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Treatment of arsenic contaminated water in a laboratory scale up-flow bio-column reactor

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Abstract

The present paper describes the observations on the treatment of arsenic contaminated synthetic industrial effluent in a bio-column reactor. *Ralstonia eutropha MTCC 2487* has been immobilized on the granular activated carbon (GAC) bed in the column reactor. The synthetic water sample containing As(T) (As(III):As(V) = 1:1), Fe, Mn, Cu and Zn at the initial concentrations of 25, 10, 2, 5, 10 ppm, respectively, was used. Concentrations of all the elements have been found to be reduced below their permissible limits in the treated water. The significant effect of empty bed contact time (EBCT) and bed height on the arsenic removal was observed in the initial stage. However, after some time of operation (approximately 3–4 days) no such effect was observed. Removal of As(III) and As(V) was almost similar after ∼2 days of operation. However, at the initial stage As(V) removal was slightly more than that of As(III). In absence of washing, after ∼4–5 days of operation, the bio-column reactor was observed to act as a GAC column reactor based on physico-chemical adsorption. Like arsenic, the percent removals of Fe, Mn, Cu and Zn also attained minimum after ∼1 day and increased significantly to the optimum value within 3–4 days of operation. Dissolved oxygen (DO) has been found to decrease along with the increasing bed height from the bottom. The pH of the solution in the reactor has increased slightly and oxidation–reduction potential (ORP) has decreased with the time of operation.

Keywords: Arsenic; Bio-reactor; Adsorption; EBCT; *Ralstonia eutropha MTCC 2487*

1. Introduction

Arsenic contaminated ground water enters into the human body and causes various types of cancers. Along with natural sources arsenic can also enter into the water stream through industrial effluents like metal-processing industries, semiconductor industries, etc., and acid mine drainage. Important role of mine drainage on the arsenic poisoning has recently been observed in Koudikasa village of Rajnandgaon district in Chhattisgarh, India [1]. Arsenic concentration in acid mine drainage is normally very high. The normal range of the concentration of As(T) in the acid mine drainage, in Carnoules Creek, France has recently been reported as 0–250 ppm [2]. The As(III) concentration in this acid drainage has also been reported to be around 60–90% of the As(T). Concentration of arsenic, copper, zinc and iron in the water of Borah and Maids creeks and drainage from Conrad mine, Australia, has recently been reported as high as 8.6, 5, 11 and 9.7 ppm, respectively [3]. A recent report uses the synthetic acid mine drainage containing 20 ppm As along with Fe, Cu, Zn, Ni, Mg and Al [4].

Considering the health impact of the arsenic poisoning in water the maximum contaminant level (MCL) of arsenic in drinking water has been reduced to 10 ppb by many countries. Similarly, the permissible limit of arsenic in industrial effluents is also low (0.2 ppm). For Cu, Zn, Fe and Mn these values are 3.0, 5.0, 3.0 and 0.2 ppm, respectively [5].

Amongst various arsenic removal methods, the bio removal process using immobilized whole bacterial cells has attracted more research interest in recent years [6]. Some of the bacteria having arsenic removal capability are *Alcaligenes faecalis*, *Agrobacterium tumafecians*, bacteria *NT26*, *Bacillus indicus*, *Bacillus subtilis*, *Corynebacterium glutamicum*, *Desulfovibrio desulfuricans*, *Galleonella ferrigunea*, *Leptothrix ocracia*, *Pseudomonas putida*, *Pseudomonas arsenitoxidans*, *Ralstonia picketti*, *Thiomonas ynys1*, *Thiobacillus ferroxidans*, etc. [7–17]. Amongst these arsenic bacteria the *D. Desulfuricans*, *G. ferri-*

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gunea, *L. ocracia*, *R. picketti*, *T. ynys1*, etc., have been exploited recently to remove arsenic in bio-column reactor [4,13,14,17]. Different types of bacteria have different types of gene. Although all the arsenic bacteria can survive in arsenic atmosphere, the bacteria type which reduces As(V) to As(III) and accumulate As(III) is specifically termed as arsenic resistant bacteria [12]. Arsenic resistant bacteria normally contain arsR and arsC gene in either plasmid or chromosome or in both and produce arsenic regulatory ArsR protein and arsenate reductase enzyme [10,12]. ArsR has specific active sites for accumulating As(III) on the cell surface [10]. Recently, arsR-arsC gene cluster has been observed in *R. eutropha CH34* [10], which is also known as *R. eutropha MTCC 2487* [18]. This strain can produce ArsR protein and arsenate reductase enzyme [10]. However, the arsenic removal by this strain is not yet demonstrated [10].

In a bio-column reactor bio-layer is developed on the solid support inside the reactor and it works as a bio-filtration unit [4]. This bio-filtration technique can be applied for treating contaminated ground water if the bacteria is indigenous to the ground water and can proved to be a cheaper option for treating arsenic contaminated industrial effluents also. For arsenic resistant bacteria like *R. eutropha*, the arsR protein of bacterial mass can capture As(III) or can convert As(V) to As(III) followed by its adsorption on the protein of the bacterial cell surface [10,19]. *R. eutropha MTCC 2487*, isolated from Zn factory wastewater, can also remove Cd, Co, Hg, Ni and Zn along with arsenic from contaminated water [18]. Hence, the use of these bacteria for the treatment of industrial effluents/acid mine drainage containing these metals appears to be promising.

