Iranian Journal of Chemical Engineering
Vol. 4, No. 1 (Winter), 2007, IAChE

Application of Bioaugmentation Technology to Improve the Activated Sludge Treatment Process in Removal of Aromatic Compounds

F. Amiri, S. Yaghmaei and S. Samie*

Chemical and Petroleum Engineering Department, Center of Excellence, Development and Strategic Plants for Bioprocess Technology, Sharif University of Technology, Tehran, Iran

Abstract

This investigation was designed to evaluate the effects of bioaugmentation on maintaining the system stability under shock loading conditions, standardizing the effluent, and improving the sludge settlement. In this study, phenol was chosen as a model of mono-aromatic compounds which are found commonly in many industrial wastewaters, especially petroleum refineries and the petrochemical industry in Iran. Impacts of bioaugmentation with the best isolated microorganism on the system performance facing sudden toxic shock were investigated after acclimatizing the system with phenol, isolating the phenol degrading microorganisms, and selecting the best phenol degrading strain. Results indicated that this method was improved the efficiency of the system under shock loading from 30% to 94% and SVI from 333 ml/g to 80 ml/g. The effluent was standardized after bioaugmentation at a minimum HRTs of 10, 10, 12 and 24 h, respectively, and at influent COD of 800, 1000, 1500 and 2000 mg/l. The system efficiency and SVI were located in an average range of 99.4-99.9% and 50-71 ml/g, respectively, and the sludge growth was good, even under high organic loading rates after bioaugmentation. In conclusion, bioaugmentation could be used as an effective and efficient method to improve a CAS process facing sudden toxic pollutant shock loading.

Keywords*: Bioaugmentation, Industrial Wastewater, Activated Sludge System, Phenol*

Introduction

The production and usage of man-made chemicals in industry has led to the entry of many dangerous compounds into the environment. One of these groups is aromatics [1]. Due to their high toxicity, recalcitrance, strong odor emission, persistence in the environment and suspected carcinogenic and mutagenic potentials for the living, aromatics pose serious ecological threats as environmental pollutants. So, their fate in the environment is of great importance [2].

Phenolic compounds, included in mono-aromatics, are among the hazardous pollutants which enter the environment easily through wastewater discharges from a variety of industries such as leather, phenol formal-

∗ Corresponding author: E-mail: yaghmaei@sharif.edu

dehyde resin, oil refineries, the petrochemical industry, coking plants, pharmaceuticals, and coal conversion [1,3,4]. Phenolic compounds are toxic to aquatic life, plants and many other organisms. When phenolcontaining water is chlorinated, toxic polychlorinated phenols result, thereby contributing to off flavors in drinking and food processing waters [2-4]. As a consequence, it is necessary to treat phenolic wastewater and reduc phenol content to the standard value of less than 1 mg/l, as recommended by environmental organizations, prior to disposal into the streams [5].

Different treatment methods are available for the reduction of phenol content in wastewater. These technologies include chlorination, ozonation, adsorption, solvent extraction, membrane process, coagulation, flocculation and biological treatment [6-9]. Physicochemical methods have proven to be costly and have the inherent drawbacks due to the tendency of the formation of secondary toxic materials such as chlorinated phenols, and hydrocarbons. Thus the biological method of treatment has turned out to be a favorable alternative for phenol degradation [6, 8].

Conventional activated sludge (CAS) systems are still widely used in wastewater treatment plants in Iran. They work generally well for easily degraded components of wastewater, but not for hazardous components, which are toxic to microorganisms, or slow to degrade. A sudden increase in recalcitrant compounds loading rate or the introduction of some unexpected recalcitrant compounds in influents may easily cause failure of the CAS system [10]. So, an efficient and reliable method to improve the removal of recalcitrant compounds is critically important to prevent toxicity discharge and system upset, especially for conventional activated sludge (CAS) systems with poor anti-shock loading capacities [6, 8].

The use of powdered activated carbon (PAC) added to the aeration basins is a common method in refineries which is seen consistently. This is occasionally used to help the biomass when there is a toxic spill. This approach helps to maintain the stability of the system under toxic shock and enhance the efficiency [6, 7]. However, the high capital and operating costs are major disadvantages of this method. On the other hand the continual use of PAC takes the spaces in the aeration basin, creates more solids, and leaves less room for a healthy active biomass [7].

The biomass is the workforce of a waste treatment system. In a dynamic state of flux, different microorganisms are dying while others grow and become more dominant. Even though the natural microbial population may develop into an acceptable one, under adverse conditions such as toxic shock, certain microbial populations may be reduced or eliminated, causing poor effluent quality. Under such conditions, wastewater treatment plants will be slow to recover [4, 11-13]. Therefore the system failure is mainly due to the low population of microorganisms capable of degrading these recalcitrant compounds and the inhibition of these pollutants to the AS in the system [1, 4, 6].

