Studies on Bacterial Growth and Arsenic (III) Biosorption Using *Bacillus subtilis*

Sk. Masud Hossain[#] and N. Anantharaman*
Department of Chemical Engineering, Mohamed Sathak Engineering College, Kilakarai 623 806, India. E-mail: skmhossain@yahoo.co.in.
*Department of Chemical Engineering, National Institute of Technology, Tiruchirapalli 620015, India. E-mail: naraman@nitt.edu

Original scientific paper
Received: July 12, 2005
Accepted: January 1, 2006

Gram-negative bacteria *Bacillus subtilis* biosorps arsenic (III) ion from its aqueous solution. The maximum biosorption of lead is $w = 97.30 \cdot 10^{-2}$ within 72 h of inoculation time with optimum pH 3.5 and optimum temperature 40 °C for $w = 500 \cdot 10^{-6}$ initial loading of arsenic ion in a shake flask (optimum $n = 60$ min^{-1}). 7 days old and $\rho = 30 \cdot 10^{-2}$ inoculum culture is used in the studies. Arsenic (III) ion is measured by using atomic absorption spectrophotometer into an air-acetylene flame and absorbance is measured at 229 nm. The maximum bacterial growth is noticed as $c = 3.90 \cdot 10^{8}$ cells mL^{-1} at optimum conditions. The bacteria can tolerate upto $w = 600 \cdot 10^{-6}$ of initial arsenic (III) ion loading. The Langmuir and Freundlich Isotherms fit the biosorption data reasonably well and played a major role in giving a better understanding of bioprocess modeling. The Monod Model for bacterial growth shows that the specific growth rate (μ) of *B. subtilis* in the initial $w = 500 \cdot 10^{-6}$ of arsenic (III) ion loading, is found to be 0.017 s^{-1}

Key words:
Biosorption, arsen, *Bacillus subtilis*

Introduction

Certain species of microorganisms have been found to adsorb surprisingly large quantities of metals.[1-6] These metals include the one involved in toxicity to humans and of commercial economic value.[1-3] The use of microorganisms to treat aqueous streams for the removal, concentration and recovery of toxic and valuable heavy metals although received increased attention in the last decades. The removal of heavy metals from municipal and industrial wastes by biological treatment systems has continued to be of interest. Bacterial surfaces have great affinity to sorb and precipitate metals, resulting in metal concentration on bacterial surface. There have been several reports[1-13] of the uptake of toxic heavy metals by bacteria so, that the metals are accumulated. Both, metabolically mediated and biosportive phenomena can occur in such systems. Biosorption is the physical/chemical process between position charged, dissolved metal species, and charged reactive cellular components. Therefore, it is not unexpected that biosorptive metal uptake is subject to environmental conditions, that affect the reaction chemistry of both the receptive sites and the metals.[1-12]

Biosorption is the passive sequestration of metals and radionuclides by interaction with live or dead biological matter and is, at present, the most practical and widely used approach for the bioremediation of metals and radionuclides.[11] Biosorption involves accumulation of metals on the surface of the cells or cell fractions by adsorption or ion exchange. All microbes, which expose negatively charged groups on their cell surface, have the capacity to bind metal ions.[1-13] Various compounds of bacterial walls sorb different metals, which later get precipitated.[1-9] Complexation with certain proteins or organic compounds presents in the cell walls. Complexolysis is a process corresponding to microbial formation of complexing or chelating agents that solubilize metal ions. As a result of that, metal-organic complexes or metal-organic chelates are formed.[1-13] The chelating compounds formed by microorganisms are organic acids (citric, oxalic, 2-keto-gluconic, tartaric, 2,3-dihydrobenzoic acid, etc) or more complex compounds such as sidrophores: $M^{+} + H^{+}L \rightarrow H^{+} + LM$, $H^{+}L + LM \rightarrow L_2M + H^{+}$ (L: organic legends). Some microorganisms produce specific proteins called Metallothionein which are induced by metal ions and these bind metal ions. A variety of precipitates and minerals have been found associated with bacterial surfaces, but the exact mechanism of precipitation and mineral formation is currently under investigations.[1-6,11]

The outer membrane and murein (the usual model for the gram-negative A 1γ chemotype has revealed about 80 distinct muorpeptides) of the gram-negative surface constitute the cell wall.[1-3] In concert the arrangement and exposure of the various polymers and macromolecules in gram-nega-

[#] For correspondence (e mail: skmhossain@yahoo.co.in)

tive walls produce a net electronegative charge, which allows these walls to interact with cations within their immediate surroundings. Metallic ions affect the packing order of the phospholipids and lipopolysaccharides of the outer membrane and even affect the bonding forces that hold the two faces of the membrane together.[1-13] In fact, it is possible that the hydrophilicity or hydrophobicity of this membrane can be moulded by its metallic ion salt form. Because lipopolysaccharide is normally partitioned to the outer membrane, these O-side chains of lipid would be first to contact exogenous metal. Lipopolysaccharide which has an abundance of phosporyl groups, has been implicated as the primary sites of metal interaction.[1-6,11,12]

