The medium was distributed 50 ml-or 90 ml per 500 ml-Erlenmeyer flasks and then sterilized by autoclaving. Agar agar 2.0% w/v was added in salt solid medium (SSM). Trace elements (Freshly prepared) consist of $MgCl_2.4H_2O$ (0.79 g); $CuSO_4.5H_2O$ (0.64 g) $ZnSO_4.7H_2O$ (0.15 g)and $FeSO_4.7H_2O$ (0.11 g) in 100 ml bidistilled water.

Isolation, identification and maintenance of culture:

bacterial strains, capable of utilizing phenol as a sole carbon source, were isolated from samples of industrial sewage. For this purpose, samples (about 2.0 litres) were obtained from wastewater of Al Alamia Company for detergents manufacturing (Finic), Al Asher Men Ramadan, Egypt.

An isolation program was initiated for phenol degrading strains in which the waste water were diluted serially, inoculated into solid media (MecConkey, Blood and Nutrient agar) and incubated at 37°C/3 days. Each isolated microorganism was inoculated into 50 ml/250 ml-flasks of mineral salt liquid medium (SLM) containing phenol at concentrations 0.5, 1.0, 1.5 and 2%, separately. The cultures were incubated with shaking (50 rpm) for 14 days at 30°C. After repeated subculturing on SLM for another 7 days, Sythetic Solid Medium (SSM) plates containing the same concentrations of phenol were inoculated from such subcultures. The plates were incubated at 30°C for 3 days. Such isolated phenol-degradable Gram negative bacterial strains were identified by API-20 E system [La Balme Les Grottes, France] and maintained on nutrient agar slants as well as nutrient broth to be stored with dimethylsulfoxide (DMSO) in refrigerator (0.09 ml/ml).

Kinetic Experiments:

Batch culture experiments were conducted in 250 ml-flasks containing 50 ml of the mineral salt liquid medium (SLM) previously described. Flasks were amended with various concentrations of phenol (100, 200, 300, 400 and 500 ug/ml) and inoculated with 5 ml of only one of the isolated bacterial strains, individually. They were incubated at 30°C for 3 days on a rotary shaker at 50 rpm. Samples of the cultures were removed periodically (every 24 hrs.) and analyzed for phenol concentration as described below. Viable bacterial count was deone every 48 hrs for growth measuring. Fifty % loss in phenol concentration in the culture is used as an indicator for the phenol-degradation activity. For turbidiemtric growth studies, 7 ml of phenolic media was dispensed for absorbance with a spectrophotometer. The A_{520} was read against a blank of uninoculated medium. All phenolic concentrations were tested for their effects on each bacterium twice, with each trial consisting of duplicate tubes per treatment.

Phenol Assay: Assay of phenol was carried out according to Martin[30] by mixing well phenol containg sample with 0.3 ml of 2% 4-amioantipyrine, 1.0 ml of 2N NH_4OH and 1.0 ml of 2% pot. ferricyanide. The obtained red color was measured spectrophotometrically at 510 nm or colorimetrically.

Retaining Phenol Degradability: The isolated phenol-degradable bacterial strains were subcultured on nutrient agar for about 10 times. Selected few colonies from each strain were inoculated, separately, in 5 ml SLM containing 100 µg/ml phenol. The tubes were incubated for 7 days at 30°C with shaking at 50 rpm. The presence of growth in the tubes, is an indicator for the retaining of phenol-degradability of that bacterial isolate.

Toxic Concentration of Phenol: About 1.0 ml cell suspension (7 x 10^4 CFU/ml) from the maintained cultures of strains No. 1, 15 and 16 were used to inoculate 50 ml SLM containing different phenol concentrations (0.2, 0.4, 0.6, 0.8, 1.0, 1.2 and 1.5 mg/ml). These set of flasks were incubated at 30°C with shaking for 7 days. The residual phenol was measured daily and the bacterial growth was visualized during the incubation period.

