J Res Health Sci, Vol 4, No 2, pp. 8-13, Autumn & Winter 2004

Evaluating the Effect of Glucose on Phenol Removal Efficiency and Changing the Dominant Microorganisms in the Serial Combined Biological System

*Movahedin H. PhD,**Shokoohi R. MS,*Parvaresh A. PhD, *** Hajia M. PhD, ** J.Jafari A. PhD

*Dept. of Environmental Health, School of Public Health, Isfahan University of Medical Sciences, Iran
**Dept. of Environmental Health, School of Public Health, Hamadan University of Medical Sciences, Iran
***Dept. of Microbiology, Baghyatollhe University, Tehran, Iran

Abstract

Background: The presence of chemical compounds has different effects on the rate of degradation of materials and on changing dominant microorganisms in the biological degradation processes. These effects are related to the type and concentration of compounds.
This study was to evaluate the effect of different concentrations of glucose as a growth substrate on phenol removal efficiency and microbial flora in a serial combined biological system.
Methods: In this study, phenol was injected to the system and then different concentrations of glucose (50, 250, and 500 mg/l) were added. At the end, phenol removal efficiency and changing of dominant microorganisms in the separated combined biological system were evaluated.
Results: The study revealed that microbial flora was changed in various concentration of glucose and degradation of phenol was better in the presence of low amount of glucose (50 mg/l) and decreased by increasing the glucose concentration. The dominant microorganisms in this research were *Moraxella* and *E.coli*.
Conclusion: Presence the low concentration of glucose will increase the phenol removal and change the dominant microorganism in biological degradation process.

Keywords: Phenol degrading bacteria, glucose, co-substrate, combined system

Introduction

Phenol is a high-priority persistent, bioaccumulative and toxic chemical compounds. PBT compounds are not readily broken down and easily metabolized in environment. They may accumulate in human body and food chains through their consumption or uptake. It may be also hazardous to human health or environment (1). Phenol is obtained from petroleum as a naturally occurring compound by the oxidation of toluene. Phenol and its derivatives are widely used in a variety of industrial units, agricultural and pharmaceutical products. The largest use of phenol is for the production of phenolic resins (2). Other indications include the manufacture of caprolacta, bisphenol A, herbicides, wood preservatives, hydraulic fluids, heavy-duty surfactants, lube-oil additives, tank linings and coatings as well as intermediates for plasticizers and other chemicals. Phenol is used medically in throat lozengess, disinfectants, and ointments; for facial skin peels (3). It can be released to the environment as air emissions and wastewater discharges from refuse combustion, brewing, foundries, wood pulping, plastics manufacturing, glass fiber manufacturing,

Correspondence: Dr Shokoohi, P.O.BOX: 689 Hamadan Iran, E-mail: Shokoohia @ Yahoo .Com

leather tanning, iron and steel manufacturing, and petroleum refining (1).

Based on many researches, phenol is toxic for animals, inducing local effects depending on the site of application as a result of primary irritation and systemic toxicity, including liver, kidney heart, and neurobehavioral effects.

Lethal Dose (LD_{50}) value is in a range of 300 to 600 mg/kg, after oral administration to mice, rats and rabbits. The dermal LD_{50} is 670 mg/kg and 850-1400 mg/kg for rats and rabbits, respectively (3). The primary concern is for acute and chronic toxicity to aquatic organisms (4). Environmental Protection Agency (EPA) recommends that the level of phenol should be lower than 0.3 mg/l in surface water to protect human, fish, water plants and animals (5).

However, phenol is a toxic matter, but some microorganisms can degrade it after adaptation. The rate of degradation can be increased by various methods such as co-metabolism. It is the transformation of a non-growth substrate in the presence of a growth substrate or another transferable compound. The non-growth substrates are those that can not support cell division. The growth substrate saves several purposes. First, it provides energy for microbial growth and maintenances. Second, it provides reducing equivalents (power), which allow degradation of non- growth substrates.

Glucose is a growth substrate when phenol is present in the wastewater because glucose stracture is simpler than phenol.

JOO-Hwa Tay showed that in up flow anaerobic sludge blanket reactor, adding glucose with 1000 mg/l to phenol solution shorted start-up period from 7 to 4 months and increased phenol removal efficiency from 88% to 98% (6).

Swaminathan K et al reported that in upflow anaerobic fixed film fixed bed reactor p-nitrophenol (PNP) was not degraded as a sole carbon source. Adding glucose as a co-substrate increased the degradation of PNP. A ratio of >1 in term of glucose to PNP could achieve 90% PNP degradation (7). Aim of this study was evaluation effect of different concentrations of glucose as a co-substrate on the phenol removal efficiency and changing microbial flora in the separated combined biological system.

