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Full Length Research Paper

Bioremediation of acid fast red dye by *Streptomyces globosus* under static and shake conditions

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Two different azo dyes known as acid fast red (AFR) and Congo red (CR) were examined for their decolorization by five strains of actinomycetes (*Streptomyces globosus*, *Streptomyces alanosinicus*, *Streptomyces ruber*, *Streptomyces gancidicus*, and *Nocardiopsis aegyptia*) under shake and static conditions. *Streptomyces globosus* decolorized AFR by 81.6% under static condition while 70.2% dye removal was achieved under shake conditions. Application of Plackett-Burman statistical design revealed that the main factors that affected biosorption capacity were the starch concentration and the inoculum size. Under static conditions, increasing the inoculum size and decreasing starch concentration increased the biosorption % up to 1.14 fold with time reduction, while increasing both the inoculum size and starch concentration under shake conditions increased the biosorption % up to 1.09 fold only. A trial for the use of potato peel for more economic biomass production of *S. globous* was carried out and (2 g/50 ml) and dried potato peel had the optimum concentration for maximum biomass production (0.3 g/50 ml) which led to considerable biosorption capacity (89.4%). Electron microscopy studies confirmed the dye removal.

Key words: Azo dyes, Biosorption, *Streptomyces globosus*, Plackett-Burman design.

INTRODUCTION

Azo dyes are widely used in industries, such as textiles, paper, plastics and leather, industries, etc, for the coloration of products. The effluents emanating from these industries often contain high concentrations of dye wastes. The release of colored wastewaters represents a serious environmental problem and a public health concern (Dos Santos et al., 2007; El Ahwany, 2008).

Dyes are generally believed to be toxic and carcinogenic or prepared from other known carcinogens. The discharge of these dye stuffs from industries into rivers and lakes results in a reduced dissolved oxygen concentration causing anoxic conditions, which subsequently affect aerobic organisms (Chander and Arora, 2007; Vijayaraghavan and Yun , 2008).

Adsorption and precipitation methods, chemical degradation or photo degradation are financially and often also methodologically demanding, time-consuming and mostly not very effective. As a viable alternative, biological processes have received increasing interest owing to their cost effectiveness and environmental friendliness (Mabrouk and Yousef, 2007).

Actinomycetes strains have been identified which decolorize effluents containing different types of reactive dyes. Adsorption of anthraquinone, phthalocyanine and azo dyes to the cells of some of the strains resulted in the decolorization of the effluents, but no degradation of the dyes was observed (Aksu, 2005).

There are large numbers of reports on the optimization of carbon and nitrogen source by the classical method of medium optimization that changes one independent variable, while fixing other variables at definite levels. Optimizing all the affecting parameters by statistical experimental design, such as Plackett-Burman methodology can eliminate the limitations of single-factor optimization process collectively (Urvish and Akshaya, 2010).

Potato peels contain considerable amounts of carbohydrate which stimulate the cells to express many hydrolytic enzymes . In addition it contains appreciable

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Abbreviation: AFR,Acid fast red; **CR**, Congo red.

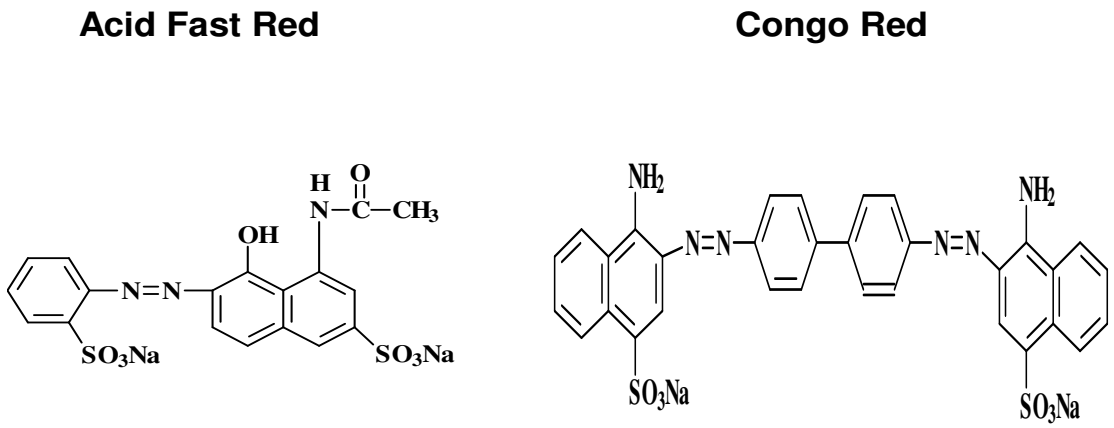

Figure 1. Chemical structure of acid fast red (AFR) and Congo red (CR) azo dyes.

