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Research Article

Effect of Different Environmental and Nutritional Factors on Biosurfactant Production from *Azotobacter chrococcum*

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ABSTRACT

Bacteria strain Azo3, which produces high amount of biosurfactant, was isolated from soil and identified as strain of *Azotobacter chrococcum* by its biochemical /physiological characteristics, this strain was selected out of 8 strains previously screened for biosurfactant production, the effects of some environmental and nutritional factors on biosurfactant production were evaluated, the maximum value of E24% was observed at pH 7 which was 68%. Among different edible and heavy oils, the sun flower oil and heavy oil 150 were the beat carbon sources in production of biosurfactant, E24% were 76.6% and 74.1% respectively and among different organic and inorganic nitrogen sources, the yeast extract was the best organic nitrogen source for biosurfactants production E24% was 83.3%, while $(NH_4)_2SO_4$ was the best inorganic nitrogen source for biosurfactants production E24% was 80%. The optimal temperature for biosurfactant production was 30 °C for 4 days in shaking incubator at 150 rpm, E24% was 89%.

Keywords: Biosurfactants, Azotobacter chrococcum, surface active molecules.

INTRODUCTION

Biosurfactants are valuable surface active molecules produced by wide variety of microorganisms. Due to its amphipathic nature, these biomolecules are capable of lowering the surface tension, interfacial tension and forming microemulsion to enable mixing of two immiscible solutions. Such properties exhibit excellent detergency, emulsifying, foaming, and dispersing traits. Some of the features, which make them promising alternatives to chemically synthesized surfactants, are their lower toxicity, higher biodegradability, greater stability at wide range of pH and temperature, and better environmental compatibility[1] Thus, interest towards these biomolecules has increased considerably in recent years, as they are potential candidates for many commercial applications in the petroleum, pharmaceuticals, biomedical and food processing industries[2].

Despite such advantages, biosurfactant have not been fully commercialized due to expensive raw material and low yields. One of the strategies to improve production is to optimize the growth media in order to get maximum production. Formulation of an optimized production medium involves selection of the right nutrients at their correct levels to provide an ideal microenvironment for supporting growth and metabolite production[3].

MATERIALS AND METHODS

Microorganism

The bacteria were isolated by using routine

microbiological techniques from the soil of corn field at College of Agriculture in Abu- Ghraib - Baghdad, These isolates were maintained at 4 °C on mannitol enrichment agar slants which containing 20 g/l mannitol, 20 g/l yeast extract, 20 g/l tryptone, 15 g/l agar[4].

Activation medium

Activation medium was composed of 0.3 g K_2HPO_4 , 0.7 g KH_2PO_4 , 0.2 g $MgSO_4.7\ H_2O$, 0.1 g $CaCl_2.2H_2O$, 0.05 g $FeSO_4.9H_2O$,0.005 g $Na_2MoO_4.2H_2O$, 20 g sucrose and 5 g yeast extract. pH was 7.3 [5].

Biosurfactant production

Cultures were grown on a minimal basal medium[4] (MB) which composed the following components in distilled water 1.5 g/l K_2HPO_4, 0.5 g/l KH_2PO_4,0.2 g/l $MgSO_4$, 0.5 g/l $(NH_4)_2SO_4$.

Emulsification index (E24%)

To determine the emulsification index, Batista *et al.*, method was applied. Centrifugation at 10,000 rpm to separate biosurfactant from microorganism cells yielded a biosurfactant cell free. A mixture of biosurfactant and crude oil (1:1) was agitated for about 2 min then stabilized for 24 h. Emulsification index (E24%) determined by measuring the column height of emulsified oil against its total height multiplied by 100 times[6].

Determination of factors affecting biosurfactant production

In all the experiments bacteria were cultured in activation medium, then fifty ml (in 250 ml flasks) of the production medium was inoculated with 0.5ml (0.04 O.D 600 nm) of activated bacterial culture and incubated at different environmental conditions to determine the optimum nutritional and environmental factors for biosurfactant production which included the following factors: pH values, different oil source, nitrogen source, temperature and incubation period. At the end of experiment, cultures broths were centrifuged at 10,000 rpm for 5 min. to separate biosurfactant from microorganism cells yielded a biosurfactant cell free. After that (E24%) was estimated.

pH values

The initial pH values of the production medium (50 mL) were adjusted with 1N HCl or 1N NaOH to three groups 5, 7 and 9 before sterilization, then inoculation was carried out by the selected *A. chrococcum* strain and incubated at 28 °C for 5 days under shaking condition.

