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Bioremediation of Arsenic (III) from Water Using Baker Yeast *Sacchromyces cerevisiae*

Debarshi Roy[1], Priya Gaur[2], Neeraj Verma[1,*], Monika Pathireddy[3], Krishna P Singh[4]

[1]Department of Biotechnology, Karunya University, Karunya Nagar, Coimbatore Tamilnadu India
[2]University School of Biotechnology, Guru Gobind Singh Indraprashta University, New Delhi, India
[3]Department of Biotechnology, Berhampur University, Odisha, India
[4]CSIR- Indian Institute of Toxicology and Research, Lucknow India
*Corresponding author: neerajvermabiotech@gmail.com

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Abstract The availability of arsenic in ground water and potable water accounts many toxic effects on human health because of its high toxicity level; it has become a global concern. Previously to decrease As (III) concentration in water various removal methods were explored. In the present study baker yeast *Saccharomyces cerevisiae*, used as a biosorbate to remediate the Arsenic (III) in the ground water, its capability was examined to sequester the metal ions from the arsenic contaminated water. Initially yeast was grown in YEPD medium and synthetic solution of As (III) was prepared in 3 different conc. 0.2 mg/l, 0.3 mg/l and 0.4 mg/l respectively. Systematic batch kinetic experiments were conducted with various process parameters such as agitation period, pH, and temperature; all the parameters were studied and found that *Saccharomyces cerevisiae* had very fine competence towards arsenic removal from the contaminated water under optimized condition of agitation period 120 hours, temperature 55ºC and pH 6 were maintained. Removal of As (III) from supernatant was analyzed using the Atomic Absorption Spectrophotometer. It has been found that the percentage removal of Arsenic species (III) increases with decrease in YPD media and *Saccharomyces cerevisiae* removed 82.2%, 87.8% and 90.46% with respect to different concentration of synthetic solution of As (III) and it is highly effective method for the As (III) removal from the contaminated water.

Keywords: *Saccharomyces cerevisiae, bioremediation, arsenic, batch kinetics, Atomic absorption spectrophotometer, Growth optimization*

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1. Introduction

A high level of Arsenic(As) toxicity has been found in many countries ground water and this contamination is a global problem in present time, which has been reported mainly in India, USA, China Bangladesh, New Zealand, Taiwan and many other countries [1,2]. There are several human activities like industrial, agriculture and sewage disposal which lead to the toxic metal pollution in environment [3]. As is a natural and pervasive element which is discharged by various anthropogenic inputs to the environment [4]. In natural profusion As ranks 20th which constitute about 0.00005% of earth's crust, 14th in seawater, and 12th in the human body [5,6]. Igneous activity of rocks also contributes considerably to the global natural emission of As [7]. Occurrence of As in ground water is mostly due to leaching of the geological materials, minerals precipitation, and dissolution of unstable As minerals adsorption-desorption, chemical transformation within the formation, and input from geothermal sources [8]. In ground water As is usually present as an oxy-anion, arsenite (H_3AsO_3) or arsenate (H_3AsO_4) [9] or both and is a general inorganic toxicants at contaminated sites [7]. Whereas As (III) is more toxic than As (V) [10] and it is an exceedingly toxicant [9] metalloid at high concentrations, extremely cautious the biota and individuals health. As contagion of soil, water, and air is a worldwide devoloping environmental complication by reason of leaching from geological accumulation, combustion of fossil fuels, wastes produced by gold mining [11] and various other reasons.