In batch study the process parameters like temperature, pH, oxidation–reduction potential (ORP), dissolved oxygen (DO), etc., are optimized, kinetic and equilibrium data are generated which can be applied to design and operate column reactor. However, without study in a column reactor the suitability of a technology cannot be established. The removal efficiency of the pollutants in a column study depends upon the empty bed contact time and bed height of the reactor. In a bio-column reactor the pH, ORP and DO of the treated water varies with time and along the height of the reactor, even when these values in inlet water are kept constant. Another important advantage of bio-column reactor is that it does not require regeneration of the adsorbent bed. However, backwashing within a certain interval is essential for the effective operation of the bio-column reactor.

In the present study, the treatment of a synthetic acid mine drainage containing As, Fe, Mn, Cu and Zn in a bio-column reactor for 15 days has been carried out. *R. eutropha MTCC 2487* has been immobilized over GAC (conditioned in metal solution) to produce bio-column reactor. The effects of empty bed contact time (EBCT), bed height and backwashing of the exhausted bed on the arsenic concentration of the treated water have been discussed. Removal of arsenic species at a constant EBCT and bed height has been discussed. The change of pH, ORP and DO with time has also been presented. It also discusses the removal of the Fe, Mn, Cu and Zn at the minimum EBCT value.

2. Materials and methods

The source of microorganism, its acclimatization to the heavy metal environment, experimental setup and experimental procedure are discussed as follows.

2.1. Source of organism

R. eutropha MTCC 2487 species was obtained from Institute of Microbial Technology, Chandigarh, India.

2.2. Acclimatization

The acclimatization of *R. eutropha MTCC 2487* in arsenic and metal environment was performed as follows.

The revived culture was first grown in nutrient broth (NB) media (13 g/l) [18] in a 250 ml conical flask. After 48 h significant bacterial growth was observed in the flask. Appropriate quantity of stock solution of arsenic was added into the flask containing NB to get a concentration of 1 mg/l of arsenic. It was kept aside, initially growth of *R. eutropha MTCC 2487* was inhibited and log phase started after 10 h. Thereafter, the arsenic was periodically added in increments of 1 mg/l in a series of 250 ml flasks till the arsenic concentration in the growth media reached 25 mg/l. The concentrations of Fe, Mn, Cu and Zn in the solution were also increased to 10, 2, 5 and 10 mg/l, respectively, in the similar manner. The NB content was decreased and arsenic and other metal content of the media were increased over a period of around one and half months. For inoculums, a further sub culturing was done and all the inoculums transfers were done in exponential phase (DO value ~0.6 at 600 nm). The temperature was maintained at $29 \pm 1\ ^{\circ}C$.

2.3. Experimental setup

The experiments related to the removal of arsenic and other metals were conducted in a bioreactor column constructed of SS pipe. The schematic diagram of the experimental setup is shown in Fig. 1, which is consisted with bio-column reactor, mixing chamber fitted with stirrer, feed tank, peristaltic pump, compressor, steam generator, rotameters and filter units for water and air. The reactor assembly is a close circuit unit. The column reactor had a working height of 100 cm, an internal diameter of 8 cm and a net empty working volume 5.03 ± 0.002 l. It was equipped with a total of four equidistant ports (excluding inlet and out let) of 1.25 cm diameter for collecting liquid samples along the height of the reactor. The top and bottom portions were connected with the main column by two flange joints, supported on SS screen (mesh no.: 16 BSS, width aperture: 1.00 mm). The final pore volume (void space) of the reactor was between 1796 and 1885 ml. The reactor was filled with granular activated carbon (GAC). The particle size and bulk density of the GAC were 2–4 mm and 40 g/100 ml, respectively. Before use, GAC was purified by soxhlet extraction with acetone/n-heptane (50:50, v/v) for 24 h and then dried [20]. Some physical properties of GAC are shown in Table 1.

Table 1
Some physical properties of GAC

Adsorbent	Elemental analysis (%)	Proximate analysis (%)	BET Surface area (m^2/g)	Micro-pore (<2 nm) volume (cm^3/g)
GAC	C: 75.06 H: 1.90 N: 0.0 S: 0.0 Others: 23.04	Ash: 2.58 Moisture: 9.71 Others: 87.71	583.23	0.2044

