Manuscript submitted to Water Air and Soil Pollution

1

2 3 Running head: Biotreatment of phenol in a bioreactor 4 Thyphen Article type: Application of environmental microbiology in organics contaminated 5 wastewater treatment 6 7 [hyphen] Biotreatment of Phenol-Contaminated Wastewater 8 (redundant with journal's requirements) Some stylesheets explicitly in a Novel Spiral Bioreactor 9 prohibit the use of words such as "novel" in the sense of "new." This word is redundant here because the stylesheet specifies that "Articles should report new science and technology knowledge." By submitting the article, you are implying that something "new" is reported therein; thus, you do not need to use "new" or a synonym 10 11 thereof in the title. 12 13 14 1. Professor 15 2. Graduate student, Department of Chemical and Environmental Engineering, University of 16 California, Riverside, USA 17 18 19 **Corresponding Author:** 20 21 Professor 22 23 E-mail: 24 25 Tel:

1 Abstract. A spiral bioreactor inoculated with microorganisms obtained from activated sludge was used to carry out a feasibility study for phenol removal. The reactor was 1 * 2 operated continuously under various phenol loadings ranging from 53 to 201.4 g m⁻³ 3 hr⁻¹, and under different hydraulic retention time (HRT) in the range of 20–180 min to 4 indicated estimate the performance of the bioreactor. Results showed that phenol removal when (redundant) 6 efficiency ranging from 82.9 to 100% can be reached as the spiral bioreactor was operated at a HRT of 1 h and a phenol loading of less than 111.9 g m⁻³ h⁻¹. For an 7 [redundant] influent phenol concentration of 201.4 g m⁻³, the removal efficiency of phenol 8 increased from 18.6 % to 76.9 % with the increase of HRT (20–120 min). The treatment of phenol in the reactor, the maximum degradation rate (Vm) was 1.82 mg maximum degradation rate (Vm) and half saturation constant (Ks) for treatment of 10 the half-saturation constart (k), 34, 95 mg l-1.

phenol in the spiral bioreactor were 1.82 mg l-1 min-1 and 34.95 mg l-1, respectively. 11 [hyphen] Moreover Besides, a first order model with a rate constant of 0.1178 min⁻¹ was established for 2 12 capable of accurately approximating diction predicting substrate conversion. The model is able to give a good approximation of 13 14 the experimental data. 15 *≫* 16 Keywords: biodegradation; kinetics; phenol; spiral bioreactor "Besides" is a colloquialism best avoided in formal academic writing. All keywords should appear in the Abstract. The circled words do not occur here.

* "Research" is an uncountable noun. Do not attempt to make it plural. However, you actually mean "researchers" here. Pardon me! 1. Introduction 2 Phenolic compounds and their derivatives are the major pollutants discharged refining petroleum, synthesizing resins, 3 from many industrial processes, including oil refineries, resins synthesis, manufacturing explosives photographic chemicals preparation, and explosives manufacture (Sittig, 1997; 5 Alemzadeh et al., 2002; Nuhoglu et al., 2005; Jiang et al., 2006; Mohanty et al., [hyphen] these pollutants 2008). Among them, phenol, a water soluble and highly mobile chemical that can 6 cause sever odor and taste problems, has early been shown to be toxic to many [reduce verbosity] [diction] 8 biochemical functions and fish life, even at low concentrations (Sufit, 1978). researchers endeavored In the recent years, researches have made great efforts to develop more efficient diction 9 10 and cost-effective control technologies for phenol treatment. Biological treatment is a 11 more preferable alternative to traditional physical and chemical control methods due efficient destruction/reduction of pollution 12 to its low cost, reliable operational stability, and pollution destruction efficiency. Althouah While phenol is considered to be inhibitory at high concentration (Yoong et al., 1997), 13 3* 14 biological treatment techniques are widely used for treatment of wastewater or soil containing moderate amount of phenol (5–500 mg 1⁻¹) (Patterson, 1985; 15 16 Bandhyopadhyay et al., 2001). [comma) Immobilized-cell technology is a recent advancement in biotechnology which 17 diction allows for compacting and maintaining a large amount of cells in biotreatment 4 18 Moreover [nospace] 5119 systems for remediating contaminants. Besides, down stream separation and Lakhwala et al. developed processing costs could be eliminated by immobilizing the microbes. In 1992, a spiral 20 to remove organic compounds Being capable of minimizing channeling bioreactor that could minimize channeling effects and maximize the 21 effects and maximizing the interaction of microbial contaminants, their microbe-contaminants interaction was developed to remove organic compounds and Syntax) 22 coordination Furthermore, this reactor achieved excellent performance. (non-parallel) diction 1 excellent performance was obtained (Lakhwala et al., 1992). The spiral reactor shows potential for kinetic studies as well as for scale up/down studies of multiphase 25 processes because it is easily reproducible and its hydrodynamics activities approach 3* Reserve usage of "while" for its temporal [time] sense. 4* Use "number of" with countable nouns; "amount 3 of" with uncountable. 5x "Besides" is a colloquialism best avoided in formal academic writing.