The biological additives industry, known as bioaugmentation, is one of the most straight forward strategies to remediate such system failures which can be used to solve problems of slow biomass recovery and supplying certain lost microbial populations. In other words, bioaugmentation is defined as the application of selected microorganisms to enhance the special microbial populations of an operating wastewater treatment facility to improve water quality. In addition, bioaugmentation provides an overall healthier biomass and has a lower operating cost in comparison to PAC method [4, 6, 10]. On the other hand, bioaugmentation is employed as a bioremediation technology to remediate the pollutants at contaminated sites such as soil and water [11].

An important step in biological wastewater

treatment is solid removal, usually through settling in a clarifier. Microorganisms form a natural biopolymer that aids in settling. Toxic shocks and system changes can result in the domination of filamentous bacteria, and a biomass population with little biopolymer which has poor settling characteristics. The traditional approach of adding organic polymers or inorganic coagulants as settling aids can be effective but expensive. By inoculating the system with organisms known to be both resistant to the toxicity and excellent floc formers, polymer demand can be greatly reduced or eliminated. Typically, the cost of bioaugmentation is significantly less than the polymer treatment [10].

Successful bioaugmentation requires total system management, and must be accomplished in a planned and controlled manner to maintain the integrity of the microbial ecosystem [13-15].

Some current researches on bioaugmentation were conducted in sequence batch reactors (SBR), a membrane separation bioreactor (MBR), and a lagoon. The operation mode of SBR, MBR and lagoon favor maintaining the supplemented culture in these systems [15-17]. However, it is more desirable for many wastewater treatment plants which have established CAS treatment systems to improve their original system capacity and reliability under occasional shock loading conditions without changing the original system or adding new facilities. Therefore it is necessary to know the efficacy of bioaugmentation in a CAS system under organic shock loadings [6].

The aim of this Study was to investigate and evaluate the potential utility of bioaugmentation with the best isolated phenol degrading microorganism in the CAS system to enhance system stability under shock loading conditions, standardizing the effluent, and improving the sludge settlement.

Experimental procedures

Reactor design, wastewater composition and experimental conditions

One rectangular 8.5-l laboratory-scale continuous flow complete mixed reactor (CFSTR) was used in this experiment (as shown in Fig.1). This glass reactor had a 7-l aeration zone, and a 1.5-l internal clarifier allowing continuous sludge recycling. Synthetic wastewater was pumped into the

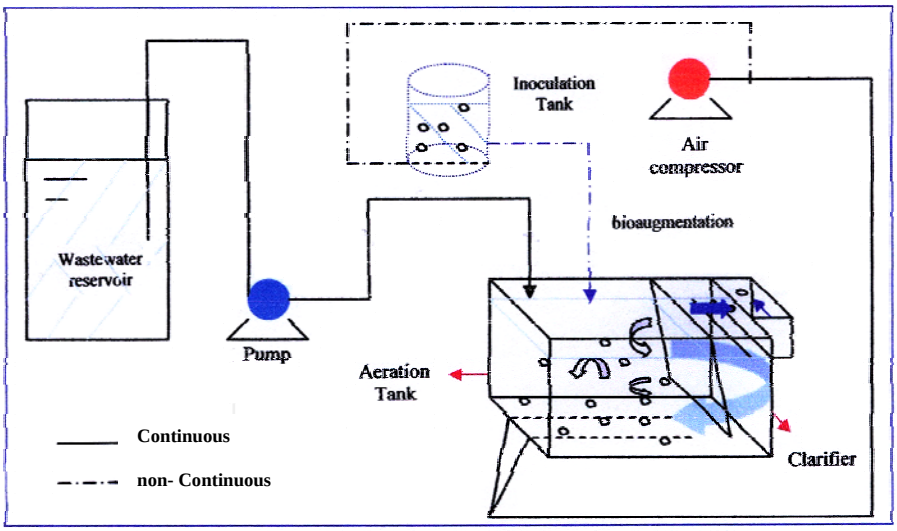

Figure 1. Schematic diagram showing the use of bioaugmentation in the CAS system

aeration zone of the reactor, and air was introduced into the reactor through diffusers placed at the bottom of the aeration zone. Synthetic wastewater was composed of phenol as the carbon source, urea (CH_4N_2O) as the nitrogen source, and ammonium phosphate ($(NH_4)_3PO_4$) as the phosphorus source. Over the acclimatization period, molasses was added to the influent as a co-substrate. Molasses and a phenol con-centration of 1g/l creates, experimentally, a COD of about 700 mg/l and 2200 mg/l, respectively. During the whole experiment, the COD: N: P ratio of the synthetic wastewater was equal to 100: 5: 1. Dissolved oxygen (DO) was kept at 2-3 mg/l, and temperature was set at 23-26 $^{\circ}$C. Sludge retention time (SRT) was controlled at about 25 days through removing a certain volume of mixed liquor from the aeration zone of the CAS reactor.