Table 1. Screening of actinomycetes strains and their biosorption % for acid fast red and Congo red.

Actinomycetes strain	Biosorption (%)			
	Acid fast red (504 nm)		Congo red (502 nm)	
	Shake	Static	Shake	Static
Streptomyces globosus	70.2	81.6	56.1	67.21
Streptomyces alanosinicus	39.5	49.77	55.3	68.17
Streptomyces ruber	68.1	79.6	60.7	72.78
Streptomyces gancidicus	69.2	80.13	42.6	58.39
Nocardiopsis aegyptia	50.5	60.1	54.4	63.43

amounts of easily utilizable sugars which encourage growth initiation, and protein, which serves as essential nitrogenous compounds. Potato peels are renewable, cheap and widely available waste in Egypt. Utilization of waste potato peels to produce biomass appears to be economic (Mabrouk and El-Ahwany, 2008).

This work attempted to formulate a suitable media using Plackett-Burman statistical design to increase biosorption of acid fast red by *Streptomyces globosus* under static and shake conditions and evaluated its biosorption potentiality by growing it on economic sources.

MATERIALS AND METHODS

Actinomycetes strains and dye

Five actinomycetes strains were used in the investigation. These were *Streptomyces globosus*, *Streptomyces alanosinicus*, *Streptomyces ruber*, *Streptomyces gancidicus*, and *Nocardiopsis aegyptia*, which were isolated from Burollus Lake and the Mediterranean Sea sediments and was kindly provided by Dr. Gehan Abou Elela (Associate professor of marine microbiology, National Institute of Oceanography and Fisheries, Alexandria - Egypt). Two dyes were used in this work; acid fast red and Congo Red (Figure 1) which were obtained from the Asma Company, Kafr El-Dawar city, near Alexandria, Egypt. Dye stock solutions were prepared in water and autoclaved for 15 min at 121°C.

Culture medium and screening test

Actinomycetes strains were cultivated on starch-nitrate medium which had the following constituents: starch, 20 (g/l); KNO_3, 1(g/l); K_2HPO_4, 0.5 (g/l); $MgSO_4.7H_2O$, 0.5 (g/l); $FeSO_4$, 0.05(g/l); and agar, 15 (g/l) (in case of solid maintenance medium). All constituents were dissolved in the Lake or sea water (pH 7). The media broths were inoculated with 1 ml of seed cultures and incubated at 30°C for six days. A screening test for the biosorption percentage of all the strains was applied for both dyes under shake and static conditions.

Measurement of decolorized dye

After centrifugation of cultures broths, cell pellets were collected from six days culture, washed twice in sterile water and 0.1 g of washed pellets was suspended in dye solution (50 mg/ml), at both static and shake conditions. All tests and their replicates were incubated at 30°C for 2 h, during which the cells were colored due to the uptake of the dye. The mixtures were centrifuged and the residual dye colors in the supernatants were measured according to the dye wave lengths (Table 1).

Biosorption percentage was calculated as follows:

$$\text{Biosorption \%} = \frac{(C_0 - C_e) \times 100}{C_0}$$

C_0 = Initial absorbance reading before decolorization and C_e = final

absorbance reading after decolorization.

The concentrations of the residual dyes in the supernatants were determined by using a standard curve. All results are the mean of replicates (El Ahwany, 2008).

Optimization experiment

The effect of medium components on dyes biosorption was studied by applying the Plackett- Burman (1946) experimental design. In this experiment, seven factors (medium components) were screened in eight combinations organized according to the Plackett Burman design matrix (Table 3). Increase of the original component level is represented by the (+) sign, while decrease of the original component level is represented by (-) sign. The main effect of each factor was determined using the following equation:

$$Exi = (Mi+ - Mi-) / N$$

Where Exi is the variable main effect, and Mi+ and Mi- are the biosorption % and the wet weight (g/ml) in the trials, where the independent variables were present in high and low concentrations, respectively, and N is the number of trials divided by two. Statistical *t-values* for equal unpaired samples were calculated using Microsoft Excel to determine the variable significance. From main effect results, an optimized medium was predicted which will give maximum biosorption %. Verification test was done to confirm the validity of the optimized medium. Results of the verification test were recorded after different time intervals.