1 * Even better than has been found toxic would be is toxic - unless you need to hedge.

1* Verisy	, the	spelling: Rues/Rus/Ruse. Compare Line 25, p. 12.		
*	1	the plug-flow operation (Kallinikos and Papayannakos, 2007). Rues and coworkers *1		
	2	(1995) using a spiral bioreactor for removal and recovery of metals from aqueous		
	3	wastes and found that the spiral bioreactor was efficient in binding metal ions from		
	4	the waste stream. Close to 90% recovery by acid wash can be achieved in the pH		
	5	range of 2.0 to 2.5. Apart from aqueous waste treatments, the spiral also had been		
	6	used to treat gaseous wastes. For instance, Shim and coworkers (1995) used a fixed-thyphen		
	7	film spiral bioreactor containing immobilized activated sludge microorganisms to		
	8	degrade ethanol vapors in the range of 600 to 7000 ppmy and a maximum elimination		
	9	capacity of 185 g ethanol h ⁻¹ m ⁻³ of reactor volume was observed. Guo et al. (2001) when		
	10	used a spiral-wound fibrous bed bioreactor to co-metabolic degrading		
	11	trichloroethylene (TCE) by Pseudomonas putida F1 and found that 98.5% TCE could		
	12	be removed over 4 hours. However, it should be note that this bioreactor was mainly		
[syntax]	13	operated under batch conditions. Whose? Their, i.e., Guo et al.?		
	14	The aim of this research was to evaluate the capacity of a mixed culture to grow		
	15	and degrade phenol in a spiral bioreactor operated under different hydraulic retention		
	16	time and various organic loading. Besides, a mathematic model capable of predicting		
	17	phenol removal efficiency and the time needed to obtain certain conversion was		
	18	successfully developed. The result obtained in present work could be a useful		
	19	reference for engineered biotreatment processes.		
	20			
	21	12-pt 11-pt 2. Materials and Methods		
2*	22	2.1. MICROORGANISM AND GROWTH MEDIUM		
	23	A mixed culture obtained from a wastewater treatment plan in central Taiwan		
	24	was acclimated in a bioreactor fed with phenol as sole carbon source. After		
	25	acclimation and incubation for a period of time, the cultures were prepared as seed		
2* Use the next-smaller font size for all except the first character in each sub-header at this level. Presumably, this should be 1/pt.				