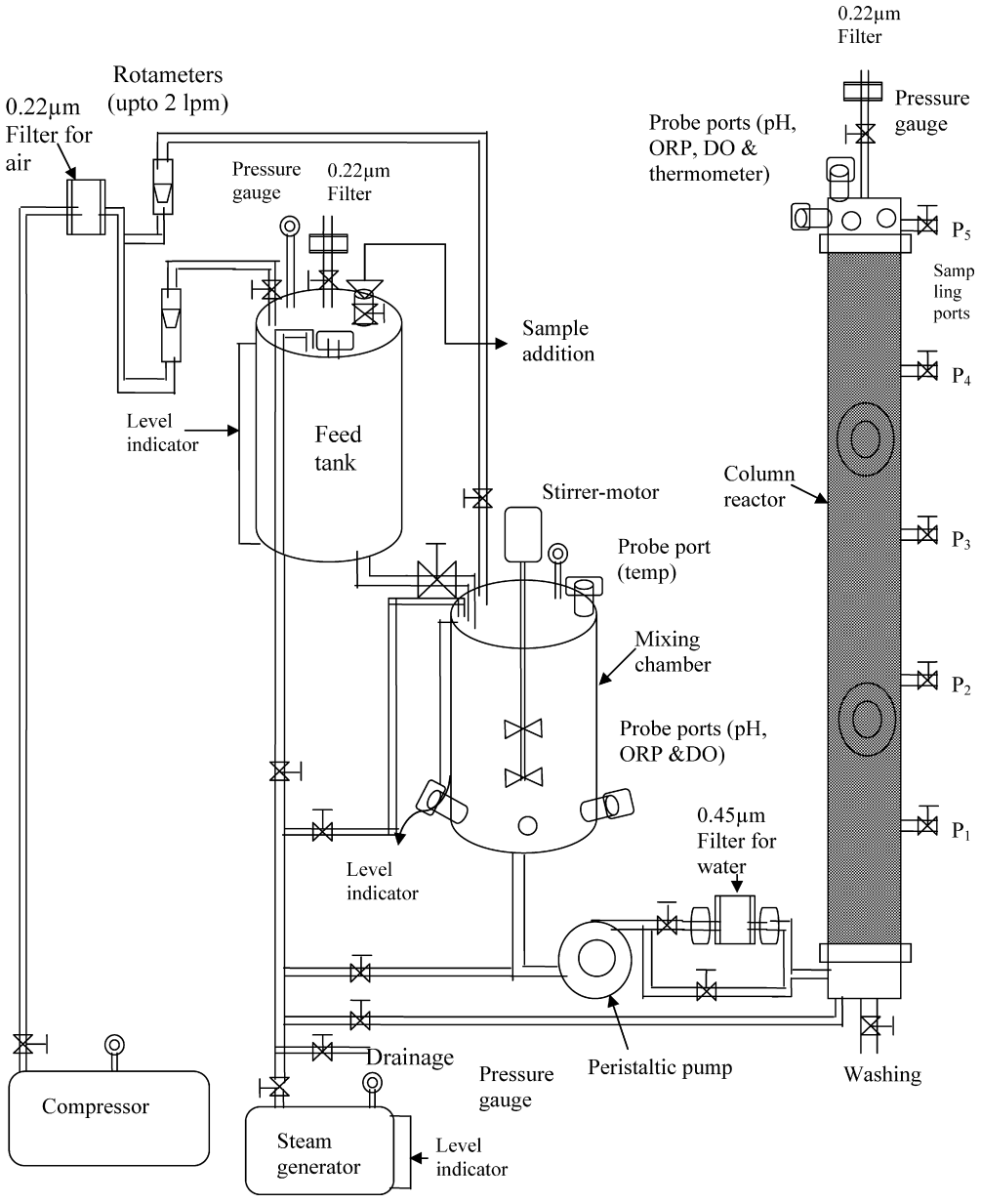

Fig. 1. Schematic diagram of the experimental setup with up-flow bio-column reactor.

2.4. Procedure

The close circuit of the reactor assembly was steam sterilized at $121 \pm 0.5\,^{\circ}C$ and 15 psig pressure for 20 min. Approximately one pore volume of the inlet substrate with an ionic strength of 9.82×10^{-3} M (composition shown in Table 2), was pumped through the reactor at the feed flow rate of 2.08 ml/min to stabilize and condition the GAC before the commencement of an experiment.

After the conditioning of GAC in the reactor, it was initially filled with the influent inoculated with acclimatized *R. eutropha MTCC 2487* containing the desired metal concentration. The

Table 2
Composition of the inlet substrate for the stabilization of GAC bed

Components	As(III)	As(V)	Fe	Mn	Cu	Zn	NB
Concentration	12.5 ppm	12.5 ppm	10 ppm	2 ppm	5 ppm	10 ppm	0.125%

influent was pumped from the mixing tank to the bottom inlet of the reactor by means of a pre calibrated variable speed peristaltic pump at 2.08 ml/min (approximately 1.6 pore volume per day) and kept as such for 3 days for the preconditioning of the bacteria. The initial pH of the solution was adjusted using 2 M HCl or NaOH to the required pH value (6.5 ± 0.1). Tri-sodium citrate was added at 1800–1850 mg/l to prevent metal precipitation. NB was supplied as energy source. The by pass line of the peristaltic pump was used to feed the bacteria containing solution (cell concentration of 2×10^8 cells/l and dry cell mass of 0.125 g/l) into the reactor. The number of cells was counted by hemocytometer and optical microscope. After 3 days of initial filling the liquid in the reactor was drained off followed by the refilling of the reactor with bacteria incubated influent. Similar refilling of the reactor was performed on 6th, 9th and 12th day to provide total 14 days period for preconditioning and bio-layer formation on the GAC bed in the column reactor. Recently, 14 days preconditioning of sulphate reducing bacteria (SRB) has been reported [4].

Continuous flow with contaminated water sample was started after 14 days and it was continued for further 15 days for each experiment. The water sample was passed through the 0.45 μm filter before entering into the column reactor [4]. Various flow rates were used to get various EBCT values (6, 12, 18 h). High purity air (passed through 0.2 μm filter) was continuously purged through the mixing and feed tanks at a rate of approximately 2 lpm to provide more oxygen for getting more DO value. The pH, ORP and DO of the inlet and outlet samples of the reactor were measured by pH, ORP and DO meters, respectively, fixed at mixing tank and top portion of the reactor. The range of operating parameters is shown in Table 3. Duplicate experiments were conducted in non-parallel manner. Control experiments containing no *R. eutropha MTCC 2487* were operated in the same manner as that of the inoculated column.

The treated samples were further filtered through 0.45 μm filters for estimation of the metal contents. As(T) in the filtrate was analyzed by Perkin-Elmer ICP-MS model ELAN-DRC-e. For arsenic speciation the treated sample was passed through a strong base anion resin column for the separation of As(III) and As(V) followed by the ICP-MS analysis. The detail procedure is mentioned somewhere [21]. The accuracy-reproducibility of ICP-MS was $98 \pm 1\%$. Concentration of Fe, Mn, Cu and Zn was determined by atomic absorption spectroscopy (AAS), GBC, Avanta, Australia. SEM photograph of GAC before and after bio-adsorption was taken by an electron microscope, LEO Electron Microscopy Ltd., England. IR spectra of the fresh GAC, spent GAC and GAC after bio-adsorption were taken by a Thermo FTIR model AVATR 370 csl coupled with EZOMNIC software version 6.2. Elemental analysis of the GAC was carried out by an elemental analyzer system (Elementar Analysensysteme GmbH, model Vario-EL V3.00). Surface area and micro pore volume of the samples were measured by N_2 adsorption isotherm using an ASAP 2010 Micromeritics instrument by Brunauer–Emmett–Teller (BET) method, using the software of Micromeritics. Nitrogen was used as cold bath (77.15 K).

