J Integr Sci Technol, 2017, 5(1), 9-14 Article

Journal of Integrated
SCIENCE & TECHNOLOGY

Simultaneous treatment of phenol and cyanide containing synthetic/simulated waste water using mixed culture immobilized on coconut shell activated carbon biomass in a packed bed bio-column reactor

Neetu Singh, Chandrajit Balomajumder

Indian Institute of Technology, Department of Chemical Engineering, Roorkee, INDIA

Received on: 19-Apr-2016 Accepted on: 11-Aug-2016 Published on: 15-Sept-2016

ABSTRACT

The removal of phenol and cyanide from synthetic/simulated waste water using a packed bed bio-column reactor was investigated. Mixed culture *of Pseudomonas putida* MTCC 1194 and *Serretia odoriferra* MTCC 5700 has been immobilized on the iron impregnated coconut shell activated carbon (Fe-CSAC) bed in the packed bed bio-column reactor. The synthetic/simulated waste water sample comprising phenol and cyanide at initial concentration 500 and 50 mg/L, respectively, was used. Concentrations of phenol and cyanide have been found to be decreased, lower than permissible limits in the treated water. The significant influence of bed height (cm) and empty bed contact time (EBCT) was examined onto the simultaneous removal of phenol and cyanide in the packed bed bio-column reactor. On the other hand, after 4-5 days of continuous treatment of phenol and cyanide the influence of process parameters was not noteworthy. Dissolved oxygen (D.O.) has been found to decline along with time and the pH of the solution in the packed bed bio-column reactor has decreased initially for 2 days but after that it became the same as the influent. Real industrial wastewater samples can be efficiently handled.

Keywords: Bio-Column Reactor, Cyanide, Mixed Culture, Phenol, P. putida, S. odorifera

INTRODUCTION

Over the past years, pollution in the environment has turn out to be a serious concern worldwide. The phenol and cyanide pollution in the environment affects the human health through the intake of polluted food and water. Poisoning through phenol and cyanide causes severe harm to animal and human health that can lead to death. Industries, which develop these pollutants, such as the iron-steel, coke, mining, petroleum refining, pesticide, paint, electroplating and explosives manufacturing considerably contribute to the pollution in the environment.[1-4] Health authorities and scientists have spent significant work in the remediation and stoppage of pollution. Since the health impact of the phenol and cyanide poisoning in water the maximum tolerance level of phenol and cyanide in drinking water has been set to 0.5 mg/L and 0.2 mg/L, respectively.[1,2] Various types of treatment technologies have been established to eliminate the contamination of phenol and cyanide in the environment through treatment of waste water. The most common treatment methods used are flocculation, coagulation, chemical oxidation, chemical precipitation, ozonation, membrane filtration and reverse osmosis.[5-11] Though, the effective costs and procedures for these treatment methods are known to be costly owing to the high manpower necessities and maintenance cost. In order to decrease the operative costs and increase the operational time of the treatment process, many batch studies have been accompanied biosorption of metals and organic compounds.[1,2,12]

Therefore, in the current study for the continuous simultaneous elimination of phenol and cyanide using bio column reactor has been carried out. A continuous up-flow packed bed bio-column was used for the biosorption process using immobilized microbial culture, then it can be worked easily, high removal percentage of contaminant can be attained. It can be simply scaled up from laboratory scale to industrial scale.[13] In literature several studies have been achieved on sole pollutants removal, but pollutants like phenol and cyanide are discharged simultaneously from industrial wastewater, such as iron-steel, coke, mining, and petroleum refining. Therefore, simultaneous removal of phenol and cyanide compounds is desired.[14] In this present study simultaneous removal of phenol and cyanide is carried out in a continuous packed bed bio column reactor since it

Prof. Chandrajit Balomajumder
Department of Chemical Engineering, Indian Institute of Technology Roorkee, Roorkee, INDIA
Email: chandfch@iitr.ernet.in, neeturbs@gmail.com

Cite as: *J. Integr. Sci. Technol., 2017, 5(1), 9-14.*

 JIST ISSN 2321-4635

http://pubs.iscience.in/jist

can be easily applied to industries level for the elimination of toxic pollutants such as phenol and cyanide.

