Effect of Factors on Activity of Bioflocculant Produced by Bacterial Strain Isolated from Waste Water Sample

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ABSTRACT

Industrial waste water, sludge and soil samples were screened for bacterial isolates capable of producing flocculants. The aim of this study is to identify the best flocculating strain. P3, S2 and W3 were the three best bioflocculant producing strains isolated out of 24 bacterial strains. The flocculating activity of strain P3, S2and W3 was assayed using kaolin clay. Strain P3 showed the best flocculating activity (96%). Optimized conditions were observed for best flocculating activity. Effect of different carbon and nitrogen sources were assayed to obtain the best suitable carbon and nitrogen sources. The best carbon source, nitrogen source preferred was ethanol and yeast +$NaNO_3$ respectively. The effect of different pH and temperature was also determined. The best flocculating activity occurred at pH7 at temperature 35^oC. Ca^+ improved the flocculating activity. The weight of the purified bioflocculant was 0.1gm. The flocculating activity of Consortia SS1 (P3+S2+W3) was 76%.

Keywords: bioflocculant, consortia.

1. INTRODUCTION

In wastewater treatment for removing suspended solids and metal ions flocculation is a common and effective technique (Deng *et al.,* 2003). Flocculants are special natural organic macromolecule substances that can flocculate cells, suspended solids, colloidal solids, etc (Zhang, 2005). They are the chemicals used in various industrial processes, like food, fermentation, waste water treatment and downstream processing etc, these all are the material separation processes so flocculants are used here. Flocculants are of three types (1) inorganic flocculants (chemical flocculants) (2) organic flocculants (chemical flocculant) (3) natural occurring flocculants (Zhang *et al*., 2007). Inorganic flocculants are aluminum sulfate and poly aluminum chloride, organic flocculants are polyacrylamide derivatives and polyethylene imine and naturaly occuring flocculants are chitosan, sodium alginate and bioflocculants.

Chemical flocculants have low cost and also have effective flocculating activity but still they are not so good because of their toxicity and nondegrability. Aluminium has found to induce alzhimer

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disease. Furthermore the acrylamide monomer is toxic as well as carcinogenic and also non-biodegradable in environment. So, bioflocculants replaced the chemical flocculants because of their degrability, non-toxicity and enviornment friendly nature (Abd-El-Haleem *et al*., 2008). Bioflocculants can be produced economically and can be recovered easily from fermentation broth. Instead of someadvantages bioflocculant have one disadvantage like they are cost effective (Zhang *et al.,* 2010). Now a days they have wide applications in many industries like waste water treatment, textiles and food industries (Salehizadeh and Shojaosadati 2001). Bioflocculants have also been used to treat dye solution (Deng, S.B., G. Yu and Y.P. 2005), inorganic solid suspension (Labille *et al.,* 2005). Flocculation is the most widely used process for treating the waste water (Salehizadeh H *et al.,* 2000).

Bioflocculants are produced by microorganisms like Bacteria, fungi and alga (Desouky *et al*., 2008). Bioflocculants are used in bioflocculation process. Bio flocculation is a simple and effective mechanism, under which living cells are used for the precipitation of suspended solids, colloids and debris. Proteins, polysaccharides, glycoprotein's, nucleic acids and some other macromolecular compounds are main constituents of Bioflocculants (Lin J and Harichund C, 2012).

2. MATERIALS AND METHODS

Bacterial strain. Bioflocculant producing strains were isolated from various waste water sources (pharmaceutical, sugar and hoggery waste water). About 24 bacterial colonies were isolated out of those 3 strains were their which produced bioflocculants. Strain P3, S2 and W3 were those bioflocculant producing strains.

Media and cultivation conditions. The medium for bioflocculant production contained (per liter) glucose, 5gm; yeast extract, 1gm; urea, 1gm ;KH_2Po_4, 0.1gm; K_2HPO4, 0.1gm; NaCl, 0.1gm; $MgSO_4.7H_2O$, 0.2gm; agar, 20gm. Slant medium contained (per liter) yeaste extract , 1gm; beef extract, 1gm; tryptone, 2gm; glucose, 10gm; $FeSO_4$, 0.02gm and agar, 20 gm. Fermentation medium contained (per liter) sucrose, 10gm; yeast extract, 1gm; urea, 1gm ;KH_2Po_4, 0.1gm; K_2HPO4, 0.1gm; NaCl, 0.1gm; $MgSO_4.7H_2O$, 0.2gm; agar, 20gm. The composition of culture medium and fermentation medium are same. The initial pH of all media was adjusted to 7.2 to 7.5 with NaOH and HCl. All the media were prepared with distilled water and sterilized at 121^0Cfor 20 min. The cultivation temperature was adjusted to 37^0C.

