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Treatment of arsenic contaminated water in a batch reactor by using *Ralstonia eutropha MTCC 2487* and granular activated carbon

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Abstract

This paper presents the observations on the bio-removal of arsenic from contaminated water by using *Ralstonia eutropha MTCC 2487* and activated carbon in a batch reactor. The effects of agitation time, pH, type of granular activated carbon (GAC) and initial arsenic concentration (As_o) on the % removal of arsenic have been discussed. Under the experimental conditions, optimum removal was obtained at the pH of 6–7 with agitation time of 100 h. The % removal of As(T) increased initially with the increase in As_o and after attaining the maximum removal (∼86%) at the As_o value of around 15 ppm, it started to decrease. Simultaneous adsorption bioaccumulation (SABA) was observed, when fresh GAC was used as supporting media for bacterial immobilization. In case of SABA, the % removal of As(III) was almost similar (only ∼1% more) to the additive values of individual removal of As(III) obtained by only adsorption and only bio-adsorption. However, for As(V) the % removal was less (∼8%) than the additive value of the individual % removals obtained by only adsorption and bio-adsorption. Percentage removal of Fe, Mn, Cu and Zn were 65.17%, 72.76%, 98.6% and 99.31%, respectively. Maximum regeneration (∼99.4%) of the used bio-adsorbent was achieved by the treatment with $5NH_2SO_4$ followed by 1N NaOH and 30% H_2O_2 in HNO_3. The fitness of the isotherms to predict the specific uptake for bio-adsorption/accumulation process has been found to decrease in the following order: Temkin isotherm > Langmuir isotherm > Freundlich isotherm. For the adsorption process with fresh GAC the corresponding order is Freundlich isotherm > Langmuir isotherm > Temkin isotherm for As(V) and As(T). However, for As(III) it was Langmuir > Temkin > Freundlich.
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1. Introduction

Arsenic enters into human body through water and causes various acute and chronic diseases. Both ground water and surface water are contaminated with arsenic by natural and anthropogenic sources, respectively. Arsenic affected ground water generally contains some cations like Fe, Mn, Ca, etc., anions like Cl^{-1}, $SO_4{}^{-2}$, $PO_4{}^{-3}$, $NO_3{}^{-1}$, etc. and its pH normally varies from 6–8. Industrial effluents like metal-processing industries, semiconductor industries, etc. and acid mine drainage contain arsenic in higher concentration. 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 [1]. The As(III) concentration in this acid drainage has also been reported to be around 60–90% of the As(T). Acid mine drainage also normally contains Cu, Zn, Fe, Mn, etc. along with arsenic [2].

Important role of mine drainage on the arsenic poisoning has recently been observed in Koudikasa village of Rajnandgaon district in Chhatrishgarh, India. The possible reasons for this arsenic poisoning has been reported as the presence of uranium mines of Atomic Energy Commission during 1982–1989 at Bodal which is about 5 km away from Koudikasa and the presence of nearby Sibnath river contaminated with gold mine drainage [3].

Amongst various treatment options the surface modified adsorbents and biological treatment with living microbes are gaining momentum in recent years for the removal of arsenic from contaminated water [4]. Some of the bacteria having arsenic removal capability are *Alcaligenes faecalis, Agrobacterium tumafecians*, bacteria *NT26*, *Bacillus indicus*, *Bacillus subtilis, Corynebacterium glutamicum, Desulfovib-*

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rio desulfuricans, Galleonella ferrigunea, *Leptothrix ocracia, Pseudomonas putida, Pseudomonas arsenitoxidans, Ralstonia picketti, Thiomonas Ynys1, Thiobacillus ferroxidans,* etc. [4–14].

Amongst these arsenic bacteria the *A. faecalis, B. subtilis,* bacteria *NT26, C. glutamicum*, etc. have been exploited recently to remove arsenic in batch reactor study [5,7,9,10]. The arsenic bacteria may be arsenic oxidizing type, iron oxidizing type, sulfate reducing type or arsenic resistant type. 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 [10]. 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 [6,10]. ArsR has specific active sites for accumulating As(III) [6] Recently, arsR–arsC gene cluster has been observed in *Ralstonia eutropha CH34* [6], which is also known as *R. eutropha MTCC 2487* [15]. This strain can produce ArsR protein and arsenate reductase enzyme [6]. However, the arsenic removal by this strain is not yet demonstrated [6]. *R. eutropha* has also the capability to grow autotropically in absence of organic source [16]. The chemoautotrophic nature of *R. eutropha* increases its potential for treating arsenic containing water where organic carbon is hardly present. Efficient removal of some heavy metals like Cd, Co, Hg, Ni and Zn from contaminated water by using *R. eutropha MTCC 2487*, isolated from Zn factory wastewater, has been well documented [15].

Arsenic removal efficiency of bacteria improves when it is immobilized on a solid support like GAC [4]. If fresh GAC is used some amount of physico-chemical adsorption may occur along with bio-adsorption/accumulation leading to simultaneous adsorption bioaccumulation (SABA). Use of spent GAC minimizes the physico-chemical adsorption as the arsenic adsorption capacity of spent GAC is very low. The removal efficiency of bio-removal process may also be dependent on the other process parameters like agitation time, pH, etc. The initial arsenic concentration also influences the % removal.

In the recent paper the capability of *R. eutropha MTCC 2487* for the treatment of arsenic contaminated water has been explored. Fe, Mn, Cu and Zn have also been included in the synthetic water sample. The effects of agitation time, pH, type of GAC and As_o on the removal of arsenic have been described. Removal of other metals and regeneration of the adsorbents have been discussed. Equilibrium isotherms for physico-chemical adsorption and bio-adsorption/accumulation have been developed and compared. The present method has also been compared with some recent works on the arsenic removal from contaminated water.

2. Theory

The mechanism of arsenic removal and the modeling of the bio-adsorption process are discussed below.