Rate of Phenol Degradation: Inoculum of 1.0 ml (4 x 10^4 CFU/ML) of the highest phenol-degradable isolate (No. 13) was used to seed 50 ml SLM containing 1.5 mg/ml phenol. The culture was incubated at 30°C for about 12 days. Every day, sample was taken for determination of the residual phenol. Viable count was also dertermined every two days.

Effect of Phenol Concentration on Biodgradation:

Bacterial strain No. 15, the most active phenol degrdable isolate, maintained on SSM containing 100 ug/ml phenol, was used. The inoculum was adjusted to optical density = 1.59 at 400 nm (viable count = 179 x 10^{10} cell/ml).

One ml was used for inoculation of 50 ml SLM-flasks containing different oncentrations of phenol (10 ,20,..... and 100 µg/ml). Waterbath shaker was used for incubation at 30°C for 5 days and samples were taken at zero time and then every 24 hrs. for measuring phenol concentration at 269 nm, O.D. of growth at 400 nm as well as bacterial viable count.

Effect of Temperature on Phenol Biodegradation: Inoculum of 2 ml strain No. 15 was used to seed 50 ml SLM containing 100 µg/ml phenol. Duplicate experiments were incubated either at room temp (25-30°C) in shaker (50 rpm); 35°C in waterbath shaker or 42°C in waterbath shaker for 5 days. Every day samples were taken for measuring phenol concentration optical density and viable count.

Effect of Time on Phenol Biodegradation: A group of flasks, each containing 50 ml SLM and 100 ug/ml phenol and inoculated with strain No. 15, were shaked at 35°C for 5 days. Every day, samples were taken for phenol assay, O.D. measuring and viable count

Effect of Organic Nutrient on Phenol Biodegradation:

Three 250 ml-flasks, each containing 50 ml SLM in addition to 100 ug/ml phenol and either 25,50 or 100 ug/ml glucose were inoculated with strain No. 15 (2 ml each). Another set of 3 flasks included yeast extract instead of glucose. All sets of flasks in addition to a control set (without organic nutrient) were incubated at 35°C in waterbath shaker for 5 days. Samples were taken every day for measuring O.D., phenol and viable count.

RESULTS AND DISCUSSION

The initiated bacterial isolation program using wastewater effluent polluted with phenol (0.04-0.07 gm %), led to 16 isolated strains of gram negative bacteria. Identification of such phenol-degrable isolates by API-20E system demonstrated both *Providencia species* (P. rettgeri[13] or *P. stuarti* [1]) and *Enterobacter cloaceae* [2]. The bacterial phenol biodegradation as a process to overcome the industrial environmental pollution, was studied. While there is no thoroughly available information about the isolation or the role of such strains in phenolic compounds degradation, similar isolates were previously reported such as *Azotobacter species* and *Arthobacter species* (10,8).

Investigations of the ability of these phenol degradable isolates to gorw in presence of phenol, revealed that 3,6,1,3 and 3 strains, out of the 16 isolates, grew well at 0.3, 0.5, 0.8, 1.0 and 1.5% w/v phenol, respectively. At phenol concentration of 2.0% w/v, none of the isolates showed growth. The three isolates tolerated 1.0% w/v phenol represented the 2 isolates of *Enterobacter cloaceae* and the sole isolate of *Providencia stuari*. All of the other isolates were *Providencia rettgeri* strains. The three isolates of *Provid. rettgeri* that did not show enhanced growth on 500 ug/ml phenol resemble thoseof *Bacillus stearothermophilus* isolated by Gurujeyalakshmi and Oriel[12] from various culture collections. This unability to grow indicates that phenol utilization of this concentration by these isolates was not a common characteristic.