1* What is a "moderate amount"? This phrase is too general in a context which should be specific. How much ? (2) Which medium was the phenol added to? You have listed 12 mediums in Lines 2-5 on this plage. Merely add 5" if you mean more than one medium. If you mean something else, let us discuss how to word it. cultures for the spiral bioreactor. The mineral medium used in the present work were Avoid redundancy as follows: K₂HPO₄, 4.27 g l⁻¹; KH₂PO₄, 3.48 g l⁻¹; (NH₄)₂SO₄, 0.34 g l⁻¹; MgSO₄ · 2 $^{7}\text{H}_{2}\text{O}$, 0.46 g 1 ; FeSO₄, 0.001 g 1 ; CaCl₂ · 2H₂O, 0.018 g 1 ; CuCl₂ · 2H₂O, 0.01mg 3 1⁻¹; CoCl₂ · 6H₂O 0.2 mg 1⁻¹; ZnSO₄ · 7H₂O, 0.1 mg 1⁻¹; MnCl₂ · 4H₂O, 0.03 mg 1⁻¹; 4 Na_2MoO_4 2H₂O, 0.03 mg I^{-1} ; and $NiCl_2 \cdot 6H_2O$, 0.02mg I^{-1} . All chemicals used were 5 6 analytical grade reagent. 2.2. BIOREACTOR SET UP See Note 2, p. 4, 7 8 Figure 1 shows the configuration of the spiral bioreactor. The reactor was made [diction] up of seven layers of polypropylene columns with an internal diameter of 120 mm and 10 a height of 22 mm. The overall height and working volume of the spiral reactor are 11 230 mm and 1 L, respectively. Each layer of column was packed with spiral-wound 12 13 bio-support to leave a space for liquid to pass through the channel. The bio-support matting (This form sounds better in diction 14 was made of filter sponge attached on a sheet of plastic mat. The artificial wastewater was prepared by the addition of moderate amount of ** 1 15 moreover phenol in to the mineral salts medium; Besides, 2% CaO2 was added to the 16 wastewater to provide sufficient dissolved O₂ (7–9 mg l⁻¹) for microbial respiration 17 and metabolic degradation of phenol. As is show in Figure 1, the influent enters the 18 center of the lowest layer column of the bioreactor and passes through the spiral 19 in more detail channel as shown in Figure 2. As it flows through the channels, phenol was oxidized 20 growing on by the microorganisms grew and attached on the bio-support sheet. After passing 21 through the seven layers of the bioreactor, treated water leaves the reactor from the avoid redundancy 22 23 top of the bioreactor. Experiments were carried out in the continuously operating bioreactor for more 24

than 300 hours. The loading of phenol in the influent was varied from 53 to 201.4 g

25

1x It is unnecessary to use the full form "spiral bioreactor" so many times - unless another reactor 2* Is "Teflon Minivert" a brand name? If so, the capitalization is appropriate; otherwise, revise to lower-case. m⁻³ h⁻¹, while the different hydraulic retention time (HRT) of the inlet stream was in the range of 20–180 min. The sampling ports were located at the inlet and exit of the 2 Periodically a 2ml sample spiral bioreactor. A liquid sample of 2 ml was taken from each sampling port using a 1 * 3 This I word is redundant unless the 4 glass gastight syringe periodically. reactor also has other types of ports. 2.3. ANALYTICAL METHODS See Note 2, p.4 5 Liquid samples taken from the bioreactor were filtered by a bio-filter (TITAN-6 Nylon -0.2 μm) to removal microorganisms and inert materials that may interference (Sorm) the analysis results. Thereof the analysis results. Thereafter, a liquid sample of 1 µl was taken from the filtered 9 sample and was then injected into a model GC-14B gas chromatograph (Shimadzu Corp., Japan) equipped with a RTX-1 capillary column (30 m×0.53 mm) and a flame 10 ionization detector by a gastight syringe equipped with a Teflon Mininert valve fitting. 2* 11 Helium (99.98% purity) was used as the carrier gas and nitrogen was used as a 12 makeup gas. Oven temperature was controlled at a constant temperature of 105°C; whereas comma [diction] while injector temperature and detector temperature were set at 200 °C and 250 °C, 15 respectively. Calibration cure were obtained using identical experimental sample 16 2.4. KINETIC STUDY [See Note 2, p.4] 17 An equation modified from Michaelis-Menten model was used in this study to [syntax] determine the kinetic parameters for phenol degradation and the degradation rate 19 could be given by delete colon 20

