Iran. J. Environ. Health. Sci. Eng., 2006, Vol. 3, No. 2, pp. 91-96

CYANIDE REMOVAL BY COMBINED ADSORPTION AND BIODEGRADATION PROCESS

*[1]**R. Roshan Dash**, [2]**Ch. Balomajumder**, [3]**A. Kumar**

[1]Department of Civil Engineering, Indian Institute of Technology, Roorkee-247667, India

[2]Department of Chemical Engineering, Indian Institute of Technology, Roorkee-247667, India

Received 11 January 2006; revised 2 March 2006; accepted 26 March 2006

ABSTRACT

Investigation of the effectiveness of simultaneous adsorption and biodegradation (SAB) process over individual processes by using microbes *Rhizopus oryzae* and *Stemphylium loti* with granular activated carbon (GAC) as adsorbent was carried out. The maximum removal efficiency of cyanide had been achieved by biodegradation alone was 83% by *R. oryzae*, while it was 90% by *S. loti* at initial pH of 5.6 and 7.2 respectively and at initial CN^- concentration of 150 mg/L. In the combined process efficiency of *R. oryzae* closer to *S. loti* (95.3% and 98.6% respectively)

Key words: Adsorption, biodegradation, cyanide, *Rhizopus oryzae*, *Stemphylium loti*

INTRODUCTION

Cyanide is present in environmental matrices and waste streams as simple and complex cyanides, cyanates and nitriles (Ebbs, 2004). Cyanide compounds are extensively used in industry and their effluents are the major source of these toxic pollutants in our environment (Desai and Ramakrishna, 1998). The release of cyanide from industries worldwide has been estimated to be more than 14 million kg/yr (Ebbs, 2004). The major industries which use cyanide are electroplating and mining (extraction of gold, silver, etc.) (Akcil, 2003; Desai and Ramakrishna, 1998; Patil and Paknikar, 2000). Due to their toxic effects, cyanide-containing effluents cannot be discharged without detoxification to the environment. US-health service cites 0.01mg/L as guideline and 0.2 mg/L as permissible limit for cyanide in effluent. Adsorption and biodegradation are two significant methods for treatment of wastewater bearing cyanide compounds. Adsorption and biological treatment either operated separately or simultaneously in one-unit results in a better removal and process performance. Microbial mass can, in some extent, adsorb the substances, but at the same time it also degrades them. On the other hand, adsorption of the substances onto adsorbent reduces the inhibitory effect of the substances for microbial mass. Accordingly, the process is expected to be more stable and the toxic compounds may be converted into less harmful substances. Usually, the substances, which are easily adsorbed, are also hardly biodegradable and vice versa. Therefore, adsorption and biodegradation successfully supplement each other in the various schemes of wastewater treatment. Attached growth processes and combined processes such as oxic/anoxic processes may prove advantageous for the cyanide detoxification (Desai and Ramakrishna, 1998; Akcil, 2003; Mosher and Figueroa, 1996). Metabolism of cyanides by strains of *Pseudomonas*, *Acinetobacter*, *Bacillus*, and *Alcaligenes* (Finnegan *et al.,*1991; Harris and Knowles, 1983; Meyers *et al.,*1991) has been mostly reported. In addition, some fungi such as *Fusarium solani, F. oxysporum, Fusarium lateritum* (Barclay *et al.,* 1998; Ezzi and Lynch, 2005), *Gloeocerospora sorghii* (Wang, 1992), *Stemphylium loti* (Fry and Millar, 1972) *Rhizopus oryzae* (Padmaja and Balagopal, 1985) have been found to utilize cyanide as a nutrient for growth. Some encouraging results were also found using combination of biosorption and biodegradation processes for removal and

*Corresponding author E-mail: *rajeshroshan77@yahoo.com*
Tel: +91 9412307539, Fax: +91 1332 276535

recovery of metal cyanides (Dursun and Aksu, 2002, Patil and Paknikar, 1999). In the present study simultaneous adsorption biodegredation (SAB) of ferrocyanide bearing synthetic solutions have been performed separately in a GAC batch reactor with or without the immobilized *Rhizopus oryzae* (MTCC 2541) and *Stemphylium loti* (MTCC 2542) on GAC. The process parameters like adsorbent dose, particle size of GAC and pH have been optimized for both compounds in batch experiments by studying the effect of process parameters on percentage removal. Percentage removal and specific uptake have been studied in the both cases of adsorption and simultaneous adsorption-biodegradation (SAB) and have been compared for both the microbes.