The three isolates of *Provid. rettgeri* that had the ability to grow on phenol at levels up to 1500 ug/ml in this study, were similar to *B. stearothermophils* strain isolated at 55°C from a river sediment by Gurujeyalakshmi and Oriel[12] that had the ability to grow and degrade phenol not only at 500 ug/ml but also at levels up to a 1500 ug/ml. Batch culture experiments were conducted to evaluate the kinetics of phenol utilization. Individual capability of the isolates to phenol degradation at concentrations 100 to 500 μg/ml after incubatioon at 39°C for 3 days with shaking, is illustrated in Table (1). All of the isolates had the ability to assemble phenol at 100 ug/ml concentration, where more than 50% of the phenol was utilized.

According to the criterion that over 50% reduction in the original phenol concentration is the indicator of phenol degradation activity, all of the isolates were active at 100 ug/ml, isolates No. 1 and 4 were active at up to 400 ug/ml, whereas, isolates No. 13, 14, 15 and 16, were active at up to 500 ug/ml phenol concentration.

Table (1) also, illustrated that *Providencia species* (represented by isolates (No. 1,2,8,13,15 and 16) were more active in phenol degradation than *Enterobacter cloaceae* (isolates No. 10 and 14). Moreover, *Provid. rettgeri* (isolates No. 15 and 16) was being more active than *Provid. stuarti* (isolates No. 8).

In a trial to correlate the rate of phenol degradation with time and viable bacterial count, one of the most potent phenol degradable isolates *(provid. rettgeri* No. 13) that had the ability to grow and degrade phenol at concentration 1.5% w/v, was used as shown in Table (2).

The results indicated that the maximum growth rate was attained after 3-4 days where about 60% of phenol had been degraded. After this period, the more phenol consumption, the low the growth rate.

One may speculate that direct proportionally was found among % phenol degradation, Time and viable bacterial count up to 3 days followed by inverse proportionally of growth rate with the two other factors 89% of phenol was degraded after about 10 days.

The rate of biodegradation was found to be proportional to the concentration of cell mass used in the test. Degradation rate were also related to the phenol concentration, however, high substrate concentrations (> 1500 μg/ml) were inhibitory, as shown in Table (3), as the removal rates were greater at low concentrations. Theseobservations indicated that the relationship between the specific growth rate and phenol concentration deviates from the classical hyperbolic function described by the Monod equation[22].

Various kinetic relationships have been devised to depict the joint dependence of specific growth rate on substrate concentration when the compound furnished for growth serves as both a substrate and an inhibitor.

The specific growth rate increases with increase in phenol concentration. Higher concentrations decrease the specific growth rate as the effects of substrate inhibition become more pronounced[21] have also stressed the importance of including substrate inhibition effects into predicitive models for describing the removal of inhibitory compounds in biological systems.[27] has previously noted that a correlation may exist between microbial growth rates and substrate affinities.

Table (1): The capability of the isolates to degrade phenol at different concentrations after 3 days incubation at 30°C water bath shaker.

Isolate		Phenol Concentrations (µg/ml)									
No	Name	100		200		300		400		500	
		D	%	D	%	D	%	D	%	D	%
1	Providencia rettgeri	87	87	158	79	264	88	221	55.3	215	43
2	Providencia rettgeri	68	68	38	19	138	46	172	43	220	44
3	Providencia rettgeri	79	79	19	9.5	166	55.3	179	44.8	220	44
4	Providencia rettgeri	78	78	108	54	184	61.3	218	54.5	203	40.6
5	Providencia rettgeri	72	72	22	11	144	48	188	47	157	31.4
6	Providencia rettgeri	64	64	14	7	104	34.7	157	39.3	185	37
7	Providencia rettgeri	61	61	121	605	134	44.7	174	43.5	129	25.8
8	Providencia rettgeri	57	57	127	63.5	136	45.3	174	43.5	137	27.4
9	Providencia rettgeri	62	62	122	61	141	47	144	36	140	28
10	Providencia rettgeri	76	76	23	11.5	91	30.3	159	39.8	180	36
11	Providencia rettgeri	71	71	21	10.5	121	40.3	180	45	185	37
12	Providencia rettgeri	79	79	19	9.5	149	49.7	187	46.8	140	28
13	Providencia rettgeri	82	82	111	55.5	182	60.7	244	61	290	58
14	Providencia rettgeri	82	82	111	55.5	152	50.7	202	50.5	255	51
15	Providencia rettgeri	91	91	160	80	224	74.7	279	69.8	360	72
16	Providencia rettgeri	92	92	190	95	187	62.3	239	59.8	305	61

D: degraded phenol concentration = original - conc. - remained estimated one.