 $\frac{C_{in} - C_{out}}{t} = \frac{V_m \times \frac{C_{in} - C_{out}}{\ln(C_{in} / C_{out})}}{K_s + \frac{C_{in} - C_{out}}{\ln(C_{in} / C_{out})}}$ (1)

22 where C_{in} and C_{out} are the phenol concentrations (g m⁻³) at the inlet and outlet of the

23 spiral bioreactor, respectively, V_m is the maximum degradation rate (g m⁻³ h⁻¹) and K_s

* Why introduce an acronym if you do not subsequently use it?

is the half-saturation constant (g m⁻³), t is the hydraulic retention time (h). While C_{in} , C_{out} , and t can be obtained from experiment data, values of V_m and K_s were calculated 2 3 numerically by fitting the biodegraation data to the solution of Equation (1) through 4 nonlinear parameter regression and a least-square minimization procedure. All of the calculations were done by using Microsoft Excel 2003 Solver. 5 6 3. Results and Discussion 3.1. REACTOR PERFORMANCE See Note 2, p.4 8 continuous operation 9 Figure 3 illustrates the performance of the bioreactor operated continuously under various experimental conditions. The hydraulic retention time and liquid flow 10 rate were controlled at 60 min and 0.001 m³ h⁻¹, respectively. High removal efficiency 11 (RE) of 100% can be reached as the phenol loadings (LO) were in the range of 5.3 to 12 is eminently capable of 69.9 g m⁻³ h⁻¹, indicating that the spiral bioreactor exhibits an excellent ability for 13 (redundant) LO phenol removal under moderate phenol loading conditions. However, the removal 14 Fredundant LO decreased efficiency dropped dramatically from 82.9% to 30.9% as the inlet phenol loading was 15 increased from 111.9 to 201.4 g m⁻³ h⁻¹. Besides, for inlet phenol concentrations below 16 201.4 g m⁻³, the removal efficiency increased with the increase of hydraulic retention 17 delete parentheses time. Note that the loading (LO) and removal efficiency (RE) are defined as 18 19

$$20 LO = \frac{Q}{V} \times C_{in} and (2)$$

 $RE = \frac{C_{in} - C_{out}}{C_{in}} \times 100\%$ (3)

24 where Q is the liquid flow rate (m³ h⁻¹), and V is effective volume (m³).

21

23

* See Note, p. 7.

3.2. EFFECT OF ORGANIC LOADING ON REMOVAL EFFICIENCY

2 Figure 4 shows the effect of organic loading on the removal efficiency and the

delete colon elimination capacity (EC) of phenol. Elimination capacity (EC) is defined as * 3

4

$$5 EC = \frac{Q}{V} \times \left(C_{in} - C_{out}\right) (4)$$

6

19

***** 21

verbosity

- [comma] was It is shown in Figure 4 that the removal efficiency is always more than 80% when the
 - 8 residence time is 60 min and the inlet phenol concentration is less than 111.9 g m⁻³.
- decreased when However, the removal efficiency dropped dramatically as the inlet phenol * 9
 - concentrations were greater than 111.9 g m⁻³. This phenomenon might be due to that 10 greater than
 - the organic loading was over the maximum tolerable value of the mixed culture and 11
- consequently caused the inhibitory effect on culture growth. Hence, the apparent 12 removal efficiency was reduced. ***** 13
- The removal efficiency was in the range of 100 to 47.2% when the spiral 14
- bioreactor was operated at an inlet phenol loading of 5.3 to 201.4 g m⁻³ h⁻¹ and a HRT 15
- [diction] of 60 min. As is demonstrated in Figure 4, the elimination capacity of the spiral **1**6
 - LO when it bioreactor is equal to phenol loading as the loading was less than 69.9 g m⁻³ h⁻¹. * 17