Reactor start-up

Inoculation sludge, used as the indigenous microorganisms in the CAS reactor, was the AS from the Gheitarieh Municipal Waste-water Treatment Plant (north-east of Tehran), which had little chance to come in contact with phenol. The initial AS concentration was 1.5 g/l in the reactor. In order to enrich the biomass, a synthetic wastewater based on molasses, urea, and ammonium phosphate at the constant COD of 150 mg/l was fed to the reactor continuously at HRT= 12 h, for about one month.

Acclimatizing the AS biomass with phenol

To acclimatize the activated sludge cells, the concentration of molasses in the reactor influent was gradually increased to COD=700 mg/l over one month. Then, a low concentration of phenol, about 5 mg/l, was added to the influent. Phenol entering into the reactor caused bulking resulting in some of the sludge from the reactor being leftover. In order to return the lost AS, semi-continuous feeding was used with molasses, urea and ammonium phosphate. To remediate the bulking issue, doubled concentrations of urea and ammonium phosphate were applied, in addition to a low concentration of $FeCl_2$ (about 2 mg/l). Continuous feeding was reused with molasses, and a usual con-centration of urea and ammonium phosphate at COD=700 mg/l and HRT=12 h after the system came back to an appropriate situation. Indeed, another influent with a phenol concentration of 5 mg/l entered the reactor at HRT=36 h. With a decrease in the amount of sludge volume index (SVI) after one week, phenol was fed to the reactor with molasses, urea, and ammonium phosphate under one influent, at HRT=12 h. Acclimatization to phenol was performed by slowly decreasing the molasses concentration and increasing the phenol in the influent. The feeding solution in water was prepared at a concentration of molasses and phenol in which the maximum COD became 700 mg/l. Acclimatization was accomplished in two months when the AS was able to use 200 mg/l phenol as the sole carbon source. After the influent was switched to phenol containing wastewater, the system performance was investigated at three HRTs of 12, 10, and 8 h.

Isolation of the phenol degrading micro-organisms

To isolate the phenol degrading microorganisms, one sample was taken from the reactor, and after microscopic studies, it was cultured in two growth mediums: 1-nutrient agar (NA) for the separation of bacteria, and 2-potato dextrose agar (PDA) for the separation of fungi and yeast. Four strains were recognized from the formed colonies: N1 & N2 from the NA culture and P1 & P2 from the PDA culture. To isolate each strain, a pure colony of each kind was separately re-cultured, and this step was repeated for four times until a pure culture for each strain was gained. It was understood from microscopic studies that N1 was cocci, N2 was bacillus, and P1 & P2 were yeast (P2 was bigger and more spherical than P1).

Also, after gram staining, N1 was recognized as gram negative, and N2 as gram positive. To finish the isolation procedure, two slants were prepared separately from each strain. In the next stage, the performance of each strain was investigated at phenol concentrations of 500, 1000, 1500, and 2000 mg/l in shaking flask experiments. The phenol specific growth medium contains (g/l): $(NH_4)_2SO_4$ 3, KH_2PO_4 0.6, K_2HPO_4 2.4, $MgSO_4.7H_2O$ 1.5, $CaSO_4$ 0.15, and $FeSO_4$ 0.03. Strain P2, which had the best phenol removal efficiency, was selected for bioaugmentation in the reactor. Now, this strain exists in the microbial collection of the biochemical and bioenvironmental research center located in Sharif University of Technology, with the identity code of BBRC-9026 (Fig. 2).

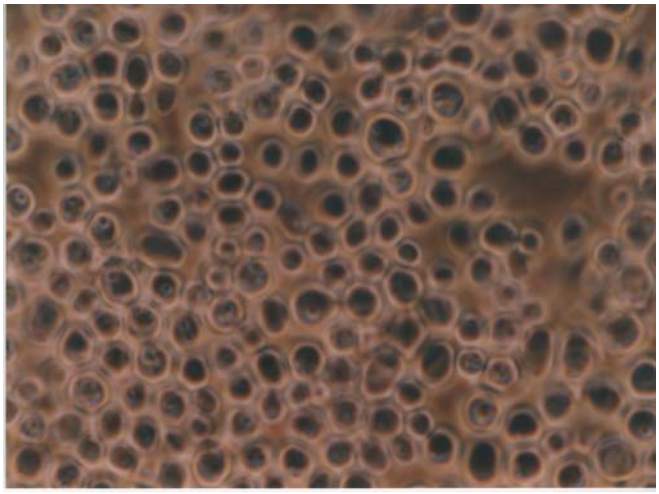

Figure 2. Microscopic image of Yeast BBRC-9026

Impact of bioaugmentation on system performance

Influent phenol (COD) concentration was suddenly increased by adding more 90 mg/l (200 mg/l) of phenol (COD) to the influent at HRT=12 h in order to investigate if the system is able to withstand sudden phenol overdose. Consequently, system failure occurred. To remediate the system upset, bioaugmentation of the best isolated micro-organism in the reactor was conducted at the next stage.