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Table (2): Phenol-Degradation produced by isolate No. 13 (Provid. rettgeri).

Time (day)	0	1	2	3	4	5	6	7	8	9	10
Phenol conc. g% w/v	1.50	1.31	1.15	0.63	0.50	0.21	0.17	0.14	0.11	0.07	0.03
% degraded phenol	0	12.7	23.3	58	66.7	86	88.7	90.7	92.7	95.3	98
viable count X 10^7/ml	0.79	16		79		73		64		51	

Organisms capable of high growth rates at high substrate concentrations typically grow less efficienctly at lower concentrations due to low substrate affinities. Alternatively, organisms that grow efficiently at low substrate concentrations generally exhibit low growth rates at high substrate affinities.

The results as shown in Table (3) revealed that more than 50% of phenol was degraded after 3 days and heavy growth took place after 4 days. The obtained data illustrated that toxic phenol concentration varies among the isolates of the same species. While, the toxic phenol concentration of *Provid. rettgeri* isolate No. 16 was 600 ug/ml, these were 1000 and 1200 ug/ml for isolates No. 1 and 15, respectively.

The findings in this study also showed that isolate No. 15 *(Provid. rettgeri)* gave the optimal growth and complete phenol utilization with 1000 ug/ml phenol concentration after 5 days. Similarly, Gurujeyalakashmi and Oriel[12] recorded 80 hrs. with *B. stearothermophilus*. This showed that *Provid. rettgeri* as well as *B. stearothermophilus* degrades phenol optimally at higher concentration than those possible for many mesophiles growth which werestrongly inhibited above 500 ug/ml phenol such as *Pseudomones putida* and *Trichosporon cutaneum*[28].

The lack of phenol hydroxylase inhibition in 1000 ug/ml phenol provides encouragement for further purification and characterization of *Providencia* enzymes. It wil also be of interest to examine such isolates for other enhanced bioconversion capabilities.

The finding that some isolates of *Provid. rettgeri*, in this study had the ability to degrade 300 ug/ml phenol completely after 2 weeks, is consistent with previous [4] finding that the anaerobic biodegradation time required for complete phenol disappearance was 2 weeks. Also, is considered comparatively a short time to that taken by bacteraial anaerobic population (4-5 weeks) as reported by Healy and Young[13] who also claimed that an evidence for the enrichment of phenol decomposers is the little lag occured after a 2nd spike of substrate addition.

In an agreement with several previous reports[13] this study illustrated that the source of the seed population, affects also the time necessary before the onset of degradation.

Concerning the retaining degradability of phenol exhibited by the isolates, the obtained data showed that only isolates No. 4,14 and 15 had the ability to grow on SLM containing 100 ug/ml phenol as a sole carbon source, whereas, the other isolates lost or unretained phenol degradability. Only 20% of phenol was degraded by isolate No. 14 (*Enterobacter cloaceae*) while 100% phenol exhausion was exhibited by isolate No. 15 *(Provid. rettgeri)*.

Isolate No. 15 *(Provid. rettgeri)*, the most potent phenol-degradable isolate in this trial, was used to study factors affecting bacterial phenol degradation. As illustrated in Table (4), the *Provd. rettgeri* was highly active during the fisrt 2 days as the optical density (O.D.) was high, increased viable count and > 70% of phenol was degraded.

Results after that, showed about 50% of the remained phenol was degraded in the 3rd day followed by reduced rat of degradation in 4th day; slight increase then constant O.D. and slight consant followed by slight decrease in viable count. These findings explained that growth phase took the first 3 days followed by the stationary phase (4th day) and finally the decline phase (5th day).