(semilar) thusian RE Besides, phenol is degraded completely and a removal efficiency of 100% also can be [syntax] ***** 18

- reached at this loading range. Therefore, this bioreactor was performed as a mass
- transfer limited system while the elimination capacity is in proportion to phenol ***** 20 shifted to a limited reaction rate when diction
 - was greater than 111.9 g m⁻³ h⁻¹. That is, the inlet loading rate was over the maximum diction 22

loading. However, the system turned to be reaction rate limited as the phenol loading

- degradation capacity of bioreactor. Hence, the elimination capacity obtained diverged ¥23
- **k** 24 gradually from the 100% degradation line. Furthermore, the removal efficiency
- dropped to a range of 45 to 50% at a phenol loading of 201.4 g m⁻³ h⁻¹. The maximum

*	1	elimination capacity of the spiral bioreactor for phenol was approximately 150–155 g
	2	m ⁻³ h ⁻¹ . 12-pt 11-pt s(?) HRT
*	3	3.3. EFFECT OF HYDRAULIC RETENTION TIME ON REMOVAL EFFICIENCY
*	4	Figure 5 shows the effect of hydraulic retention time on the removal efficiency of
[diction]	5	the spiral bioreactor. The investigation was carried out at an inlet phenol concentration
*	6	of 201.4 g m ⁻³ and hydraulic retention time from 20 to 180 min (corresponding liquid
	7	volume flow rate from 0.003 to 0.0003 m ³ h ⁻¹). As the reactor was operated at a
Ą	₹ 8	hydraulic retention time from 20 to 60 min, the removal efficiency was only in the
	9	range of 18.6 to 46.2%. It is surmised that this result was attribute to the reason that
	10	this operation condition could not provide sufficient time for phenol to pass through
[dietion]	11	the biofilm and to react. This speculation could be proofed as the liquid retention time
*	12	doubled. Removal efficiency increased from 46.2 to 76.9% as the hydraulic retention
	13	time was extended from 60 min to 120 min.
*	14	However, as can be seen in Figure 5, the removal efficiency increased only
*	15	slightly when the hydraulic retention time is greater than 120 min. For instance, as the
. *	16	hydraulic retention time increased from 120 min to 180 min, removal efficiency only
[syntax]	17	increased from 76.9% to 82.2%. This indicated that there must be something must have
	18	happened in the bioreactor and limited the removal ability of the bioreactor. After a
[diction]	19	serious of tests, it is found that this was due to the shortage of dissolved oxygen for
	20	aerobic degrading phenol. It is found that the effluent contained only 0.72 mg l ⁻¹
	21	oxygen whereas the influent contained 7–9 mg l ⁻¹ oxygen. Hence, for the bioreactor
	22	operated at an inlet phenol concentration of 201.4 g m ⁻³ and hydraulic retention time
	23	of 180 min, dissolved oxygen was the rate-limited factor.
	24	3.4. KINETICS

* See Note, p.7.

25

The kinetic parameters for the mixed culture degrading phenol in the spiral

- bioreactor were 1.82 mg l⁻¹ min⁻¹ and 34.95 mg l⁻¹ for the maximum degradation rate
- 2 (V_m) and half-saturation constant (K_s), respectively. The low value of half saturation
- [reduce redundancy]

redundance

3 constant indicates that the mixed culture has a high affinity to phenol. That is, the

- 4 bioreactor could provide a large surface for microbe-contaminants contact and