Major part of world population completely relies on ground water for drinking purpose thus extremely prone to diseases compelled by As contamination [12]. Presence of As in water is also caused by mineral precipitation, dissolution of not fixed As minerals, [13] desorption, chemical transformation inside the formation, and contribution from geothermal sources. Though there are several important factors which influence the ground water As concentration but some of the majorities involved out of them are pH, Eh, solution composition, competing and complexing ions, aquifer mineralogy,

reaction kinetics, and hydraulics of ground water system. [14] In the manufacturing units of the agricultural products, pesticides, pigments, dyes, semi conductors, glass, pharmaceuticals production units, workers are more prone to exposed to air borne As contamination [15]. Persistent contact to As is linked with a broad variety of neurological disorder [16], cardiovascular disease [17,18], dermatologic [19], and carcinogenic effects [20], Peripheral neuropathy [21], diabetes [22], ischemic heart disease [23] melanosis and keratosis [24,25] and impairment of liver function [26]. Previously several techniques have been evolved for As removal, most frequently using absorbents such as activated carbon, aluminum oxide [27,28], co-operative with iron oxide to form sludge's [29], sorption onto iron-oxide-coated polymeric materials[30], Electrocoagulation [31], by nanoparticle [32]. To remove the stress of heavy and toxic metals, environment friendly approach must be applied and use of naturally occurring microbe must be emphasized [33]. Bacteria, yeast, fungi, algae all of them can be used for remediation processes and it is always recommended that microbe used for bioremediation must have natural decontamination process and the method should be cost-effective [34]. Moreover, microorganism can detect very less concentration of toxic metals in water which serve as an added return to the remediation process [35]. Heavy metals such as Pb, Au, Co, Cu, Fe and their respective cations can be competently removed from water and other aqueous environments by *Saccharomyces cerevisiae* [36,37,38]. Metal uptake or metal sequestration mechanism by *Saccharomyces cerevisiae* depends upon whether cell is dead or inactive in which it function through passive mode without energy requirement or living/active cell in which it operates by transportation of metal and is metabolism dependant [38]. Recently, it has been shown that heat killed *Saccharomyces cerevisiae* at 45˚C preserve flocculation ability in heavy metal contaminated water [39] and reveal superior metals uptake capacities [40]. As for now it is implicit that As contamination in environment can be fatal. In this present work As removal through natural activity of *Sacchromyces cerevisiae* and microorganism metal interaction was investigated. Firstly, the growth of *Sacchromyces cerevisiae* was optimized using various parameters like temperature, pH, and agitation time in different combination of experiments. Secondly, the interaction of microorganism with As (III) and its successive removal from water has been investigated. Subsequent remediation of the As (III) species was done by setting up a protocol as shown in Figure 1 by optimizing the aforementioned conditions. The present study also examines the effect of nutrient limitation in the similar set of As removal experiments so as to see the growth of microorganism in As environment. This work further review that use of *Sacchromyces cerevisiae* is safe and ideal for remediation of As.

2. Materials & Methods

2.1. Collection of Strain and Media Preparation

Saccharomyces cerevisiae, used in this work was acquired from Institute of Microbial Technology (IMTECH) Chandigarh, India. The strain obtained was preserved at 4°C. The YEPD medium Yeast Extract (10.0g/l), Peptone (20.0g/l), Dextrose (20.0g/l) was prepared in Erlenmeyer flasks as per the guidelines of Microbial Type Cell Culture [41,42] and were sterilized using autoclave at121°C, 15 psi and 15minutes. The aseptic conditions were maintained at every step of the experiment.