The initial pH (6.5 ± 0.1) of the solution favors the precipitation of metals and the precipitation increases with time. To reduce the chance of such precipitation of metals, the sample was freshly prepared after each 12 h and added in the feed tank. To identify the initial precipitation of the elements the nominal concentrations of metals (before entering into the bio-column) were compared to their initial measured concentrations as shown in Table 4. It was observed that all the metals were partly removed from the water by initial precipitation. However, in all the cases it was $< 20\%$. Backwashing of the column was done by using limited amount of treated water (∼3 l) [13].

3. Results and discussion

Effect of EBCT and bed height on the removal of total arsenic, removal of arsenic species, regeneration and backwashing of the reactor bed, change of pH and ORP with time, removal of

Table 3
Range of operating parameters for the column reactor operation

Temperature (°C)	pH	Air flow (lpm)	Feed flow rate (ml/h)	EBCT (h)	GAC size (mm)	Water filter size (μm)	Air filter size (μm)
29 ± 1	6.5–7.5	2	102–306	6–18	2–4	0.45	0.2

Table 4
Comparison of the nominal and initially measured concentration of various metal ions in the sample

Components	As(III)	As(V)	Fe	Mn	Cu	Zn	pH
Concentration initially measured (ppm)	12.5	12.5	10	2	5	10	6.5
Concentration nominal (ppm)	12.4	12.2	9.4	1.8	4.3	8.2	6.5
% Variation	0.8	2.4	6.0	10	14	18	–

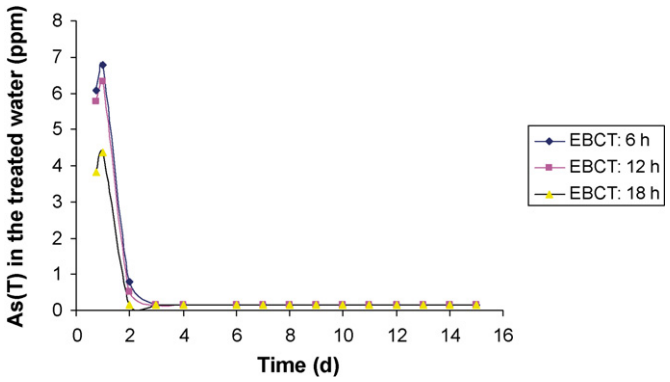

Fig. 2. Effect of EBCT on the arsenic removal in the bio-column reactor (sample collected at the top of the reactor, initial arsenic concentration (As_o): 25 ppm).

other metals and the comparison of the recent method with some recently reported techniques are mentioned in the subsequent sections.

3.1. Effect of EBCT

EBCT highly influences the performance of a bio-column reactor. Longer EBCT provides more contact time as a result the out let concentration of the pollutants becomes less. However, more EBCT value also reduces the treatment capacity of the reactor. Wide range of EBCT values (6 min to 18 h) have been used in literature [13,17]. Normally, lower EBCT is used for the treatment of ground water with indigenous bacteria. In this process a bio-layer is developed on the adsorbent bed by passing the tap water for 3–4 months, no inoculated media is required is this case. However, longer EBCT is normally used for the treatment of industrial effluents, where bacteria inoculated media is used to develop the bio-layer on the adsorbent bed. In the present study, three EBCT (6, 12 and 18 h) have been chosen and the effect of EBCT on the arsenic concentration in the treated water, collected from top of the reactor has been shown in Fig. 2. From Fig. 2 it is evident that for all the EBCT values the arsenic concentration in the treated water increases initially and after ~2 days it starts to decrease. After day 3 the arsenic concentration in the treated water reduces to ~0.15 ppm, which is below the MCL of arsenic in industrial effluents. Maximum concentration of arsenic in the treated water is found after ~1 day of operation, which reduces gradually with operation time. This indicates that the bacteria take some time to adjust in the continuous operation of the reactor. Similar observation has been reported recently during the arsenic removal in a bio-column reactor using SRB [4]. With the increase in EBCT value, the contact time of the water sample with the bio-layer increases. Due to this reason arsenic concentration in the treated water using an EBCT value of 18 h is less than those obtained by EBCT values of 12 and 6 h. For lower EBCT values, the water sample gets lower contact time with adsorbent and at the initial stage of operation, it leaves the reactor before the bacteria of the bio-film cope up with the continuous operation. Hence, at the initial stage of operation the arsenic removal is less. With the increase in time bacterial mass accommodate themselves in the continuous mode of operation, as a result of the observed effect of EBCT on the arsenic removal becomes negligible after ~2–3 days. Hence, 6 h EBCT is sufficient for the bio-treatment process.

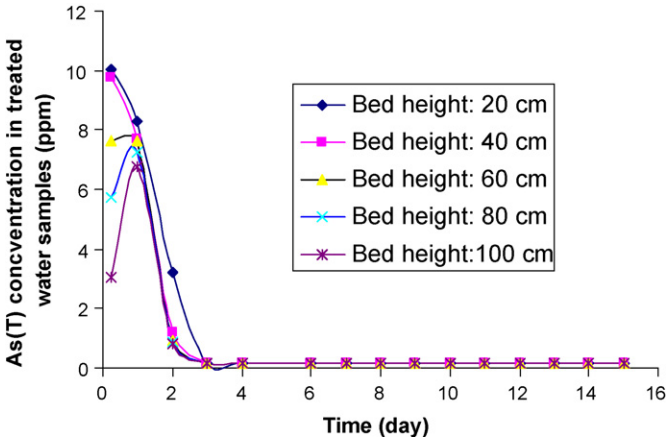

Fig. 3. Effect of bed height on the arsenic removal in the bio-column reactor (EBCT: 6 h, As_o: 25 ppm).