Following the enrichment of yeast BBRC-9026 in two steps, first in the yeast-malt growth medium (YM) and second in the phenol specific growth medium (with phenol concentration of 500 mg/l), 700 ml (about 10% (v/v) of aeration tank) of microbial suspension was prepared with a concentration of 10^6 cell/ml in the phenol specific growth medium. This was then gradually added to the reactor from a 1- l vessel (Fig. 1).

System performance was investigated at influent COD concentrations of 800, 1000, 1500, and 2000 mg/l, and four HRTs of 24, 12, 10, and 8 h after bioaugmentation. Indeed, microscopic observations were performed during the experiments.

Abiotic losses

In order to estimate the amount of volatilization or stripping of phenol from the activated sludge system, an investigation was performed in a cell free system at COD=1000mg/l and HRTs of 8, 10, 12, and 24 h, at the same conditions with other experiments.

Analytical methods

Phenol concentration, MLVSS, COD, and SVI were determined by following the standard methods [18].

The effluent phenol concentration was analyzed using a UV-Vis spectrophotometer (Model Spectronic 21D) in UV range. The maximum wavelength was found to be 274nm, but the color of the growth medium in shaking flask experiments created difficulty in measuring the phenol concentration in the samples in the UV range. Therefore, the phenol concentration was estimated by a direct photometric method based on rapid condensation with 4-aminoantipyrene, fol-lowed by oxidation with potassium fer-ricyanide under alkaline conditions to give a red color product.

Results and Discussion

As shown in Figs. 3-4, feeding of phenol in the influent caused an apparent decrease in COD removal efficiency, and a great increase in SVI in the acclimatization period. It is mainly due to the toxicity of phenol to the un-acclimatized AS, while continuing the AS acclimatization with phenol caused a gradual enhancement in COD removal efficiency and a gradual decline in SVI. This phenomenon could be explained by the induction of necessary enzymes for phenol degradation in the microorganisms which could tolerate phenol during acclimatization [3]. Therefore, the AS found the ability to consume phenol as a substrate through adaptation, which resulted in the reduction of the toxicity exerted by phenol and the improvement of the sludge settling.

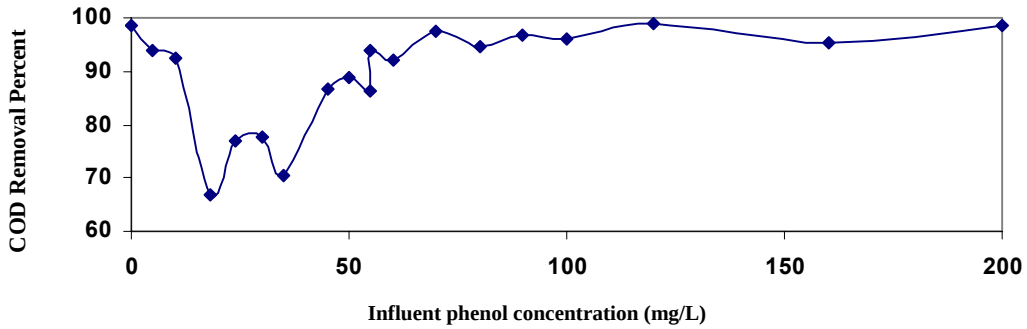

Figure 3. COD removal percent changes in acclimatization period with gradual increasing of influent phenol concentration (in presence of molasses), at HRT=12 h