The biosorption of heavy metal ions from aqueous solution by bacteria is effected not, only, by the surface properties of the organism but also by various other physico-chemical parameters of metal ion solution. The present batch investigations were undertaken to develop an effective bacterial treatment (biosorption) of arsenic (III) ion in aqueous solution using *Bacillus subtilis.* Aerobic studies were conducted to optimize bioprocess parameters,[15-18] such as biosorption time, initial arsenic (III) loading, pH, temperature and shaking speed, using aerobic batch suspension culture of the acidophilic, mesophilic, gram-negative bacteria *B. subtilis.*

Experimental procedure

Collection of microorganism and growth

The acidophilic, mesophilic *Bacillus subtilis* (MTCC-1427), was procured from Institute of Microbial Technology (CSIR), Chandigarh, India. The slant cultures were prepared with prescribed growth medium containing beef extract 1.0 g, yeast extract 2.0 g, peptone 5.0 g, agar 15.0 g, sodium chloride 5.0 g, and distilled water 1.0 L. It was sterilized in an autoclave maintained at 102.52 kPa for 15 min. The slant cultures were maintained at constant temperature of 37 °C in an incubator. The stock cultures were subcultured every two weeks.

Culture media preparation

The following constituents were used for culture media preparation for the bacteria per liter: KH_2PO_4 – 20 g, $MgSO_4 \cdot 5H_2O$ – 5 g, $CaCl_2$ – 1 g, $CaSO_4 \cdot 5H_2O$ – 0.1 g, $ZnSO_4 \cdot 7H_2O$ – 0.1 g $CuSO_4 \cdot 5H_2O$ – 0.1 g, $AlK(SO_4) \cdot 12\ H_2O$ – 0.01 g, H_3PO_3 – 0.01 g, $Na_2MoO_4 \cdot 2H_2O$ – 0.01 g, glucose – 10 g, and peptone $w = 0.1 \cdot 10^{-2}$.

General method

The biosorption of arsenic (III) ion was studied in batch system. Experiments were carried out in 1L Erlenmeyer flasks containing 250 mL of the medium. 250 mL of $w = 400 \cdot 10^{-6}$ of arsenic (IV) ion solution, and $w = 30 \cdot 10^{-2}$ inoculum was taken to the flasks. Inoculum was taken from a seven days old culture. The initial biomass (*B. subtilis*) concentration[19] was $2.0 \cdot 10^8$ cells mL^{-1} and mass of dry biomass was 3.6 g L^{-1}. The aerobic condition of the system was maintained by putting nonabsorbent cotton to the mouth of the flasks.[15-18] The dissolved oxygen (DO) level is measured to $w = 6.4 \cdot 10^{-6}$ in the broth.[19] The flasks were incubated in a constant temperature water bath[15-18] maintained at 30 °C with constant shaking (M = 20). The initial pH was maintained at 2.5 by using 0.1 mol L^{-1} H_2SO_4 acid and/or 1 (mol L^{-1}) $CaCO_3$ slurry.

Effect of time and initial arsenic (III) loading on biosorption

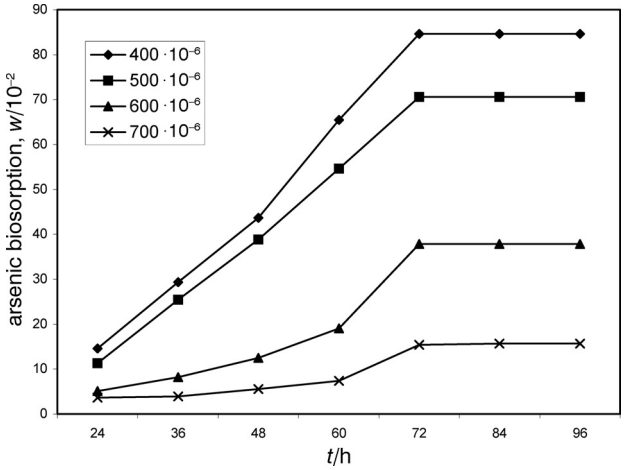
The general method was repeated for w = 500, 600 and 700 $\cdot 10^{-6}$ of initial arsenic (III) ion loading, respectively. Flasks were taken out on a regular basis[15-18] i.e. after t = 24, 36, 48, 60, 72, 84, and 96 h of inoculation, respectively, followed by analysis for arsenic (III) in solution.[20,26] The bacterial growth was also measured for every biosorption time.[19] To assess the extent of chemical reaction a set of experiments was carried out under sterile conditions (without microbe). The fraction of arsenic (III) ion biosorption and bacterial growth are shown in figures 1 and 2, respectively.

Effect of pH on arsenic (III) biosorption

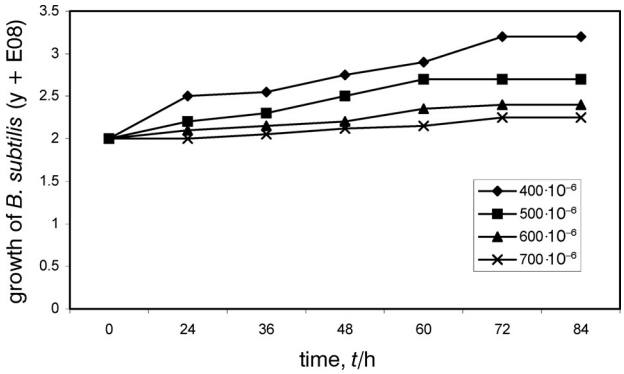
The general method was repeated for various pH values such as 1.5, 2.5, 3.5, and 4.5, respectively, for w = 500 $\cdot 10^{-6}$ initial arsenic (III) ion loading. The broths were taken out after 72 h of inoculation (optimum time) and were analyzed for arsenic (III) ion in solution. The bacterial growth was also measured for every pH value.[19] The fraction of lead biosorption and bacterial growth is shown in figures 3 and 4, respectively.