Materials and Methods

Physical properties of system The system was combined biological processes including activated sludge and biofilter reactors as a series incidentally. Type of hydraulic flow is continuous and incidentally some of sedimented microorganism in sedimentation tank returns to entrance of system. Physical properties and environmental condition of system are as follows: Capacity of system=25 l; Hydraulic detention time=7 h; Concentration of phenol= 500 mg/l; Efficiency of phenol removal=99.9%;Dissolved oxygen=1.5-2 mg/l; pH= 7.5 and T= 20-30°C. Schematic and flow diagram of system is shown in Fig. 1.

Source of Microorganisms Primary source of microorganisms that was provided from biological sludge of municipal wastewater treatment plant was added to the reactor with continuous dry milk solution as a substrate and suitable growth condition for one month. Then microorganisms were grown on the media as biofilm. In the next step, phenol was added in 0.1 mg/l concentration as well as dry milk solution. Then phenol concentration gradually was increased and concentration of dry milk was decreased. Finally, phenol was used as an only substrate source.

Sampling for phenol analyses For this purpose, samples were collected from effluent of system. Each sample was 50 ml. Phenol concentration was determined by spectrophotometric method.

Sampling for identification of microorganisms For this purpose, samples were collected from sedimented microorganisms from the sedimentation tank. The volume of each sample was 50 ml and was analyzed three times to meet high accuracy and confidence.

Microbial identification

Direct microscopic examination Samples were taken by a standard loop and put on the

slide from each sampling bottle. The smears were stained by the Gram staining method and microbial morphology observed by the light microscope.

Collected samples were shaken carefully before microscopic examination and isolation. Fifty ml of each collected sample were used for culture. Following enrichment and differential culture media were used for primary isolation including blood agar, Eosin Methylen Blue (EMB) and Brain Heart Infusion (BHI) agar. Anaerobic condition for culturing fungi was not provided in this study. All types of isolated colonies were separated and subcultured for next purification.

Organisms grown in the lowest dilution of the plating technique were considered to be dominant bacteria in each condition.

Biochemical tests were used for identification after microscopic examination. Various microorganisms were isolated on the used media. Isolated bacteria were identified by these tests: Catalase, SIM, Urea, OF (glucose), Citrate, TSI susceptibility to polymyxin, Gelatin, DNase, Manitol, Maltose and Esculin hydrolysis.

Glucose Addition First, glucose was added with 50mg/l concentration to phenol solution. Then efficiency of system was assessed for 3 days. In this time, removal efficiency of phenol was determined and recorded, and also microbial flora was identified. By stabilization the effective conditions at reactor outlet, remaining and degradation rate were determined. Then concentration of glucose solution was increased to 250 and 500 mg/l, respectively and experiments were repeated.

Results

Phenol removal efficiency was determined in different glucose concentrations. Collected data determined that maximum phenol removal efficiency was accessible at the 50 mg/l of glucose concentration. It was decreased by increasing glucose as well as in the absence of the glucose (Fig.2).

The observed microorganisms were different related to different concentrations of glucose and included gram negative and gram positive bacilli, cocobacilli, spirochetes, fungi, gram positive cocci and gram negative comma form bacilli in direct sample smears by microscopic examination (Table 1).

Separated and identified bacteria in variety of glucose concentrations are shown in Table 3. The data show that *Pseudomonas aeruginosa, Moraxella* sp, *Brevundiomonas* sp, *Pseudomonas alcaligenes* and *Acinetobacter* sp were identified in the absence of glucose, and *E.coli* as well as *Neisseria weaveri* were not detected. But in the presence of 500 mg/l of glucose, *E.coli, P. aeruginosa* and *Moraxella* sp were identified (Table 2).

Moraxella was a dominant microorganism in the absence and presence of 50 mg/l of glucose, while *E. coli* was dominant in the other concentrations of glucose (250 and 500) mg/l.

Table 1: Observed microorganisms in direct sample smears by microscopic examination (Phenol concentration was 500 mg/l)

	Glucose concentration (mg/l)			
	0	50	250	
Gram negative bacilli and cocobacilli	+	+	+	+
Gram positive bacilli	+	+	+	+
Spirochetes	+	+	+	-
Fungi	+	+	+	-
Gram positive cocci	+	+	+	+
Gram negative comma form bacilli	+	+	-	+

Table 2: Separated bacteria in variety of glucose concentration separated BF/AS (Phenol concentration was 500 mg/l)

Type of microorganism	Glucose concentration (mg/l)			
	0	50	250	500
E.coil	-	+	+	+
Pseudomonas aeruginosa	+	+	+	+
Moraxella sp.	+	+	+	+
Brevundiomonas sp.	+	+	-	-
Neisseria weaveri	-	+	+	-
Pseudomonas alcaligenes	+	+	-	-
Acinetobacter sp.	+	-	-	-

