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Identification of Phenol Degrader Microorganisms in the Integrated BF/AS Combined System

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Abstract: Chemical disposal and toxic pollutants are always one of the main environmental pollutants of water sources distributed from industrial wastewaters. The most suitable method for treatment such these wastewaters is biological systems. Effective microorganisms variety obviously depend on the type of pollutant, system and environmental conditions. Identification of the most effective microorganism is necessary for determination of optimum conditions, controlling method and monitoring of bioreactor to access the maximum efficiency and improvement of operation. The objective of this study is identification of effective microorganisms in phenol treatment procedure in combined biological system of biofilter and activated sludge Some amount of biological sludge was provided from domestic wastewater treatment plant as a primary source of microbe and added to the designed reactor. Then samples were collected after growth of microbial mass. Phenol concentration and environmental condition (i.e., dissolved oxygen and pH) were stabilized after gradually adaptation of the system to phenol. All samples were collected by sterile glass container. These samples were cultured on enrichment media and identified by various differential tests. Identification results proved phenol degrader bacteria are aerobe, nonfrementer and of negative for glucose test. These isolated bacteria were *Pseudomonas aerginosa*, *Pseudomonas alcaligenes*, *Moraxella* sp., *Acinetobacter* sp. and *Brevundiomonas vesicularis*. All these biodegrader microorganisms used only phenol for the both carbon and energy source, since phenol, nitrogen and phosphorus were the only available substrate in this system. Phenol was degraded at completely aerobic condition and dissolved oxygen concentration was sufficient.

Key words: Phenol degrader, microorganisms, combined system

INTRODUCTION

Phenol is a toxic aromatic hydrocarbon, which Federal Water Pollution Act (FWPA) was putted in list of priority pollutants (Sullivan *et al.*, 2001). This compound and it's derivatives have been used in many industries such as production of resin, color, pesticide, pharmacy, petroleum, coal mine, steel and aluminum industries. These industries can cause environmental pollution and especially on water sources due to unhealthy discharge of wastewater (Eulabingham *et al.*, 2001; Patterson, 1975). Many methods have been used for phenolic wastewater treatment which some of most the important methods are chemical oxidation, adsorption, biological treatment and some combination of these methods (Freeman, 1989). Biological system is more used in comparison with to other mentioned methods above because of its special advantages such as more compatibility to environment. In the other word, use of biological system is safer than other processes in environment (Freeman, 1989). In absorption process, pollutants temporarily absorb on the absorber without any changes and it is possible that under some condition desorbtion process can emit pollutants to the environment. Next advantage that can be mentioned is usually no need to apply any hazardous chemical compounds. Therefore, effluent and sludge produced discharge have less bad effects than chemical process on the acceptor sources in this process. The main advantage of attached growth systems is its more possibility of presence variety types and number of microorganisms. Therefore their tolerance, flexibility and reliability are high when high organic compound are loaded, but possibility of effluent standard approach is higher in suspended growth system. Therefore combined biological treatment was used to remove of phenol due to approach to these two advantages in this investigation (Rehm and Reed, 1999).

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It is clear that microorganisms and their varieties are responsible for conversion of interested pollutant matter in biological treatment systems. Thus, identification microbial varieties are useful to have effective treatment system for designers and their operators, also researchers specially environmentalists and biotechnologists.

Many researchers studied and identified phenol degrader microorganism in different types of wastewater treatment systems, and activated sludge (Tchobanoglous and Urton, 2003). But so far, identification of phenol degrader microorganism has not been studied in combined biological BF/AS treatment systems.

MATERIALS AND METHODS

Physical properties of system: The system is a combined biological process includes activated sludge and biofilter reactors as a series. Type of hydraulic flow is continuous and incidentally some of sediment microorganism in sedimentation tank returns to entrance of system. Physical characteristic and environmental condition of the system include:

Capacity of system = 25 l
Hydraulic detention time = 7 h
Concentration of phenol = 500 mg L^{-1}
Efficiency of phenol removal = 99.9%
Dissolved oxygen = 2 mg L^{-1}, pH = 7.5 and T = 30°C
The schematic and flow diagram of bioreactor was showed in Fig. 1.