3.2. Effect of bed height

The contact time of the water sample collected at various heights along the reactor bed of the bio-column reactor varies even if the flow rates of the influents remain constant. Fig. 3 shows the effect of bed height on the arsenic concentration in treated water at the EBCT of 6 h. Bed heights 1–5 correspond to the height of sampling port P_1 to P_5, respectively.

From Fig. 3 it is evident that the arsenic concentration of the treated water collected from port P_4 and P_5 reduces to ~0.15 ppm within 2 days. However, the arsenic concentration of the samples collected from port P_1, P_2 and P_3 after 2 days are more than 2 ppm. These values reduce to ~0.15 ppm after 3 days. At the initial stage, the bacteria take some time to adjust themselves in the continuous mode of operation of the reactor. With the decrease in the bed height, the contact time reduces, which leads to the lower arsenic removal. After some time of operation the bacteria is adjusted in the reactor and no effect of bed height is observed on the arsenic concentration of the treated water. It was also observed that with the increase in bed height from the bottom, the DO value of the water decreases. Fig. 4

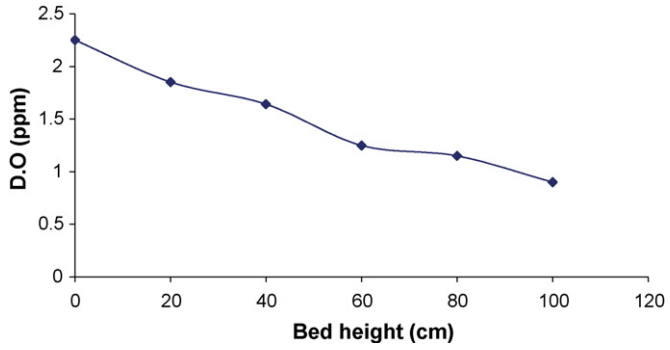

Fig. 4. Change of DO along with the bed height of the bio-column reactor.

shows the change in DO of the solution along with the reactor bed height after 2 days of operation.

From Fig. 4 it is evident that the dissolved oxygen is continuously consumed by the bacteria along the bed height, which is obvious for aerobic bacteria. From Fig. 4 it is also evident that the DO value reduces from ~2.25 ppm at the inlet of the reactor to ~1 ppm at the top (outlet) of the reactor. It is also evident that the DO value changes in small amount due to its shift from port P_3 to port P_5. At port P_3 the DO value is ~1.25 ppm. This may be due to the change in cell concentration along the height. The cell concentrations in solution at port P_3 and port P_5 after 3 days of operation are almost similar (~8×10^8 cells/l), which is greater than the cell concentration of the inlet solution (~2×10^8 cells/l). Increase in cell concentrations along the height of the bio-column reactor has also been reported recently [17]. Due to the increase in bacterial cell concentration along the height of the reactor the DO value decreases. However, after P_3 the increase in bacterial concentration in the reactor is negligible as a result there is very less difference in the DO value at P_3 and P_5. It is also evident from Fig. 3 that after around 3 days, arsenic concentration in the treated water collected from all the sampling ports is similar. This indicates that the bacterial cells are properly adapted in the reactor environment throughout the length of reactor, within this time period. The influent water enters the reactor at the bottom. Therefore, the bacteria in the lower part of the reactor may take less time to adjust themselves in the continuous mode of operation of the reactor than those of the upper part bacteria. Absence of initially increasing parts of the curves corresponding to bed height of 20 and 40 cm (Fig. 3) indicates that 6 h of operation time may be sufficient for the adjustment of the bacteria present in the lower part of the reactor (up to 40 cm of bed height).

3.3. Removal of arsenic species

After ~2–3 days of operation of the bio-column reactor more than 99% of arsenic is removed from the water. It indicates that both the arsenic species are removed by the bio-adsorption process. Fig. 5 shows the removal of arsenic species (sample collected at P_5) with an EBCT value of 6 h. From Fig. 5 it is evident that after ~2 days of operation the concentration of As(III) and As(V) are almost equal in the treated water. It indicates that both the arsenic species are equally adsorbed by the bio-adsorbent

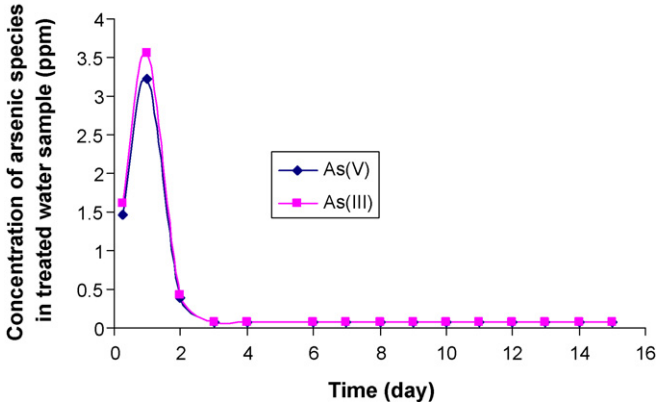

Fig. 5. Removal of arsenic species in the bio-column reactor (As_o: 25 ppm, As(III):As(V) = 1:1).

after ~2 days of operation. However, at the initial stage As(V) shows slightly more removal than As(III). This may be explained as follows.