2.1. Mechanism of arsenic and metals removal

Gram negative bacteria, which expose negatively charged groups on their cell surface, have the capacity to bind metal ions. Various compounds of bacterial walls sorb different metals, which later get precipitated [9]. Another important route for the capturing of metals/metalloid is complexolysis by which metal/metalloid ions are solubilized in the microbial formation of complexing or chelating agents [9]. Some microorganisms produce specific proteins, which are induced by metal/metalloid ions and bind these ions [9]. ArsR is such a protein, which contains a very specific binding site towards As(III) and can discriminate effectively against phosphate, sulfate, cobalt and cadmium [17]. Production of ArsR is also inducible by As(III) concentration [17]. In case of arsenic resistant bacteria the removal of As(V) may occur through the initial conversion of As(V) into As(III) by the arsenate reductase and the subsequent sequestration by ArsR [17]. Therefore, in living cell system the removal of arsenic increases with the increase in the concentration of bacterial mass. In neutral pH range As(V) exists as negatively charged moiety in the solution. Hence, some extent of As(V) may be co-precipitated with the metals like Fe and Mn or may be adsorbed on the positive sites of the adsorbent by physico-chemical adsorption.

2.2. Modeling

The synthetic solution contains arsenic along with Fe, Mn, Cu and Zn. In the experimental pH range, all the metal ions except arsenic species exist as positively charged ions where as arsenic exists as negatively charged moiety. Hence, there is no competition amongst the other metals and arsenic to occupy the same active sites of the adsorbent. Similarly, for bioconsumption of different metals, different enzyme systems are active. Therefore, the overall process can be modeled by mono component isotherms rather than multi component isotherms. Langmuir, Freundlich and Temkin isotherms are commonly used mono component isotherms to describe the arsenic removal by physico-chemical adsorption as well as bio-adsorption processes [9,18].

The Langmuir isotherm theory is based on the assumption that adsorption is a first-order chemical process and monolayer of adsorbed material is formed onto a series of distinct sites (unisite) on the surface of the solid. The mathematical expression of Langmuir isotherm is mentioned below:

$$q_e = \frac{q_{max} b C_e}{(1 + bC_e)} \quad (1)$$

The q_{max} (mg/g) and b (l/mg) are the Langmuir constants related to the capacity and energy of adsorption.

Freundlich isotherm is developed on the basis of the formation of monolayer due to adsorption onto a rough heterogeneous surface (multi sites). It can be presented by the following equation.

$$q_e = K_f C_e^{1/n} \quad (2)$$

Where K_f ((mg/g)/(mg/l)$^{1/n}$) and n are the Freundlich isotherm constants related to the adsorption capacity and degree of favourability of adsorption, respectively.

Temkin isotherm is derived using molecular statistical theory. It is also applicable for heterogeneous surface and can be expressed as follows:

$$q_e = B_1 \ln(K_T C_e) \quad (3)$$

Where K_T (l/mg) is equilibrium binding constant corresponding to the maximum binding energy and B_1(mg/g) is related to the heat of adsorption.

To determine the best-fit isotherms the above isotherm equations are converted to linear form and the value of correlation coefficient (R^2) is determined. Marquardt's percent standard deviation (MPSD) error function may also be generated as follows to find out the best-fitted isotherm [19]:

$$\text{MPSD} = 100\sqrt{\frac{1}{(n-p)}\sum_{i=1}^{i=n}\left(\frac{(q_{e,exp}-q_{e,calc})}{q_{e,exp}}\right)_i^2} \quad (4)$$

The MPSD error function is similar in some respects to geometric mean error distribution modified according to the number of degrees of freedom of the system. n and p in the above equation are the number of experimental data points and number of parameters in the isotherm equation.

3. Materials and methods

3.1. Microorganism and growth medium

R. eutropha MTCC 2487 species was obtained from Institute of Microbial Technology, Chandigarh, India. Nutrient broth (N.B.) culture media was prepared as per the guidelines of microbial type cell culture (MTCC).

3.2. Acclimatization

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

The revived culture was first grown in N.B. media in a 250 ml conical flask. After 48 h the synthetic medium in the flask had turned milky indicating significant bacterial growth in the flask. Appropriate quantity of stock solution of arsenic was added into the flask containing N.B. media to get a concentration of 1 mg/l of arsenic. Initially growth of *R. eutropha MTCC 2487* was inhibited and the growth started after 10 h. There after, the arsenic was periodically added in increments of 5 mg/l in a series of 250 ml flasks till the arsenic concentration in the growth media reached 200 mg/l. The content of N.B. was decreased over a period of one month to direct the bacteria towards the consumption of metals and arsenic. *R. eutropha* shows autotrophic nature in absence of organic source only [16]. Therefore, the N.B. concentration was reduced gradually to direct the bacteria to perform autotrophic growth. For inoculum, a further sub culturing was done and all the inoculum transfers were done in exponential phase (D.O. value ~0.6 at 600 nm) [20]. The temperature was maintained at 29 ± 1 °C.

3.3. Adsorbent and standard solution preparation

GAC and other chemicals were of analytical reagent (AR) grade. The GAC having bulk density of 40 g/100 ml was ground and sieved to 2–4 mm particle size fraction with standard testing sieve. Analytical grade sodium arsenate ($Na_2HAsO_4{\cdot}7H_2O$) and sodium arsenite ($NaAsO_2$) (s.d.fine chemicals, India) were used to prepare stock solutions of 1000 mg/l As(V) and As(III), respectively. Milli-Q water with resistivity of 18.2 MΩ-cm (Q-H_2O, Millipore Corp.) was used to prepare the solutions. Secondary As standards (10 mg/l) were prepared by diluting the stock solutions. Various arsenic solutions were prepared from secondary standard solutions of As(V) and As(III) and filtered through a 0.45 μm membrane. Stock iron solution was prepared by adding calculated amount of ferrous sulfate to get 200 mg/l Fe(II) solution. Stock solutions (100 mg/l) of manganese, copper and zinc were prepared by adding calculated amount of mangenus sulfate, cupric sulfate and zinc sulfate, respectively.