Different oils sources

Fifty ml of production medium containing six different oils 1 % (heavy oil 40, heavy oil 60, heavy oil 150, olive oil, corn oil and sun flower oil) then the media were sterilized, inoculated with the selected *A.chrococcum* strain and incubated at 28 °C for 5 days under shaking condition.

Nitrogen sources

Fifty ml of production medium was supplemented with different organic and inorganic nitrogen sources 1% (meat extract, peptone, yeast extract, $NaNO_3$, NH_4Cl and $(NH_4)_2SO_4$) then the media were autoclaved, inoculated with the selected *A. chrococcum* strain and incubated at 28 °C for 5 days under shaking condition.

Temperature

Fifty ml of production medium after sterilization were inoculated with the selected *A. chrococcum* strain and incubated under shaking condition for 5 days at different temperatures; 25, 30 and 35 °C.

Incubation period

Fifty ml of production medium was inoculated with the selected *A. chrococcum* strain and incubated under shaking condition for different incubation periods 1, 2, 3, 4, 5 and 6 days.

RESULTS AND DISCUSSION

Isolation and identification of the biosurfactant producing strain

The reason we choose *A. chrococcum* are first, *A. chrococcum* is a rhizosphere bacterial strain that able to produce biosurfactant constitutively from water soluble substrate without any addition of inducer substrate; second, *A. chrococcum* known have the ability to utilized free nitrogen from the air; and third, the ability to produce biosurfactant from water soluble substrates is preferred because single-phase fermentation is simpler than biphasic fermentation that usually occurred when hydrocarbon based substrates were applied.

In this study, a total of 8 bacterial strains have been isolated from the soil. All those isolates revealed mucoid colonies when grown on enrichment agar medium. These bacterial isolates

were screened for their ability to produce biosurfactant, strain Azo3 showed the highest biosurfactant production E24% was 68% .Thus it was selected for the further steps of this study.
Strain Azo3 was identified as strain of *A. chrococcum* by its biochemical and physiological characteristics[7].

Determination of factors affecting biosurfactant production

Environmental factors and growth conditions such as pH, temperature, nitrogen source, and incubation period affect biosurfactant production through their effects on cellular growth or activity. Some environmental factors as well as nutritional requirements were investigated to optimize the conditions of biosurfactant production from *A. chrococcum*. These factors which included the following:

Effect of pH values

The pH of the medium plays an important role in biosurfactant production [8]. Jagtap *et al.*, showed that maximum production of bioemulsifier was at pH 7 though less activity was at pH 6, 8 and 9[9]. Similarly, our data showed that pH 7 was the optimum one for production of biosurfactant by *A. chrococcum*. E24% was 68% (figure 1).

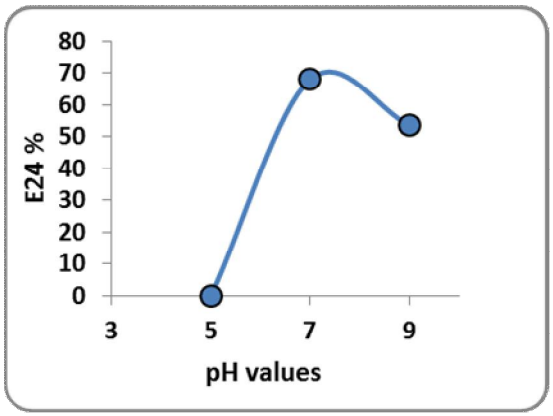

Fig. 1: Effect of pH on biosurfactant production by *A. chrococcum*

In the study Karanth *et al.*, they found that the type , quality and quantity of biosurfactant are influenced by culture conditions such as pH and many other conditions[10].

The initial pH of the culture medium determines the electric charge of the cells and the oxidation–reduction potential which can affect nutrient absorption and enzymatic reaction [11].In the case of strain *A. chrococcum*, pH 7.0 is the optimum for the biosurfactant production, which is a neutral pH and can save large numbers of acid and alkali used to adjust pH. This value was chosen as the initial pH in the following studies.

Effect of different oils sources

The type, quality and quantity of biosurfactant are influenced by the nature of carbon source. Media containing edible oils and heavy oils were used for production of biosurfactant by *A. chrococcum* .In general all kind of oil were found suitable for production of biosurfactant by *A. chrococcum* , among different edible and heavy oils ,the sun flower oil and heavy oil 150 were the beat carbon sources in production of biosurfactant, E24% were 76.6% and 74.1% respectively (figure 2).
These results indicate that *A. chrococcum* have ability to utilize different carbon sources for biosurfactant production, *A. chrococcum* is a bacterium with a broad metabolic diversity, this feature enables it to degrade numerous highly resistant substrates [12], or to synthesize different compounds[13].