It is a well-know fact that in a bio-column reactor a bio-film is formed on adsorbent surface. In the present case, formation of a bio-layer on the GAC bed is evident by comparing Fig. 6(a and b). *R. eutropha* in this bio-layer may produce ArsR protein, which may capture As(III). It can also produce arsenate reductase enzyme, which may convert As(V) to As(III) followed by adsorption on ArsR protein on bacterial cell surface [10,11]. A broad band around the wave number of 1400 cm^{-1} in spectra (C) in Fig. 7 indicates the presence of proteins and lipids on the surface of the GAC after bio-removal [22–23]. This accumulation of protein and lipids on the surface of GAC is due to the formation of bio-layer on it. A small peak is observed in the spectra C at the wave number of 780 cm^{-1}. However, the peak at the wavelength of 885 and 820 cm^{-1} are negligible. This indicates the presence of As(III) in the bio-adsorbent (after bio-adsorption) [24]. This also supports the mechanism of As(III) accumulation on the cell surface and conversion of arsenate to arsenite by arsenate reductase. However, the extent of removal of As(V) by its conversion to As(III) and subsequent sequestration is not clear. It may be due to the complex nature of As(V) adsorption, no single mechanism can explain adsorption of arsenic from water [25].

At the initial stage bacteria take some time for adaptation hence capture of As(III) and As(V) on bacterial protein may

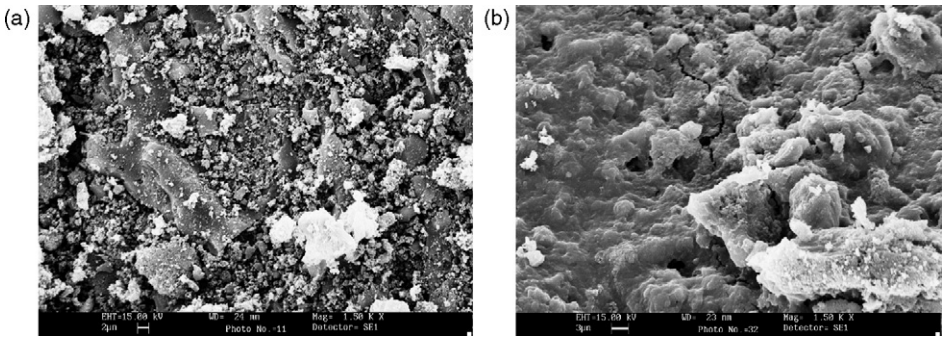

Fig. 6. SEM micrographs of GAC. (a) Before bio-adsorption/accumulation and (b) after bio-adsorption/accumulation, each shown at a magnification of 1500×.

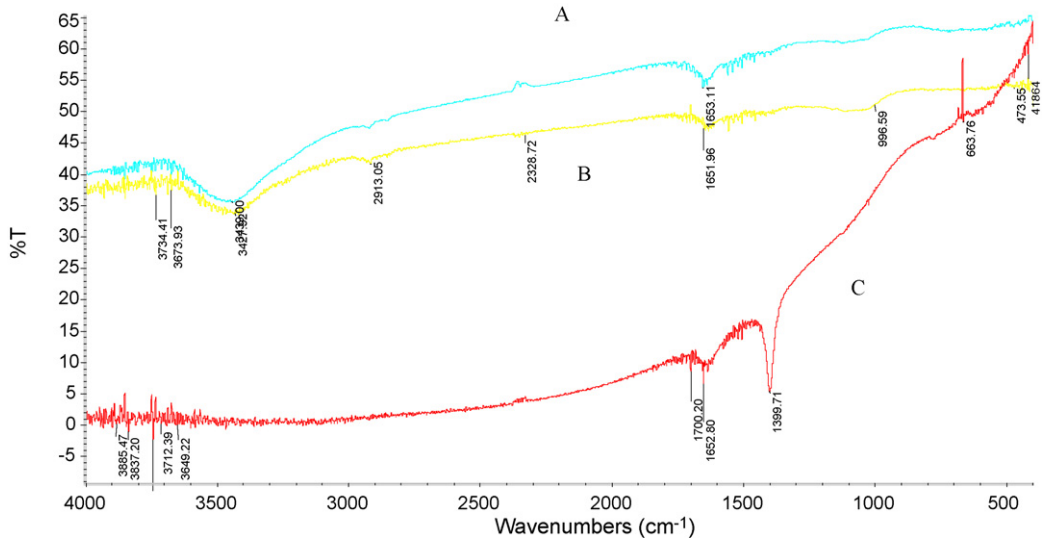

Fig. 7. FTIR spectra of GAC (A), GAC after adsorption (B) and GAC after bio-adsorption/accumulation (C).

be less. However, adsorption or to some extent precipitation may occur with As(V) as it exists as negatively charged moiety in the experimental pH range. As a result removal of As(V) is slightly more than that of As(III) at the initial stage. After around 2 days of operation *R. eutropha* may get fully adapted in the environment resulting almost equal removal of both arsenic species.