3.4. Procedure

Synthetic solutions were prepared by adding predetermined amount of secondary standard solutions of As(III), As(V), Fe, Mn, Cu and Zn. The initial pH of the solutions was maintained by using 0.1 M tris buffer for pH 6 and above. The pH of the 0.1 M tris buffer was 10 and it was reduced to pH 6 by adding 0.5 M HNO_3. The initial pH value of the solution was adjusted to 4 and 5 by a mixture of acetic acid (0.2 M) and acetate (0.2 M) solution [21]. The As(III) to As(V) ratio in the synthetic wastewater was 1:1. The spent GAC was prepared by the pretreatment with sufficient amount of synthetic water sample containing arsenic, Fe, Mn, Cu and Zn followed by washing and drying. The physical properties of the fresh GAC and spent GAC are shown in Table 1. For bio-adsorption study each experiment was carried out in conical flask (batch reactor) containing spent GAC and synthetic water inoculated with *R. eutropha MTCC 2487*. For simultaneous adsorption bioaccumulation study spent GAC was replaced by fresh GAC. The batch reactor was kept at 150 rpm in a shaker incubator. The concentrations of Fe, Mn, Cu and Zn in the synthetic solution were 10, 2, 5 and 10 mg/l, respectively. The As_o value was changed from 0–220 ppm at 29 ± 1 °C. To avoid the biological contamination the synthetic solution excluding As(III) was steam sterilized to 120 °C for 15 min. The As(III) solution of required concentration and volume was passed through 0.45 μm filter and added to the sterilized solution in laminar hood under aseptic condition. Each 5 ml of inoculum from 2 days old culture was added with each 45 ml of sample to get a final cell concentration of 2×10^8 cells/l and dry cell mass of 0.125 g/l (at the starting of bio-adsorption). The number of cells was counted by hemocytometer and optical microscope. IR spectra of the fresh GAC, spent GAC and GAC after bio-adsorption have been taken by a Thermo FTIR model AVATR 370 csl coupled with EZOMNIC software version 6.2. Elemental analysis of the GAC and spent GAC was

Table 1
Physical properties of GAC and spent GAC

Adsorbent	Elemental analysis (%)	Proximate analysis (%)	BET Surface area (m^2/g)	Micro-pore 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
Spent GAC	C: 73.39 H: 1.37 N: 0.40 S: 0.35 Others: 24.49	Ash: 3.13 Moisture: 8.35 Others: 88.52	359.24	0.0775

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). All the experiments were repeated thrice and average results have been reported. Control experiments were also done for bioprocesses using fresh GAC and spent GAC without bacteria. Control experiment with fresh GAC presents the physico-chemical adsorption studies on fresh GAC. The range of operating parameters is given in Table 2.

3.5. Arsenic speciation and analysis of metals

To determine the removal of metals from the water, the treated samples were further filtered through 0.45 μm filters for the estimation of the metal contents. As(III) and As(V) in the filtrate were analyzed by modified Edward's methods [22,23]. For the separation of As(V) from the solution containing As(III) and As(V), 20 ml sample was passed through a resin column containing 20 ml strong base anion resin (AG1X8) under gravity with a flow rate of 2.5 ± 0.25 ml/min (for optimum separation). Length and diameter of the resin column were 200 and 12 mm, respectively. The flow rate was maintained by a nozzle feed control system. The temperature and pH of the inlet water sample were 29 ± 1 °C and 2 ± 0.1, respectively. Analysis of the arsenic was done by ICP-MS, model ELAN-DRC-e. The foreword power (radio frequency power) and extraction voltage (radio frequency voltage) of the ICP-MS were 1100 W and 200 V, respectively. The pressure of argon gas was 4–5 kg/cm^2 with plasma gas flow rate of 15 L/min. The nebulizer gas flow and auxiliary gas flow rate were 0.93 and 1.2 l/min, respectively. The arsenic detection limit of ICP-MS was 1.6×10^{-6} g/l (parts per billion). As(T) was determined without separation of As(V) in the resin column and As(V) was measured by the difference between As(T) and As(III). The effect of Fe and Mn on the separation of As(V) from the solution containing As(V) and As(III) were minimized by using interactive correlation model [23]. Concentration of Fe, Mn, Cu and Zn was determined by atomic absorption spectroscopy (AAS), GBC, Avanta, Australia.

Table 2
Range of operating parameters used in the study

Operating parameters	Range
Temperature (°C)	29 ± 1
pH	4–7
Adsorbent dose (g/l)	16
Adsorbent particle size (mm)	2–4
Agitation time (h)	0–120
As_o (ppm)	0–220
N.B. concentration (g/l)	1.25

4. Results and discussions

The effect of agitation time, pH, GAC type and As_o on the arsenic removal, regeneration of the adsorbent, equilibrium isotherms of the bioprocess and the importance of the present study are discussed in the subsequent sections.

4.1. Effect of agitation time on the removal of arsenic species

Fig. 1 shows the increase in % removal of arsenic species with agitation time in the bio-removal process using fresh GAC as support media. From Fig. 1 it is evident that the % removal of both As(V) and As(III) increases with agitation time and after around 75 h of agitation time the increase in % removal of As(III) is very less. However, for As(V) it increases slightly even after

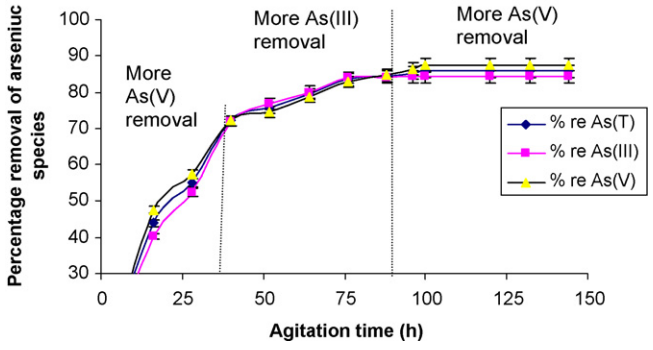

Fig. 1. Effect of agitation time on the removal of arsenic species (As_o =15 ppm, pH 6.5 ± 0.2).