Isolation of bacteria. Serial dilution was done for the isolation of strains. After that the pure colonies was obtained on agar plates. Three bioflocculant producing strains were obtained. Each colony was inoculated into 250-ml flasks containing 50ml fermentation medium. The strains were

incubated for 48 h at 37^0C with shaking speed 250rpm. The strain with highest flocculating activity (P3) was selected. Identification was done on the basis of morphological identification, biochemical characterization.

Determination of flocculating activity. The flocculating activity was measured using a kaoline clay suspension. 0.5 gm kaoline clay was suspended in 100 ml distilled water, and 1 ml of the liquid bioflocculant was mixed thoroughly with 45 ml of the kaoline suspension. Then, 4.5 ml of 1% $CaCl_2$ solution was added to the mixture. The mixture was stirred with a vortex mixer and left standing for 5 min at room temperature. The optical density (O.D.) of the supernatant and the blank control where distilled water was used instead of the supernatant was measured at 600 nm. The flocculating activity was defined and calculated as follows

Flocculating activity = (A-B)/A* 100.

Where A and B are the optical densities at 600 nm of the control and the sample, respectively

Effect of Different Parameters on Flocculating Activity. Following variables were assayed to check the nutritional and environmental factors affecting bioflocculant production: incubation period (1–5 days), carbon source (glucose, fructose, sucrose, lactose, galactose, mannose, maltose, starch, sodium acetate, citric acid, glycerol and ethanol), nitrogen source (yeast extract, beef extract, peptone, urea, glutamic acid, ammonium sulphate, ammonium nitrate, ammonium chloride and sodium nitrate), initial pH (pH5-12) and incubation temperature (20, 25, 30, 35 and 40°C).

Bioflocculant purification. The purification and characterization of the bioflocculant was performed using the method described by (Chang *et. al*. (1998) and (Chen *et. al*. 2002). Fermentation culture was prepared based on the optimal culture conditions determined earlier. After three days of cultivation, the culture was centrifuged at 6000 rpm for 30 min and at 4^0C to remove cells. One volume of distilled water was added to the supernatant and centrifuged again for 15 min to remove insoluble solutes. Two volumes of cold ethanol were added to the supernatant, and the solution was mixed and left standing at 4ºC for 12 hr. The resultant precipitate was vaccum dried to obtain the crude bioflocculant.

Multiple microorganisms consortia. Strains that have flocculants producing microorganisms were screened in lab. These strains were used to construct multiple microorganism consortia producing bioflocculant of high flocculating activity. Combination of two or three strains were their to construct the multiple microorganism consortia producing bioflocculant.

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Results and discussion. Waste water, sugar waste water and pharmaceutical waste water was taken and check for their flocculating activity. W3, S2 and P3 were the best flocculating producing strains; out of them the P3 showed excellent flocculating activity. P3 had 75% flocculating activity. P3 showed the best flocculating activity so we considered the pharmaceutical waste water for the study of different factor i.e. temperatures, pH, carbon sources and nitrogen sources on Bioflocculating activity.

3. FACTORS AFFECTING THE BIOFLOCCULANT PRODUCTION

The bioflocculant production is affected by many factors, such as the constituents of the culture medium and environmental conditions (He *et al*., 2004)

The pH of the reaction mixture is a key factor influencing the flocculating activity. The flocculating activity increased gradually when the pH value went up and reached its maximum at pH 7.0.

At low pH the, the absorbance of H^+ ions tend to weaken the bioflocculant- kaolin complex formation process and a similar effects is also observed at high pH value (He *et al.,*2010).The pH stability of biopolymer solution was observed at the pH ranges 3-9 and reached maximum stability at pH 7. It is demonstrated that bioflocculant solution is suitable to be applied in neutral, weakly acid and weakly alkaline circumstances. Gao *et al.* (2006) and He *et al.,(*2010) reported similar optimal pH value (7.0) for the activity of the bioflocculants produced by *Vagococcus sp*. and a mutant *Halomonas sp*., respectively.