Effect of temperature on arsenic (III) biosorption

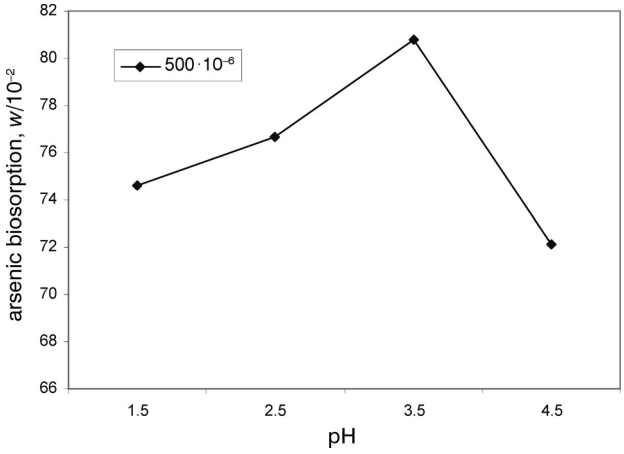
The general method was repeated for various temperatures such as T = 20, 30, 40, and 50 °C, respectively for w = 500 $\cdot 10^{-6}$ initial arsenic (III) ion loading. The pH value was maintained at 3.5 (optimum). The broths were taken out after 72 h of inoculation (optimum biosorption time) and were analyzed for arsenic (III) ion in solution. The bacterial growth was also measured for every temperature value.[19] The fraction of lead biosorption and bacterial growth are shown in figures 5 and 6 respectively.



F i g . 1 – *Effect of initial arsenic (III) mass fraction and time*



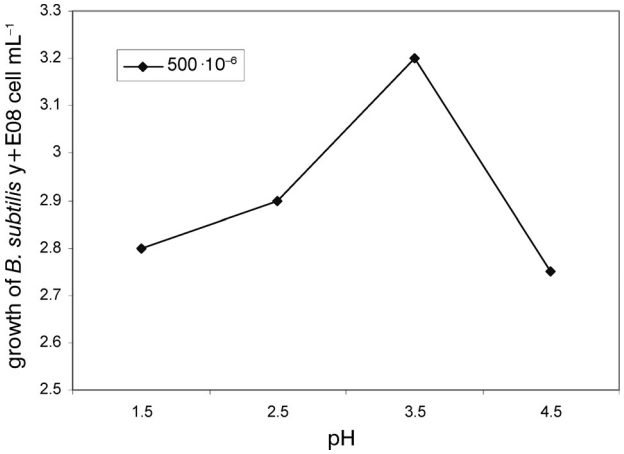
F i g . 2 – *Effect of initial arsenic (III) mass fraction and time on bacterial growth*



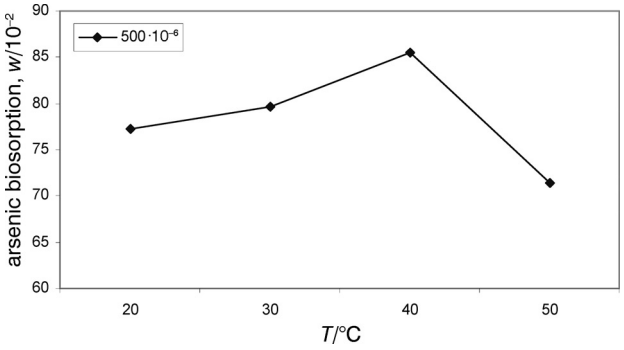
F i g . 3 – *Effect of pH on arsenic (III) biosorption*

Effect of shaking on arsenic (III) biosorption

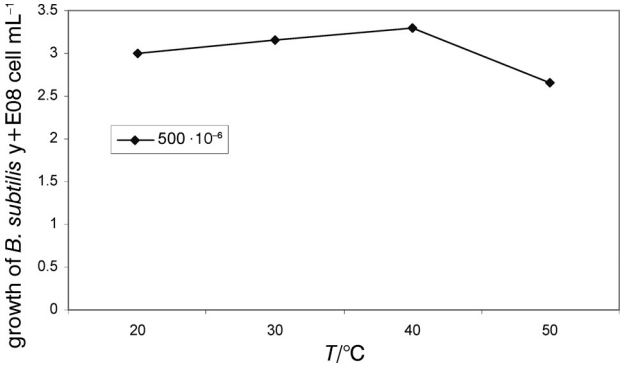
The general method was repeated for different shaking speed such as n = 20, 40, 60 and 80 min^{-1}, respectively, for $w = 500 \cdot 10^{-6}$ initial arsenic (III) ion loading. The pH value was maintained at 3.5 (optimum). The temperature was maintained at 40 °C (optimum). The broths were taken out after