Biosorption capacity was calculated according to the following equation:

$$\text{Biosorption capacity (mg/ml) by cells} = \frac{\text{Concentration of acid fast red biosorbed}}{\text{mg biomass}}$$

Biomass production using agricultural waste

A trial for using potato peels for more economic biomass production of *S. globous* was carried out. Optimized media (free of starch and KNO_3) with different concentrations (0.75, 1, 2, 3, 4 and 5 g/50 ml) of dried, finely grounded potato peel were prepared. Biomasses were collected after six days incubation, washed twice with sterile distilled water and were subjected to dye solution (50 mg/ml). Results were recorded in the static condition (Mabrouk and El Ahwany, 2008).

Chemical analysis of potato peels

Oven dry weight of potato peels was used to estimate its content of ash (Browne and Zerban, 1948), protein (Daughton et al., 1984), carbohydrate (Dubois et al., 1956), and easily utilizable sugars (Miller, 1959).

Transmission electron microscope (TEM) studies

Fresh samples of *S.globosus* were fixed using a universal electron microscope fixative as described by McDowell and Trump (1976). Series dehydration steps were followed using ethyl alcohol and propylene oxide. The sample was then embedded in labeled beam capsules and polymerized. Thin sections of cells with adsorbed dye were cut using LKB 2209-180 ultra microtome and stained with a saturated solution of urinyl acetate for half hour and lead acetate for 2 min (McDowell and Trump 1976). The procedure was applied to the control cells not exposed to dye solution and to dye-exposed cells to observe the location of the dye. Electron micrographs were taken using a transmission electron microscope (JEM-100 CX Joel), at the Electron Microscope Unit, Faculty of Science, Alexandria University, Egypt.

RESULTS

Screening tests

Acid fast red and Congo red were screened for their biosorption % by five actinomycetes strains under static and shake conditions. The results in Table 1 revealed that the highest biosorption percentages were achieved under static conditions. Moreover, *S. globous* was the most efficient strain that gave the highest biosorption % (81.6) with acid fast red (50mg/l) which was selected for use in the examination of different factors controlling biosorption %.

Plackett-Burman optimimization experiments

Growth factors and their levels (Table 2) which affect acid fast red biosorption by *S. globous*, were screened using the Plackett-Burman design. The results achieved from the several combinations matrix of Plackett-Burman design (Table 3) revealed that, biosorption % under static conditions was higher than that obtained under shake conditions. Table 4 records the quantitative results of the Plackett-Burman design for *S. globosus* biomass (g/ml) and its corresponding dye removal (biosorption) (mg/ml). In spite that the results clarified that acid fast red biosorption capacity under static condition was higher than that under shake condition, it was noticed that, the biomass production was mostly the reverse.

Calculation of the biosorption % main effect (under static and shake conditions) and its corresponding t-value (Table 5) revealed that inoculum size and starch concentration were highly significant variables.

Figure 2 illustrates the factors which had positive and negative main effects under static conditions. The predicted optimum medium resulted from this experiment was as followed: starch, 10 (g/l); KNO_3, 0.5 (g/l); K_2HPO_4, 0.1 (g/l); $MgSO_4.7H_2O$, 1.0 (g/l); $FeSO_4$, 0.08 (g/l); culture volume, 75 ml and with 2 ml inoculum size of six days culture old. The inoculum size contained 10^6 cfu/ml. On the other hand , Figure 3 illustrates the positive and negative main effects under shake conditions, and the predicted optimized medium (g/l) was as followed: starch, 30 (g/l); KNO_3, 2 (g/l); K_2HPO_4, 0.1(g/l); $MgSO_4.7H_2O$, 1(g/l); culture volume, 75 ml with 2 ml inoculum size of six days culture old.

The interaction effect between starch concentrations and inoculums sizes was plotted to illustrate their effect on biosorption % under static conditions (Figure 4). The figure showed that, increase in the inoculum size with the low concentrations of starch gave maximum dye biosorp-

Table 2. Screening for growth factors affecting acid fast red biosorption by *S. globosus* and their levels in the Plackett-Burman experiment.