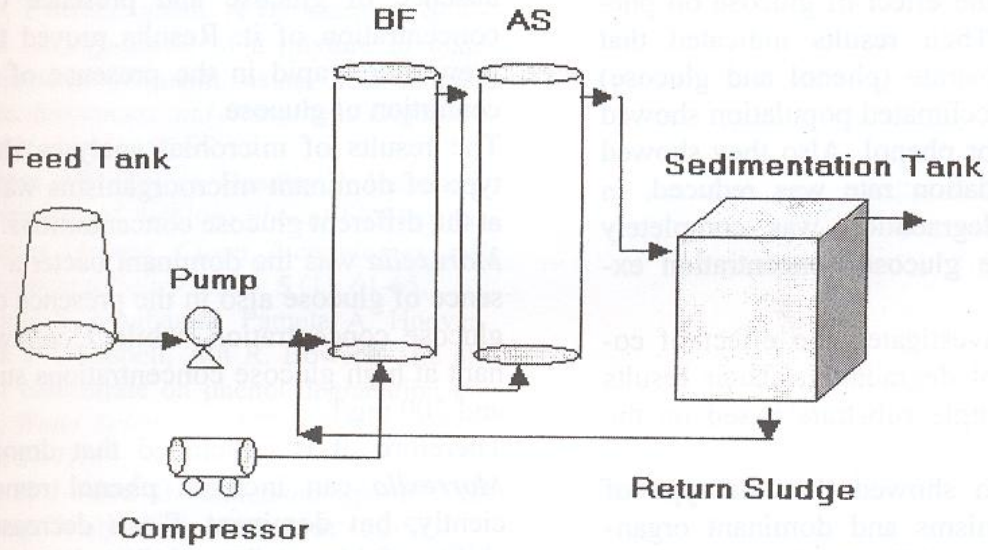

Fig. 1: Schematic and flow diagram BF/AS separated combined system

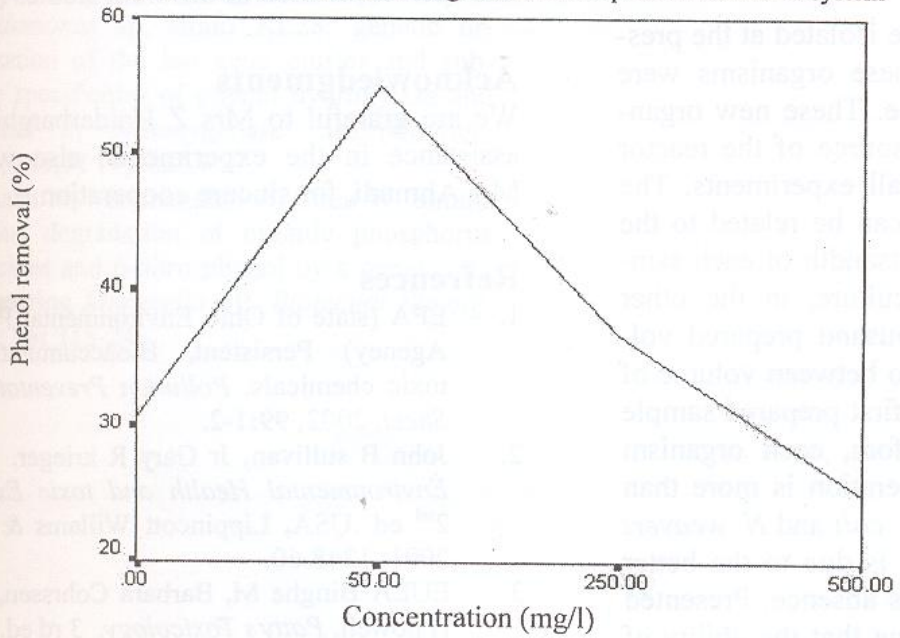

Fig. 2: Phenol removal efficiency at different glucose concentrations (concentration of phenol was 500 mg/l)

Discussion

In this research the effect of different concentrations of glucose as a co-substrate was assessed on the phenol removal efficiency and changing microbial flora in the separated combined biological system. The results of investigation showed that use of low concentration (50 mg/l) of glucose increased phenol removal efficiency and decreased when concentration of glucose increased to 125, 250, and 500 mg/l, respectively.

Kar et al showed the effect of glucose on phenol degradation. Their results indicated that when a mixed substrate (phenol and glucose) was used, phenol acclimated population showed initial preference for phenol. Also they showed that phenol degradation rate was reduced, in addition phenol degradation was completely inhibited when the glucose concentration exceeds 2 mg/l (8).