It is obvious that the increase in phenol concentration led to slight decrease in the rate of phenol degradation but increase in growth optical density and bacterial viable count in the first 3 days. Herein, the higher phenol concentrations yielded increase in O.D., rate of phenol degradation and bacterial viable count up to approximate exhaustion of the substrate where drop of phenol degradation and viable count, was taken place.

Thus, the specific growth rate of the cultures was found to be increased with increasing phenol concentrations, a finding consistent with that of Klecka and Maier[29]. However, substrate concentrations greater than 1500 ug/ml significantly decreased the specific growth rate.

Results in Table (5) illustrated that the optimal temperature for bacterial phenol-degradation was 35°C in waterbath shaker as the growth rate was profuse and the degradation of phenol was high. At 27°C, the phenol biodegradation was also moderately high while at 42°C, the biodegradation was about to be lost with constant O.D. and decreased viable count followed by bacterial cells death.

There is no exact relationship between the rate of phenol biodegradation and temperature but it seems that

Table (3): Degradation and toxic phenol concen... by Provid. rettgeri (isolate No. 1,15 and 16).

Phenol Concn. (µg/ml)	Residual Phenol Concentration (µg/ml) / Time (day)																	
	1			2			3			4			5			6		
	Isolate No																	
	1	15	16	1	15	16	1	15	16	1	15	16	1	15	16	1	15	16
200	150	165	150	87	105	75	53	41	50	35	13	45	15	0	15	10	0	15
400	320	305	340	210	197	195	110	55	110	65	34	85	65	19	45	50	0	35
600	550	500	450	420	263	310	325	150	250	170	50	115	104	0	95	63	0	79
800	790	710	800	510	320	800	270	210	800	103	120	750	89	40	750	75	0	735
1000	1000	875	1000	1000	420	1000	990	240	1000	990	53	1000	970	0	1000	970	0	108
1200	1180	1150	1200	1180	1000	1200	1160	1000	1200	1160	1000	1200	1160	1000	1175	1160	1000	1175
1500	1500	1450	1500	1450	1450	1500	1450	1450	1500	1450	1450	1500	1440	1420	1500	1440	1400	1500

Table (4): Effect of phenol concentration on bacterial phenol-degradation growth optical density and bacterial viable count.

At zero time			Time (day)														
			1			2			3			4			5		
C	V $\times10^{5}$	D $\times10^{-3}$	C %	V $\times10^{6}$	D $\times10^{-3}$	C %	V $\times10^{8}$	D $\times10^{-3}$	C %	V $\times10^{10}$	Dx 10^{-3}	C %	V $\times10^{12}$	D $\times10^{-3}$	C %	V $\times10^{9}$	D $\times10^{-3}$
10	73	110	51	68	140	75	5.2	153	85	2.2	166	95	0.25	165	95	63	167
20	21	111	62.5	24	154	83	59	166	89	3.1	167	93.5	0.49	172	95.5	9.2	175
30	43	110	47	15	218	82	310	236	85.7	190	244	91.7	15	251	94.3	8×10^{3}	251
40	31	113	51.3	630	273	83.3	450	296	96	39×10^{2}	315	96	240	323	98	22×10^{3}	323
50	23	110	33.6	3400	324	71	18×10^{2}	347	77	43×10^{2}	359	81	270	367	84.6	18×10^{4}	369
60	112	115	40.5	5400	359	65	73×10^{2}	369	76.7	62×10^{3}	393	87.8	17×10^{2}	409	88.3	16×10^{4}	405
70	75	110	35.9	43×10^{3}	395	61.4	77×10^{2}	399	68.6	59×10^{3}	405	75	870	413	81.4	19×10^{4}	402
80	141	109	35	56×10^{3}	409	58.1	38×10^{3}	435	68.8	33×10^{3}	458	77.5	760	477	78.8	47×10^{4}	472
90	161	114	41.1	15×10^{4}	429	50	13×10^{4}	453	70	22×10^{5}	480	85	16×10^{4}	489	88.9	52×10^{6}	491
100	72	115	38	53×10^{4}	445	64	43×10^{6}	472	87	62×10^{6}	492	92.5	15×10^{6}	499	59.1	39×10^{9}	496

C: % degraded phenol concentratin (µg/ml), D: optical denstiy at 400 nm and V: viable count.

