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BIOSORPTION OF As (III) BY *ASPERGILLUS NIGER* X300 USING COMPLEX NUTRIENT MEDIUM

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ABSTRACT: An experimental study was carried out to select a suitable complex nutrient medium for growth and As(III) biosorption by an As(III) resistant strain *Aspergillus niger* X300 .For this purpose different complex nutrient were examined one by one. The recommended complex nutrients (gm%) were corn steep liquir, 20; rice bran extract, 10; paddy soak liquir, 10; wheat bran extract, 15 beef extract, 30, meat extract, 2 0 potato scale extract, 15 and malt extract, 30.

INTRODUCTION: Microorganisms require nutrients as a source of energy and supply of constituents for their growth. Surveying the nutritional capabilities of a fungus is an endless task since every chemical found in living organism and a wide variety of manufactured and inorganic materials are potentially useful for satisfying the need of different fungi [1]. But such chemical nutrients are not cost effective.

Several naturally occurring by products like corn-steep liquor, rice bran extract, paddy soak liquir ,wheat bran extract, beef extract, meat extract, potato scale extract and malt extract etc. could be used for growth and metabolites production of different microorganism [2-7].

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Besides serving as carbon and nitrogen sources, complex nutrients contain a wide range of vitamins, amino acids, apart from the characteristic stimulator or inhibitor in them.

In the present study, the effect of a host of complex nutrients which are readily available in our country, have been probed into.

MATERIALS AND METHODS:

Microorganism: An experimentally developed As (III) resistant strain *Aspergillus niger* X_{300} was used throughout the study [8].

Estimation of As (III): The concentration of As(III) in the broth was estimated by the method as reported by Cernansky *et al*., 2007 [9].

Estimation of dry cell / spore weight: Fungal cells /spores were filtered using Whatman No.1 filter paper and heated at 70^{o}C until it becomes dry and its weight was estimated by electronic weighing machine (ECELON MS- 2690) [10].

Statistical analysis: All data were expressed as Mean± SEM. Data were analysed by one way ANOVA followed by Dunnett'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, with pH, 4.5; temperature, 30^{o}C, spore density, 7x 10^{8} spores or cells/ml; shaker speed, 200 rpm; volume of medium, 100ml; age of inoculum, 7 days and incubation period, 7 days [11].

Composition of synthetic medium: The synthetic medium composed of glucose , 12% ; $(NH_4)SO_4$, 0.8% ; K_2HPO_4 , 0.01% ; KH_2PO_4 , 0.01% ; $CaCl_2.2H_2O$, 0.03%; NaCl, 0.02% ; $MgSO_4.7H_2O$,0.03 µg/ml ; $MnSO_4.4H_20$, 0.02 µg/ml; $FeSO_4.7H_20$,0.02 µg/ml and thiamine-HCl ,0.02µg/ml.

Preparation of complex nutrient media:

Preparation of rice bran and wheat bran extracts: 40 gm of each material was taken into 250 ml of warm distilled water separately. The suspension was kept at 28^{o}C for 48h. The extracts were filtered separately through cotton and evaporated to dryness under vacuum to recover solid content.

(i) **Preparation of corn steep liquir:** About 150 gm of corn was taken into 300 ml distilled water and kept it for 24h at 28^{o}C. The extract was filtered through cotton and evaporated to dryness under vacuum to recover solid content.

(ii) **Preparation of paddy soak liquor:** 100 gm paddy was added to 250 ml of distilled water and kept it at 28^{o}C for 24h. The extract was filtered through cotton and evaporated to dryness under vacuum to recover solid content.

(iii) **Preparation of potato-scale extract:** 50 gm of soybean was poured into 250 ml of distilled water and allowed it to swallow for 24h at 28^{o}C. The soaked water was extracted thoroughly and filtered through cotton. It was then dried for determination of solid content.

All the above mentioned complex nutrients were solid content in a sterile condition, but meat extract, beef extract and malt extract were added directly to the synthetic medium[12].

The effects of varying concentrations of each of the complex nutrients were examined on growth and As (III) biosorption by the mutant *Aspergillus niger* X_{300}.

RESULTS AND DISCUSSION: The effect of individual complex nutrients and their combined effect on growth and biosorption of As (III) by *Aspergillus niger* X_{300} were depicted in the following **Tables 1- 9**.