MATERIALS AND METHODS

Chemicals

All the chemicals used in the experiment were of analytical grade. Granular activated carbon was sieved to various fractions of particle range 1.4-2 mm, 2-4 mm and 4-5 mm with standard testing sieve and used as adsorbent and as the support medium for bio layer formation. The bulk density of GAC was 0.4 g/mL. Purification of the adsorbent was carried out by soxhlet extraction with acetone/ n-heptane (50:50, v/v) for 24 h and dried at 110 °C. Cyanide solution was prepared by dissolving 2.7 g of $K_4[Fe(CN)_6].3H_2O$ in 1 L of double distilled water to yield a stock solution containing 1 mg (CN^-)/mL.

Microorganisms and growth conditions

Freeze-dried/lyophilized culture of *Rhizopus oryzae* (MTCC 2541) and *Stemphylium loti* (MTCC 2542) species was obtained from Institute of Microbial Technology (IMTECH), Chandigarh, India. Cultures were revived in the growth media as per the guidelines of IMTECH at 25 °C. *R. oryzae* (MTCC 2541) was grown on Potato Dextrose Agar (PDA) plates and in an enrichment medium containing Potato Dextrose Broth at a pH of 5.6, where as S. *loti* (MTCC 2542) culture was revived in Soya broth and Soya agar plates at pH 7.2. The pH was adjusted with concentrated and diluted H_2SO_4 and NaOH. *R. oryzae* and *S. loti* was incubated at 25 °C for 5 days and for 7 days respectively. *R. oryzae* and *S. loti* cultures prepared as above were transferred to flasks containing potato dextrose (PD) broth and Soya broth respectively with 10-30 mg/L CN^- for adaptation. Two replications were maintained for each concentration of cyanide as well as for the control.

Biodegradation studies

Microorganisms were transferred (in 1:20 ratio) into the growth medium containing ferrocyanide ions as the only source of nitrogen with minimal medium (1% glucose, 0.5% KH_2PO_4, 0.5% K_2HPO_4, 0.05% $MgSO_4.7H_2O$, 0.01 NaCl, 0.013% $CaCl_2.2H_2O$, 0.02% $FeSO_4.2H_2O$, 0.01% $CuSO_4$, 0.2% $ZnSO_4.7H_2O$, 0.002% $MnSO_4.4H_2O$, 0.005% biotin). The biodegradation medium was prepared by mixing the ferrocyanide solution autoclaved separately and the sterilized solution containing minimal salt medium. The pH of the final solution was adjusted to the desired value by using sterile dilute and concentrated H_2SO_4 or NaOH solutions. Sterilization of the medium was performed in an autoclave at 121 °C for at least 20 min. All batch experiments were performed in 250 mL conical flasks containing 100 mL of the growth medium and incubated at 25 °C in rotary incubator shaker at 140 rpm for 120 h. After incubation, the contents of the flasks were analyzed for residual cyanide concentration.

Adsorption and SAB study

It was found that 10 g/L GAC and 2-4mm size particles have optimum adsorption capacity for adsorption of cyanide. Adsorption studies were carried out in a 250 mL conical flask with 2-4 mm size and 10 g/L GAC with cyanide concentration of 150 mg/L in a rotary incubator shaker at 140 rpm. For SAB study 150 mg/L ferrocyanide solutions with 10 mg/L GAC and microbial mass (1:20 ratio) was taken in a 250 mL conical flask and incubated at 25 °C in rotary incubator shaker at 140 rpm for 120 h. After incubation, the contents of the flasks were analyzed for residual cyanide concentration.