Factor (g/l)	Symbol	Level		
		-1	0	+1
KNO_3 (g/l)	KN	0.5	1	2
K_2HPO_4 (g/l)	K_2	0.1	0.5	2
$MgSO_4.7H_2O$ (g/l)	Mg	0.1	0.5	2
Culture volume (ml)	CV	25	50	75
$FeSO_4$ (g/l)	Fe	0.0	0.05	0.08
Inoculum size (ml)	IS	0.5	1	2
Starch (g/l)	Star	10	20	30

Table 3. The applied Plakett-Burman experimental design for the seven culture variables with its biosorption (%).

Trial	Factor							Biosorption %	
	KN	K2	Mg	CV	Fe	Is	Star	Static	Shake
1	-1	-1	-1	1	1	1	-1	86	66.4
2	1	-1	-1	-1	-1	1	1	80.2	80
3	-1	1	-1	-1	1	-1	1	79.2	66.5
4	1	1	-1	1	-1	-1	-1	81.4	60.6
5	-1	-1	1	1	-1	-1	1	78.5	74
6	1	-1	1	-1	1	-1	-1	80.5	62.2
7	-1	1	1	-1	-1	1	-1	84.5	65.4
8	1	1	1	1	1	1	1	81.5	75
9	0	0	0	0	0	0	0	82.1	70.4

Table 4. Quantitative results of the Plackett-Burman design for *S. globosus* biomass and dye biosorption.

Trial	Response			
	Wet weight (g/ml)		*Acid fast red biosorption capacity (mg/ml)*	
	Static	Shake	Static	Shake
1	0.17	0.24	42.85	31.0
2	0.27	0.24	40.1	37.5
3	0.16	0.18	39.6	31.1
4	0.21	0.23	40.7	27.0
5	0.18	0.23	39.3	34.2
6	0.19	0.22	40.4	28.0
7	0.16	0.17	42.14	29.7
8	0.24	0.27	40.7	35.5
9	0.21	0.21	41.1	32.2

tion % while under shake conditions (Figure 5) increase of the inoculum sizes with increased concentrations of starch gave maximum biosorption %.

Verification experiment

To verify the results obtained from the statistical analysis of Plackett-Burman design, a verification tests were performed in duplicates using the predicted optimized media against the basal condition media under static condition (Table 6). At different time intervals, the results obtained revealed that, after only 1 h, biosorption % was elevated to 1.14 fold increase than that in the case of basal condition with time reduction (from 2 h to 1 h) which

Table 5 . Statistical analysis of the Plackett- Burman experimental design results.

Variable	Static condition		Shake condition	
	Main effect	t-value	Main effect	t-value
KNO_3 (g/l)	-1.15	-0.60	1.37	0.26
K_2HPO_4(g/l)	0.35	0.17	-3.78	-0.76
$MgSO_4.7\ H_2O$ (g/l)	-0.45	-0.23	0.77	0.14
Culture volume (ml)	0.75	0.38	0.47	0.09
$FeSO_4$ (g/l)	0.65	0.33	-2.47	-0.48
Inoculum size (ml)	3.15	2.12	5.87	1.27
Starch (g/l)	-3.25	-2.24	10.23	3.29

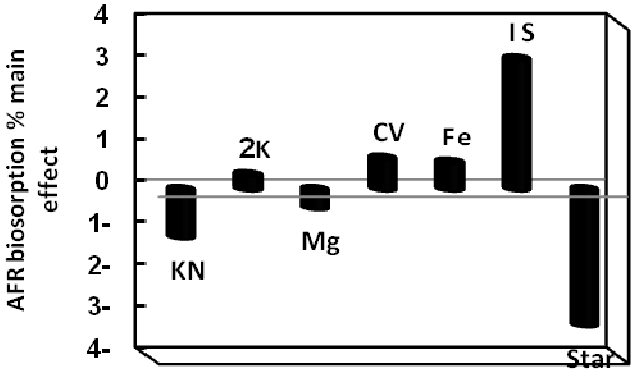

Figure 2. The main effects of different factors affecting acid fast red biosorption (%) by *S. globosus* under static conditions.