 Moreover [verbose]
- 5 reaction. In addition, it is observed that the maximum degradation rate increased with
- * 6 Lincreasing HRT or decreasing phenol loading.
 - As can be seen in Figure 5, the data obtained from experiment result seems can
 - 8 be best approximated by an exponential line. Hence, it is suggested that a first-order
 - 9 reaction model may be suitable for describing the kinetic behavior under the given
- 10 experimental conditions

$$\ln\left(\frac{C_{in}}{C_{out}}\right) = K \cdot t, \quad \text{comma} \tag{5}$$

- 12 Where K is the first order reaction rate constant (h⁻¹) and can be obtained by direct
- linear plot of $ln(C_{in}/C_{out})$ versus t and has a value of 0.1178 h⁻¹ in this study.
- 14 Rearranging Equation (5) after combined it with Equation (3), an equation capable of
- * 15 predicting the removal efficiency of phenol under various HRT may be given as:

17
$$RE = (1 - e^{-Kt}) \times 100\%$$
 (6)

- 19 The solid line shown in Figure 5 is the result of model predictions. As can be seen in
 - this figure, the stimulated results compared favorably with the experimental data [VT]
- obtained. Therefore, the proposed model could be utilized to predict phenol
- syntax 22 degradation in the spiral bioreactor successfully.

23 (Here you are generalizing.)

24

16

18

2* See Note, p.7. 4. Conclusions 1 [redundant] The elimination capacity of the reactor and the pollutant-loading rate play 2 An appropriate important roles in the design and operation of a bioreactor. The size of a bioreactor fro 3 the removal of certain pollutants is primarily depend on these two parameters. Such 4 demonstrates endear is presently is in progress. This study shows that a laboratory-scale spiral bioreactor was effective for removal of phenol from wastewater. The removal 2* 6 7 efficiency in the bioreactor decreased with an increase in the inlet liquid flow rate or in the inlet. Moreover, | [subscript?] 8 the inlet phenol concentration. Besides, the low value of Ks indicated that the spiral hyphens bioreactor was capable of providing large surface for phenol and biofilm interaction. 9 Furthermore, the first order model could be utilized to predict phenol removal 10 efficiency in the spiral bioreactor successfully. 12 Use headline grammar (i.e., omit "the") in lists. Nomenclature 13 phenol concentrations at the inlet (g m⁻³) 14 C_{in} phenol concentrations at the outlet (g m⁻³) 15 C_{out} first-order reaction rate constant (h⁻¹) 16 K half-saturation constant (g m⁻³) 17 K_s the liquid flow rate (m³ h⁻¹) 18 0 hydraulic retention time (h) 19 effective volume (m³) 20 Vmaximum degradation rate (g m⁻³ h⁻¹) 21 22 Acknowledgment 23 The authors wish to express appreciation to Dr. Cheryl J. Rutledge for her 24 25 editorial assistance. notice Department of English,
Dayen University, 26

1* "Such endear" is ungrammatical. Furthermore, any form containing "endear" does not make good sense here. Another word is needed, or the entire sentence should be deleted.

1 * Why are different-sized fonts used in this list?

2* Remember: It is redundant to say 'A Comparative Study of... in a title. Avoid this error in your own titles.

See Note, p.1.

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 We See Note 1, p. 4.

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1* Use headline grammar (i.e., omit "the") in captions - except when complete sentences are needed. A caption should be as brief as possible - not verbose or redundant.

11* Figure captions 3* See Note, p. 1 Figure 1. Schematic diagram of the novel spiral bioreactor. 2 3 4 Figure 2. Cross-section of the spiral bioreactor and mechanics of flow in the spiral redundant bioreactor. (a: Inside view of the spiral bioreactor; b: Wastewater enters . 5 redundant (delete period) bioreactor via the center of the reaction layer for layers 1, 3, 5 and 5; c: 6 Wastewater enters bioreactor via the edge of the reaction layer for layers 2, 7

8 4 and 6)

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Figure 3. Reactor performance for continuous operation under various experimental conditions. (I: Acclimation period; II: Inlet phenol loads of 5.3–69.9 g m⁻³ h⁻¹, HRT of 60 min, Avg. removal of 100%; III: Inlet phenol loads of 111.9–201.4 g m⁻³ h⁻¹, HRT of 60 min, Avg. removal of 82.9–47.2%; IV: Inlet phenol loads of 201.4 g m⁻³ h⁻¹, HRT of 20, 40, 60, 120, 180 min, Avg.