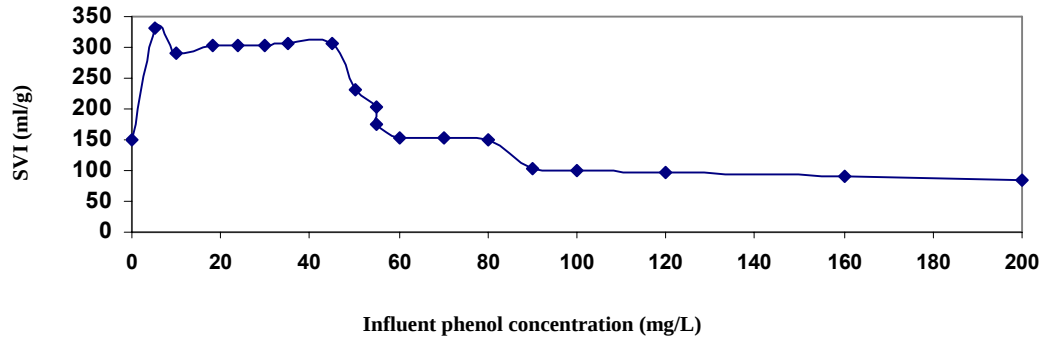

Figure 4. Sludge volume index changes in acclimatization period with gradual increasing of influent phenol concentration (in presence of molasses), at HRT=12 h

Fig. 5 illustrates the system performance in absence of a co-substrate (molasses) with a gradual increase in phenol concentration at three HRTs of 12, 10, and 8 h. In every increase, system conditions were considered as well as the amount of MLVSS, F/M, and SVI to avoid system shocking. In this way, the phenol concentration was increased to 700 mg/l at HRTs of 12, 10, and 8 h.
The system efficiency was found to be more than 90%, which shows the high ability of the acclimatized sludge to degrade phenol. However, the standardized effluent was not achieved, and this situation became worse at higher effluent phenol concentrations (more than 550 mg/l), especially at HRT=8 h. It is mainly due to increasing the organic loading on the system, and the disability of the AS to handle this situation properly.

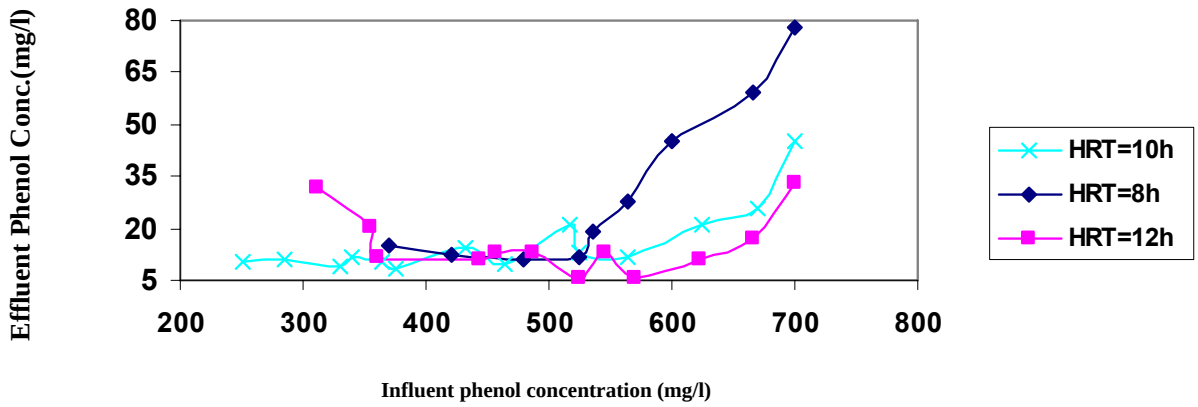

Figure 5. Effluent phenol concentration. vs. influent phenol concentration. in the acclimatized CAS system (in absence of molasses), at HRTs of 12, 10, and 8 h

System resistance under phenol shock loading was investigated by the sudden addition of 90 mg/l (200 mg/l) of phenol (COD) to the influent at HRT=12 h. As indicated in Fig.6, this increase caused a sudden and severe decline in phenol removal percent to 30%, and obvious enhancement in SVI to 333 ml/g. This situation continued for 8 days. So despite the fact that the acclimatized AS system had high phenol removal efficiency, it was not stable under shock loading condition. The system failure was considered mainly due to the low population of microorganisms capable of degrading high concentrations of phenol and the inhibition of phenol to the AS in the system. Therefore, bioaugmentation technology was used to recover system upset. Bioaugmentation was done on the ninth day which caused an apparent recovery in the system behavior. Phenol removal efficiency increased to 94% and SVI decreased to 80ml/g until the 15^{th} day.
It is obvious that the entry of a superior strain to the system had positive effects on handling the shock loading condition. It was not only capable of degrading high phenol concentrations, but also an excellent floc-former (Fig. 6).