F i g . 4 – *Effect of pH on bacterial brow th*



F i g . 5 – *Effect of temperature on arsenic (III) biosorption*

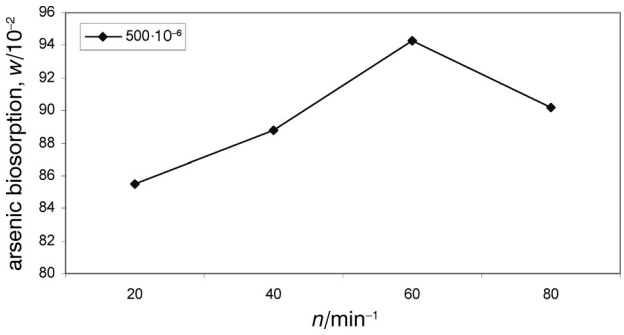


F i g . 6 – *Effect of temperature on bacterial growth*

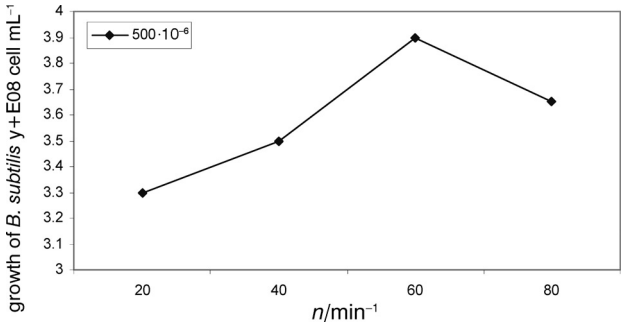
72 h of inoculation (optimum time) and were analyzed for arsenic (III) ion in solution. The bacterial growth was also measured for every shaking value.[19] The fraction of arsenic (III) biosorption and bacterial growth are shown in figures 7 and 8, respectively.

Lackey's drop method for microbial counting

Exactly 0.1 mL volume of the sample was put by using a calibrated medicinal dropper onto a glass slide. A cover slip of known area was placed, avoiding any air bubble. The slide was put under a



F i g . 7 – *Effect of shaking speed on arsenic (III) biosorption*



F i g . 8 – *Effect of shaking speed on bacterial growth*

microscope and measured the width of the high power microscopic field. Suppose the area visible at one time is one micro transect. Now, the slide moved from one corner to another counting planktons in each microscopic field visible. Several fields were counted by moving the slide in horizontal and vertical directions. Counting must be quick to avoid drying of the sample. The phytoplankton calculated as follows: Number of planktons per mL = (No. of organisms counted in all fields · Area of cover slip, mm^2) / (Area of one macroscopic field, mm^2 · No. of field counted · volume of sample in the cover slip)

Determination of arsenic (III) using atomic absorption spectrophotometer

At the end of the specific biosorption time, 100 mL of the solution were taken out of the flasks and centrifuged at $n = 1000$ min^{-1} for 10 min in Sorvall RC-5 super speed centrifuge at room temperature to remove biomass and unsolubilized materials and filtered the solution.[15-18] An arsenic hollow cathode lamp was placed in the operating position, adjusted the current to 2-3 mA, and selected the arsenic line at 229 nm using the appropriate monochromator slit width. The appropriate acetylene gas was supplied to the burner following the instructions detailed for the instrument, and adjusted the operating conditions to give a fuel- lean air- acetylene flame. The chelate was extracted with ammonium pyroline dithiocarbamate into methyl isobutyl ketone for low levels of arsenic. The organic layer was aspirated into the air-acetylene flame and recorded the absorbance of three readings for each sample. Between each solution, de-ionized water was aspirated in the burner. Finally, the absorbance of the test sample was read. A calibration curve is plotted by aspirating into the flame samples of solutions containing known solutions of the arsenic (III), measuring the absorption of each solution, and then constructing a graph in which the measured absorption is plotted against the concentration of solutions. Using calibration curve, the concentration of the relevant arsenic (III) in the solution is interpolated from the measured absorbance of the test solution.

Results and Discussion

Effect of initial arsenic (III) loading and time on biosorption

The effect of initial arsenic (III) ion loading and biosorption time with *B. subtilis* is shown in figure 1. For initial arsenic (III) ion loading, w = 400, 500, 600, and 700 · 10^{-6} of its aqueous solutions are experimented. The permissible limit of arsenic (III) ion discharge into water stream is w = 200 (Indian Standard). Therefore, the above mentioned mass fraction of arsenic (III) ion are undertaken for bacterial treatment. From the figure 1, it is shown that the biosorption of arsenic (III) ion is measured after 24 h of inoculation. This period is avoidance of lag-phase for growth of bacteria and adaptation to the environment of solid substrate i.e. arsenic (III) ion.[15-18] The biosorption is maximum after 72 h of inoculation by the bacteria as equilibrium is reached nearly at this contact time. The biosorptions are w = 92.60, 72.65, 40.75 and 18.55 · 10^{-2} for w = 400, 500, 600 and 700 · 10^{-6} of initial arsenic (III) ion loading after 72 h of inoculation respectively (Fig 1). It can be concluded that the tolerance limit for the bacteria is $w = 600 \cdot 10^{-6}$ of initial arsenic (III) ion loading, as biosoption is w = 40.75 · 10^{-2} at this initial loading. The biosoption of arsenic (III) is $w = 18.55 \cdot 10^{-2}$ at $w = 700 \cdot 10^{-6}$ of initial loading, which is very low. The growth and activity of bacteria are demised maximally at w = 700 · 10^{-6} initial arsenic (III) ion loading (Fig. 2). The rate of arsenic (III) ion binding with bacteria is more at initial stages, which gradually decreases and remains almost constant after an optimum time. At equilibrium, removal of arsenic (III) ions attains a constant value, because adsorption and desorption balance each other. The bacterial growth decreases with increase of initial arsenic (III) ions loading (Fig. 2). The cell growth increases with increase of