Table (5); Effect of temperature on phenol-biodegradation, growth optical density and bacterial viable count (100µg/ml phenol).

Temperature °C	Time (day)																	
	0			1			2			3			4			5		
	C %	D $\times10^{-3}$	V $\times10^{6}$	C %	D $\times10^{-3}$	V $\times10^{8}$	C %	D $\times10^{-3}$	V $\times10^{10}$	C %	D $\times10^{-3}$	V $\times10^{11}$	C %	D $\times10^{-3}$	V $\times10^{12}$	C %	D $\times10^{-3}$	V $\times10^{13}$
27	0	32	109	28	202	176	56	219	27	73	273	27	75	303	148	79	309	22.6
35	0	32	109	33	192	103	75	229	155×10^{2}	89	325	$\times10^{3}$	90	351	164×10^{3}	94.5	356	15×10^{3}
42	0	33	112	15	18	25×10^{-3}	20	3	121×10^{-7}	20	3	-	25	3	-	26	3	-

there is an optimum temperature specified for a certain microorganism for maximum phenol biodegradation activity.

As it is shown in this study (Table 5), the optimum temperature of *Provid. rettgeri* is 35°C. The results showed also that 7°C decrease in this temperature led to slight decrease in the rate of phenol biodegradation and decrease in bacterial viable count and slight increase in O.D. while, 7°C increase, led to sharp decrease in the biodergradation rate and bacterial viable count upt o the 3rd day. This was followed by slight decrease at low temperature and sharp decrease at higher one.

The effect of time on bacterial phenol biodegradation is shown in Table (6). At first phenol utilization was low, then about 65% of phenol was consumed on the 2nd day. After 4 days, approximately, all phenol was utilized with constant O.D. and decreased viable count.

Table (6): Effect of time on bacterial phenol degradation, growth optical density and bacterial viable count (100 µg/ml)

Indicator	Time (days)					
	0	1	2	3	4	5
Phenol conc. (µg/ml)	100	91	35	13	5	2
% degraded phenol	0	9	65	87	98	98
$O.D.x10^{-3}$	85	339	409	510	500	480
vible count $x10^6$	1.36	$21x10^3$	$56x10^9$	$37x10^{11}$	$114x10^8$	$231x10^8$

The increase in incubation time led to an increase in the rate of phenol biodegradation, optical density and bacterial viable count up to the point of substrate exhaustion (about 3 days). Herein, the increase in time led to the decrease in the rate of phenol biodegradation and viable count with constant O.D.

As shown in Table (7), the organic nutrient either glucose or yeast extract increased bacterial growth during the first 2 days is accompanied by little phenol degradation while in the following 3 days, degradation of phenol has been increased especially in the case of yeast extract.

It was noted also that the increase in organic nutrient concentration led to decrease in the rate of phenol biodegradation with increase in cell mass up to the 3rd day. After that, higher concentrations yielded higher rates of phenol biodegradation and the vice versa with the bacterial viable count. The higher the organic nutrient concentration, the higher the growth optical density.

An understanding of the kinetics of phenol biodegradation provides a foundation for process analysis and design for the optimum removal of the compound in waste water treatment systems.

The low growth rate, cell yield and effects of substrate inhibition indicate that relatively long cell residence times will be required for the efficient removal of phenol. Mean cell residence time is inversely proportional to the net specific growth rate.

Further applications of the kinetic parameters in conjuction with predictive models, such as the one described by Rozich et al.(21)), may reveal additional process control strategies for the optimum performance of waste water treatment facilities.