Analyses

Parameters such as pH, total cyanide content, optical density (550 nm) and/or bacterial cell count were monitored periodically. The initial and residual cyanide was determined by pyridine barbituric acid colourimetric method (575 nm) after distillation as described in Standard Methods (APHA, 2001). All the batch experiments were carried out in duplicate and repeated twice to confirm the results.

RESULTS

Growth of microbes in culture media

Microorganism growth in the enrichment medium was determined by measuring the absorbance of the broth at 550 nm using standard curve of absorbance against dry cell weight. Fig. 1 and Fig. 2 indicate that growth of *R. oryzae* and *S. loti* in enrichment medium. But in the medium with cyanide the growth was delayed for about 12 h and the bacterial density was low.

Biodegradation study

Fig. 3 shows the percentage removal of ferrocyanide with respect to time for both the cultures. Here it was found that the percentage removal efficiency of *S. loti* is more as compared to *R. oryzae*. From the data it can be interpreted that maximum efficiency of cyanide removal achieved was 83% by *R. oryzae*, while it was 90% by *S. loti* at initial pH of 5.6 and 7.2 respectively and at initial CN^- concentration of 150 mg/L. Biodegradation rate was determined from the slope of ferrocyanide consumption with time plot at the exponential growth region.

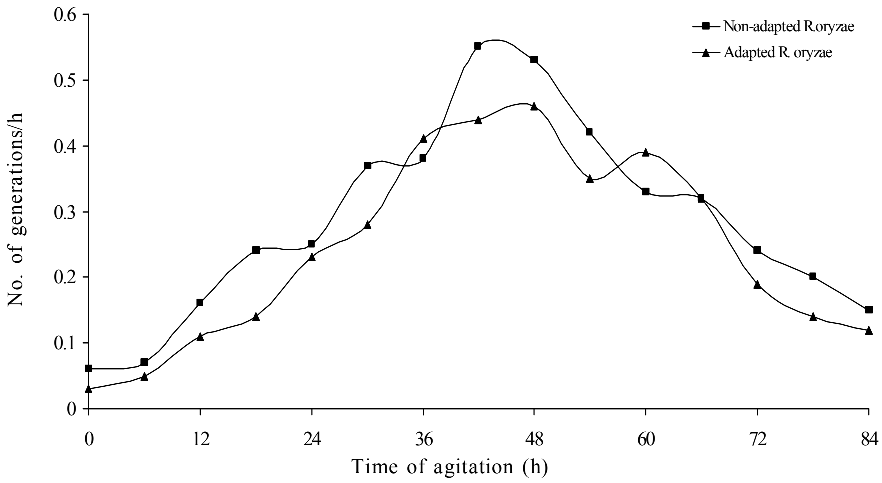

Fig. 1: Growth rate (No. of generations/h) of *R. oryzae* vs. time of agitation

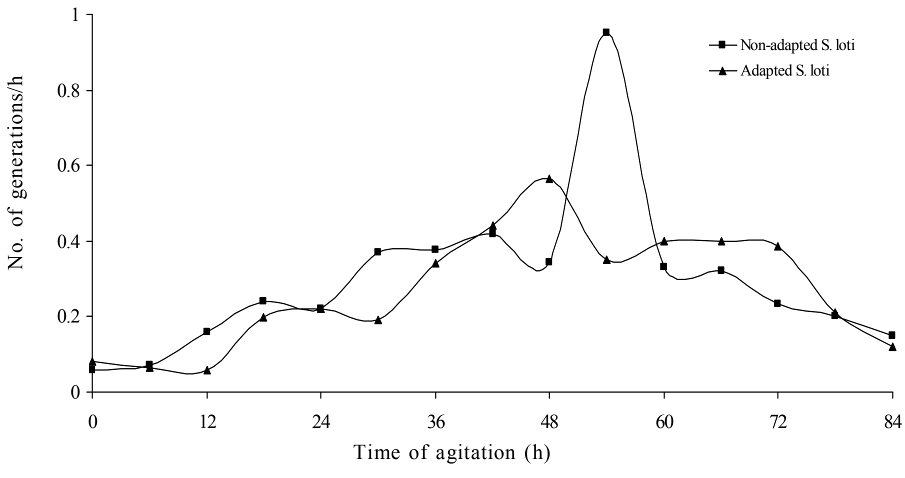

Fig. 2: Growth rate (No. of generations/h) of *S. loti* vs. time of agitation

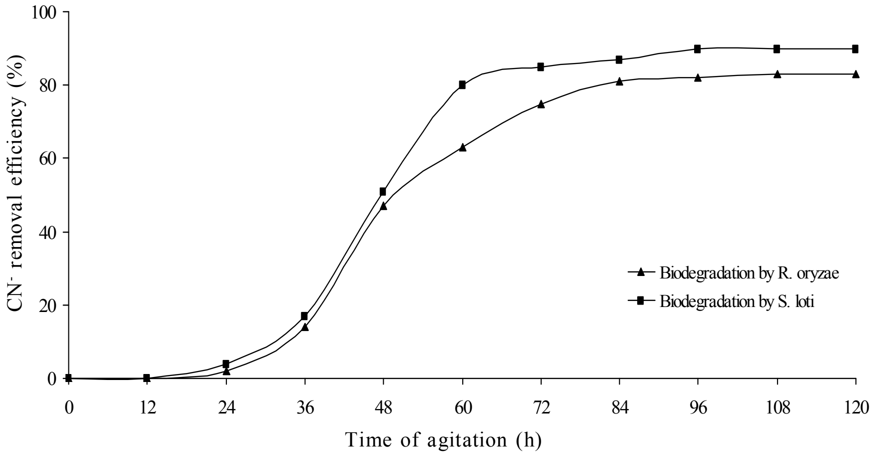

Fig. 3: CN^- removal efficiency through biodegradation by *R. oryzae* and *S. loti*

Adsorption and SAB study

Fig. 4 shows the percentage of cyanide removal with the in duration of shaking. SAB study was conducted to evaluate the combine process efficiency and compare its data to adsorption and biodegradation. The experimental parameters were kept constant. Fig. 5 shows the percentage of ferrocyanide removal versus time by SAB process. In the combined process *R.. oryzae* had efficiency closer to *S. loti* (95.3% and 98.6% respectively) as compared to biodegradation process alone. Again in the SAB process more than 90% removal efficiency was observed at only 48-60 h of agitation.

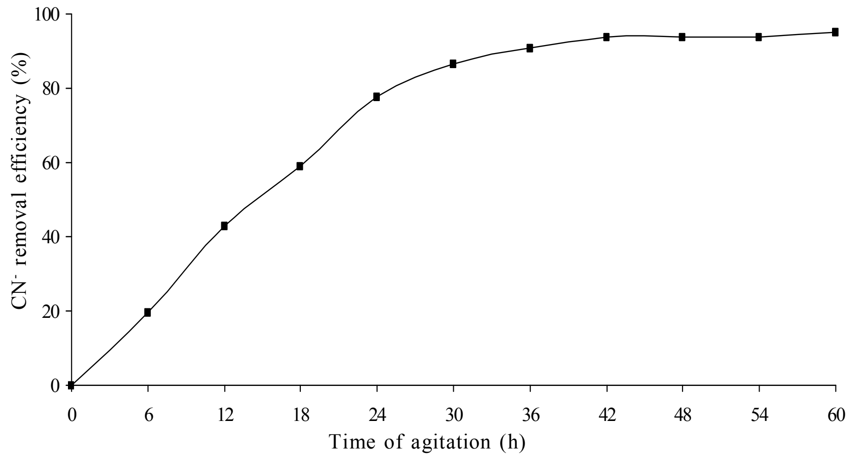

Fig. 4: CN^- removal efficiency through adsorption vs.
(particle size of GAC = 2-4mm, GAC dose = 10 mg/L)