Some of the bacteria which degrade cyanide are *Pseudomonas sp.*,[15] *Escherichia coli*,[16] *Acinetobacter sp.*,[17] and *Alcaligenes Bacillus pumilus*,[18] and phenol degrading microorganism are *Bacillus stearothermophilus*,[19] *Pseudomonas putida*,[20] *Pseudomonas flurorescens*,[21] and *Acinetobacter sp.*[22]

The benefit of using a bio column packed bed reactor is that it does not need regeneration of the biosorbent bed, for the reason that the pollutant adsorbed by the packing material is consumed by the microbes.[23] In a bio column packed bed reactor a thin layer of microbes (biolayer) is established onto the surface of biosorbent bed which acts as a biofiltration unit. The appropriateness of technology established in batch study is tested in a continuous bio-column reactor. Systematic backwashing is a significant process for packed bed biolayer reactors that is essential to avoid clogging, to keep an active biofilm, to avoid the rise of filamentous bacteria, and to improve the mass transfer of pollutants to the biofilm.[24,25] On the other hand, back washing also decreases the amount of active biomass in the bio column reactor, which could possibly lead to unsteady reactor performance.[26]

The main aim of this study is to determine the capability of mixed culture of *Pseudomonas putida and Serretia odorifera* immobilized on coconut shell activated carbon biomass for simultaneous removal of phenol and cyanide. The influence of (empty bed contact time) EBCT, bed height onto the percentage removal of phenol and cyanide was examined. The effect of pH, dissolved oxygen (D.O.) and growth of biomass was evaluated. The backwashing of bio column reactor was also performed.

Materials and Method

The source of microorganism, acclimatization of microorganism, experimental setup and experimental procedure are discussed as follows.

Source of microorganism

Pseudomonas putida MTCC 1194 species was obtained from Institute of Microbial Technology, Chandigarh, India. The strains were revived according to the instructions given by MTCC.[27] Cultures were kept on agar plates till further use and were sub cultured after every 30 days. All inoculations were performed in aseptic conditions in laminar air flow unit (Rescholar Equipment, INDIA). The strains *Serratia odoriferra* MTCC 5700 were isolated from coke waste water in laboratory by reported method.[28]

Growth and acclimatization of mixed culture

The growth mediums for mixed culture are as follows: Na_2HPO_4, KH_2PO_4, NaCl, NH_4Cl, $MgSO_4.7H_2O$, and Glucose. The acclimatization of mixed culture was carried out at different concentrations of phenol and cyanide, respectively. For acclimatization the bacterium was grown at different concentrations of phenol and cyanide in ratio of 10:1.[29] Based on the steel industry wastewater composition phenol and cyanide in synthetic/simulated wastewater was taken in (10:1) ratio for the reason that normally the concentration of phenol in waste effluent is more than cyanide.

Experimental setup and procedure

The experimental setup used for the simultaneous removal of phenol and cyanide was as described by Mondal et al.[23] A schematic diagram of the experimental setup is given in Figure 1, which is contained with bio-column reactor, feed tank, mixing chamber with stirrer, compressor, peristaltic pump, and filter units for air and water. The reactor assembly is a close circuit unit. The bio column packed bed reactor comprises of stainless steel pipe of 93.1 cm height, 8 cm internal diameter and 5 L net empty working volume. It was equipped with five equidistant ports of 1.25 cm diameter for collecting samples along the height of the bio-column reactor. The S.S reactor was steam sterilized at 121^{o}C and 15 psig pressure for 30 minutes. The bio-column reactor was filled with coconut shell activated carbon biomass immobilized with mixed culture of *P. putida* and *S. odorifera.* Coconut shell activated carbon biomass was washed with double distilled water in several time and then dried in hot air oven at 60 ^{o}C for 12 h. When all the volatile material and moisture was removed from coconut shell activated carbon biomass, it was filtered to attain a constant particle size. Growth medium was pumped through the bio-column reactor at the feed flow rate of 133.3 mL/h for the conditioning of biosorbent bed. After the conditioning of the packed bed, it was filled with mixed culture of *P. putida* and *S. odorifera* and left for two days for the immobilization of microbes onto the surface of biosorbent bed.

When the bacterium *mixed culture* was immobilized onto the biosorbent surface in packed bed bio-column, desired concentration of phenol and cyanide was passed through the bio-column.