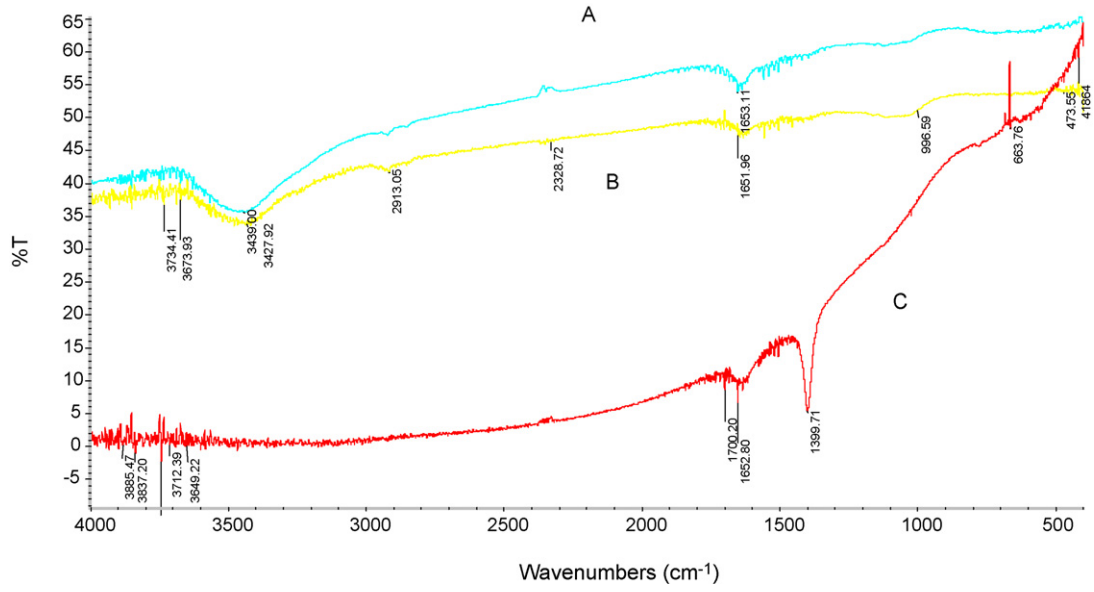

Fig. 2. FTIR spectra of GAC (A), GAC after adsorption (B) and GAC after bio-adsorption/accumulation (C) (As_o = 15 ppm).

96 h. After around 100 h of agitation time the increase in % removal of As(V) remains almost constant. Another important observation is that the % removal of As(V) is slightly higher than that of As(III) when agitation time is less than around 35 h and the % removal of As(III) is equal or slightly more than that of As(V) within the agitation time range of 35–90 h. It seems that the As(III) accumulation starts to predominate after some time of the bacterial growth (~30 h) and is continued for a long time, as a result, within the agitation time of 35–90 h, the % removal of As(III) is more than that of As(V). Similar observation on the As(III) accumulation by *B. subtilis* (more removal after 24 h of inoculation) has been reported [9]. Very less increase in the % removal of As(III) after around 75 h of agitation time may be due to the death of the bacterial mass, which ceases the accumulation of As(III) by ArsR protein. Maximum removal of As(III) by arsenic resistant bacteria *B. subtilis* has also recently been reported after 72 h of incubation [9]. Dead biomass can adsorb arsenic species until the equilibrium is reached. Hence, the % removal of As(V) increases up to around 100 h as As(V) is a negatively charged moiety in the operating pH range.

Comparing the FTIR spectra of the fresh GAC (A), GAC after adsorption (B) and GAC after bio-adsorption (C) in Fig. 2, it seems that some changes occur in the functionality of the GAC surface after bio-adsorption. A broad band around the wave number of 1400 cm^{-1} indicates the presence of proteins and lipids on the surface of the GAC after bio-removal [24,25]. This accumulation of protein and lipids on the surface of GAC may be 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) [26]. 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 [27].

4.2. Effect of pH on removal of arsenic species

The most important parameter of an adsorption process as well as bioprocess is pH. Fig. 3 shows the effect of pH on the % removal of arsenic species during bio-adsorption/accumulation using GAC at the As_o concentration of 15 ppm. It is evident that the % removal of all the arsenic species decreases with the decrease in pH from 7 to 4 and As(V) shows more removal than As(III). In bio-adsorption/bioaccumulation process As(III) is directly captured by ArsR protein and As(V) is converted to As(III) by arsenate reductase followed by adsorption/precipitation on ArsR protein. Therefore, the extent of relative removal of As(III) and As(V) will depend on the relative concentration of arsenate reductase enzyme and ArsR protein produced. If arsenate reductase production be more than the

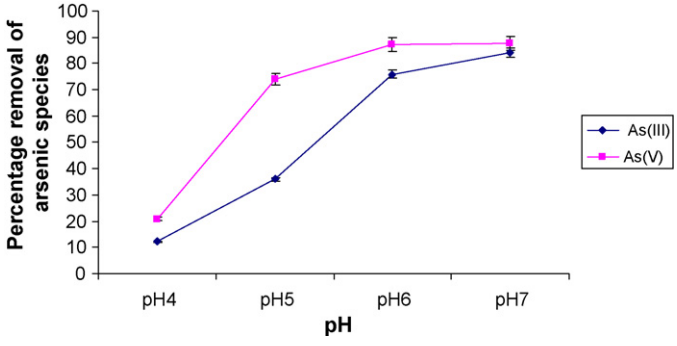

Fig. 3. Effect of pH on the removal of arsenic species (As_o = 15 ppm).

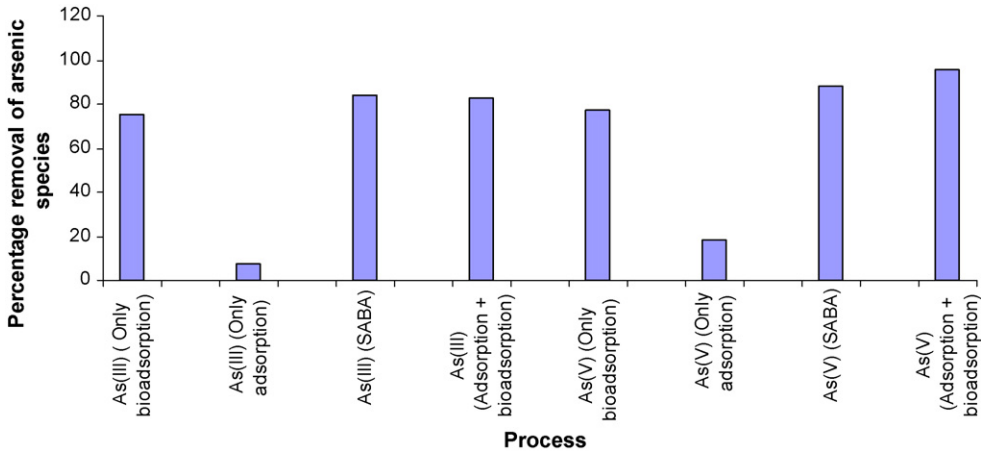

Fig. 4. Effect of GAC type on the removal of arsenic species.

production of ArsR protein then the As(V) removal can be more than As(III). However, hardly any report on this matter is available so far. Alternatively, As(V) may also be adsorbed to some extent by the GAC (at the initial stage) [28] and by the metal ions adsorbed on the bio-layer (in advanced stage), as As(V) has negative charge in the experimental pH range. It can also be co-precipitated in small extent along with metal ions. Due to these reasons the percentage removal of As(V) is more than that of As(III).