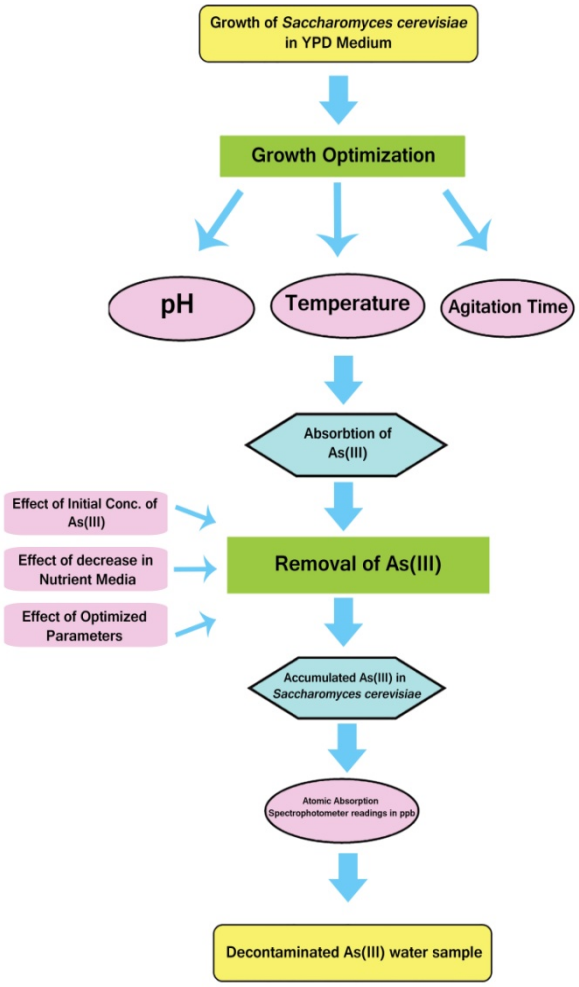

Figure 1. General schematic representation of biosorption process

2.2. Growth and Culture Conditions

The lyophilized culture of microorganism was revived in 100 ml Erlenmeyer flask containing sterilized YEPD medium and incubated at 25°C at150 rpm for 24 h. For the considerable growth of the organism 250 ml of the culture flask is kept for 72 h in incubator wherein the media in the flask turned milky followed by a pellet formed at the bottom indicating significant growth of the *Saccharomyces cerevisiae* in the flask.

2.3. Preparation of Synthetic As Solution

The As (III) synthetic solutions were made by adding predetermined quantity of As in As free water. The concentrations of the solution were prepared as 0.2 mg/l, 0.3 mg/l, and 0.4 mg/l. Aliquots of the solution subjected to the pH adjustment using 1M NaOH to obtain the desired pH, mixture were stirred continuously. Once the desired pH was achieved solution was stirred for one minute before addition of the YEPD Medium.

2.4. Batch Experiments Studies

The biosorption studies were carried out by batch experiments which were carried out using *Saccharomyces cerevisiae*, the yeast which was grown initially in YEPD media was allowed to grow in As environment by transferring the inoculums which was in exponential phase to the synthetic As (III) water prepared of desired concentration. Flasks were kept on rotating shaker with constant shaking. The experiment was carried out at different values of pH (2.0, 4.0, 6.0, and 8.0), different temperature ranges (35ºC, 45ºC, 55ºC) and various agitation periods were kept in hours as (24, 48, 72, 96, and 120). From each set of experiment samples were taken and analyzed using spectrophotometer at 600 nm [43] for the measurement of As (III) removal from the sample.

2.5. Media Variation

The YEPD medium suitable for the growth of *Saccharomyces cerevisiae* has been subjected to the one half (1/2) and one third (1/3) variation in its components which are Yeast Extract, Peptone and Dextrose in the As environment. The subsequent removal of As due to this media variation has been studied and explained.

2.6. Analysis of the Final As Concentration

The *Saccharomyces cerevisiae* cells grown in the combination of As (III) 0.5 mg/l, 1.0 mg/l and 1.5 mg/l respectively in the YEPD media after biosorption was then tested for the remaining As (III) concentration using Atomic Absorption Spectrophotometer [44] at Griffith India Pvt. Ltd. Bhubaneswar, Orissa, India.

3. Result & Discussion

3.1. Growth Optimization of *Saccharomyces cerevisiae*

Table 1. Percentage removal of As (III) by *Sacchromyces cerevisiae* with respect to initial concentration and final concentration

As species	Microorganism	Initial Conc. (mg/l)	Final Conc. (mg/l)	Conc removed (mg/l)	% of removal
As III	*S.cerevisiae*	0.2	0.062	0.138	69%
As III	*S.cerevisiae*	0.3	0.063	0.237	79%
As III	*S.cerevisiae*	0.4	0.0284	0.171	85.5%