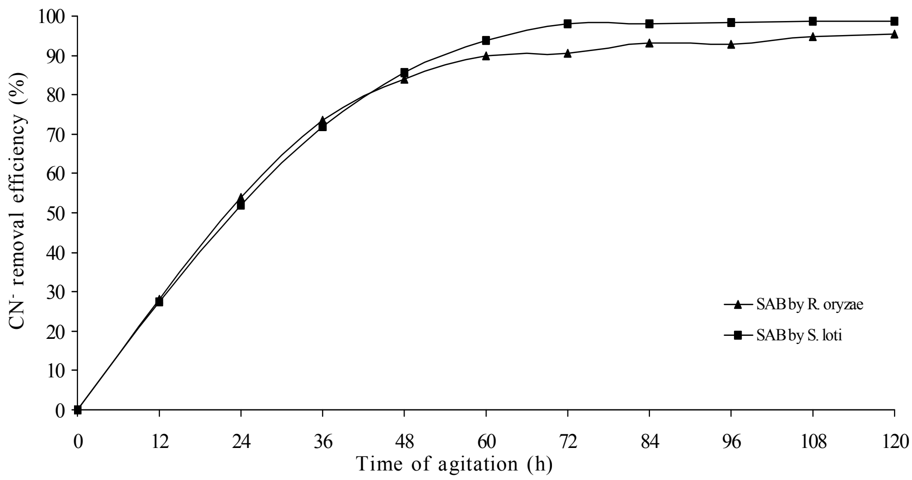

Fig. 5: CN^- removal efficiency through SAB process by *R. oryzae* and *S. loti* immobilized on granular activated carbon (GAC)

DISCUSSION

In Fig. 1 and Fig. 2 the delay in the growth of *R. oryzae* and *S. loti* in the presence of cyanide ions indicate the intial toxicity of cyanide to the microbes, but the growth after that may be due the adaptation of microbes to ferrocyanide. Due to the presence of ferrocyanide the death phase of the microbe comes faster as compared to the growth in enrichment medium. It was also observed that the cell concentration in initial phase of growth was less in medium containing ferrocyanide as compared to enrichment media (Dursun *et al.*, 1999). From Fig. 3 it can be observed that ferrocyanide was used as sole nitrogen source in these experiments. The toxicity of cyanide compounds restricts microbes for using them as primary carbon source for growth. Since the amount of nitrogen needed for growth is less than the requirement for carbon, it could be easier to utilize ferrocyanide as a source of nitrogen in the presence of another source of carbon and energy (Dursun and Aksu, 2002). Diffusion of CN^- from the bulk solution to the active surface sites was appeared to be the slowest step from Fig. 4. This process is dependent on the concentration gradient between those two points and the thickness of the diffusion layer, which is a function of the agitation process (Guo *et al.*, 1993). From Fig. 5 it was observed that adsorption occurred in the first phase followed by biodegradation. Due to the delayed growth of microbes in the culture media adsorption occurred in the first phase. It was observed from this figure that the removal efficiency of SAB process is more as compared to adsorption or biodegradation alone presented in Fig. 3 and Fig. 4. Many treatment processes have been used and others are under investigation including biological methods for treatment of cyanide and its safe disposal. The main objectives of this research were to determine the biodegradation ability of microorganisms for ferrouscyanide complex ions in the presence of glucose and to evaluate its efficiency in SAB process. The results showed the efficient action of the microorganism in the removal of very stable ferrouscyanide complexes at lower concentrations with SAB process. It was found that SAB process is a suitable methodology with higher efficiency and effectively can be used. The results are encouraging, therefore it can be concluded that simultaneous adsorption and biodegradation (SAB) is a better treatment option when compared to adsorption or biodegradation alone. The study therefore, is a pointer towards alternative possibilities of toxicity removal through bioremediation and their respective merits. In the future plan of work, SAB process with packed bed and effect of bio-regeneration of GAC on removal efficiency can be carried out.