Microorganisms provided and their adaptation procedure with phenol: Primary source of microorganisms that were provided from biological sludge of municipal wastewater treatment plant, added to the reactor with continuous dry milk solution as substrate and suitable growth condition for one month. Then Microorganisms were grown up to form biofilm on the media. In the next step, phenol was added in 0.1 mg L^{-1} concentration as well as dry milk solution. Then phenol concentration gradually was increased and concentration of dry milk was decreased. Finally phenol was as an only substrate source in the reactor after 3 months with nitrogen and phosphorus as necessary nutrients.

Sampling: In this study samples were collected in a sterile glass bottle from sediment microorganisms from after nine month. Each sample was analyzed 3 times to meet high accuracy and confidence.

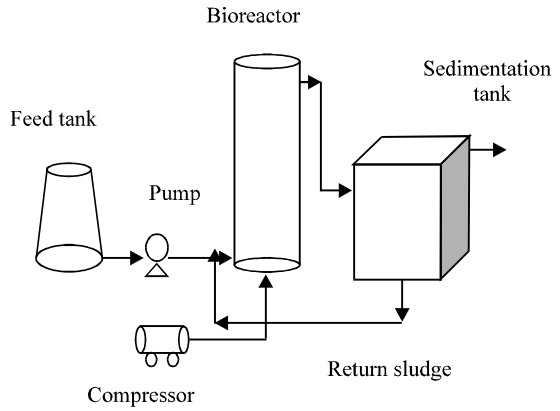

Fig. 1: Schematic and flow diagram BF/AS integrated combined system

Microorganism identification

Primary isolation: Blood agar, Eosin Methylen Blue (EMB) and Brain Heart Infusion (BHI) agar cultures were used for primary isolation. All type of colonies was separated and sub cultured for next purification and biochemical identification tests.

Applied differential tests for identification: Biochemical tests were used to identification after direct observation. Various microorganisms were isolated on blood agar, Eosin Methylen Blue (EMB) and Brain Heart Infusion (BHI) agar. Following tests were used to Identify isolated bacteria: Catalase, SIM, Urea, OF (glucose), Citrate, TSI susceptibility to polymyxin, Gelatin, Dnase, Manitol, Maltose tests and Aesculin hydrolysis (Koneman *et al*., 1997; MacFaddin, 2000).

RESULTS

Microorganism's adaptation results: Provided results revealed microorganisms can be adapted during 3 month adaptation period with phenol so that they use phenol as a only substrate source.

Microbial analysis results in studied system

Microscopic examination of the direct sample smear: Microscopic study of the sample revealed following organisms were presented: different type of gram-negative bacilli and coco bacilli, fungi, amoeba, and spirochetes.

Isolation and identification results: Selective and differential applied media proved following organisms (Table 1):

1-*Pseudomonas aeruginosa*
2-*Pseudomonas alcaligenes*
3-*Acinetobacter*
4-*Moraxella*
5-*Brevundiomonas vesicalaris*

Table 1: Characteristics of identified phenol degrading bacteria in BF/AS

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Other tests
P. aeroginosa	GNB	GP	+	+	+	K/K	+	-	-	+	S	O	+	-	GROWTH AT 42°C Sensitive to CB
Moraxella	Cocci	YC	-	+	+	K/K	-	-	-	-	S,W	-	-	-	Sensitive to penicillin
B. vesicularis	GNB	YC	+	+	+	K/K	-	-	-	+	S	-	+	+	Oxidative on sugar
P. alcaligenes	GNB	+	+	+	+	K/K	-	-	-	+	S	-	-	-	-
Acenitobacter	GNB	+	+	+	-	K/K	-	-	-	-	ND	-	-	-	Resistant to sugar