Growth of bacteria is highly sensitive to pH and the favourable pH for the growth of *R. eutropha* is 6.5–7. Therefore, the % removal of As(III) is maximum at pH 7. With the decrease in pH, the growth of the bacterial cells reduces, which decreases the % removal of As(III) and As(V). At pH 4, the growth of the bacteria was less as a result the percentage removal of both As(V) and As(III) was slightly higher than that of the corresponding values obtained by physico-chemical adsorption under the exper-

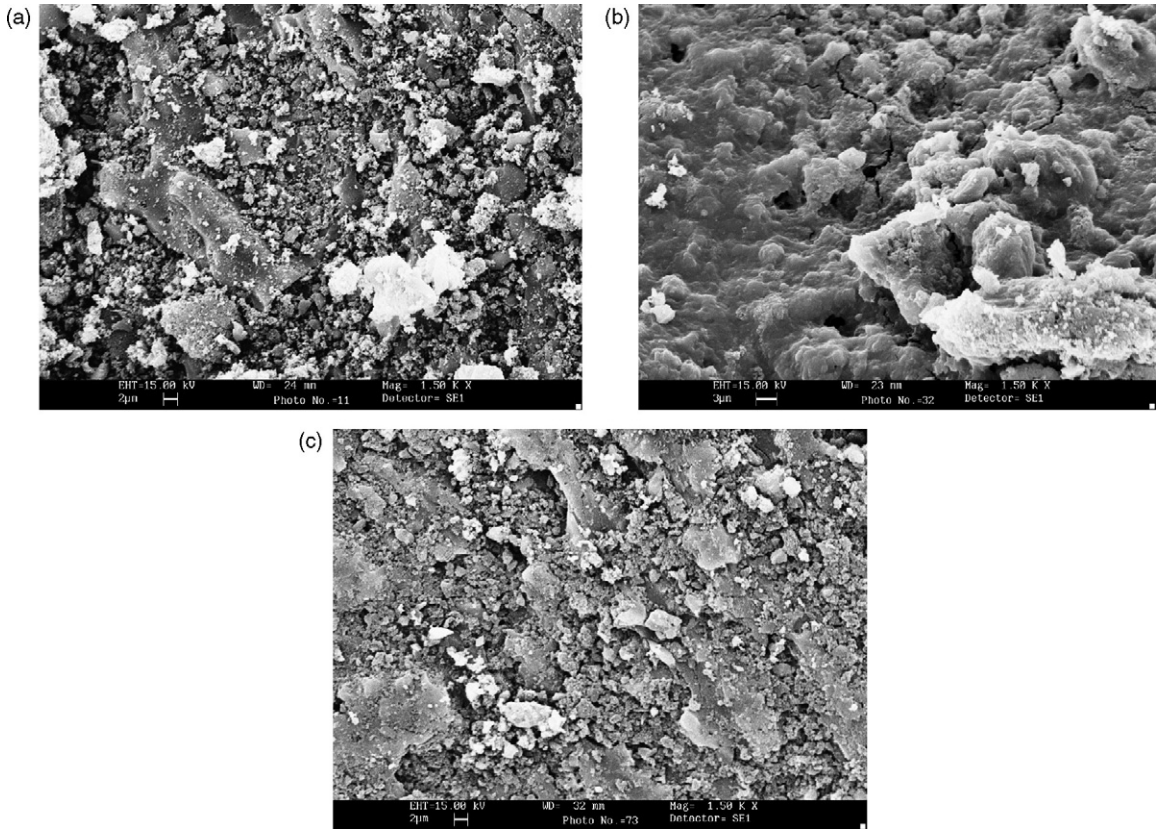

Fig. 5. SEM micrographs of GAC; (a) before bio-adsorption /accumulation, (b) after bio-adsorption /accumulation and (c) after adsorption (spent GAC), each shown at a magnification of 1500×, (As_o = 15 ppm).

imental conditions. At pH ≤ 3 no growth of bacteria was observed.

4.3. Effect of GAC type on the removal of arsenic species

The contribution of the physico-chemical adsorption (only adsorption) and the bio-adsorption/accumulation (only bio-adsorption) on the overall SABA process are shown in Fig. 4. The control experiment using fresh GAC was considered as only adsorption process. The bio-adsorption using spent GAC was considered as only bio-adsorption (after eliminating control experiment) process. The dose of fresh GAC and spent GAC was 16 g/l. From Fig. 4 it is evident that the SABA process gives more arsenic removal than the bio-removal process using spent GAC. It is also evident that the % removal of As(III) in SABA is slightly more (∼1%) than the additive value of the individual % removals obtained by only adsorption and only bio-adsorption processes. However, for As(V), the % removal in SABA process is less (∼8%) than the additive value of the individual % removals obtained by only adsorption and only bio-adsorption processes. This can be explained as follows.

Two factors, i.e., bio-adsorption and only adsorption may play role on the removal of arsenic by using *R. eutropha MTCC 2487* and GAC. In case of bio-adsorption process the arsenic species is adsorbed on the cell surface of the bacterial mass of bio-layer formed on the GAC surface. Formation of bio-layer, at the initial stage, is dependent on the porosity of the solid support [29]. More porosity gives more layer formation. However, bio-film structure is mainly influenced by the substrate concentrations [30]. Fresh GAC has more porosity and surface area than spent GAC (Table 1). The SEM micrographs of fresh GAC and spent GAC as shown in Fig. 5(a and c), respectively, also support the partial coverage of active sites on spent GAC. Therefore, at the initial stage, formation of bio-layer on fresh GAC may be more than the spent GAC. Consequently, the overall removal of arsenic by only bio-adsorption in case of SABA may be slightly more than that of bio-adsorption using spent GAC. Due to this reason more removal of both As(III) and As(V) may be obtained in SABA. Moreover, in case of SABA the contribution of only adsorption is also more due to more surface area of fresh GAC. Thus, the removal of As(III) and As(V) by SABA is more than the bio-removal with spent GAC.