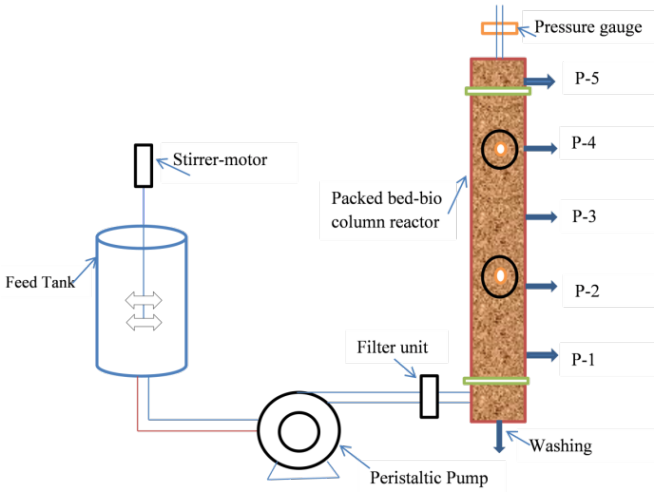

Figure 1. Schematic diagram of continuous packed bed bio-column reactor.

Results and Discussion

Study of biosorption of phenol and cyanide using immobilized *mixed culture* packed bed bio-column analyses the effect of operating parameters such as: EBCT and bed height on the elimination of phenol and cyanide,

backwashing of the reactor bed, change of pH and O.D. with time, elimination of other metals ions and the comparison of the new technique with some recently stated techniques are revealed in the following sections.

Effect of Operating Parameters

Effect of bed height

To Study to effects of bed height on the bio-removal of phenol and cyanide from synthetic/simulated wastewater, continuous experiments have been accompanied in a bio-column reactor with *mixed culture* immobilized on Fe-CSAC bed as shown in Figure 1. Treated water samples collected from port P1 to P5 after certain interval of reactor run have been analyzed for phenol and cyanide. Influent flow rate to the reactor has been keep up at 400 ml/h with corresponding EBCT value of 5h. The effect of bed height on bio-removal of phenol and cyanide by *mixed culture* immobilized on Fe-CSAC is shown in Fig 2(a) and 2(b), respectively. From Figure 2, it could be revealed that for first 2-3 days of bio-column reactor run for all bed height values, concentration of phenol and cyanide in effluent decreases in the initial stage of the run in the following order:

P5 <P4 <P3 <P2 <P1

However after a run of 7 days, phenol and cyanide concentration in the treated effluent collected from all the 5 ports becomes constant and less than the regulatory levels (0.5 mg/L for phenol and 0.2 mg/L for cyanide).

This statement can be described as follows:

At the initial stage, the bacteria take some time to adjust in the continuous mode of operation of the bio-column reactor. Hence, its efficiency decreases initially for some time until it is fully adjusted into the new environment. Therefore, the phenol and cyanide concentration in the treated water collected from various ports increases initially. With the increase in the bed height, the contact time of water sample with bacteria increases, which leads to the increase in phenol and cyanide removal. Due to this reason, the residual concentration of phenol and cyanide in treated water increases in the order as mentioned above for first 2-3 days of reactor run. After some time of operation, the bacteria are adjusted in the reactor and no effect of bed height is detected on the phenol and cyanide concentration of the treated water. It was observed that after 7 days of operation, the effect of bed height value on remaining concentration of phenol and cyanide becomes negligible which can be attributed to the adaptation of bacteria to the continuous mode of operation. Also the flow rate at the outlet was unstable when the bed depth was too high due to the higher flow resistance, which has resulted from a tighter packing of the longer bed comprising more amount of adsorbent. The increase in capacity of removal is due to the increase in travel time with increase in bed height and more volume phenol and cyanide passed through the packed bed. Though an increase in the bed depth increases the volume of phenol and cyanide adsorbed, too high bed depth is not beneficial for a single column: otherwise the multiple-bed may be designed. The comparable statement has been made by Mondal et al.,[23] for the treatment of arsenic contaminated water.