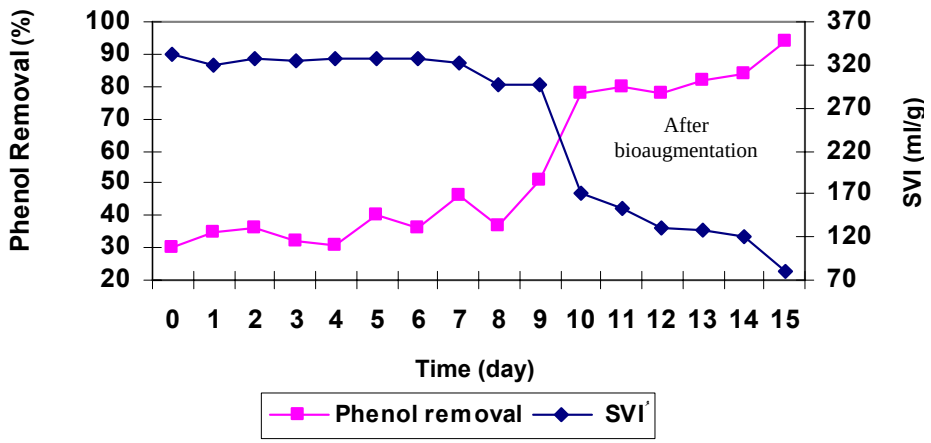

Figure 6. Phenol removal percent and SVI changes vs. time, under shock loading condition, before and after bioaugmentation at HRT=12 h

Bioaugmentation is not done permanently, but due to system upsets and influent composition changes, a maintenance dosage is required to maintain the desired population diversity. Under practical conditions, wastewater generally is a mixture of multiple organic pollutants. Therefore, it is preferable to utilize mixed cultures than pure strains in a real wastewater treatment plant. Mixed cultures, which contain a variety of microorganisms, generally have wider ranges of substrate for utilization [4, 10].

As shown in Figs. 7-8 and as was expected, the system became resistant under the sudden increase of COD. After bioaugmentation, system efficiency was raised to over 99%. More importantly, effluent was standardized at HRTs of 10, 10, 12, and 24 h for influent COD concentrations of 800, 1000, 1500, and 2000 mg/l, respectively. There were slight changes in the effluent phenol concentration, even when the organic loading rate was enhanced by decreasing HRT or increasing the influent phenol concentration. This shows the ability of the AS in degrading high phenol concentrations after bioaugmentation.

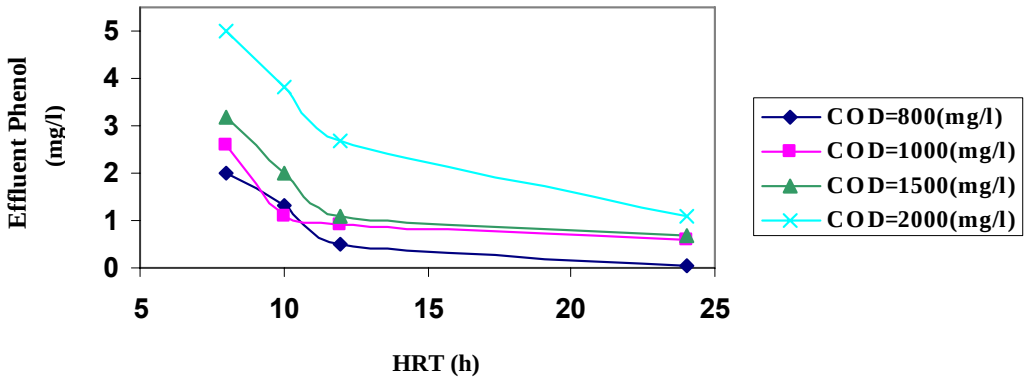

Figure 7. Effluent phenol concentration vs. HRT at different influent COD, after bioaugmentation

SVI increased at each COD by changing HRT from 24 to 12 h. This enhancement was expected because of a high decrease in HRT, and so a high increase in the organic loading rate. The positive point is that, despite this alteration, SVI remained in an acceptable range. On the other hand, SVI values improved at lower HRTs and also higher CODs (1500 & 2000 mg/l). It could be explained by more domination of the bioaugmented strain, which is a good floc-former, on the system at the higher organic loading rates (It was confirmed by microscopic observation).

By and large, after bioaugmentation neither sudden decrease in HRT, nor sudden increase of the influent phenol concentration, had an adverse effect on system performance, and there were no sharp changes in SVI and effluent phenol concentration values. The high stability and capacity of the system are good results of bioaugmentation.

The average growth of activated sludge versus HRT after bioaugmentation was much higher than that before bioaugmentation, considerably at HRT=8 h (Fig. 9). Low growth rate and a decline in average MLVSS at HRT=8 h before bioaumentation were the results of inhibitory effects exerted by increasing the phenol loading rate on sludge growth. However, there was no serious symptom of phenol inhibition on activated sludge growth by decreasing HRT after bioaugmentation. This was due to the domination of the bioaugmented strain (It was confirmed by microscopic observation), which could grow well in high organic loading conditions.