O: Oxidative, W: Weak, GP: Green Pigment, YC: Yellow Colony, ND: Not Done, GNB: Gram Negative Bacilli, S: Sensitive
1: Morphology, 2: Blood Agar, 3: EMB, 4: Catalase , 5: Oxidase, 6: TSI, 7: Citrate, 8: Urease, 9: Indole, 10: Motility, 11: Polymixin, 12: Glucose, 13: Jelatinase, 14: Aesculin, +: Present, -: Absent

Table 2: Characteristics of unknown phenol degrading bacteria in BF/AS system

Morphology	Blood Agar	EMB	Catalase	Oxidase	TSI	Citrate	Urease	Indole	Motility	Polymixin	Glucose	Jelatinase	Aesculin
GNSB	+	+	+	+	K/K	-	-	+/ W	+	S	-	-	-

W: Weak, GNSB: Gram Negative Short Bacilli, S: Sensitive, VW: Very Weak, V: Variable

Besides of these bacteria another organism was isolated that could not be identified because of the unavailability some differential test. Applied tests and their results are mentioned in Table 2.

Comparisons the isolated organisms after adaptation with isolated organisms in primary source: Samples were taken before and during adaptation period. Various microorganisms were observed on microscopic examination but some of them were not isolated, because in this study only phenol adaptation of bacteria were considered to be studied. First experiments on isolation proved the persistence of Bacillus, *E. coli neisseria*, and Flavobacterium in primary source while they were not isolated after using phenol as the only carbon source.

DISCUSSION

Provided results confirmed following points. First, biological treatment system proved possibility of adaptation of microorganisms to use phenol as a substrate source, resulting used up wastewater and contaminated source. Second, dry milk solution that was used in the biological treatment system as a primary substrate, replaced gradually by the phenol, sample was taken after 9-month adaptation. This means all identified bacteria used phenol as the only substrate source since they do not have spores. All microorganisms were aerobic; meaning the amount of oxygen dissolved was sufficient for degrading phenol and growth of bacteria in bioreactor. Additionally, this point indicated metabolic pathway of phenol by microorganisms and product degraders. All identified bacteria were gram negative and non-glucose frementors.

Two types of identified microorganisms were *P. aeroginosa* and *P. alcanigens*. The ability of *Psuedomonas* sp. to degrade phenol was previously reported by Cerniglia and Crow. They reported *P. putidai* has ability to degrade phenol (Riser-Roberts, 1992).

German and his colleagues identified a *Psuedomonas* sp. from activated sludge system that had been used for treatment of phenolic wastewater, but they did not determine what was species of *Psuedomonas* (German and Ariel, 1996). El-Sayed *et al.* (2003) found a new phenol degradation bacterium with high biodegradation activity were isolated as *Burkholderiacepacia* PW3 and *Pseudomonas aeruginosa* AT2 (Shimazu *et al.,* 2001).

Psuedomonas sp. have high ability for degrading organic contaminants such as phenol. These bacteria are gram negative that does not ferment glucose. Some of these bacteria are capable to use more than 100 organic substances as a carbon source. The high ability of these organisms is not just due to production of catabolic enzymes, but depend to regulate pathway too. Incidentally Jeong *et al.* (2003) showed phenol degradation pathway in *Pseudomonas* sp. strain KL28 (El-Sayed *et al.*, 2003). The ability of Moraxella has been confirmed for degrading phenol by different researchers. Recently Shmazu and his colleagues have enhanced its ability in degrading of organo-phosohorus by use genetic engineering method (Jeong *et al.,* 2003).

At last the other isolated bacteria *Brevendimonas vesicularis* has not been reported as phenol-degraded organisms yet. *Brevudimonas* has capability to degrade the herbicides 4-(2,4-dichlorophenoxy) butric acid and 4-(4-chloro-methyphenoxy) butyric acid (Smejkal *et al.*, 2003).

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