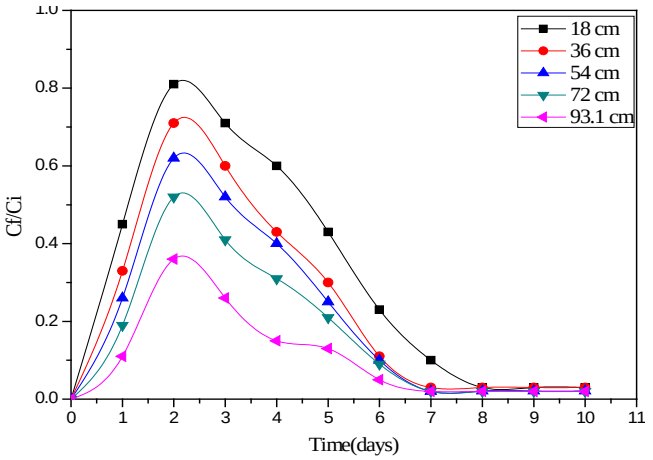

Figure 2(a) Effect to bed height on the bio-removal of phenol by *mixed culture* (Process conditions : temp: 30±1^{0}C, initial phenol concentration: 500 mg/L), initial , pH : 8 ± 0.1, run time : 0 to 10 days).

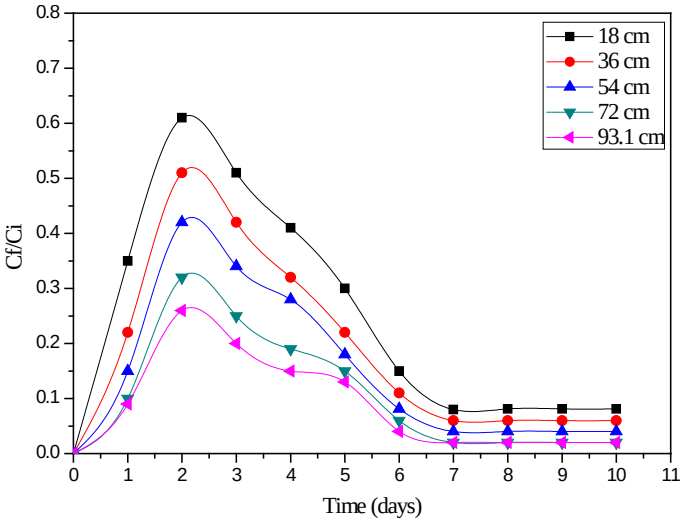

Figure 2(b) Effect to bed height on the bio-removal of cyanide by *mixed culture* (Process conditions : temp: 30±1^{0}C, initial cyanide concentration: 50 mg/L), initial , pH : 8.1 ± 0.1, run time : 0 to 10 days).

Effect EBCT on bio-removal of phenol and cyanide

To Study the effects of EBCT on the bio-removal of phenol and cyanide from synthetic/simulated coke plant effluent, experiments have been accompanied in the a bio-column reactor with mixed culture of *P. putida* MTCC 1194 and *S. odoriferra* MTCC 5700 immobilized on Fe-CSAC bed as shown in Figure 3(a) and 3(b). Samples collected at a regular interval of time from port P5 of bio-column rector run up to 150 hour have been analyzed for phenol and cyanide. Various flow rates rates 400 ml/h, 200 ml/h and 133.33 ml/h with corresponding EBCT values of 5, 10 and 15 h, respectively were used. It is revealed from figure 3 that from all the EBCT values, concentrations of both phenol and cyanide increases initially and reaches its maximum value and decreases thereafter with run time. This statement is attributed to the fact that the microbes take some time to adjust in the continuous operation of the bio-column reactor

as well as growth of bio-layer of on Fe-CSAC. The comparable statement has been made by Mondal et al.,[23] for the treatment of arsenic contaminated water. Therefore, residual concentration of phenol and cyanide initially increases for two days of reactor run time. During initial stage, up to the run time of 40-50 hour the phenol and cyanide concentration increases with the decrease in EBCT value. However, within 5 days of operation the concentration of both phenol and cyanide in treated water reaches the regulatory levels (0.5 mg/L for phenol and 0.2 mg/L for cyanide) for all EBCT values. This is due to the fact that at higher EBCT value, the contact time of the sample with the bio-layer is more, which reduces with the decrease in EBCT value.[30]