Formation of bio-layer on the surface of adsorbent reduces its surface porosity as the bacterial mass partially occupies the void spaces of the adsorbent surface. Moreover, the bio-layer formed on the adsorbent surface covers the active sites and prevents the pollutants to come in contact with the active sites of the adsorbent [31]. Comparing SEM micrographs as shown in Fig. 5(a–c), it is evident that most of the active sites of GAC are covered due to the formation of bio-film on it. Due to these reasons formation of bio-layer reduces the physico-chemical adsorption capacity of adsorbents. Therefore, the formation of bio-layer on the fresh GAC has two opposite effects on the removal of arsenic species, i.e, increase in bio-adsorption capacity and the decrease in the only adsorption capacity. For As(III), these two opposite effects may cancel each other as the removal of As(III) by only adsorption is less as it exists as neutral species

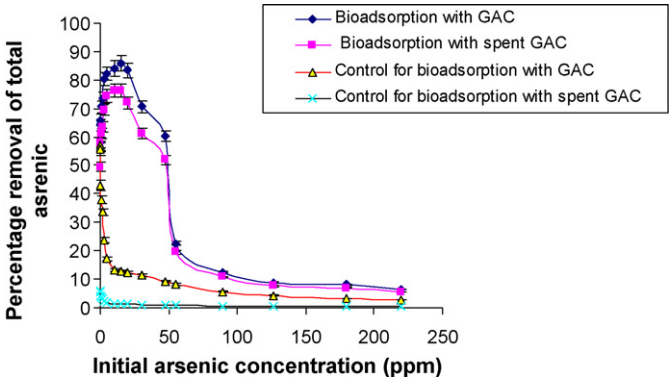

Fig. 6. Effect of initial arsenic concentration on the percentage removal of arsenic (pH 6.5 ± 0.2).

in experimental pH range. Consequently, the over all As(III) removal by SABA process is almost similar to the additive effects of only adsorption and only bio-adsorption. However, for As(V), these two effects may not cancel each other as fresh GAC can adsorb considerable amount of As(V) in absence of bio-film. The As(V) adsorption capacity (by only adsorption) of fresh GAC can be reduced due to the formation of bio-layer [31]. Consequently, the over all impact of these two opposite effects (increase in bio-adsorption and decrease in only adsorption capacity of GAC) leads to the As(V) removal, which is less than the additive effects of only adsorption and only bio-adsorption processes. Hence, it seems that in SABA process the adsorption and bio-adsorption/bioaccumulation perform simultaneously and synergistically. Although there is hardly any report on the SABA process for arsenic removal but recently, the adsorption and biodegradation of organic compounds have been found to be performed synergistically in simultaneous adsorption biodegradation process [32].

4.4. Effect of initial arsenic concentration on arsenic removal

Percentage removals of total arsenic by bio-adsorption/bioaccumulation process using fresh GAC and spent GAC are shown in Fig. 6. Initial arsenic concentration in the solutions varied from 0–220 ppm. The ratio of the initial concentration of As(III) and As(V) was 1:1 for all the solutions. The agitation time was 100 h and each experiment was repeated thrice. The control experiments show the arsenic removal by only adsorption. It is evident that for only adsorption the % removal of arsenic decreases with the increase in As_o but for bio-adsorption/bioaccumulation process it increases and reaches maximum at the As_o value of around 15 ppm, which is followed by gradual decrease. At the lower value of As_o (<0.2 ppm) the % removal of arsenic for physico-chemical adsorption is similar to that of bio-adsorption.

At higher As_o value (>90 ppm), the % removal of arsenic for both the adsorption and bio-adsorption using fresh GAC are less and almost equal. Within the As_o range of 1–50 ppm, the % removal of arsenic by bio-adsorption/accumulation is much higher than that of physico-chemical adsorption. It is also evident

that the arsenic removal by the bacteria gradually increases with the increase in the As_o value at the lower concentration of As_o. This is possible in the following cases alone or in their combined effect.

(i) If the arsenic induces *R. eutropha* to synthesize arsenate reductase to convert As(V) to As(III) and subsequent sequestration.
(ii) If the arsenic induces *R. eutropha* to synthesize ArsR protein for the accumulation of As(III).

It has been reported that *R. eutropha MTCC 2487* produces ArsR protein and arsenate reductase enzyme, but there is no report available on the As(III) accumulation mechanism of this particular strain as it has not yet been used for arsenic removal [6]. However, *B. subtilis*, an arsenic resistant bacterium, also produces arsenate reductase in presence of arsenate and the activity of this is also inducible [9]. The above observation also supports the inducible nature of arsenic on this strain for arsenic accumulation. Presence of arsenic in the media may stimulate the cells to produce ArsR protein responsible for accumulation of As(III) and arsenate reductase responsible for the conversion of As(V) to As(III). At very less As_o value (0.1–0. 2 ppm) the bacterial system produces less amount of protein and enzyme. With the increase in As_o the release of protein and enzyme increases which results in more removal of arsenic. However, beyond the As_o value of 20 mg/l the bacterial cells reach their maximum limit to sequester the arsenic and further increase in As_o acts as inhibitor. Consequently, the % removal of both As(III) and As(V) decreases. The substrate inhibition effect at higher arsenic concentration (>20 ppm) has also been reported recently [33].

4.5. Removal of other metals

Under the optimum process conditions the removal of Fe, Mn, Cu and Zn were 65.17%, 72.76%, 98.6% and 99.31%, respectively, as shown in Fig. 7. *R. eutropha* belongs to the cation diffusion facilitator protein family of bacteria. It has an amino-terminal streptavidin-tagged protein $C_{ZC}D$, which binds Zn and Cu [34]. Mn may be taken up by Mg transport system [35]. Mechanism of iron take up by this strain is not clear. However, iron normally follows siderophore-mediated uptake [36]. Fe, Mn, Cu and Zn may be used as nutrients for *R. eutropha MTCC 2487*. The consumption of the above stated heavy metals as constituent of growth media by *R. eutropha JMP134* has also been reported [37]. Some amount of metals like Fe, Mn and Cu may also be precipitated in the operating pH range.

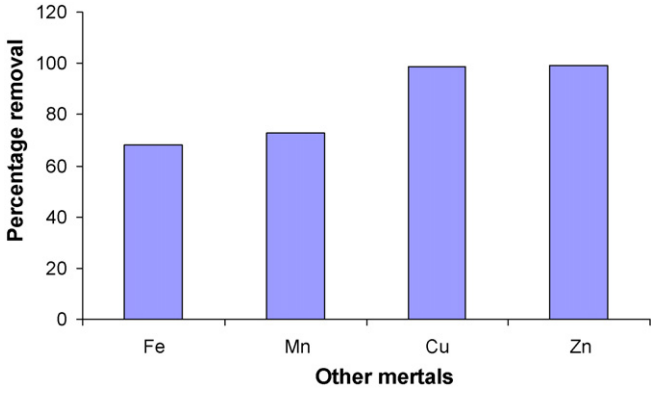

Fig. 7. Removal of other metals (pH 6.5 ± 0.2).