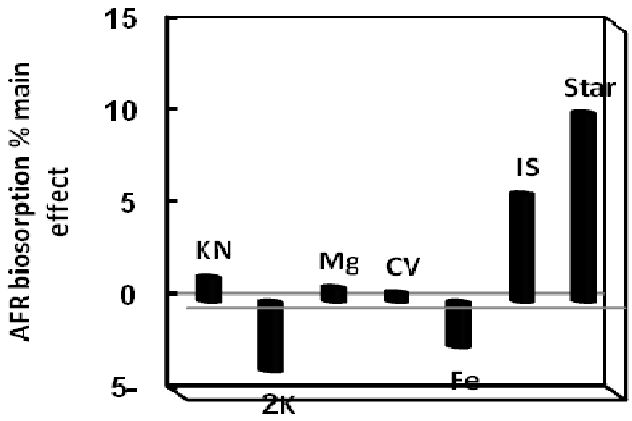

Figure3. The main effects of different factors affecting acid fast red biosorption (%) by *S. globosus* under shake conditions.

indicated the validity of the design while under shacked condition, biosorption % was elevated to only 1.09 fold increase after 2 h of contact (data not shown). These results recommend the application of static condition in AFR biosorption by *S.globosus*.

Transition electron microscope (TEM) studies

Figure 6 shows the TEM micrographs of the native and dye exposed cells of *S. globosus*. The control cells (a) appeared with regular cell wall and no dense areas were seen. Cells exposed to acid fast red dye (b) under static conditions showed dense dark areas distributed inside the cell.

Potato peel as an economic source

Chemical analysis of potato peels (Table 7) revealed that, carbohydrates constituted the highest % of its dried materials (64.47%). The protein was 13.52% while all other components were minors.

Different potato peel concentrations were tested as an economic source for *S. globosus* biomass production for AFR biosorptionl process. Figure 7 shows that, the use of 2 g of dried potato peel was the optimum concentration for biomass production under static conditions (0.3 g/50ml), and led to maximum dye removal (89.4%), while more increase in potato peel concentrations gave lower biomass production, and less biosorption efficiency.

DISCUSSION

Azo compounds constitute the largest and the most diverse group of synthetic dyes and are widely used in a number of industries (Pandey et a., 2007; El Ahwany 2008). Azo dyes can still be removed from wastewater by the microbial biomass via the process of biosorption. Biosorption by actinomycetes strains is also becoming a promising azo dyes removal process from wastewaters (Aksu, 2005; Abou-Elela, 2006). This study provides evidence that metabolizing cells of *S. globosus* biomass are capable of removing color from the solutions of the two tested azo dyes; Congo red and acid fast red. Acid fast red was removed with the highest decolonization percentage (82%) under static condition.

The results proved that static conditions are the most

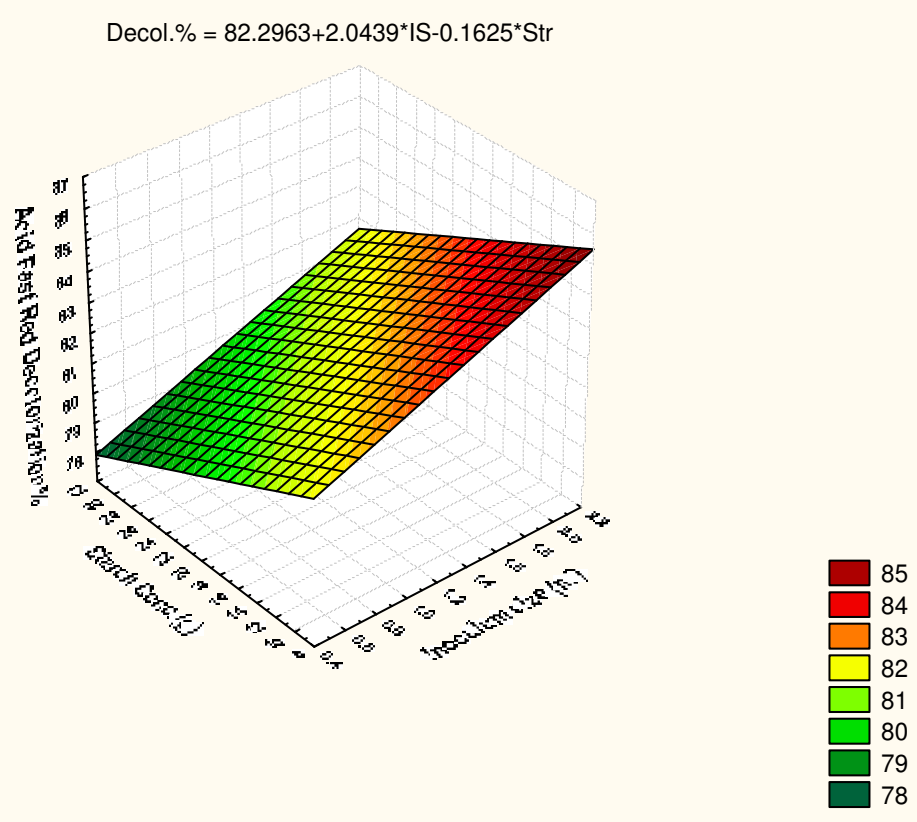

Figure 4. Interaction effect between inoculum sizes and starch concentrations on AFR biosorption (%) under static conditions**.**