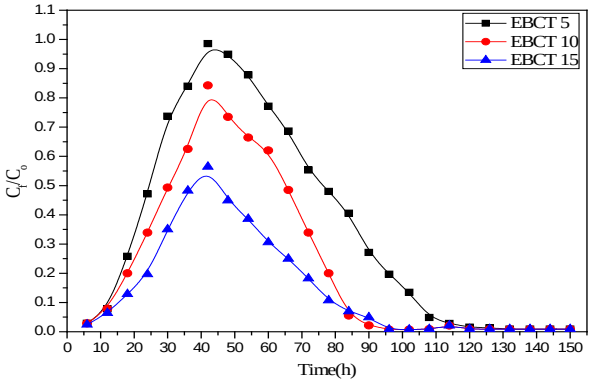

Figure 3(a) Effect of EBCT on the removal of phenol by *mixed culture* (Process conditions : temp: 30±1^{0}C, initial phenol concentration: 500 mg/L), initial pH : 8.0 ± 0.1, run time : 0 to 150 hours).

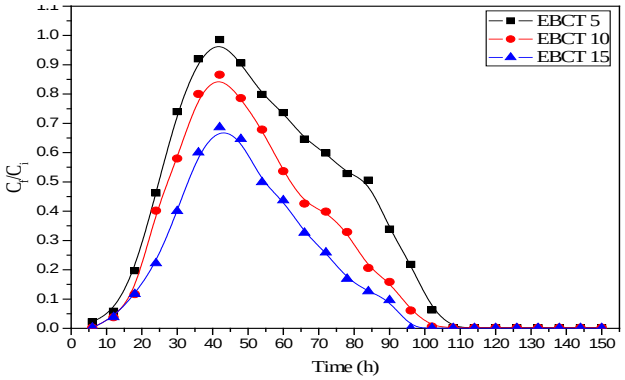

Figure 3(b). Effect of EBCT on the removal of cyanide by *mixed culture* (Process conditions : temp: 30±1^{0}C, initial cyanide concentration: 50 mg/L), initial pH : 8.0 ± 0.1, run time : 0 to 150 hours).

The lower EBCT values resulted in higher residual concentrations this is due to the fact that lower EBCT corresponds to less contact time between pollutant and the microbes. Therefore, the possibility for the accumulation of phenol and cyanide by the mixed culture decreases with the decrease in EBCT value. At initial stage of operation, with lower EBCT value, the water sample may leave the reactor before the microbes of the bio-film manage with the continuous operation. Therefore, at the initial stage of operation the phenol and cyanide removal is less. With the increase in run time bacterial mass accommodate them in the continuous mode of operation; as a result the observed effect of EBCT on the phenol and cyanide removal becomes negligible after 4-5 days. Additionally, at industrial scale lower EBCTs are desired due to economical reasons. Therefore an EBCT value of 5 h was found to be satisfactory for the continuous bio-treatment of phenol and cyanide.

Change in D.O. with the run time

To study the change in D.O. with the run time, samples were collected at port P5 for 10 days. The change in D.O. of the effluent with run time is depicted in Figure 4. It is revealed from figure 4 that with the increase in contact time, the D.O. value of the treated water reduces. This statement can be recognized to the continuous intake of dissolved oxygen by the aerobic bacteria (mixed culture) along with the contact time. The D.O. value decreases from 7.7 to 4 mg/L 0 to 10 days. This may be owing to the change in concentration of cell along with time. The cell concentrations in treated water samples collected at port P5.

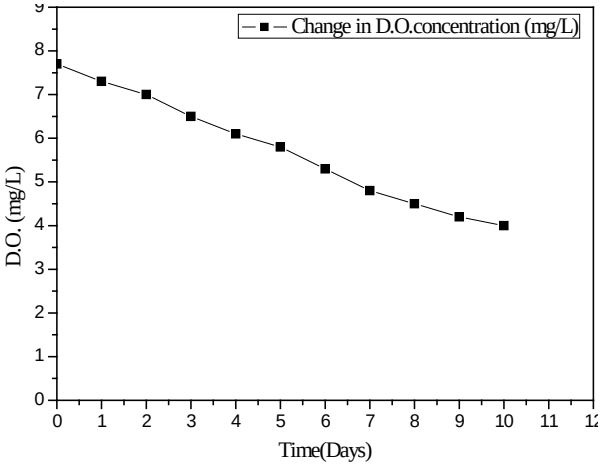

Figure 4. Change in D.O. of the treated water sample with run time in continuous column reactor packed with *mixed culture* immobilized on Fe-CSAC (Process conditions: Initial pH 8, Temperature 30 ^{o}C, Initial concentration 500 mg/L phenol and 50 mg/L cyanide, Run time 10 days)

Changes in pH with run time.