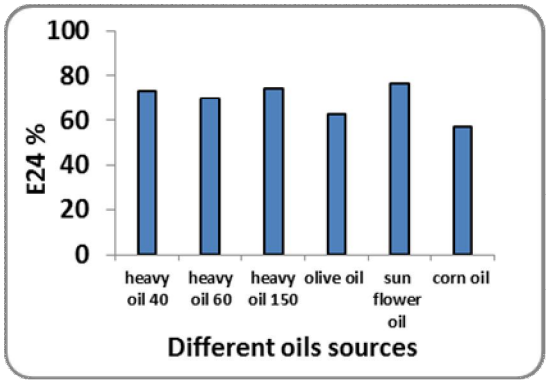

Fig. 2: Effect of different oils sources on biosurfactant production by *A. chrococcum*

Efect of different nitrogen sources

In addition to carbon sources, nitrogen sources play an important role in the production of biosurfactants, because the bacteria require nitrogen to complete its metabolic pathways, among different organic and inorganic nitrogen sources the results shows that yeast extract was the best organic nitrogen source for biosurfactants

production by *A. chrococcum* E24% was 83.3%, while $(NH_4)_2SO_4$ was the best inorganic nitrogen source for biosurfactants production by *A. chrococcum* E24%was 80% (figure 3).
These results are similar to those reported by Banat *et al.*, who found that ammonium sulfate is considered as good nitrogen source for good production of biosurfactant from *Serratia marcescens*[14].

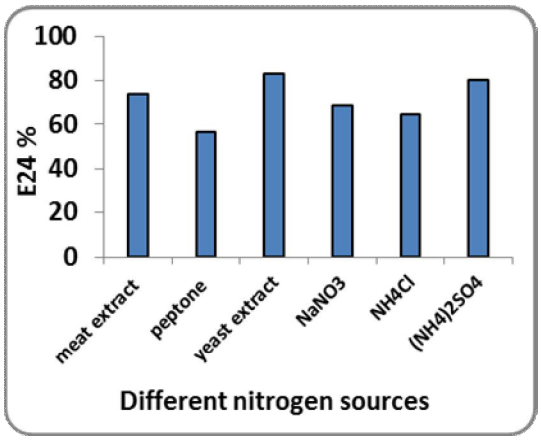

Fig. 3: Effect of different nitrogen sources on biosurfactant production by *A. chrococcum*

The maximum value of biosurfactant production may be attributed to the presence of ammonium sulfate; this salt is inorganic nitrogen source, very soluble and utilized easily as nitrogen source for cell metabolism, growth enhancing and may play a significant role in the pathways of biosurfactant or extracellulary secretion[15] .

Effect of temperature
Various culture temperatures were tested in order to investigate their effect on the biosurfactant production. When the culture temperature was 30 °C, the E24% was 85.2% (figure 4). The optimal temperature for biosurfactant production was 30 °C, which was used in the following studies. The metabolism of microorganisms has direct relationship with culture temperature[11]. Maximum enzymatic activation can only be obtained at an optimum temperature[16]. A lower culture temperature might make strain *A. chrococcum* hibernate partially, and its enzyme system for biosurfactant production couldn't be activated completely. On the other hand, a higher culture temperature may have an adverse effect on the nucleiu acid and the enzyme system of the strain.

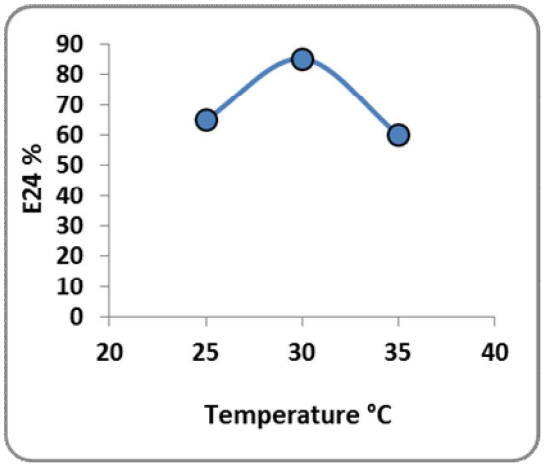

Fig. 4: Effect of temperature on biosurfactant productionby *A. chrococcum*

Effect of different incubation period
biosurfactant production by *A. chrococcum* was followed for 6 days, continuous increasing in the production of biosurfactant was observed during the first 4 days, the maximum value of E24% was 89% at the fourth day, then it decreased to 72% after 6 days (figure 5), these results indicate that the biosurfactant production from *A. chrococcum* takes a long time to reach its maximum value, and it may indicate that the bacteria need the biosurfactant during the different stages of their life.