3.4. Regeneration of the rector by backwashing

In a bio-column reactor using *R. eutropha*, As(V) may be converted to As(III) by arsenate reductase enzyme followed by the accumulation on the protein (ArsR) of bacterial cell surface [10,11]. Other metals may be adsorbed on the negative sites of the gram-negative *R. eutropha*. With increase in reaction time more metals and arsenic are adsorbed/precipitated on the cell surfaces and produces excess dead biomass, which blocks the pore volume (void space) of the reactor. Excess biomass and heavy metal precipitates can potentially create a problem by clogging the pore space of the reactor. Complete clogging can be avoided by intermittent flashing of the column by increasing the influent up-flow velocity [4] or by backwashing [13]. The effect of washing on the arsenic removal capacity of the reactor, with EBCT of 6 h is shown in Fig. 8. It also compares

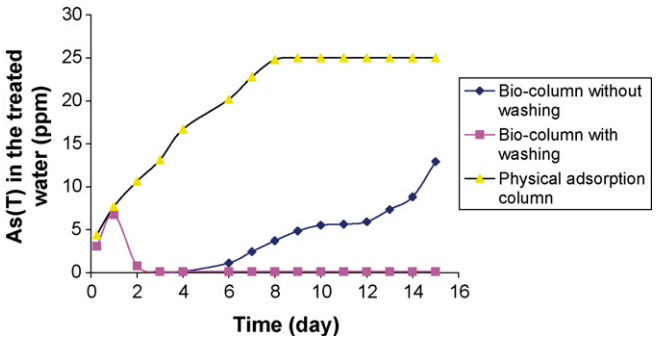

Fig. 8. Effect of backwashing on the arsenic removal in the bio-column reactor.

the removal capacity of a column reactor with GAC working on the basis of physico-chemical adsorption. From Fig. 8 it is evident that the bioprocess improves the capacity of the reactor dramatically if the backwashing is applied. However, after some days of operation without washing, the whole bed becomes exhausted resulting no removal of arsenic. Hence, after 4–5 days of operation the bio mass and heavy metal precipitates must be cleaned by backwashing to get the effective removal of the arsenic. Recently, backwashing after 3 days has been reported for the treatment of arsenic contaminated ground water with iron oxidizing bacteria [13]. In that experiment no Cu and Zn were used and the Fe and Mn concentration were also less. In spite of more concentration of the heavy metals the requirement of backwashing after 4–5 days, in the present study, may be due to the low flow rate of influent water. In the previous experiment the flow rate was 111–560 ml/min but in the present experiment it is 1.8–5.2 ml/min.

At the starting of the reaction, up to around 1 day, both bio-adsorption column and physico-chemical adsorption column give similar removal of arsenic. Thereafter, the concentration of As(T) in the treated water for bio-column reactor reduces significantly whereas for physico-chemical adsorption column this arsenic concentration gradually increases. This observation indicates that the bacteria take some time to adjust in the new environment.

3.5. Change in pH and ORP

Another important observation of the experiment is the slight increase of pH with time as shown in Fig. 9. The pH in Fig. 9 corresponds to the top of the reactor and EBCT value is 6 h. From Fig. 9 it is evident that initially the pH decreases slightly and starts to increase after 1 day of operation of the reactor, which gives the indication for the adaptation of the bacteria in the new environment. After around 6 days of operation the pH reaches 7.5. Simultaneous decrease in ORP from around 260 mV to around −280 mV was observed within this time period. Sim-

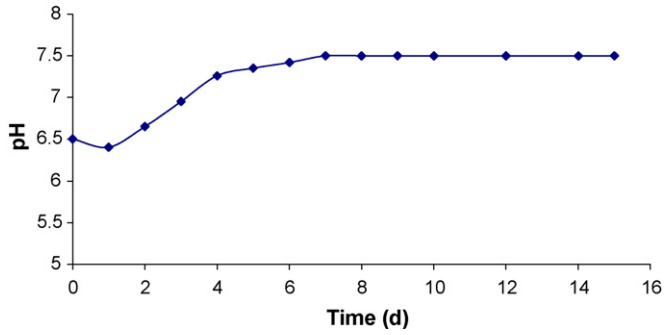

Fig. 9. Change of pH with the time of operation in the bio-column reactor.

ilar change in pH was reported recently on the arsenic removal study by using SRB in bio-column reactor [4]. The mechanism for such slight increase in pH is not well understood. However, recently it has been reported that the reaction of molecular oxygen at the surface of the carbon results complex C_xO or C_xO_2. This adsorbed oxygen complex, in neutral solution, is sufficiently active to cause an oxidation of water as per the following reaction [20,26].

$$C_xO + H_2O = C_x + 2OH^{-1}$$

The hydroxyl ion may combine with H_2O resulting in a net increase in the pH of the solution [26].

3.6. Removal of other metals

Residual concentration of Fe, Mn, Cu and Zn in the treated water sample with the EBCT value of 6 h is shown in Fig. 10. The residual concentrations are with reference to the treated water collected from the sampling port P_5 (top). From Fig. 10 it is evident that the residual concentration of Fe, Mn and Cu increase at the starting of the continuous reaction and reach their maximum value after ~1 day of operation. Thereafter, the residual concentrations dramatically decrease and after 2–3 days of operation the minimum residual concentration of the metals are obtained, which virtually do not change up to 15 days. However, for Zn no such initial increase in residual concentration is observed. It is also evident that under the operating conditions, the removal of Zn is maximum, followed by Cu, Mn and Fe. *Ralstonia* belongs

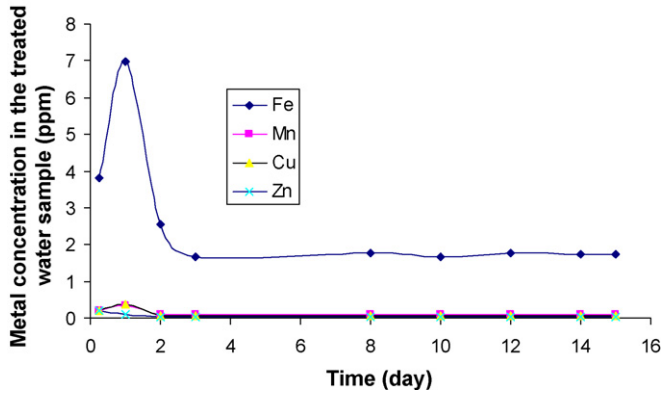

Fig. 10. Removal of Fe, Mn, Cu and Zn in the bio-column reactor (initial concentration of metals, Fe_o: 10 ppm, Mn_o: 2 ppm, Cu_o: 5 ppm, Zn_o: 10 ppm).