To find out the change in pH value of the treated water the samples were collected at the top of the reactor (P5) for 10 days of reactor run. The changes in pH value of the treated water with reactor run time are shown in Figure 5.It could be noted from figure 5 that initially the pH of wastewater decreases slightly, but after one day of operation the pH increased and then became constant with rest of the time. This is due to fact that the initial decrease in pH may be attributed to adaptation of the bacteria in the environment.[31] The initial decrease in pH may be recognized to the adaptation of microbes to the toxic environment.[31] Increase in pH after a day of the run can be attributed to the formation of ammonia due to the nitrogenous activity of mixed culture. The reduction in pH may be owing to the fact that as the microbes used for the toxic pollutant removal was aerobic, as a result, they consumed the oxygen available at the surface of biosorbent for the biodegradation of pollutant. The bacterium utilized for the biotreatment of phenol formed the complex of oxygen and carbon as phenol was used as toxic pollutant. The complex of oxygen and carbon was

combined with water generating hydroxyl anions, then these hydroxyl ions combined with water to produce H^+ ions; then, the pH of the effluent was increased.[23]

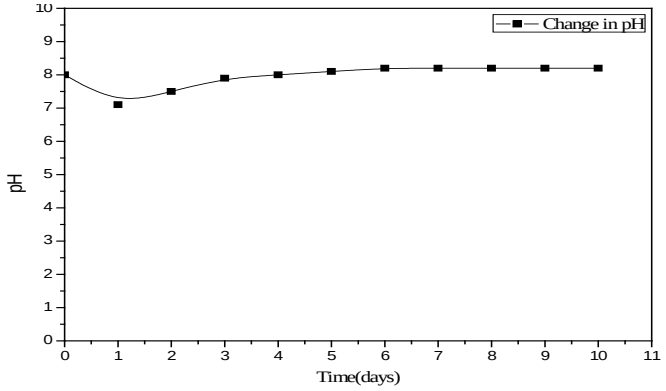

Figure 5. Change in pH of the treated water sample with run time in continuous column reactor packed with *mixed culture* immobilized on Fe-CSAC (Process conditions: Initial pH 8, Temperature 30 ^{0}C, Initial concentration 500 mg/L phenol and 50 mg/L cyanide, Run time 10 days)

Growth of biomass with time

The growth of biomass with reactor run time are shown in Figure 6. From figure 6, it is evident that the growth of biomass initially reduced and then started increasing after 3 days h of contact time at all EBCT values. This is due to fact that the increase in biomass concentration with run time is due to the utilization of both phenol and cyanide as carbon and nitrogen source respectively by *mixed culture.*

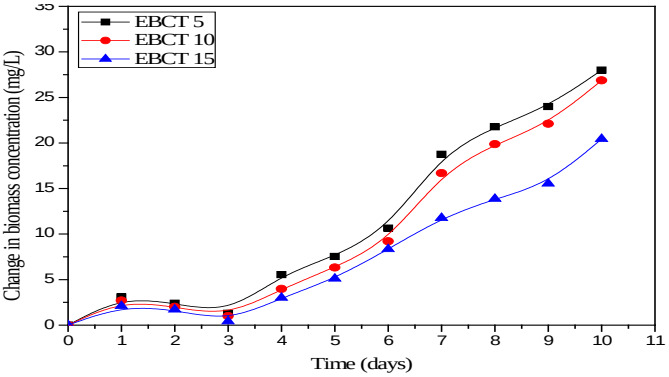

Figure 6. Growth of biomass with run time in continuous bio-column reactor packed with *mixed culture* immobilized on Fe-CSAC (Process conditions: Initial pH 8, Temperature 30 ^{o}C, Initial concentration 500 mg/L phenol and 50 mg/L cyanide, Run time 10 days)