Growth of *Saccharomyces cerevisiae* was tested under batch conditions by studying three parameters significant for its growth; agitation time, pH and temperature. pH is a significant factor for maintaining the surface characteristics of the biosorbent, sustain the chemical properties of biosorbate [45] and a vital controlling aspect in the biosorption process. The effect of pH ranging from 2-8 was studied by the series of batch experiments with different initial As (III) concentration 0.2 mg/l, 0.3 mg/l and 0.4 mg/l. It was observed that *Saccharomyces cerevisiae* showed maximum growth at pH 6 and growth was decreased at pH 8, so the optimum growth of *Saccharomyces cerevisiae* occurs at pH 6. *Saccharomyces cerevisiae* contains chitin- chitosan units and amino acids like hystidine, which serve as a matrix of –COOH and –NH2 groups. This matrix interacts with As ion and this interaction depends on the pH of the solution which finally leads to removal of As ions [36]. So pH of the solution must be appropriate for interaction and it was studied that biosorption of Cd(II), Cr(III), Cu(VI), Pb(II) Zn(II) by *Saccharomyces cerevisiae* is dependent on optimum pH above 5 [46].

The effect of temperature ranging from 35-55ºC was studied by the series of batch experiments with different initial As (III) concentration 0.2 mg/l, 0.3 mg/l and 0.4 mg/l. With the increase in temperature the growth of *S.cerevisiae* was also increased and reached to maximum growth at 55ºC. Similarly, the effect of agitation time i.e. 24-120 hrs on the growth of biosorbent with three different initial concentration of As (III) 0.2 mg/l, 0.3 mg/l and 0.4 mg/l was studied. O.D.at 600 nm was noted for every set of experiment. It was evident that as the agitation time was increased the growth of *Saccharomyces cerevisiae* also increased and showed the maximum growth at 120 h. Both agitation time and temperature affects the biomass generation. *Saccharomyces cerevisiae* was grown in synthetic As (III) water of desired concentration i.e. 0.2 mg/l,0.3 mg/l,0.4 mg/l of As (III) specie at the optimized conditions that is pH 6, temperature 55ºC and agitation time 120h. After 120 h the final conc. of As (III) was measured and the experiment was done in triplicate. The experimental data reveal that maximum amount of As (III) removed by *Saccharomyces cerevisiae* was 85.5% at 0.4 mg/l as illustrated in Table 1. The final concentration of As (III) was calculated to be 0.0284 mg/l. As shown in the graph Figure 2 variation in the initial concentration of As (III) the % removal of As (III) species also varies. As the initial concentration from 0.2 mg/l to 0.4 mg/l increases the removal of the As (III) also increases. At high metal ion concentration, the amount of metal ions sorbed was more than at low metal concentration because more binding sites were free for interaction [36].

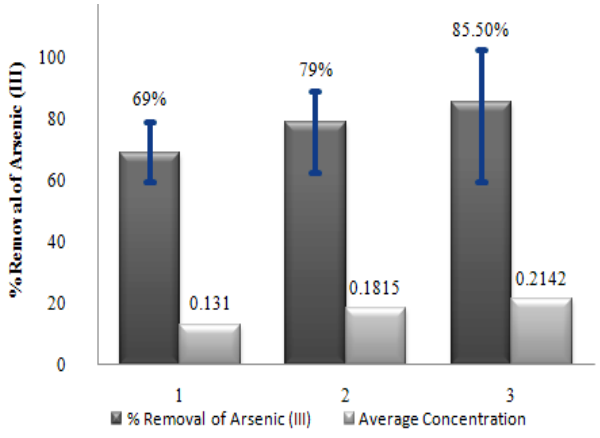

Figure 2. Percentage removal of As (III) with respect to decrease in nutrient media, increase in initial As (III) concentration and average conc (In graph:1-0.2 mg/l;2-0.3 mg/l;3-0.4 mg/l)