biosorption time and attains maximum of $2.70 \cdot 10^8$ cells mL^{-1} for $w = 500 \cdot 10^{-6}$ of initial arsenic (III) ions loading after 72 h of inoculation (Fig. 2). After 72 h of biosorption time, the growth of bacteria remains constant with constant arsenic (III) ions biosorption (Figs. 1 & 2). $w = 500 \cdot 10^{-6}$ of arsenic (III) ion is taken as initial loading with 72 h as optimum biosorption time (contact time) for further studies, respectively.

It is evident from the figure 1 that as the initial mass fraction of arsenic (III) ions increases the removal efficiency by the bacteria decreases. This is because of microbial populations in the broth can effect arsenic (III) ion removal. The bacterial growth decreases with increase of initial arsenic (III) ion loading (Fig. 2). The maximum cell growth was found as $3.2 \cdot 10^8$, $2.7 \cdot 10^8$, $2.4 \cdot 10^8$ and $2.25 \cdot 10^8$ cells mL^{-1} for $w = 400$, 500, 600 and $700 \cdot 10^{-6}$ of initial arsenic (III) ion loading, respectively (Fig. 2). As can be seen at the early stage of biosorption, which coincided with lag-phase of bacterial growth, the biosorption of arsenic (III) was slow (Fig. 1). The extent of lag-phase was dependent on initial mass fraction of arsenic (III) ion and medium initially containing high fraction of arsenic (III) showed longest lag-phase with less biosorption of metal ion (Figs. 2 & 3). The transition of bacterial (*B. subtilis*) activity from the lag-phase to exponential phase of growth led to a notable increase in biosorption of arsenic (III), which proceeded the same way, until it reaches equilibrium of biosorption (optimum). It should be pointed out that application of arsenic (III) ions at higher concentrations increased only the extent of lag-phase (Fig. 2).

There is a real danger that arsenic (III) ions may poison the environment, stopping biological (metabolic) activity, and microbial growth.[8,9] More recently greater attention has been given to decoupling the propagation of the microbial sorbent from conduct with the metal – contaminated streams to circumvent the toxicity problem.[8-10] Arsenic (III) ion has a poisonous effect on growth of bacteria *B. subtilis* beyond a certain fraction. Arsenic (III) metal ions accumulate on cell surface that slows the rate of growth and halts due to nutrient exhaustion and toxified metal ion medium. Exact criteria are more difficult to ascertain: growth of nutrient limited populations does slow somewhat before total exhaustion and the growth rate of a poisoned population may become imperceptibly slow.[8,9,23] It has been inferred in several instances, that the biosorption of metal results from the lack of specificity in a normal metal transport system and that, at high concentrations, metals may act as competitive substrates in a transport system.[22]

Effect of pH on arsenic (III) biosorption

The effect of pH on biosorption of arsenic (III) ions with *B. subtilis* is shown in figure 3. The arsenic (III) ion biosorption is measured after 72 h of inoculation (optimum time). An increase in arsenic (III) ion removal with increase in pH of the medium was observed for the arsenic (III) ion upto pH value of 3.5. It is observed that the maximum $w = 83.45 \cdot 10^{-2}$ removal of arsenic (III) ion occurred at pH 3.5 for $w = 500 \cdot 10^{-6}$ initial arsenic ion loading (Fig. 3). At pH values of 1.5, 2.5 and 4.5, the biosorption of arsenic (III) ion was noticed as $w = 78.55$, 80.70, and $74.45 \cdot 10^{-2}$ for $w = 500 \cdot 10^{-6}$ of initial arsenic (III) ion loading, respectively (Fig. 3). With increase in pH (beyond 3.5), the biosorptions of arsenic ions sharply declines (Fig. 3). Hence, pH value of 3.5 is the optimum and taken for further studies. The tolerance of the bacteria to most metals in low pH media probably results from effective competition by H^+ ions for negatively charged sites at the bacterial surface. These results suggest that the biosorption of arsenic (III) ion to the biomass be mainly due to oppositely charged ionic attraction. Therefore, as pH decreases the cell surface becomes more positively charged, reducing attraction between biomass and metal ions.[1-10] In contrast, higher pH results in facilitation of metal uptake, since the cell surface is more negatively charged. An optimum pH of 3.5 value for the adsorption of arsenic (III) ions was found in the studies. At pH of 3.5 value, neutralization of positive and negative ions occurred. However, it is needless to mention, that *B. subtilis* is a gram-negative, acidophilic, mesophilic and chemoautotrophic bacteria.