Magbanua et al investigated the effect of co-substates on phenol degradation. Their results showed *qm* was single substrate based on the Monod model (9).

Collected data also showed that the type of isolated microorganisms and dominant organisms were changed at different glucose concentrations.

E. coli and *N. weaveri* were isolated at the presence of glucose, while these organisms were not observed in its absence. These new organisms were isolated while source of the reactor was the same during the all experiments. The only possible explanation can be related to the growth condition. One thousandth of each sample was chosen for the culture, in the other hand, one part of first thousand prepared volume was cultured. The ratio between volume of sample for the culture and first prepared sample was one thousand. Therefore, each organism could be grown if its numeration is more than 1000 per ml. Isolation of *E. coli* and *N. weaveri* in the presence of glucose is due to the better growth condition than in its absence. Presented results in Table 2, determine that the ability of phenol degrading bacteria will be increased in low concentration of glucose as a co-substrate, and this has not been reported before. Tarighian et al used glucose as a co-substrate for degradation of phenol and 4-chloro phenol by a pure strain of *Pseudomonas putida*. They showed phenol was metabolized according to the Monod model. Their data showed glucose acting as the primary growth co-substrate (10).

Results of this research also determined that dominant microorganisms were different in the absence of glucose and presence of various concentration of it. Results proved that *E.coli* grew more rapid in the presence of high concentration of glucose.

The results of microbial analyses showed the type of dominant microorganisms was changed at the different glucose concentrations.

Morexella was the dominant bacteria in the absence of glucose also in the presence of 50 mg/l glucose concentration, while *E.coli* was dominant at high glucose concentrations such as 250 and 500 mg/l.

Therefore, it is concluded that dominance of *Morrexlla* can increase phenol removal efficiently, but dominant *E.coli* decrease it. The ability of *Moraxella* and *Pseudomonas* species has been confirmed in different studies (11-13).

Acknowledgments

We are grateful to Mrs Z Haidarbarghi for her assistance in the experiments; also we thank Mrs Ahmadi, for sincere cooperation.

Refrences

1. EPA (state of Ohio Environmental Protection Ageney) Persistent, Bioaccumulative and toxic chemicals. *Pollution Preventation Fact Sheet*. 2002; **99:**1-2.
2. John B sullivan, Jr Gary R krieger. *Clinical Environmental Health and toxic Exposures*. 2nd ed .USA, Lippincott Willams & wilkins; 2001: 1248-60.
3. EULA Bingha M, Barbara Cohrssen, Charles H powell. *Patty's Toxicology*. 3 rd ed. Canada john wiley & sohn. 2001; **4:** 383-98.

4. Michael J Derelanko, Mannfred A Hollinger. *Handbook of toxicology*. 2nd ed. CRC press; 2001.
5. M Habeck. *Toxic: phenol*. Eco-USA.2003.
6. JOO-Hwa Tay. Imroved anaerobic de- gradation of phenol with supplemental glucose. *Journal of Environmental Engineering*. 2001; **127** (1): 38-45.
7. Swaminathan K, Subrahmanyam PV. Biodegradation of P- nitrophenol in anaerobic fixed film fixed bed reactor. *Indian J Environ Health*. 2002; **44** (1): 8-11.
8. S Kar, T Swaminathan, A Baradarajan. Studies on biodegradation of a mixture of toxic and nontoxic pollutant using *Arthrobacter* species. *Bioprocess and Biosystems Engineering*. 1996; **15** (4):195-99.
9. Tarighian AR, Hill G, Headley J, Pedras S. Enhancement of 4-chloro phenol biodegradation using glucose. *Clean Technologies and Environmental Policy*. 2003; **5** (1): 61-65.
10. Benjamin S. Magbanua, Pamela A. Hoover, Patrick J. Campbell, Aln R. Bowers. The effect of cosubstrate on phenol degradation kinetics. *Water Science and Technology*. 1994; **30** (9): 67-77.
11. Rehm H J Reed G. *Biotechnology*. 2nd ed. Germany. Wiely-VCH. 1999; **11a:** 150-57.
12. Jeong JJ, Kim JH, Kim CK, Hwang I, Lee K. 3- and 4-alkylphenol degradation pathway in *Pseudomonas* sp. strain KL28: genetic organization of the lap gene cluster and substrate specificities of phenol hydroxylase and catechol 2, 3-dioxygenase. *Microbiology*. 2003; **149**(Pt 11): 3265-77.
13. Shimazu M, Mulchandani A, Chen W. Simultaneous degradation of organic phosphorus pesticides and p-nitro phenol by a genetically engineering *Moraxella* SP. *Biotecnol Bioeng*. 2001; **76:** 318-24.