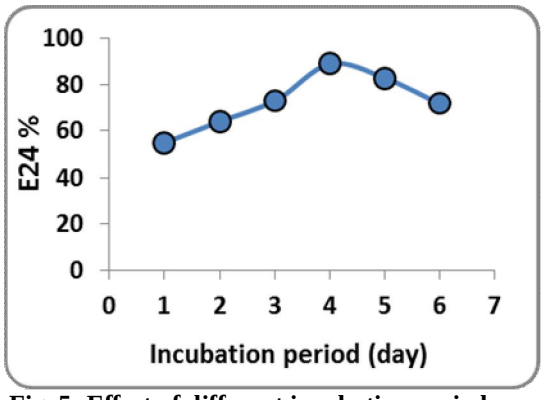

Fig. 5: Effect of different incubation period on biosurfactant production by *A. chrococcum*

CONCLUSION

Bacteria strain Azo3 was isolated from soil, which produces high amount of biosurfactant, this strain able to utilize different carbon and nitrogen source for biosurfactant production, biosurfactant production by Azo3 influenced by environmental condition and media composition.

REFERENCES

1. Desai JD and Banat IM. Microbial production of surfactants and their commercial potential. Microbiology and Molecular Biology Reviews. 1997;61(1):47-64.
2. Parveen J, Wan M and Md ZA. Optimum Medium Components for Biosurfactant Production by *Klebsiella pneumoniae* WMF02 Utilizing Sludge Palm Oil as a Substrate. Australian Journal of Basic and Applied Sciences. 2012;6(1):100-108.
3. Mukherjee S, Das P, Sivapathasekaran C and Sen R. Enhanced production of biosurfactant by a marine bacterium on statistical screening of nutritional parameters. Biochemical Engineering Journal. 2008;42:254-260.
4. Qomarudin H, Edwan K, Zeily N and Wisjnuprapto. Application of Biosurfactant Produced by *Azotobacter vinelandii* AV01 for Enhanced Oil Recovery and Biodegradation of Oil Sludge, International Journal of Civil & Environmental Engineering. 2012;10(1):7-14.
5. Thompson JP and Skerman VBD. Azotobacteraceae: the taxonomy and ecology of aerobic nitrogen-fixing bacteria. Academic Press, Inc. (London), Ltd., London. 1979.
6. Batista SB, Mounteer AH, Amorim FR and Totola MR. Isolation and characterization of biosurfactant/ bioemulsifier-producing bacteria from petroleum contaminated sites. Bioresource Technology. 2006;97:868–875.
7. Garrity GM. Bergey`s Manual of Systematic Bacteriology. 2th ed.vol 2.part B. Springer. USA, 2005.
8. Gobbert U, Lang S and Wagner F. Sophorose lipid formation by resting cells of *Torulopsis bombicola*. Biotechnol. Lett. 1984;6:225– 230.
9. Jagtap S, Yavankar S, Pardesi K and Chopade P. Production of bioemulsifier by *Acinetobacter* species isolated form healthy human skin. Indian Journal of Experimental Biology. 2010;48:70-76.
10. Karanth NGK, Deo PG and Veenading NK. Microbial production of biosurfactants and their importance. Curr Sci. 1999;77(1):116-126.
11. Salehizadeh H and Shojaosadati SA. Extracellular biopolymeric flocculants: recent trends and biotechnological importance. Biotechnol Adv. 2001;19:371–385.
12. Moreno J, De la T, Ramos-Cormenzana A and Vela GR. Growth and nitrogenase activity of *Azotobacter vinelandii* on soil phenolics acids. Journal of Applied Bacteriology. 1990;69:850–855.
13. Revillas J, Rodelas B, Pozo C, Martı´nez-Toledo MV and Gonzalez-Lopez J. Production of B-group vitamins by two Azotobacter strains with phenolic compounds as sole carbon source under diazotrophic and azodiazotrophic conditions. Journal of Applied Microbiology. 2000;89:483–493.
14. Banat IM, Makkar RS and Cameorta SS. Potential commercial applications of microbial surfactants. Appl Microbiol Biotechnol. 2000;53:495 – 508.
15. Entissar FA. Production and Characterization of Bioemulsifier from Locally Isolated *Serratia marcescens* S10, M.Sc.Thesis. College of Science, Baghdad University.Iraq. 2010.
16. Nakata K and Kurane R. Production of an extracellular polysaccharide bioflocculant by *Klebsiella pneumoniae*. Biosci Biotechnol Biochem. 1999;63:2064–2068.