Control kept in the experiment was As (III) water without inoculum of *Saccharomyces cerevisiae*. The control shows 0% removal of As (III). *Saccharomyces cerevisiae* was grown in an environment with decrease in YEPD media and increase in As (III) concentration at

optimized conditions like agitation time maintained at 120 h, temperature at 55ºC and pH at 6. It was observed that as the As (III) concentration was increased and simultaneously the YEPD media decreased, the percentage removal of As (III) was also increased and maximum concentration of 1.5 mg/l. Firstly, when the YEPD media was maintained at the composition of 5g of yeast extract, 5g of peptone and 10g of dextrose and As conc. maintained at 0.5 mg/l then the % removal of As was found to be 82.2.Secondly, when the YEPD media was maintained at 2.5g of yeast extract, 2.5g of peptone and 5g of dextrose and the As(III) concentration at 1.0 mg/l in that case the % removal of As was found to be 87.8. Finally, when the YEPD media maintained at 1.25g of yeast extract, 1.25g of peptone, 2.5g of dextrose and the As conc. at 1.5 mg/l then the % removal of As was found to be 90.46 as illustrated in Table **2**. So, from the above observation increase in the initial As (III) concentration with decreasing YEPD media lead to the increase in % removal of As (III). The maximal removal was shown at 1.5 mg/l.

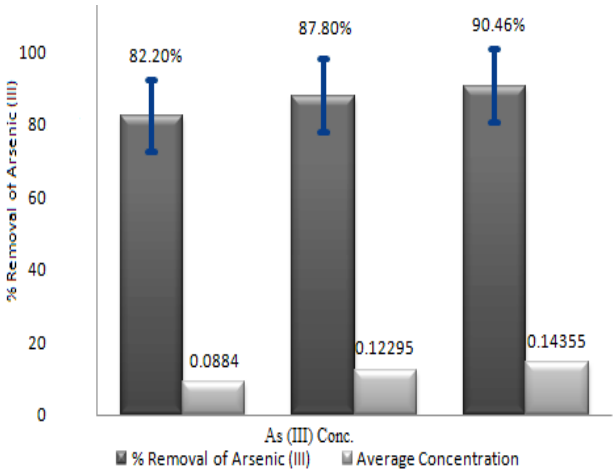

Figure 3. Percentage removal of As (III) with respect to varying initial concentration and the average concentration

There occurs no difference in growth if YEPD media is decreased. So, even in the As (III) environment the growth of *Saccharomyces cerevisiae* is not affected with decreased nutrient media and efficient removal of As(III) from synthetic As (III) water was observed as in Figure 3.

3.2. Effect of Agitation Time on the Removal of As (III)

Removal of As (III) increases with the agitation time as shown in the graph Figure 4 even after 96 h the removal increases slightly but it is more when the agitation time varies from 96-120 h. After 120 h the removal of As (III) remains almost constant i.e. 84%. It seems that the As (III) accumulation starts to predominate after some time of the *Saccharomyces cerevisiae* growth around 30 h of agitation and is continued for a long time; as a result, within the agitation time of 96-120 h, the % removal of As (III) is more because more of the As ions are exposed for removal.

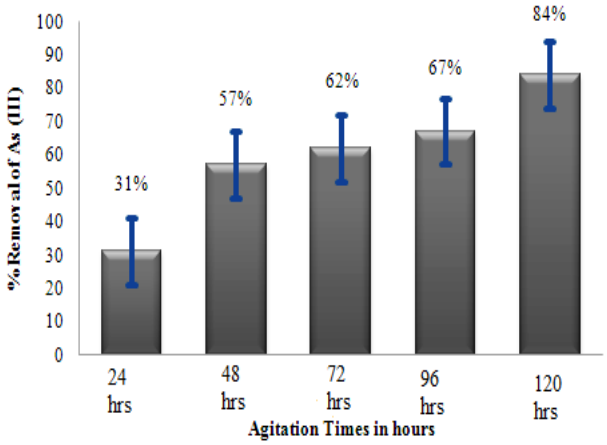

Figure 4. Percentage removal of As (III) with respect to agitation time

3.3. Effect of pH on the Removal of As (III)

The most important parameter of the adsorption process is pH. These occurs on the different pH adsorption environment according to different heavy metals and because of this pH, binding with metal ions and chemical interaction with yeast cells takes place. It is evident that the % removal of all the As species increases with the increase in pH from 2 to 6. The maximum removal of As 66% was shown at pH 6 as shown in the Figure 5 In bio-adsorption bioaccumulation process As (III) is directly captured by ArsR protein [47, 48]. Therefore, the extent of relative removal of As (III) will depend on the relative concentration of arsenate reductase enzyme and ArsR protein produced.