The effect of the temperature on kaolin clay mixture was tested. The highest flocculating activity was achieved at 35^0C (96%). Bioflocculants containing sugar as the main flocculation components are heat stable, and their flocculating activity could retain more than 50%.

The main backbone of the bioflocculant is made up of polysaccharide and high temperature may cause degradation of this polysaccharide chain of bioflocculant that reduces its flocculation efficiency. This thermal behaviour of the bioflocculant is supported by other studies (Gong *et al.,* 2008)

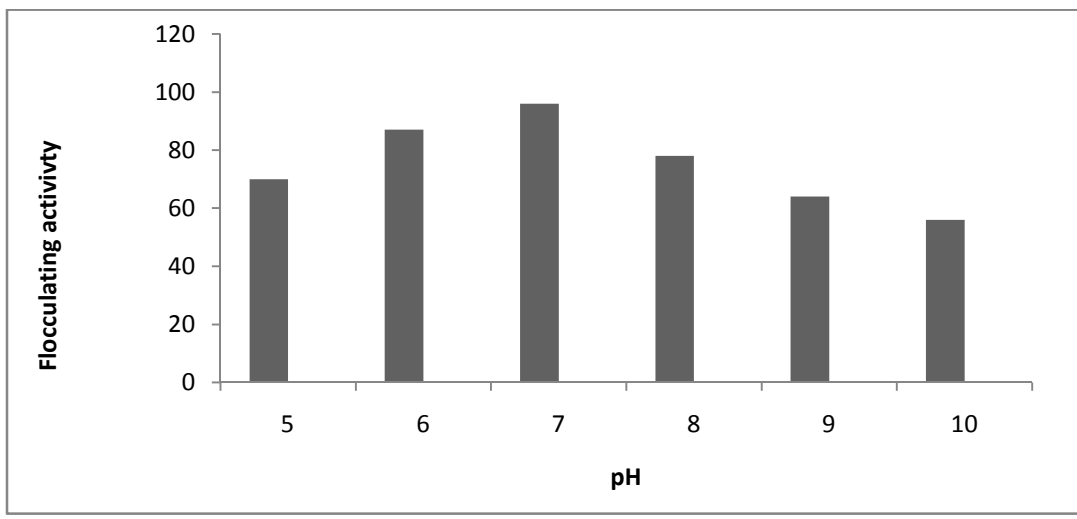
The effect of bioflocculant dosage showed flocculation activity over 90% in the range of 1-3mgl^{-1} the maximum flocculating activity of 96% was observed at an optimum bioflocculant dosage of 2$mgl^{-1.}$

The divalent cation (Ca^{2+}) was more effective than others. Ca2+ could destabilize and bridging. However, trivalent cations could change the surface charge of kaolin particle and cover particles and less absorb sites induce the low flocculation activity (Gong *et.al ,2008).* It was studied that bioflocculants produced by *Citrobacter* sp. TKF04 (Fujita *et al.,* 2000) and *Bacillus* sp. F19 w (Zheng *et al*., 2008) were capable of flocculating kaolin clay without metals.

The highest effect of Ca^{2+} is one of the significant characteristics because trivalent cations are difficult to remove and can cause environmental problems.Fe^{3+} form the gelatinous precipitate, which was more difficult to remove. Similarly, Al^{3+} gives rise to an environmental problem; therefore $CaCl_2$ has own coagulant aid. Increase in the positive charge density over the clay particle surface may be giving the same influence of the trivalent cations presence that inhibits the biopolymer efficiency (Gong *et al.,* 2008).

It has been well documented that changing the carbon and nitrogen sources highly influences bacterial growth and bioflocculant production (Sheng *et al.,* 2006).. Ethanol was the favoured carbon source for bioflocculant production in the industrial scale. Multiple nitrogen sources were better than a single nitrogen source. The medium containing yeast extract and $NaNO_3$ was the most favourable for production of bioflocculant as they caused the highest bioflocculant activity.

Effect of pH. The best flocculating activity was shown at pH 7. So, pH 7 was considered as standard for optimization of other factors.



Effect of temperature. The highest flocculating activity of P3 strain was shown at 35^0C.

