



## Research Paper

# OPTIMIZATION OF PHYSICAL CONDITIONS FOR AS(III) BIOSORPTION BY *ASPERGILLUS NIGER* X<sub>300</sub>

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An experimental study was conducted to optimize different physical conditions for As(III) bio sorption by an As(III) resistant strain *Aspergillus niger* X<sub>300</sub>. Maximum biosorption was resulted with initial pH , 4.5; temperature , 30 °C; spore density , 7 X 10<sup>8</sup>; shaker speed , 200 rpm ; volume of medium , 100 mL ; age of inoculum , 7 days and incubation period , 7 days.

**Keywords:** Experimental, Optimize, Physical, As (III) , Biosorption

## INTRODUCTION

Bio-sorption is a metabolically passive process which is dependent on kinetic equilibrium and the composition of sorbent cellular surface chemistry (Loukidou *et al.*, 2003). Adsorbing elements are adsorbed onto the cellular surface. As it is determined by equilibrium, it is greatly influenced by pH, concentration of biomass and other physical conditions that alter the biomass (Murugesan *et al.*, 2006; Mamisahebei *et al.*, Velasque and Dussan, 2009). Among the many abiotic factors (physical conditions) fungal growth is significantly affected by pH and temperature (Roberts and Cambell, 1977). Considering the reviews, my present study was intended to optimize different Physical conditions namely initial pH, temperature, spore density, agitation/aeration, volume of medium, age of inoculum and incubation period one by one to improve the bio sorption of As(III) by

experimentally developed resistant fungus *Aspergillus niger* X<sub>300</sub> (Ganguly, 2013).

## MATERIALS AND METHODS

**Microorganism:** An experimentally developed As(III) resistant strain *Aspergillus niger* X<sub>300</sub> was used throughout the study (Ganguly , 2013).

### Medium Composition Used for Fungal Growth:

The growth medium was composed of: glucose, 10%; urea, 0.8%; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.03%; KH<sub>2</sub>PO<sub>4</sub>, 0.1%; K<sub>2</sub>HPO<sub>4</sub>, 0.1% ; CaCl<sub>2</sub>, 0.3%.

### Medium Composition for Maintenance

**Medium:** Synthetic maintenance medium contains: glucose, 0.1%; urea , 0.6%; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.01%; KH<sub>2</sub>PO<sub>4</sub>, 0.1%; K<sub>2</sub>HPO<sub>4</sub>, 0.1%; CaCl<sub>2</sub> , 0.2% and agar , 4% as solidifying agent.

**Estimation of As(III) :** The concentration of As(III)

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in the broth was estimated by the method as reported by Cernansky *et al.* (2007).

**Estimation of Dry Cell/Spore Weight:** Fungal cells /spores were filtered using Whatmen No.1 filter paper and heated at 70 °C until it becomes dry and its weight was estimated by electronic weighing machine (ECELON MS- 2690).

**Statistical Analysis:** All data were expressed as Mean± SEM. Data were analyzed by one way ANOVA followed by Dunett's *post hoc* multiple comparison test considering  $p < 0.05$  as significant and  $p < 0.01$  as highly significant (using Prism 4.0).

Experiments were carried out in six sets using 250 mL Erlenmeyer Conical flask using 50 mL growth medium, pH 5.0, temperature, 28 °C, spore/cell density  $5 \times 10^7$  cells or spores/mL, shaker speed, 150 rpm, age of inoculum, 7 days and incubation period, 7 days.

## RESULTS AND DISCUSSION

Tables 1 to 7 showed the optimization of different physical condition for this bio sorption.

From the tables, it can be tentatively concluded that the fungus *Aspergillus niger* X<sub>300</sub> grew best with pH, 4.5, temperature, 30 °C, spore density,  $7 \times 10^8$  spores or cells/mL; shaker speed,

**Table 1: Effect of Initial pH**

Initial pH	Initial concentration of As(III) [mg/L]	Final concentration of As (III) [mg/L]	Dry cell/spore weight (gm/L)
7.0	1500	672.7±6.136 **	6.3±0.431 **
6.5	1500	657.8±4.212 **	6.5±0.661 *
6.0	1500	642.3±4.313 **	6.6±0.731 *
5.5	1500	598.7±3.683 *	6.9±0.613 *
5.0 (Control)	1500	566.4±4.683	7.3±0.683
☒4.5	1500	495.8±3.661 *	8.3±0.832 **
4.0	1500	542.4±6.136 *	7.7±0.661

Note: (values were expressed as Mean ± SEM , where n=6 ; \* $p < 0.05$  and \*\* $p < 0.01$ , ☒ Stands for maximum bio sorption)

**Table 2: Effect of Temperature (°C)**

°C	Initial Concentration of As(III) [mg/L]	Final Concentration of As(III) [mg/L]	Dry Cell/Spore Weight (g/L)
25	1500	**696.2±3.396	**6.0±0.661
26	1500	**631.6±4.136	**6.3±0.432
27	1500	*591.2±6.213	7.0±0.361
28 (Control)	1500	566.4±4.683	7.3±0.683
29	1500	**511.2±3.163	*8.1±0.832
☒30	1500	**476.6±4.163	**8.6±0.881
31	1500	**496.2±3.683	**8.9±0.763

Note: Values were expressed as Mean ± SEM , where n=6 ; \* $p < 0.05$  and \*\* $p < 0.01$ , ☒ Stands for maximum bio-sorption.