Table 2. Percentage removal of As(III) by Sacchromyces cerevisiae with respect to decrease in nutrient media (YEPD) and increase in As(III) concentration under optimized conditions pH-6, Temperature 55˚C and agitation time 120 hrs. Working volume-250 ml

Serial no.	As species	Micro-organism	YEPD media composition(gm/L)			Initial conc.(mg/l)	Final conc.(mg/l)	Conc. removed(mg/l)	% of removal
			Yeast extract	Peptone	Dextrose				
1	As(III)	*Saccharomyces cerevisiae*	5g	5g	10g	0.5 mg/l	0.0884	0.411	82.2%
2	As(III)	*Saccharomyces cerevisiae*	2.5g	2.5g	5g	1.0 mg/l	0.12295	0.878	87.8%
3	As(III)	*Saccharomyces cerevisiae*	1.25g	1.25g	2.5g	1.5 mg/l	0.14355	1.357	90.46%

3.4. Effect of Temperature on Removal of As Species

The temperature was maintained at 35ºC, 45ºC and at 55ºC. It was observed that as the temperature was increasing the % removal of As (III) is also increasing as shown in Figure 5 Temperature plays a vital role in the was found at 55ºC.

3.5. Analysis of Accumulated As in *Saccharomyces cerevisiae*

Supernatant collected from the culture broths of *Saccharomyces cerevisiae* was tested for the As (III)

concentration using Atomic absorption spectrophotometer for the determination of As (III) in the yeast supernatant during biosorption examining studies. Final As concentration 0.008 mg/l, 0.122 mg/l and 0.143 mg/l for 0.5 mg/l, 1.0 mg/l and 1.5 mg/l of initial As concentration respectively was observed in the sample collected.

4. Conclusion

This work has assured the capability of the *Saccharomyces cerevisiae* with respect to removal of As (III) from the contaminated water. When the optimized conditions were kept at maximum removal was 85.5% and with the change in initial As (III) concentration maximum removal was observed at 1.5 mg/l which was calculated to be 90.46%. The decrease in nutrient media does not actually affect the growth of *Saccharomyces cerevisiae*, the components of YEPD medium provide energy to the cells for better and efficient removal but overall the removal of As (III) was not affected. The atomic adsorption spectrophotometry revealed the concentration of As (III) accumulated during the process of removal in the cells of the *Saccharomyces cerevisiae.* Remediation of As (III) is essential as it is noxious and yeast cells suspended in medium can retain their capability to remove As (III) ions from contaminated water as it is examined in this study. So, though several attempts have been made to remove As species via various physico-chemical techniques but the *Saccharomyces cerevisiae* removal of As (III) leads into an economical and effective pathway for bioremediation of As.

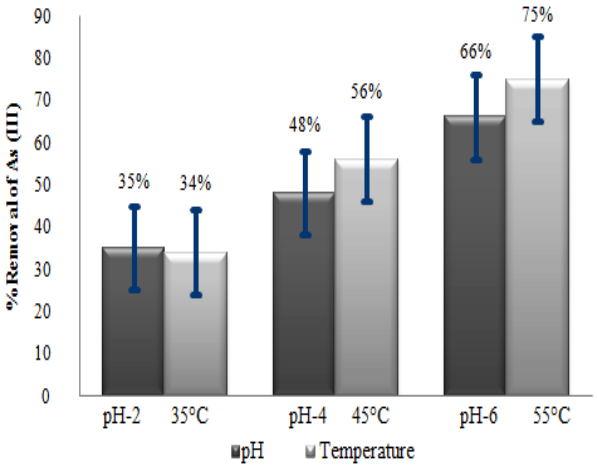

Figure 5. Percentage removal of As (III) with respect to pH and percentage removal of As (III) with respect to temperature

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