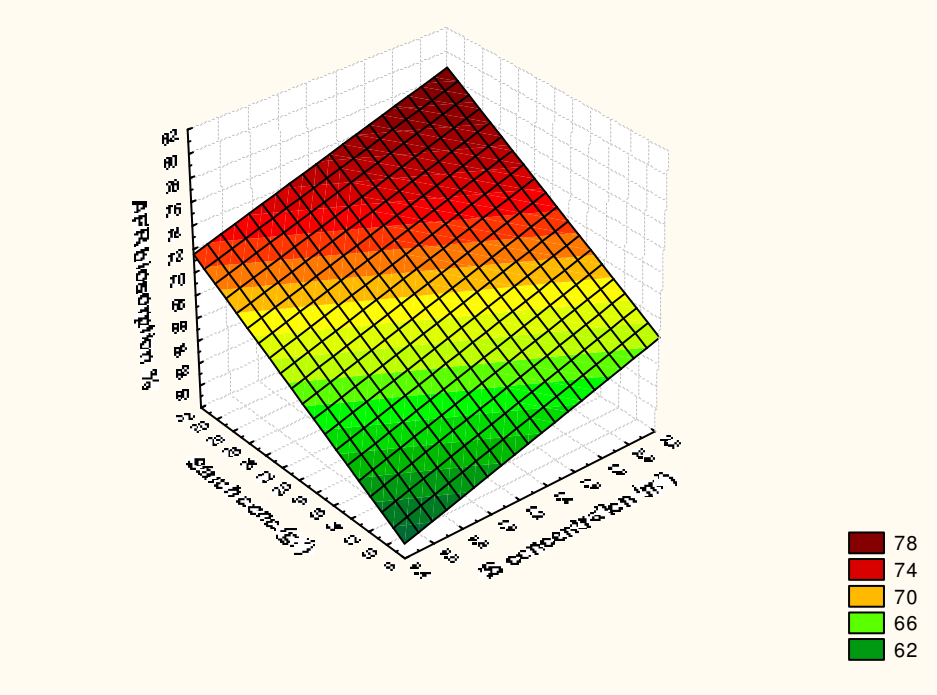

Figure 5. Interaction effect between inoculum sizes and starch concentrations on AFR biosorption (%) under shake conditions.

Table. 6. Verification experiment for AFR biosorption (%) by *S. globosus* cells which were grown on basal versus optimized medium under static condition.

Contact time (min)	[1]AFR Biosorption (%)	
	Basal	Optimized[2]
30	78.1±1.0	85±0.6
60	79.2 ±1.5	90±2.1
90	80.4±1.1	86±1.2
120	81.7±0.5	84±1.9
150	76.2±1.3	82±1.5

[1] Results were obtained under static condition.
[2] an optimum medium formula was predicted according to the results obtained from the Plackett-Burman experiment.

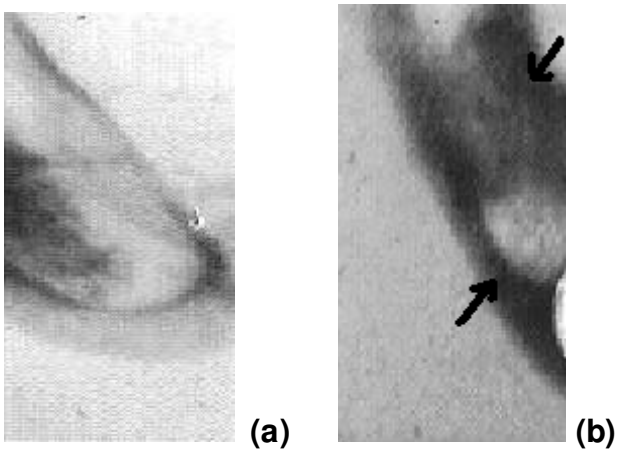

Figure 6. Transmission electron micrographs showing (a) *S. globosus* cells in absence of dye and (b) *S. globosus* cells in dye solution after 1 h contact.