The bacterial growth at different pH values with arsenic (III) ion is shown in figure 4. The bacterial growth reached its maximum value of $3.20 \cdot 10^8$ cells mL^{-1} with maximum biosorption of arsenic (III) ion at pH value of 3.5 (Figs. 3 & 4). The bacterial growths are found as $2.8 \cdot 10^8$, $2.9 \cdot 10^8$ and $2.75 \cdot 10^8$ cells mL^{-1} for pH values of 1.5, 2.5 and 4.5, respectively (Fig. 4). Variation in pH of the medium result in changes of the activity of the microbes and, hence, the microbial growth rate as well as the arsenic (III) ions biosorption. Microbes are very active over a certain pH range. The optimal pH for growth may be different from that for biosorption of arsenic (III) ions. When pH differs from the optimal value, the maintenance energy requirements increase.[23,24] In biosorption of arsenic (III) ion from aqueous solution pH sharply decreases with time, which results in suppression of bacterial activity. In order, to avoid this negative effect lime and / or calcite was added to the medium to maintain constant pH.

Effect of temperature on arsenic (III) biosorption

The effect of temperature on biosorption of arsenic (III) ion with *B. subtilis* is shown in figure 5. However, the effect of temperature on activity of bacteria depends on a number of factors, e.g., pH, substrate loading (arsenic fraction), ionic concentrations of medium, etc. The optimum temperature is 40 °C at which the maximum arsenic (III) ion biosorption occurred. The biosorption is $w = 90.65 \cdot 10^{-2}$ at optimum temperature of 40 °C for $w = 500 \cdot 10^{-6}$ of initial arsenic (III) ion loading (Fig. 5). The biosorptions of arsenic (III) ion are observed as $w =$ 82.70, 85.30, and $77.55 \cdot 10^{-2}$ for temperatures of 20, 30 and 50 °C, respectively, for $w = 500 \cdot 10^{-6}$ of initial arsenic (III) ions loading (Fig. 5). With increase in temperature (beyond 40 °C), the biosorption is decreased for arsenic (III) ions with *B. subtilis* (Fig. 5). The bacterial growth with different temperatures is shown in figure 6. The bacterial growth is noticed maximum of $3.30 \cdot 10^8$ cells mL^{-1} at temperature of 40 °C (Fig. 6). The bacterial growths are found as $3.0 \cdot 10^8$, $3.15 \cdot 10^8$ and $2.65 \cdot 10^8$ cells mL^{-1} at temperatures of 20, 30 and 50 °C for $w = 500 \cdot 10^{-6}$ initial arsenic (III) ions loading, respectively (Fig. 6). The rates of aerobic biological process increase with temperature, and oxygen demand increases accordingly at high-temperature conditions, where dissolved oxygen is least soluble.[22-24] In aerobic biological process, the limited solubility of oxygen is of great importance because it governs the rate at which oxygen will be absorbed by the medium. The oxygen consumption by bacteria may exceed the rate of oxygen supply, leading the oxygen limitation. When oxygen is the rate limiting factor for aerobic process, specific growth rate varies with dissolved oxygen (DO) concentration. Rate of oxygen transfer has a big impact on bacterial aerobic biosorption process.[22-24]

Mesophiles grow best within a temperature range[23,24] of 20 to 50 °C. Every type of microbe has an optimum, minimum and maximum growth temperature. Temperatures below the optimum for growth depress the rate of metabolism of cells. Above the optimal temperature, the growth rate decreases and thermal death may occur.[23,24] At high temperature (beyond 40 °C), death rate exceeds the growth rate in the studies, which causes a net decrease in the concentration of viable cells (Fig. 6). However, it is needless to maintain that the temperature optimum for growth and biosorption may be different.

Effect of shaking on arsenic (III) biosorption

The effect of shaking speed on biosorption of arsenic (III) ion with *B. subtilis* is shown in Fig. 7. The maximum arsenic (III) ions biosorption is noticed as $w = 97.30 \cdot 10^{-2}$ at agitation speed of $n = 60$ min^{-1} (Fig. 7). The biosorption of arsenic (III) ion is noticed as $w = 90.65$, 93.40 and $89.75 \cdot 10^{-2}$ at shaking speed of $n = 20$, 40 and 80 min^{-1}, respectively. The biosorption of arsenic (III) ion increases with increase in agitation upto 60 min^{-1}, then it declines. This is because the binding of arsenic (III) metal ions to the bacterial surface is highest as well as cell population is maximum at this optimum shaking speed. The bacterial growth is maximum of $3.90 \cdot 10^8$ cells mL^{-1} at $n = 60 \cdot 10^{-2}$ (Fig. 8). The bacterial growth was noticed as $3.3 \cdot 10^8$, $3.5 \cdot 10^8$, $3.65 \cdot 10^8$ and $3.0 \cdot 10^8$ cells mL^{-1} for shaking speed of 20, 40. 80 and 100 min^{-1}, respectively (Fig. 8). Increase in mechanical forces can disturb the elaborate shape of enzyme molecule of the bacteria to such a degree that denaturation of the protein occurs and deactivates the bacterial growth.[22-23]

Interpretation of experimental data with adsorption isotherms

The application of biosorption technique in the commercial scale requires proper quantification of the sorption equilibrium for process simulation. The Langmuir and Freundlich isotherms[21] are more frequently used to give the sorption equilibrium.