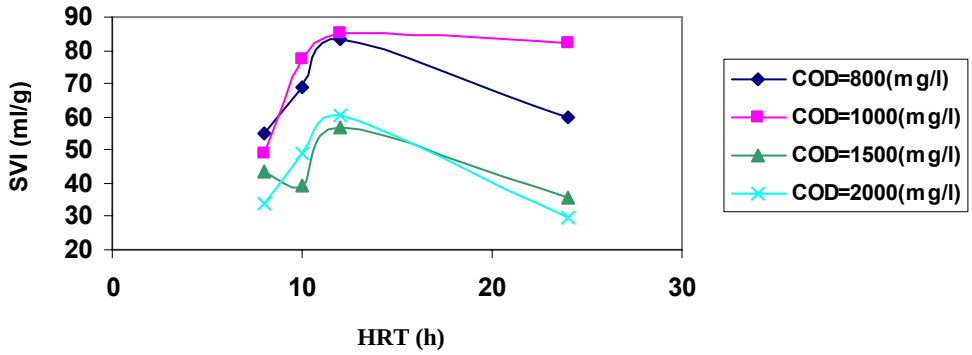

Figure 8. SVI changes vs. HRT at different influent COD, after bioaugmentation

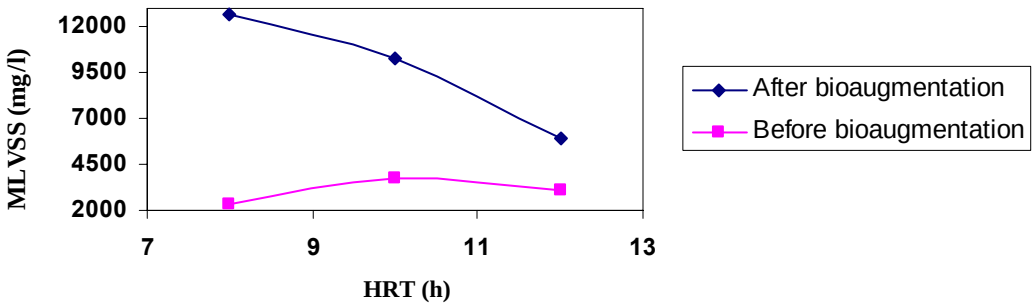

Figure 9. Comparison of average MLVSS vs. HRT, before and after bioaugmentation

As indicated in Fig. 10, sludge settlement before bioaugmentation was not acceptable. It was almost out of the range of 50-150ml/g. While after bioaugmentation the SVI level declined and was located averagely in the range of 50-71 ml/g. This was because of the good floc-forming capability of the bioaugmented strain, which resulted in enhancing settle-ability.

Therefore, forming activated sludge with good settlement quality is one of the bioaugmentation advantages.

It was found from cell free experiments that phenol stripping is less than 10%, even at HRT=24 h. According to Stenstrom et al. [9], this value should be less in the presence of acclimatized AS. As reported through the modeling results, volatilization of xylenol and compounds with similar Henry's law constants (~ 10^{-5}), such as phenol and cresol, is insignificant in the presence of high rates of biodegradation in activated sludge systems with common aeration rates. Typically, a mass fraction of less than 10^{-4} to 10^{-5} percent will occur with effective biodegradation.

In addition, Stenstrom et al. [9], following the extraction of hydrophobic mono-aromatic compounds such as xylenol, cresol, and phenol adsorbed onto the surface of the suspended solids in the activated sludge, by using a modified method from Warner [19], found that adsorption of these compounds to sludge particles does not occur significantly and can be ignored.

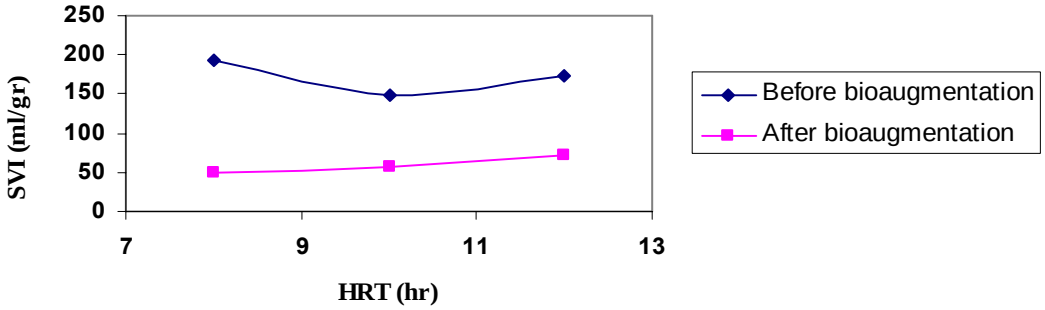

Figure 10. Comparison of average SVI vs. HRT, before and after bioaugmentation

Conclusions

The CAS system was not well suited to treat wastewater stressed by occasional increases in the loading rate of hazardous and recalcitrant organic compounds such as phenol. Application of bioaugmentation resulted in reducing the inhibitory action of phenol to AS activity, increasing system stability during the shock loading condition, standardizing the effluent, and improving AS settling.