Regeneration of the reactor by backwashing

In a packed bed bio-column reactor with increase in time more phenol and cyanide are adsorbed/precipitated on the microbes surface and creates additional dead biomass, which blocks the void space (pore volume) of the reactor. Additional biomass and precipitates can possibly generate a problem by blockage the pore space of the packed bed bio-reactor. Clogging can be escaped by alternating flashing of the packed bed column by backwashing[32] or by increasing the influent flow rate.[31] After some days of continuous operation without washing, the entire packed bed turns out to be tired resulting in no removal of phenol and cyanide. Therefore, after 4–5 days of continuous operation the biomass and precipitates must be removed by backwashing to acquire the effective elimination of the phenol and cyanide.

CONCLUSION

From the above considerations the following conclusions are drawn:

1. The packed bed bio-column reactor is accomplished to decrease the concentration of the phenol and cyanide in the effluent water below their permissible limit.

2. Removal of phenol and cyanide was attained ina continuous packed bed bio-column reactor packed with mixed culture of *P. putida* and *S. odorifera* immobilized onto Fe-CSAC biomass.

3. At the initial stage, the bed height and EBCT have noteworthy effect on the removal of phenol and cyanide from the contaminated water. On the other hand, after some time of continuous operation (about 6-7 days) such effect is minor under the experimental conditions.

4. D.O. reduces along the time of the continuous operation in packed bed bio-reactor, which supports the aerobic nature of the microbes.

5. pH of the solution drops initially for the first day and increases within small range.

6. Packed bed bio-column reactor must be backwashed for effective continuous operation.

REFERENCES

1. G. Busca, S. Berardinelli, C. Resini, L. Arrighi. Technologies for the removal of phenol from fluid streams: A short review of recent developments. *J. Hazard. Mater*. **2008**, 160, 265–288.
2. R.R. Dash, C. Balomajumder. A. Kumar Removal of cyanide from water and wastewater using granular activated carbon. *Chem. Eng. J.* **2009**, 146, 408–413.
3. Z. Aksu, J. Yener. A comparative adsorption/biosorption study of mono-chlorinated phenols onto various sorbents. *Waste Manage.* **2001**, 21, 695–702.
4. J.D. Desai, C. Ramakrishna. Microbial degradation of cyanides and its commercial application. *J. Sci. Ind. Res.***1998**, 57, 441-453.
5. K. Juttner, U. Galla, H. Schmieder. Electrochemical approaches to environmental problems in the process industry. *Electrochim. Acta.* **2000**, 45, 2575–2594.
6. M. Tomaszewska, S. Mozia,W. Morawski. Removal of organic matter by coagulation enhanced with adsorption on PAC. *Desalination.***2004**, 162, 79–87.
7. Z. Lazarova, S. Boyadzhieva. Treatment of phenol-containing aqueous solutions by membrane-based solvent extraction in coupled ultrafiltration modules. *J. Chem. Eng.* **2004**, 100, 129–138.
8. W. Kujawski, A. Warszawski, W. Ratajczak, T. Porebski, W. Capala. Removal of phenol from wastewater by different separation techniques. *Desalination*. **2004,** 163, 287–296.
9. S.A.K. Palmer, M.A. Breton, T.J. Nunno, D.M. Sullivan, N.F. Surprenant. Technical Resource Document: Treatment Technologies for Metal/Cyanide-Containing Wastes. Volume III, US EPA Rept. No. EPA-60O/S2-87/106, **1988**.
10. M.M. Botz. Overview of cyanide treatment methods,in: Mining Environmental Management, Min. J. Ltd., London, UK, **2001**.