REFERENCES

Akcil, A., (2003). Destruction of cyanide in gold mill effluents: biological versus chemical treatments. Biotechnol. Adv., **21**: 501-511.

APHA (2001). Standard methods for the examination of water and wastewater. American Public Health Association, 13th Ed. Washington DC.

Barclay, M., Hart, A., Knowles C. J., Meeussen, J. C. L. and Tett, V. A., (1998). Biodegradation of metal cyanides by mixed and pure cultures of fungi. Enzyme and Microbial. Technol., **22**: 223-231.

Desai, J. D., Ramakrishna, C., (1998). Microbial degradation of cyanides and its commercial application. J. Sci. Ind. Res., **57**: 441-453.

Dursun, A. Y., Aksu, Z., (2002). Effect of internal diffusivity of ferrous (II) cyanide complex ions in Ca-alginate immobilized *Pseudomonas fluorescens* gel beads on the biodegration rate. Process Biochem., **37**: 747-752.

Dursun, A.Y., Çalik, A., Aksu, Z., (1999). Degradation of ferrous (II) cyanide complex ions by *Pseudomonas fluorescens*. Process Biochem., **34**: 901–908.

Ebbs, S., (2004). Biological degradation of cyanide compounds. Biotechnol., **15**: 1-6.

Ezzi, M. I., Lynch, J. M., (2005). Biodegradation of cyanide by *Trichoderma* sp. and *Fusarium* sp. Enzyme Microbial. Technol., **36**: 849-854.

Finnegan, I., Toerien, S., Abbot, L., Smit, F., Raubenheimer, H. G., (1991). Identification and characterisation of an *Acinetobacter* sp. capable of assimilation of a range of cyano-metal complexes, free cyanide ions, and simple organic nitriles. Appl. Microbial. Biotechnol., **36**: 142-144.

Fry, W. E., Millar, R. L., (1972). Cyanide degradation by an enzyme from *Stemphylium loti*. Arch. Biochem. Biophys., **151**: 468-474.

Guo, R., Chakrabarti, C. L., Subramanian, Kunnath S., Ma X., Lu Y., Cheng J., Pickering W. F., (1993). Sorption of low levels of cyanide by granular activated carbon. Water Environ. Res., **65**: 640-644.

Harris, R., Knowles, C. J. (1983). Isolation and growth of a *Pseudomonas* species that utilises cyanide as a source of nitrogen. J. Gen. Microbiol., **129**: 1005-1011.

Meyers, P. R., Gohool, P., Rawling, D. E., Wood, D. R. (1991). An efficient cyanide degrading *Bacillus pumilus* strain. J. Gen. Microbiol., **137**: 1397-1400.

Mosher, J., Figueroa, B. L., (1996). Biological oxidation of cyanide: A viable treatment option for the minerals processing industry. Minerals Eng., **9**: 573-581.

Padmaja, G., Balagopal, C., (1985). Cyanide degradation by *Rhizopus oryzae*. Can. J. Microbiol., **31**: 663-669.

Patil, Y. B., Paknikar, K. M., (1999). Removal and recovery of metal cyanides using a combination of biosorption and biodegradation processes. Biotechnol. Lett. **21**: 913-919.

Patil, Y. B., Paknikar, K. M., (2000). Development of a process for biodetoxification of metal cyanides from wastewater. Process Biochem., **35**: 1139-1151.

Wang, G., Matthews, D. E., Vanetten, H. D., (1992). Purification and characterisation of cyanide hydratase from the phytopathogenic fungus *Gloeocerospora sorghii*. Arch. Biochem. Biophys., **298**: 569-575.