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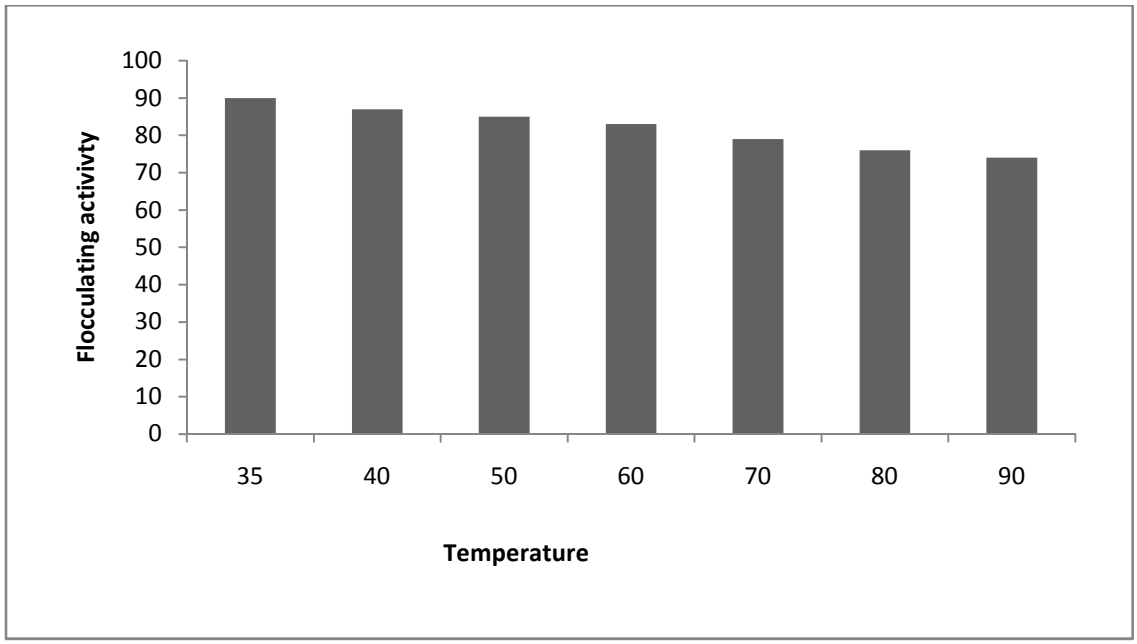
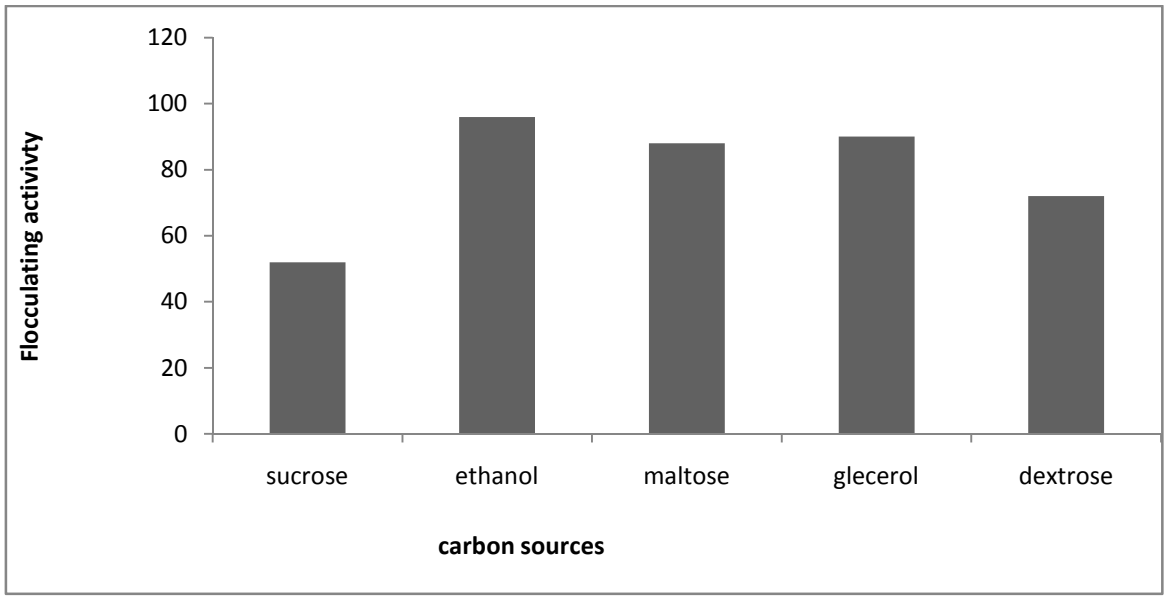
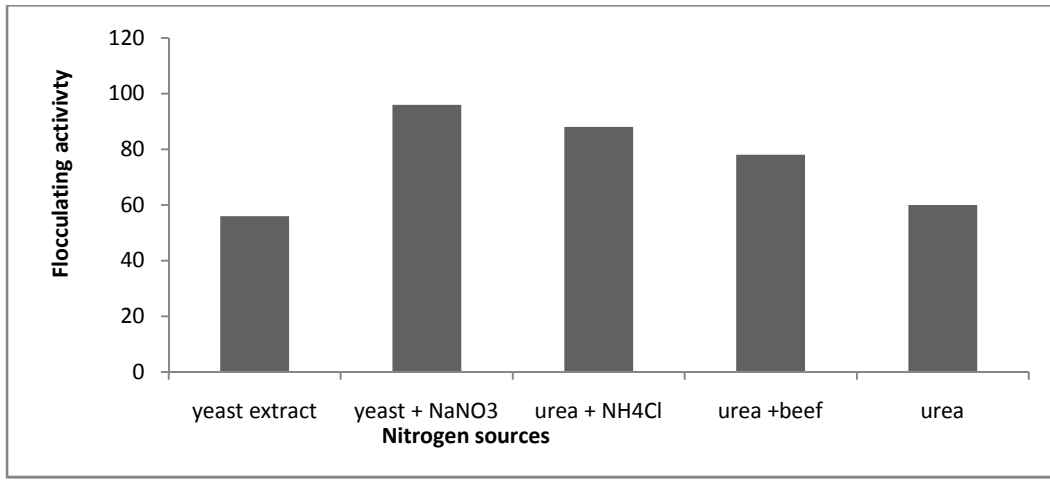