Langmuir isotherm

The Langmuir isotherm[21] is applied for biosorption equilibrium and can be represented as:

$$\frac{\gamma_{eq}}{\gamma_{a,eq}} = \frac{1}{\gamma_0 b} + \frac{\gamma_{eq}}{\gamma_0}$$

where:

γ_{eq} – equilibrium mass concentration of arsenic (III) ion in solution, mg L^{-1}

$\gamma_{a,eq}$ – mass concentration of arsenic (III) ion adsorbed at equilibrium, mg L^{-1}

γ_o and b – Langmuir constants

The linear plot (Fig. 9) of γ_{eq} vs $\gamma_{a,eq}$ shows that biosorption of arsenic (III) ion follows the Langmuir isotherm. The values of γ_o and b are calculated from the linear plot (Fig. 9). Hence, $\gamma_o = 97$ and $b = 2.95$, are obtained respectively.

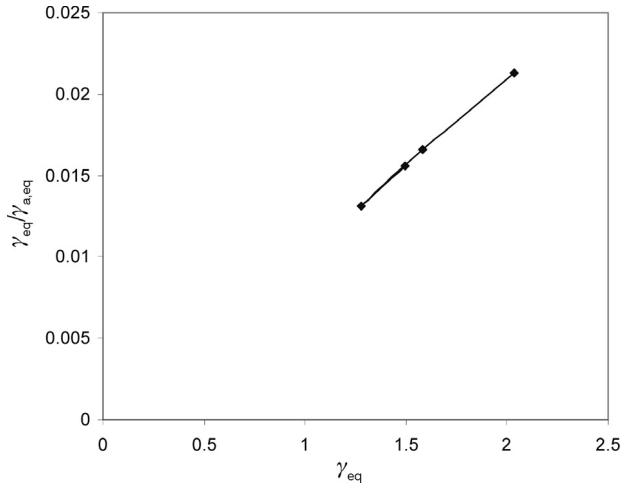
Freundlich isotherm

The Freundlich isotherm[21] is widely used for model of adsorption of heavy metal from an aqueous medium and represented as:

$$\log \gamma_{a,eq} = \log (m_{As}/m_{ad}) = \log K_f + (1/K_n) \log \gamma_{eq}$$

where:

m_{As} – mass of arsenic (III) ion adsorbed at equilibrium, mg



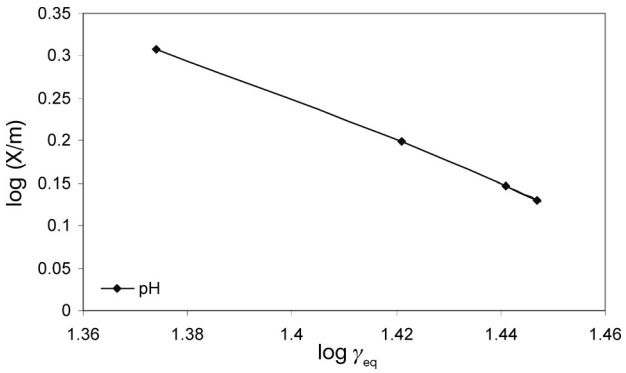
F i g . 9 – *Langmuir isotherm for arsenic (III) biosorption*

m_{ad} – mass of adsorbent (dry biomass), mg

γ_{eq} – equilibrium mass concentration of arsenic (III) ion in solution, mg L^{-1}

K_f and K_n – Constants.

The values of K_f and K_n are obtained as 0.32 and 11 respectively from the linear plot (Fig. 10).



F i g . 1 0 – *Frendlich isotherm for cadmium biosorption*

Interpretation of bacterial growth data with Monod model

The bacterial growth in the presence of arsenic (III) ions at initial loading of 500 ppm at optimum conditions is shown in figure 11. For bacterial specific growth rate determination against limiting substrate glucose concentration using Monod Growth Model,[22] is as follows:

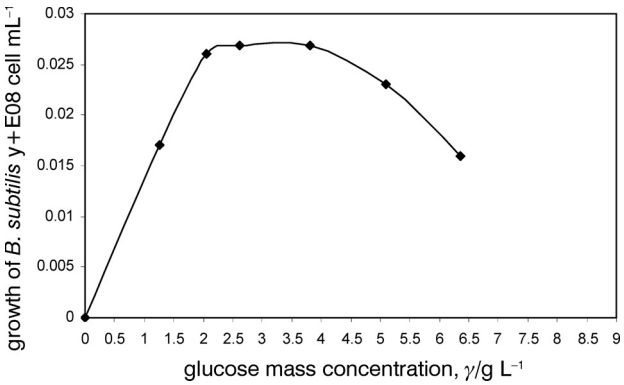
$$\mu = \mu_{max}\, \gamma_S / (K_S + \gamma_S)$$

where

K_s – Limiting substrate glucose mass concentration at which the specific growth rate (μ) is half of maximum value (μ_{max}) i.e. $\mu = \mu_{max}/2$ at $K_s = \gamma_S$.

γ_S – limiting substrate glucose mass concentration

From figure 11, the value of K_S is 7.55 g L^{-1} (upto straight line on the curve). It is calculated from figure 11 that $\mu = 0.017$ s^{-1} at $K_S = 7.55$ g L^{-1}. The specific growth rate (μ) of the bacteria *B. subtilis* in $w = 500 \cdot 10^{-6}$ of initial arsenic (III) loading at optimum parameters is found to be 0.017 s^{-1}.