Table (7) : Effect of organic nutrients on bacterial phenol-degradation, growth optical density and bacterial viable count (100 µg/ml).

Organic nutrient (µg/ml)	Time (day) 0			1			2			3			4			5		
	C %	D $x10^{-3}$	V $x10^4$	C %	D $x10^{-3}$	V $x10^{10}$	C %	D $x10^{-3}$	V $x10^{13}$	C %	D $x10^{-3}$	V $x10^{15}$	C %	D $x10^{-3}$	V $x10^{15}$	C %	D $x10^{-3}$	V $x10^{13}$
Control	0	79	12	11	219	2.15	67	419	27	88	502	139	94.5	500	21.5	98	500	7.9
Glucose 25	\0	63	13	35	281	31.1	61	408	66	75	442	154	85	490	1650	90	467	$x10^2$
50	0	74	15	37	334	237	43	444	$57x10^2$	63	462	$x10^3$	78	513	$x10^2$	87	487	130
100	0	91	67	9	374	271	51	467	$x10^3$	65	557	$x10^3$	81	540	35	90	447	147
Yeast extract 25	0	138	25	36	409	0.215	55	429	21.5	72	512	9.6	86	444	1.03	87	374	193
50	0	141	37	30	522	35	56	514	179	82	574	110	90	506	113	[illegible]	486	[illegible]
100	0	155	75	7	491	720	52	666	$x10^2$	65	524	$x10^2$	95	591	1250	95	563	8.5

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التحلل البيولوجي لمركب الفينول ببكتريا البروفيدنشيا رتجيرى

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فى هذا البحث أمكن عزل سلالات من ميكروب البروفيدنشيا والانتيروباكتر من المخلفات السائلة لمصنع انتاج الفينيك بمدينة العاشر من رمضان. وقد أظهرت الدراسة أن قدرة تلك العزلات على تحليل مركب الفينول أو النمو فى وجوده تختلف من سلالة لأخرى. فقد أظهرت ٩ عزلات قدرة على تحمل الفينول حتى تركيز ٥ر٠ جرام ٪ و ٤ أخرى حتى تركيز ١ جرام ٪، أما الثلاثة الباقية فقد تحملت حتى تركيز ٥ر١ جرام ٪. وقد وضح من الدراسة أن بكتريا البروفيدنشيا أقوى وأنشط فى تكسير مركب الفينول عن بكتريا الانتروباكتر ثلاثة من أقوى العزلات على تحليل وتكسير الفينول ثم اختبارها فى تركيزات ٦٠٠، ٨٠٠، ١٠٠٠ ميكروجرام/مل فينول، فوجد أن ٨٤٪ و ٨ر٨٨٪ و ١٠٠٪ من الفينول قد تحللها والتخلص منها بعد ٥ أيام فقط. هذا وقد تم دراسة بعض العوامل التى تؤثر على العملية البيولوجية للتخلص من الفينول مثل التركيز الأولى للمادة الملوثة، درجة الحرارة، الزمن اللازم للعملية وكذا تأثير وجود مواد عضوية فى بيئة التكسير.

وأوضحت الدراسة أن معدل النمو يتناسب عكسياً مع معدل التكسير وأن التركيزات العالية من المواد الأولية توقف نمو ونشاط ميكروب التكسير. ولكن معدل التكسير يزيد مع التركيز المتوسط المحتمل بالنسبة للميكروب المؤثر. كما أوضحت الدراسة أن الزمن الأنسب لاتمام عملية التكسير قد تراوحت بين ٣-٥ أيام وكذا درجة الحرارة المناسبة بالنسبة للميكروب البروفيدنيشيا هى ٣٥° م فى حمام مائى هزاز. أما عن وجود المواد العضوية بوسط التكسير فقد أوضحت الدراسة أنها تزيد معدل النمو إلا أنها تؤخر عملية التكسير.