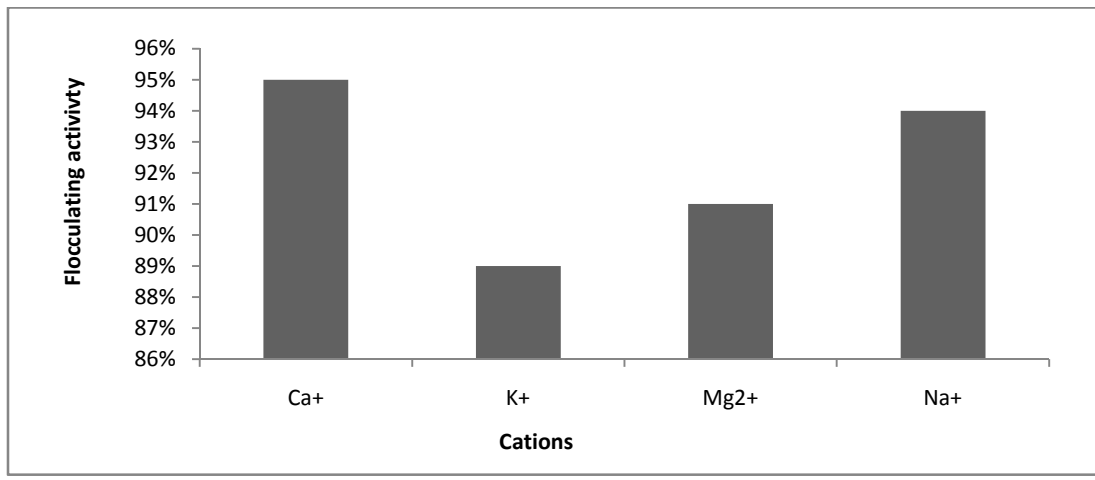
Effect of carbon sources. The best flocculating activity were observed when ethanol was used as carbon source.



Effect of nitrogen sopurces. The best flocculating activity were observed when yeast + $NaNO_3$ was used as nitrogen source.



Effect of cations. The best flocculating activity was shown at Ca^{+}.



Multiple microorganism consortia. Multiple microorganism consortia were constructed in which 3 strains were selected out of 6 best strains and combined cultivation occurred. Now the flocculating activity of their cell-free supernatant was investigated. Strain MM1 (P3+S2+W3) has the flocculating activity 70%.

4. CONCLUSION

The flocculating activity of P3, S2 and W3 were 96%, 78% and 87% respectively.The following suggestions are made to guide prospect for future studies: Development of process conditions for large scale production of the bioflocculants, Identification of bacteria and study of flocculant stability and toxicity.

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