Figure 4. Effect of organic loading on phenol removal efficiency and elimination capacity (EC) of the spiral bioreactor.

removal of 18.6-82.2%)

Figure 5. Effect of hydraulic retention time on phenol removal efficiency of the spiral bioreactor and simulative result of the mathematic model.

2* In this case, my Note on p.7 is overruled by a prescription under "Figures" in the stylesheet: "... legend (without abbreviations)..."

3* Remember to indicate in the margins: "Insert Figure _ about here."

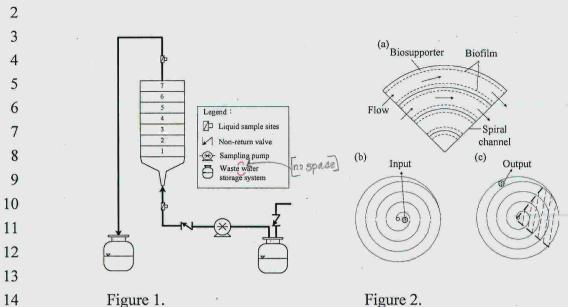


Figure 1.

Figure 2.

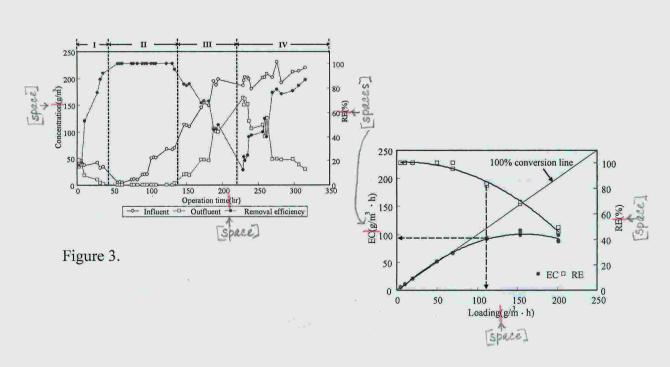
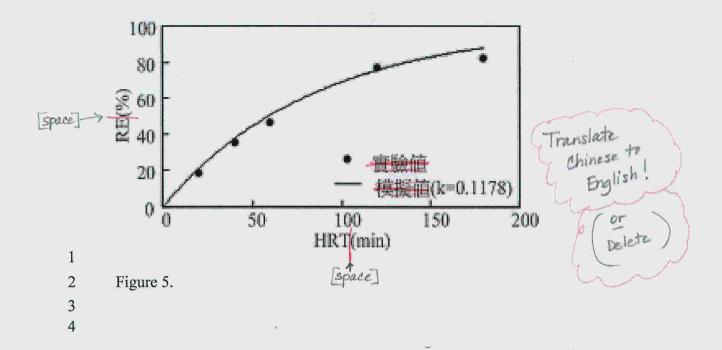


Figure 4.



Dear Editor:

titled

Contaminated Wastewater in a Novel Spiral Bioreactor" submitted for publication in Water Air and Soil Pollution. The aim of this research was to evaluate the capacity of a mixed culture to grow and degrade phenol in a spiral bioreactor operated under different hydraulic retention time and various organic loading. Besides, a mathematical model capable of predicting phenol removal efficiency and the time needed to obtain certain conversion was successfully developed. The result obtained in present work could be a useful reference for engineered biotreatment processes.

The original paper was not published elsewhere. The experiments been properly conducted with controls, replication at procedures. The article also is of international value. The authors all consent to submit the manuscript to the Journal, and all agree to transfer the copyright to the publisher.

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Sincerely,