Table 7. Proximate chemical composition of potato peel.

Component	Dry weight (%)
Moisture	11.2
Ash	7.56
Sugars	3.45
Carbohydrates	64.47
Protein	13.52

t-value significant at the 1% level = 3.70; t-value significant at the 5% level = 2.45; t-value significant at the 10% level = 1.9; t-value significant at the 20% level = 1.37

powerful tool in removing azo dyes. These results agreed with that of El Ahwany (2008), in the study on decolonization of fast red acid by metabolizing cells of *Oenococcus oeni* ML34 where dye removal was also under static conditions. Sani et al. (1998) disagrees with the results of this study. Decolonization rates for all the dyes in static condition were found to be less than the shake culture and also were dependent on biomass concentration.

Increase in the inoculums size led to an increase in biosorption capacity. These results agreed with that of Mohana et al. (2008) and Mabrouk and Yusef (2008); they used response surface methodology for optimization of medium for decolonization of textile dye direct black 22 by a novel bacterial consortium. Their results revealed that decolonization values above 80% were observed when high concentration of glucose and inoculum size was applied. Statistical analysis also revealed that low starch addition (10g/l) had a significant effect. The results of this study, also agreed with that of Abedin (2008); addition of low concentration of starch (5 g/l) gave crystal violet decolonization of 96% by *F.solani* while, previous results indicated that the availability of a supplementary carbon source seems to be necessary for faster growth and decolorization (El-Sersy, 2001; Mohana *et al*., 2008).

The results revealed that, high concentration of K_2HPO_4 and low concentration of KNO_3 increased the availability of dye removal by *S.globosus,* but Abedin (2008) reported that, Malachite Green removal by *F. solani* increased by increasing the concentration of $NaNO_3$ and K_2HPO_4. In addition, increasing K_2HPO_4 and decreasing $MgSO_4$ concentrations in this study enhanced dye decolourization. On the contrary, increasing K_2HPO_4 and $MgSO_4$ concentrations led to a positive effect on AFR decolorization by *B.subtilius* (Mabrouk and Yusef , 2008).

The verification test revealed that, after only 1 h contact of *S. globosus* biomass with dye solution, a higher decolorization of 90% was achieved. Longer contact time revealed low decolorization which may be due to desorption of dye (Acemioglu et al., 2010).

Potato peels are agro-industrial by-products that could have good biotechnological potential application. Nevertheless such waste was not tested extensively in previous studies (Mabrouk and El Ahwany, 2008). Therefore, it was used for economic studies as a carbon and nitrogen source replacement for starch and KNO_3.

It is worthy to mention that a powerful removal of AFR (89.4%) by *S. globosus* biomass (0.3 g wet weight/ ml)

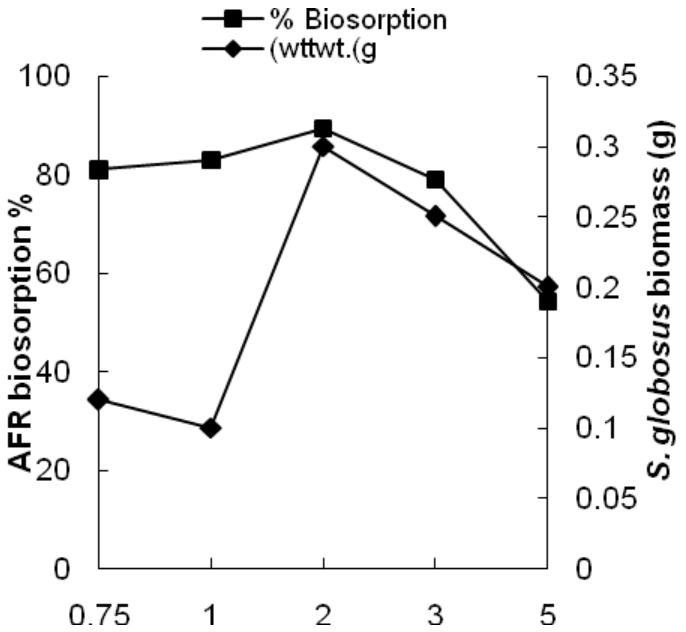

Figure 7. Effect of different concentrations of potato peel on growth of *S. globosus* and the equivalent AFR biosorption (%).

was achieved after 1 h which may be promising for using in industrial bioremediation.

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