4.6. Regeneration of adsorbents

In bio-adsorption/accumulation process heavy metals are adsorbed/accumulated on the bio-film produced on GAC or spent GAC. With the time of agitation, the bacterial cells consume more arsenic and die. Consequently, a considerable amount of biomass is produced and the adsorbent losses its activity. Loss on adsorption capacity is also achieved due to the blockage of pores of the adsorbents due to the adsorption of metals/metalloids and growth of biomass. Therefore, regeneration of the adsorbent is required. Some regenerating solvents like1N NaOH, 5N H_2SO_4 and 30% H_2O_2 in 0.5M HNO_3 have recently been used for the regeneration of spent adsorbents [27,38]. Regeneration is also possible to some extent by washing the spent adsorbent with distilled water [38]. In the present case the bio-adsorbents (after use) were regenerated up to 88% by washing with distilled water for further use in bio-adsorption processes. However, for the use in only adsorption process the regeneration by washing with distilled water was only ∼10% and ∼18% for spent GAC and GAC, respectively. For regeneration with the regenerating solvents the metal loaded bio-adsorbents (cells and GACs) were collected by centrifugation and were suspended in 25 ml of the above-mentioned desorbing solution [39]. The regeneration of the bio-adsorbents using 1N NaOH, 5N H_2SO_4 and 30% H_2O_2 in 0.5M HNO_3 were around 96.5%, 99.4% and 95%, respectively (tested up to third cycle) for the application in bio-adsorption as well as only adsorption processes. During desorption, using acid and base, the biomass are removed from the GACs. In addition to that, the occupied active sites of spent GAC (occupied during physico-chemical adsorption on fresh GAC) also partially get free. Due to this reason the % regeneration is more for all the desorbing agents. The desorption of arsenic by 5N H_2SO_4 is performed due to the formation of neutral H_3AsO_3 and H_3AsO_4, which are not adsorbed on to the positive surface of activated carbon in this lower pH (<1). At the pH > 13, the surface of the GAC becomes negative and hence 1N NaOH can desorb arsenic. Use of 30% H_2O_2 in HNO_3 also produces H_3AsO_3 (As(III) species) but in this case As(III) may partially be converted to As(V) which may be adsorbed on the surface of the GAC. Amongst the above-mentioned regenerating solvents maximum regeneration of the exhausted $CaCl_2$ impregnated rice husk carbon has recently been observed by using 5N H_2SO_4 [27].

4.7. Equilibrium isotherms

The experimental specific uptake (q_e) of As(III), As(V) and As(T) for only adsorption on GAC and bio-adsorption on fresh GAC at various As_o values (5, 10, 15, 20, 30 and 47 ppm) are shown in Fig. 8. The specific uptakes for adsorption and

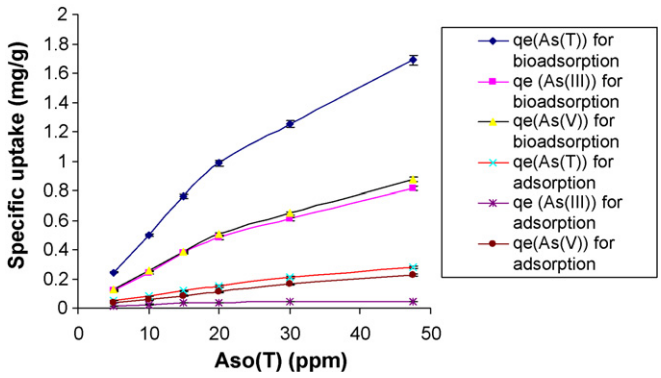

Fig. 8. Experimental specific uptake at various initial arsenic concentrations for various arsenic species (pH 6.5 ± 0.2, agitation time 100 h).

bio-adsorption/accumulation, at various As_o values are calculated by various isotherm equations and are compared with the experimental specific uptake values. The constants of various isotherm models, correlation coefficients and the MPSD values on the measurement of the specific uptakes using these models are shown in Table 3.

From Table 3 it is evident that the R^2 value is minimum for Temkin isotherm for all the arsenic species in case of bio-adsorption/accumulation and for Freundlich isotherm these values are maximum for all the arsenic species. Comparing the R^2 value and the MPSD of the isotherm models it is evident that for all the arsenic species the Temkin isotherm predicts the specific uptake more accurately followed by Langmuir and Freundlich isotherm.

In case of bio-adsorption/accumulation, arsenic is adsorbed on the active sites of the proteins on the cell surface of the bacterial film produced on GAC. It has recently been reported that protein shows highest affinities for the surface arrangement, which best match its own distribution of functional sites, resulting in a distribution of binding energies [18]. Due to this reason the Temkin isotherm predicts better the bio-adsorption/accumulation process followed by Langmuir and Freundlich isotherm. The bio-adsorption process has also recently been modeled using Temkin, Langmuir and Freundlich isotherms [18,39,9].

In case of only adsorption, the predictions on the specific uptake of As(V) and As(T) by Freundlich isotherm are better. The order of fitness of the isotherms is Freundlich isotherm > Langmuir isotherm > Temkin isotherm. However, for As(III) the order of fitness is Langmuir isotherm > Temkin isotherm > Freundlich isotherm. It appears that due to the adsorption of arsenic and other metals, perhaps both the heterogeneity and roughness of the surface increase, which lead to the better fit of Freundlich isotherm to explain the As(V) adsorption phenomena. The As(III) adsorption capacity of GAC is very less and the active sites for adsorption of As(III) may be different from those of As(V) and other metal ions as As(III) exists as neutral species in the experimental pH range. Due to this reason the order of fitness of the isotherms for explaining the adsorption phenomena of As(III) and As(V) may differ.