It can be concluded that, bioaugmentation is a practical means to increase the resistance of a CAS system against shock loadings of recalcitrant compounds, and to remove hazardous compounds according to standards recommended by the Iran Environmental Organization. Using this method has no need to change the CAS system construction, so it can be used simply and widely in wastewater treatment units in Iran.

Abbreviation

AS	Activated Sludge
CAS	Conventional Activated Sludge
COD	Chemical oxygen demand
MLVSS	Mixed liquor suspended solid
SVI	Sludge volume Index

References

1. Cardinal, L.J., Stenstrom, M.K., "Enhanced Biodegradation of polyaromatic hydrocarbons in the activated sludge process", Research Journal Water Pollution Control Federation, 63 (7), 950 (1997).
2. Arutchelvan, V., Kanakasabai, V., Elangovan, R., Nagarajan, S., Muralikrishnan, V., "Kinetics of high strength phenol degradation using *Bacillus brevis*", Journal of Hazardous Material, Article in Press, (2005).
3. Kumar, A., Kumar, Sh., Kumar, S., "Biodegradation Kinetics of phenol and catechol using *Pseudomonas putida* MTCC1194", Biochemical Engineering Journal, 22,151 (2005).
4. Quan, X., Shi, H., Liu, H., Lv, P., Qian, Y., "Enhancement of 2, 4- dichlorophenol degradation in conventional activated sludge systems bioaugmented with mixed special culture", Water Research, 38, 245 (2004).
5. Environmental Standards, Environmental Protection Organization, (1378).
6. Yaghmaei, S., "Experimental comparison of two modifications of activated sludge for treatment of furfural- containing wastewater", Iranian Journal of Chemical Engineering, 2 (1), 3 (2005).
7. Ng, A., "Nitrification enhancement in the powdered activated carbon- activated sludge process for the treatment of petroleum refinery wastewaters", J. Water Pollut. Control Fed., 56 (1987).
8. Yaghmaei, "Studies on Treatment of Carbonaceous Wastewater by Fixed Film Aeration Tank", Scientia Iranica, 9, 47 (2002).
9. Stenstrom, M.K., Cardinal, L., Libra, J., "Treatment of Hazardous Substances in Wastewater Treatment Plants", Environmental Progress, 8(2), 107 (1989).
10. Wastewater bioaugmentation resource website: www.bioaugmentation.com
11. Amiri, F., Yaghmaei, S., Samie, S., "Bioaugmentation Technology and its Application in Bioremediation", Iranian Journal of chemical engineers (Persian), 5 (22), 6 (1385).
12. Wilderer, P.A., Rubio, M.A., Davids, L., "Impact of the addition of pure cultures on the performance of mixed culture reactors", Water Res, 25, 1307 (1991).
13. Sahinkaya, E., Dilek, F. B, "Biodegradation of 4-chlorophenol by acclimated and Unacclimated activated sludge- Evaluation of biokinetic coefficients", Environmental Research, 99, 243 (2005).
14. Dybas, M.J., Hyndman, D.W., Heine, R., Tiedje, J., Linning, K., Wiggert, D., Voice, T., Zhao, X., Dybas, L., and Criddle, C.S,"Development, operation, and long-term performance of a full-scale bio-curtain utilizing bioaugmentation", Environ. Sci. Technol., 36, 3635 (2002).
15. Hung, Y.T., Horsfall, F.L., Wong, J., "Bioconversion of accumulated sludge with bacterial augmentation process in aerated lagoon for municipal wastewater treatment". Int J Environ Stud, 28, 41(1989).
16. Yu, Z., Mohn, W.W., "Bioaugmentation with resin-acid- degrading bacteria enhances resin removal in sequencing batch reactors treating pulp mill effluents", Water Res, 35, 883 (2001).
17. Bouchez, T., Patureau, D., Wagner, M., Delgenes, J.P., Moletta, R., "Successful and unsuccessful bioaugmentation experiments monitored by fluorescent in situ hybridization", Water Sci Technol; 41(8), 61(2000).
18. APHA, AWWA and WPCF, Standard Methods for the Examination of Water and Wastewater", 18th ed., American public Health Association, Washington, DC, (1994).
19. Warner, J. S., Analytical procedures for determination organic priority pollutants in municipal sludge, EPA 600/280-030, USEPA, Cincinnati, OH, (1980).