F i g . 1 1 – *Monod growth model on bacterial growth and rate*

Conclusion

Biosorption of arsenic (III) ion by *Bacillus subtilis* is shown to be an effective bacterial bioprocess. The maximum biosorption of arsenic (III) ions to be obtained is upto $97.30 \cdot 10^{-2}$ for $500 \cdot 10^{-6}$ of initial arsenic (III) ion loading by 72 h inoculation. The optimum pH is observed as 3.5 and optimum temperature is noticed as 40 °C for maximum ($w = 97.30 \cdot 10^{-2}$) biosorption of arsenic (III) ions in the present studies. The optimum shaking speed is noticed as 60 min^{-1} at which the maximum bacterial growth is observed as $3.90 \cdot 10^8$ cells per mL at optimum biological process conditions. Langmuir and Freundlich isotherms fit the arsenic (III) ion biosorption data well and played a major role in giving a better understanding of biosorption modeling. The Monod model for microbial growth shows that the specific growth rate (μ) of *B. subtilis* at optimum bioprocess parameters is found to be 0.017 s^{-1}.

List of symbols

b – constant

C – number concentration, cell mL^{-1}

c – concentration, mol L^{-1}

K – saturation constant, g L^{-1}

m – mass, mg

n – shake speed, min^{-1}

T – temperature, °C

t – time, s

w – mass fraction, 10^{-2}

Greek letters

γ – mass concentration, mg L^{-1}
μ – specific growth rate, s^{-1}
ρ – volume fraction, 10^{-2}

References

1. *Sharma, P., Verma, A*., Indian. J. Microbiol. **31** (1) (1991) 1.
2. *Beveridge, T. J., Murray, R. J. E*., J. Bacteriol. **141** (1980) 876.
3. *Beveridge, T. J*., Can. J. Micribiol. **34** (1988) 363.
4. *Beveridge, T. J*., Int. Rev. Cytol. **72** (1981) 229.
5. *Beveridge, T. J., Koval, S. F.*, Appl. Environ. Microbiol. **42** (1981) 325.
6. *Macaskie, L. E., Dean, A. C. R., Cheetham, A. K., Jakeman, R. J. B., Skarmulis, A. S*., J Gen. Microbiol. **133** (1987) 939.
7. *Tuovinen, O. H., Niemila, S. I., Gyllenberg, H. G*., J. Microbiol. Serol. **37** (1971) 389.
8. *Ray, P., Mishra, A. K*., J. Appl. Microbiol. **47** (1979) 289.
9. *Barkey, T., Schaefer*, J. Curr. Opin. in Microbiol. **4** (2001) 318.
10. *Mann, H*., Biosorption of Heavy Metals, C R C Press, Boston, USA, 1990.
11. *Browning, E*., Chromium in Toxicity of Industrial Metals, 2nd ed, Butterworth and Company, London, UK, 1959.
12. *Schiewer, S., Volesky, B*., In by Lovley D R (Ed), Environmental microbe-Metal Interactions, ASM Press, Washington DC, USA, 2000, pp. 329-356.
13. *Langley, S., Beveridge, T. J*., Appl. Environ. Microbial. **65** (1999) 489.
14. *Macaskie, L. E., Dean, A. C. R*., Environ. Technol. Lett. **5** (1984) 177.
15. *Hossain, S. M., Das, M., Anantharaman, N., Ibrahim, S. H*., Indian Chem. Engr. **45** (2003) 232.
16. *Hossain, S. M., Das, M.., Anantharaman, N., Begum, K. M. M. S., Ibrahim, S. H*., Indian J. Chem. Technol. **11** (2004) 116.
17. *Hossain, S. M., Das, M., Anantharaman, N., Ibrahim, S. H*., The Journal of The Institution of Engineers (India), Chemical Engg Divn, **85** (2004) 7.
18. *Hossain, S. M*., Indian. J. Environ. Protec. **23** (2003) 1396.
19. *Hossain, S. M., Das, M., Anantharaman., N*., Indian Chem. Engr. **47** (2005)
20. *Trivedy, R. K., Goel, P. K*., Chemical and Biological Methods for Water Pollution Studies, Environmental Publications, Karad, India, 1986, pp. 125-126.
21. *De, A. K*., Environmental Chemistry, 4th ed, New age International Publishers, New Delhi, India, 2000, pp. 262.
22. *Treybal, R. E*., Mass Transfer Operations, McGraw-Hill Book Company, Singapore, 1981.
23. *Bailey, J. E., Ollis, D. F.,* Biochemical Engineering Fundamental, McGraw-Hill Inc, New York, USA, 1981.
24. *Shulter, M. L., Kargi, F.,* Bioprocess Engineering Basic Concept, Parentice-Hall of India Pvt Ltd, New Delhi, India, 2000.
25. *Pelczar, M. J., Chan, E. C. S., Kring, N. R.,* Microbiology, 5-th Ed, Tata McGraw-Hill Publishing Co Ltd, New Delhi, India, 2004.
26. *Jeffery, G. H., Bassett, J., Mendham, J., Denney, R. C.,* Vogels Textbook of Quantitative Chemical Analysis, 5th ed, Longman Scientific and Technical UK Ltd, ELBS, England, UK, 1989.