4.8. Advantage of the new method

Important features of some recently reported arsenic removal techniques like use of bacterial whole cells, biomass and surface modified adsorbents, including the present study are summarized in Table 4. It is difficult to compare the various techniques

Table 3
Isotherm constants and MPSD values on the measurement of specific uptake by various isotherms (As_o: 5–47 ppm)

Process	Isotherm	Parameters	As(T)	As(III)	As(V)
Bio-adsorption with GAC	Langmuir	q_{max} (mg/g)	5.485	2.779	2.689
		b (l/mg)	0.059	0.101	0.141
		R^2	0.95	0.95	0.95
		MPSD	7.65	7.78	7.71
	Freundlich	K_f ((mg/g)/(mg/l)$^{1/n}$)	0.392	0.266	0.321
		n	1.745	1.71	1.791
		R^2	0.87	0.87	0.86
		MPSD	9.79	9.63	9.99
	Temkin	K_T (l/mg)	2.110	3.716	4.897
		B (mg/g)	0.479	0.236	0.241
		R^2	0.98	0.98	0.98
		MPSD	4.74	4.69	4.87
Adsorption with GAC	Langmuir	q_{max} (mg/g)	0.3708	0.0811	0.2624
		b (l/mg)	0.040	0.098	0.089
		R^2	0.98	0.99	0.94
		MPSD	3.97	2.98	6.69
	Freundlich	K_f ((mg/g)/(mg/l)$^{1/n}$)	0.019	0.011	0.022
		n	1.385	0.098	1.294
		R^2	0.99	0.90	0.98
		MPSD	1.62	6.12	3.21
	Temkin	K_T (l/mg)	0.323	1.274	0.614
		B (mg/g)	0.097	0.015	0.080
		R^2	0.94	0.96	0.90
		MPSD	8.61	3.37	14.23

Table 4
Comparison of the present method with some latest literature on arsenic removal

Adsorbent/biomass	As_o (ppm)	As species	pH	Isotherm model	Capacity (mg/g)	Ref.
B. subtilis	500	As(III)	3.5	Langmuir	97	[8]
A. facealis strain O1201	10–500	AS(III)	4–9	–	–	[4]
CASO1	100–1000	As(III)	3–8	–	–	[12]
			6			
L. nigrescens	50–60	As(V)	2.5	Langmuir	45.2	[41]
Modified calcined boxite	0.5–8	As(V)	6–8	Langmuir	1.57	[42]
Modified calcined boxite	0.5–8	As(III)	6–8	Langmuir	1.37	[43]
Tea fungal biomass	2.2	As(III)	7.2	Freundlich	1.11	[44]
		As(V)			4.95	
Mixed rare earth	50	As(V)	6.5	Langmuir	2.95	[45]
Coconut shell carbon	0–200	As(V)		Langmuir	2.4	[46]
Activated carbon from olive pulp and olive stone carbon	5–20	AS(III)	7.0	Langmuir	1.39	[47]
Fresh GAC & immobilized *R. eutropha MTCC 2487* (SABA)	5–47	As(III)	6–7	Langmuir	2.78	Present study
		As(V)			2.69	
GAC	5–47	As(III)	6–7	Langmuir	0.37	Present study
		As(V)				
Immobilized *R. eutropha MTCC 2487* (only bio-adsorption)	5–47	As(III)	6–7	Langmuir	88	Present study
		As(V)			93	

due to diversity in applied experimental conditions. However, somebody may get some idea about the performance of these various techniques. From Table 4 it is evident that the *B. subtillis* has slightly more arsenic adsorption capacity (∼97 mg/g) than the present study. In the present study, the capacity of immobilized *R. eutropha* for As(III) and As(V) adsorption are 88 and 93 mg/g, respectively. This may be due to the difference in dry mass of bacteria present in unit volume of the solution. In the present study, the inoculum was only 2 days old and the dry cell mass of the solution (at the start of agitation) was 0.125 g/l. Whereas, in the previous study (Ref. [9]), 7 days old inoculum was used and the dry cell mass in the solution was 3.6 g/l. Other bacteria like *A. faecalis strain O1201* and *CASO1* have been used to find out their arsenite oxidizing properties only, no systematic study has been performed for arsenic removal using these bacteria. It is also evident that the As(III) adsorption capacity of the adsorbent (GAC & immobilized biomass) is more than that of the tea fungal biomass. However, As(V) removal is more for tea fungal biomass. Although *L. nigrescens* shows more As(V) removal capacity but it has not been used for As(III) removal. The present adsorbent (GAC & immobilized biomass) is also superior to some recently reported surface modified adsorbents like modified calcined boxite, coconut shell carbon, activated carbon from olive pulp and olive stone carbon, mixed rare earth, etc. as mentioned in Table 4.

It is evident that at the end of one treatment step with As_o value of 15 ppm the residual arsenic concentration in the treated water is higher than the legislative limits of 0.2 ppm. Hence, at least two steps of treatment are required to reduce the residual arsenic concentration below the legislative limit from an As_o value of ∼15 ppm. However, this problem may be solved in column study as in column reactor the unit volume of liquid comes in contact with large amount of adsorbents.

It is also evident that the bio-adsorbent is not efficient at As_o value > 50 ppm. Therefore, the high arsenic containing acid mine drainage should be treated through some pretreatment steps like coagulation-precipitation or lime softening. Outlet solutions from these pretreatment steps can be effectively treated by the bio-adsorbents as a polishing stage of 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, and (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 there of are used [40]. In the present case, the eluted solution can be dried and the solid can be stabilized or can be disposed in secured landfill.

5. Conclusions

From the above discussions the following conclusions are made.

1. Bio-adsorption/accumulation process is superior to the adsorptive method when the initial arsenic concentration is less (<50 ppm). At higher arsenic concentration the bioprocess gives similar removal of arsenic to that of adsorption.
2. With the decrease in pH the % removals of both As(III) and As(V) decrease. The optimum pH for arsenic removal is 6–7.
3. The optimum agitation time for As(III) removal is around 75 h and for As(V) it is around 100 h.
4. Arsenic removal is inducive to the initial concentration of arsenic.

5. In case of SABA, adsorption and bio-adsorption occurs simultaneously and synergistically.
6. Maximum regeneration of the adsorbent is obtained using 5N H_2SO_4.
7. The present adsorbent (GAC & immobilized biomass) is also superior to some recently reported surface modified adsorbents like modified calcined boxite, coconut shell carbon, activated carbon from olive pulp and olive stone carbon, mixed rare earth, etc.
8. Temkin isotherm predicts the specific uptake more accurately in case of bio-adsorption of all types of arsenic species.

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