to the cation diffusion facilitator protein family of bacteria. It has an amino-terminal streptavidin-tagged protein $C_{ZC}D$ that binds Zn and Cu [27]. That is why the removal of Zn and Cu is very high. Mn may be taken up by Mg transport system [28]. Mechanism of iron take up by this strain is not clear. However, iron normally follows siderophore-mediated uptake [29] as a result it gives less removal of Fe. From Fig. 10 it is also evident that the residual concentration of all the metals (except Mn) is below their respective MCL values for effluent waters.

3.7. Advantage of the new method

Important features of some recently reported bio-column reactor studies on arsenic removal are summarized in Table 5. The type of bacteria, influent arsenic concentrations and other experimental conditions were different in these experiments. Hence, it is difficult to compare the efficiency of these technologies. However, the comparison can give an idea about various technologies reported in these experiments. From the above table it is evident that the present method using *R. eutropha MTCC 2487*, gives better removal of arsenic. In this case the residual arsenic concentration is lower than the permissible limit of arsenic for effluent water. The EBCT value is also less in the present experiment. In reference [14] the oxidation of As(III) was examined rather than the removal of arsenic. Like the present experiment, acid mine drainage was treated in ref. [4]. However, sulfate-reducing bacteria was used in that experiment. The present experiment has also been conducted with more initial arsenic concentration than that of ref. [2]. Another important report on the removal of arsenic using immobilized bacterial mass has been reported recently [23]. Iron oxidizing bacteria, indigenous to the groundwater, were used to remove arsenic from contaminated ground water in ref. [23]. The bio-column reactor using *G. ferruginea* was capable to handle water sample with higher velocity. However, the concentration of arsenic and Mn was very less in that experiment. Therefore, the use of the arsenic resistant bacteria, applied in the present study is more efficient for treating acid mine drainage.

Another important technique for removing arsenic from contaminated water is adsorption on surface modified adsorbents. Some of the recently reported adsorbents used for removing arsenic in column reactors are aluminum-loaded Shirasu-zeolite, iron-oxide-coated polymeric materials, iron oxide coated cement, bead cellulose loaded with iron oxyhydroxide, MnO loaded polystyrene resin, etc. [30–34]. These surface modified adsorbents require less contact time and can remove arsenic even at a higher velocity. However, the major drawback of these adsorbents is the need of regeneration, which decreases the cost effectiveness of the process. In some cases the adsorbent losses its mass due to regeneration also. In the present case, regeneration of the bed is possible by only backwashing the bed with water. Therefore, the present process can also compete with the surface modified adsorbents.

Acid mine drainage normally contains high amount of arsenic (0–250 ppm) and its pH is also less. Therefore, pretreatment steps like coagulation–precipitation or lime softening is necessary to reduce the eventual arsenic concentration to a lower value

Table 5
Comparison of the present work with the literature

Ref.	As_o (ppm)	Contact time (min)	Flow rate (ml/min)	Flow velocity (cm/min)	pH	ORP (mv)	DO (ppm)	As_e (ppm)
[2]	20	840	2.61	0.8	4–8	−218	Anaerobic	>4
[9]	0.030–0.160	6–12	560–111	8.3–16.7	7	−300 to 320	0.9–3.7	<0.01
[10]	–	1062	0.6	–		–	Anaerobic	–
[14]	100	420	0.75	–	6-8	–	–	>20
Present study	25	360–1080	1.7–5.1	0.8–2.4	6-7	−280 to 260	1.0–2.25	<0.15

As_e: Arsenic concentration in the treated water.

(<50 ppm) prior to bio-removal process. The pH of the solution can also be increased during the pretreatment steps and the pretreated samples can be effectively treated by the bio-adsorbents as a polishing stage of water treatment.

Another problem is the disposal of the eluted solution. At present the most attractive option for dealing with arsenic wastes is encapsulations of the material, usually through stabilization/solidification techniques and disposing of the treated wastes in secured landfills. USEAP has recognized stabilization process as best demonstrated available technology (BDAT) for land disposal of most toxic elements. The solidification process for arsenic contaminated solids can be done by fixation with (i) Portland cement, (ii) Portland cement and iron(II), (iii) Portland cement and iron(III), (iv) Portland cement and lime, (v) Portland cement, iron and lime, (vi) Portland cement and fly ash, (vii) Portland cement and silicates. Presently, it has been reported that stabilization/solidification of arsenic is most successful when cement, cement and iron, cement and lime, or combination of these are used [35]. In the present case the eluted solution can be dried and the solid can be stabilized or can be disposed in secured landfill.

4. Conclusion

From the above discussions the following conclusions are drawn:

1. The bio-column reactor is capable to reduce the concentration of the pollutants in the effluent water below their permissible limit.
2. Bio-column reactor must be backwashed for effective continuous operation.
3. At the initial stage, the EBCT and bed height have significant influence on the removal of arsenic from the contaminated water. However, after some time of operation (approximately 3–4 days) such influence is negligible under the experimental conditions.
4. Both As(III) and As(V) are removed equally after ∼2 days of operation. However, at the initial stage the removal of As(V) is slightly more than that of As(III).
5. DO reduces along the bed height of the reactor, which supports the aerobic nature of the bacteria.
6. pH of the solution slightly decreases initially for the first day and increases within small range (6.5–7.5) and ORP decreases from 260 to −280 mV.

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