11. R.R. Dash, A. Gaur, C. Balomajumder. Cyanide in industrial wastewaters and its removal: A review on biotreatment. *J. Hazard. Mater*. **2009**, 163, 1–11.
12. H. S. Lee, J. H. Suh. Continuous Biosorption of Heavy Metal Ions by Ca-loaded Laminaria japonica in Fixed Bed Column. *Korean J. Chem. Eng*. **2000,** 4, 477.
13. Z Aksu, F. Gönen. Binary biosorption of phenol and chromium (VI) onto immobilized activated sludge in a packed bed: prediction of kinetic parameters and breakthrough curves. *Sep. Purif. Technol*. **2006**, 49, 205–216.
14. N. Singh, C. Balomajumder. Simultaneous biosorption and bioaccumulation of phenol and cyanide using coconut shell activated carbon immobilized Pseudomonas putida (MTCC 1194). *J. Environ. Chem. Eng.* **2016**, 4, 1604–1614.
15. A. Akcil, A.G. Karahan, H. Ciftci, O. Sagdic. Biological treatment of cyanide by natural isolated bacteria (*Pseudomonas* sp.). *Min. Engg.* **2003**, 16, 643–649.
16. M.M. Figueira, V.S.T. Ciminellei, M.C. deAndrade, V.R. Linardi. Cyanide degradation by an *Escherichia coli* strai. *Cana. J. Microbio.* **1996**, 42, 519–523.
17. Finnegan, S. Toerien, L. Abbot, F. Smith, H.G. Raubenheimer. Identification and characterization of an *Acinetobacter* sp. capable of assimilation of a range of cyano-metal complexes. Free cyanide ions, and simple organic nitriles. *App. Micro. Biotech.***1991**, 36,142–144.
18. P.R. Meyers, P. Gohool, D.E. Rawling, D.R. Wood. An efficient cyanide degrading *Bacillus pumilus* strain. *J.Gen. Microb.***1991**, 137, 1397–1400.
19. G. Gurujeyalakshmi, P. Oriel. Isolation of phenol-degrading Bacillus stearothermophilus and partial characterization of the phenol hydroxylase. *Appl. Environ. Microbiol*. **1989**, 55, 500.
20. P. J. Allsop, Y. Chisti, M. M. Young and G.R. Sullivan. Dynamics of phenol degradation by *Pseudomonas putida*. *Biotechnol. Bioeng*. **1993,** 41, 572.
21. L. G. Torres, A. Sanchez-de-la-Vega, N.A. Beltran, and B.E. Jimenez. Production and characterization of a Ca-alginate biocatalyst for removal of phenol and chlorophenols from wastewaters. *Process Biochem.* **1998**, 33, 625-634.
22. O. J. Hao, M. H. Kim, E. A. Seagren, H. Kim. Kinetics of phenol and chlorophenol utilization by Acinetobacter species. *Chemosph*. **2002**, 46, 797.
23. P. Mondal, C. B. Majumder and B. Mohanty. Treatment of arsenic contaminated water in a laboratory scale up-flow bio-column reactor. *J. Hazard. Mater.* **2008**, 153,136-145.
24. J. C. Brown, V. L. Snoeyink, M. J. Kirisits. Abiotic and biotic perchlorate removal in an activated filter. *J. Am. Water Works Assoc.* **2002,** 94, 70–79.
25. P. Laurent, A. Kihn, A. Andersson, P. Servais. Impact of backwashing on nitrification in the biological activated carbon filters used in drinking water treatment. *Environ. Technol.* **2003,** 24, 277–287.
26. G. Nakhla, S. Farooq. Simultaneous nitrification-denitrification in slow sand filters. *J. Hazard. Mater.* **2003,** 96, 291–303.
27. MTCC Guide lines, http://www.imtech.res.in/mtcc/bacteria.htm.
28. B. Agrawal, C. Balomajumder. Coke waste water: A source of phenol and cyanide assimilating bacteria. *J. Sci. Tech. Manegement*, **2012,** 5(1).
29. N. Singh, B. Agarwal, C.Balomajumder. Simultaneous treatment of phenol and cyanide containing aqueous solution by adsorption, biotreatment and simultaneous adsorption and biotreatment (SAB) process. *J. Environ. Chem. Eng.* **2016**, 4, 564–575
30. M.H. El-Naas, S. Al-Zuhair, S. Makhlouf. Continuous biodegradation of phenol in a spouted bed bioreactor (SBBR). *Chem. Eng. J.* **2010**, 160, 565-570.
31. T. Jong, D.L. Pany. Removal of sulfate and heavy metals by sulfate reducing bacteria in short term bench scale up-flow anaerobic packed bed reactor runs. *Water Res.* **2003**, 37, 3379–3389.
32. Katsoyiannis, A. Zouboulis, H. Althof, H. Bartel. Arsenic removal from ground waters using fixed bed up flow bioreactor. *Chemosphere*. **2002**, 47, 325–332.