TABLE 1: EFFECT OF CORN-STEEP LIQUIR

Concentration (gm%)	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry cell/spore weight (gm/L)
Control	1500	1500	0.0
5	1500	**721.4±3.613	**6.7±0.731
10	1500	**322.6±6.236	**11.0±0.636
15	1500	**220.9±5.116	**11.4±0.736
⋕20	1500	**194.2±4.384	**12.3±0.661
25	1500	**210.1±3.614	**12.1±0.536

(Values were expressed as Mean ± SEM, where n=6; **p<0.01, ⋕ stands for maximum bio sorption)

TABLE 2: RICE BRANE EXTRACT

Concentration (gm%)	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry Cell / spore weight (gm/L)
Control	1500	1500	0.0
5	1500	**832.6±7.363	**5.3±0.461
⋕10	1500	**621.3±6.113	**6.1±0.736
15	1500	**691.2±4.142	**5.9±0.771

(Values were expressed as Mean ± SEM, where n=6; and **p<0.01, ⋕ stands for maximum bio sorption)

TABLE 3: PADDY SOAK LIQUIR

Concentration (gm%)	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry cell/spore weight (gm/L)
Control	1500	1500	0.0
5	1500	**1321.2±7.316	**1.6±0.661
⋕10	1500	**1104.6±4.836	**3.1±0.873
15	1500	**1196.9±6.890	**3.0±0.913

(Values were expressed as Mean ± SEM, where n=6; **p<0.01, ⋕ stands for maximum bio sorption)

TABLE 4: WHEAT BRANE EXTRACT

Concentration (gm%)	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry Cell / spore weight (gm/L)
Control	1500	1500	0.0
5	1500	**1421.2±7.339	**1.3±0.661
10	1500	**1400.7±6.769	**1.2±0.769
15	1500	**1397.1±6.613	**1.2±0.864
20	1500	**1410.6±7.361	**1.2±0.464

(Values were expressed as Mean ± SEM, where n=6; **p<0.01, ⋕ stands for maximum bio sorption)

TABLE 5: EFFECT OF BEEF EXTRACT

Concentration (gm%)	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry Cell / spore weight (gm/L)
Control	1500	1500	0.0
5	1500	**1103.6±7.636	**3.1±0.661
10	1500	**921.3±6.864	**3.6±0.641
15	1500	**678.4±6.616	**6.2±0.786
20	1500	**422.3±8.646	**8.7±0.666
25	1500	**376.2±6.661	**10.9±0.743
30	1500	**221.2±7.366	**11.4±0.431
35	1500	**299.7±7.913	**11.6±0.467

(Values were expressed as Mean ± SEM, where n=6;* *p<0.01, ⋕ stands for maximum bio sorption)

TABLE 6: EFFECT OF MEAT EXTRACT

Concentration (gm%)	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry Cell / spore weight (gm/L)
⋕Control	1500	1500	0.0
5	1500	**1130.4±7.693	**3.3±0.436
10	1500	**722.6±6.613	**6.7±0.661
15	1500	**621.4±7.777	**6.3±0.864
20	1500	**332.7±7.911	**11.0±0.916
25	1500	**370.6±4.326	**10.8±0.686

(Values were expressed as Mean ± SEM, where n=6;* *p<0.01, ⋕ stands for maximum bio sorption)

TABLE 7: EFFECT OF POTATO SCALE EXTRACT

Concentration (gm%)	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry Cell / spore weight (gm/L)
Control	1500	1500	0.0
5	1500	**1324.1	**1.6
10	1500	**1227.0	**2.8
15	1500	**1184.4	**3.1
20	1500	**1201.6	**3.0

(Values were expressed as Mean ± SEM, where n=6; **p<0.01, ⋕ stands for maximum bio sorption)

TABLE 8: EFFECT OF MALT EXTRACT

Concentration (gm%)	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry Cell / spore weight (gm/L)
Control	1500	1500	0.0
5	1500	**1376.4±7.331	**1.4±0.613
10	1500	**1221.2±4.616	**1.6±0.463
15	1500	**927.6±6.821	**3.6±0.716
20	1500	**723.1±7.164	**6.7±0.661
25	1500	**432.6±8.913	**8.8±0.531
⋕30	1500	**221.3±6.661	**11.3±0.684
35	1500	**237.4±7.832	**11.5±0.586

(Values were expressed as Mean ± SEM, where n=6;* *p<0.01, ⋕ stands for maximum bio sorption)

TABLE 9: COMPARISON OF As(III) BIOSORPTION BY *ASPERGILLUS NIGER* X300 BETWEEN SYNTHETIC MEDIUM AND COMPLEX NUTRIENT MEDIUM

Media	Initial concentration of As (III) [mg/L]	Final concentration of As (III) [mg/L]	Dry Cell / spore weight (gm/L)
Control	1500	1500	0.0
Synthetic medium	1500	**89.3±4.384	**15.1±0.613
Mixed Complex nutrient medium	1500	**132.7±7.616	**12.8±0.861

(Values were expressed as Mean ± SEM, ** p<0.01 when compared to control)

In the present study , it was found that ,even though the synthetic medium was proved to be potentially more effective in As(III) biosorption by the mutant than complex nutrient medium but considering the cost effectiveness, a complex nutrient medium composed of (in gm%) corn steep liquir, 20; rice bran extract, 10; paddy soak liquir, 10; wheat bran extract, 15 beef extract,30,meat extract, 20 potato scale extract, 15 and malt extract, 30 can strong be recommended for satisfactory growth and As(III) biosorption by the As(III) resistant strain *Aspergillus niger* $X_{300.}$

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