**Table 3: Optimization of Spore Density (Spores/mL)**

Spores/mL	Initial Concentration of As(III) [mg/L]	Final Concentration of As (III) [mg/L]	Dry Cell/Spore Weight (g/L)
$3 \times 10^6$	1500	**521.8±3.961	8.3±0.661
$5 \times 10^7$ (Control)	1500	476.6±4.163	8.6±0.881
$6 \times 10^8$	1500	*432.4±4.613	8.9±0.731
$8 \times 7 \times 10^8$	1500	*401.2±3.921	*9.1±0.683
$9 \times 10^{11}$	1500	**422.6±3.613	*9.3±0.661

Note: Values were expressed as Mean ± SEM , where n=6 ; \*p<0.05 and \*\*p<0.01, ☒ Stands for maximum bio sorption.

**Table 4: Effect of Agitation/Aeration**

Shaker Speed (rpm)	Initial Concentration of As(III) (mg/L)	Final Concentration of As(III) (mg/L)	Dry Cell/Spore Weight (gm/L)
50	1500	**522.6±6.213	*8.3±0.861
100	1500	**464.2±4.831	*8.5±0.832
150 (Control)	1500	401.2±3.921	9.1±0.683
☒200	1500	*372.8±7.613	*9.9±0.661
250	1500	*591.9±6.136	*9.7±0.862

Note: Values were expressed as Mean ± SEM , where n=6 ; \*p<0.05 and \*\*p<0.01, ☒ Stands for maximum bio sorption.

**Table 5: Effect of Volume of Medium**

Volume of Medium	Initial Concentration of As(III) (mg/L)	Final Concentration of As(III) (mg/L)	Dry Cell/Spore Weight (gm/L)
25	1500	*416.3±6.133	*9.3±0.732
50 (control)	1500	372.8±7.613	9.9±0.661
75	1500	**333.2±6.113	*10.4±0.763
☒100	1500	**301.6±8.616	**10.8±0.669
150	1500	**321.4±4.663	*10.5±0.613

Note: Values were expressed as Mean ± SEM , where n=6 ; \*p<0.05 and \*\*p<0.01, ☒ Stands for maximum bio sorption.

200 rpm; volume of medium, 100 mL; age of inoculum, 7 days and incubation period, 7 days. Papaigianni (2007) reported the optimum pH for *Aspergillus niger* was 4.5, required dissolved oxygen for its maximum growth. Majumder *et al.* (2010)

claimed that a mutant strain *Aspergillus niger* 14/20 grew best with pH 5 at 30 °C. As the biosorption is directly proportional to the fungal growth, maximum As (III) biosorption was obtained with the above mentioned physical conditions.

**Table 6: Effect Of Age Of Inoculum**

Age of Inoculum (days)	Initial Concentration of As(III) (mg/L)	Final Concentration of As(III) (mg/L)	Dry Cell/Spore Weight (g/L)
1	1500	**1332.6±4.316	**1.6±0.161
2	1500	**922.4±6.613	**3.6±0.642
3	1500	**821.2±5.163	**4.2±0.432
4	1500	**527.4±6.111	**8.3±0.331
5	1500	**419.3±5.213	**8.8±0.683
6	1500	**376.4±7.161	**9.9±0.731
7 (control)	1500	301.6±8.616	**10.8±0.669
8	1500	*316.6±6.316	**10.9±0.661

Note: Values were expressed as Mean ± SEM , where n=6 ; \*p<0.05 and \*\*p<0.01, ☒ Stands for maximum bio-sorption.

**Table 7: Optimization of Incubation Period**

Incubation Period(Days)	Initial Concentration of As(III) (mg/L)	Final Concentration of As(III) (mg/L)	Dry Cell/Spore Weight (g/L)
3	1500	1122.4±7.313	2.9±0.613
4	1500	774.3±6.224	4.6±0.661
5	1500	521.6±5.313	8.4±0.461
6	1500	326.2±6.316	11.1±0.663
7 (control)	1500	301.6±8.616	10.8±0.669
8	1500	301.6±6.116	10.8±0.591

Note: Values were expressed as Mean ± SEM , where n=6 ; \*p<0.05 and \*\*p<0.01, ☒ Stands for maximum bio-sorption.

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