ORIGINAL ARTICLE

Bio Surfactants Production by *Pseudomonas Aeruginosa* using Agricultural Resources

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ABSTRACT

Aim: To yield bio surfactants from Pseudomonas aeruginosa on substrate optimization of 0.75g using agricultural resource and to produce Bio surfactants using low cost materials.
Study design: Observational type of Descriptive study.
Place and duration of study: The present study was piloted at Institute of molecular biology and biotechnology in University of Lahore. Duration of the study was two years.
Methods: The 0.75gsize of sample taken of inoculum from growing culture of Pseudomonas aeruginosa was isolated and collected from industrial area of District Kasoor and flasks were then placed into an orbital shaker at speed of 120rpm. Sterile screw capped bottles were used to collect the samples, and aseptic measures were taken. For further use, 4^{o}C was the temperature of sample storage.
Results: The surface tension was 62.9, 53.8, 49.8 and 47.6 mN/m at time one, two, three and four days respectively at 37°C - constant temperature and 0.75g with 1ml inoculum size the molasses were used.
Conclusion: After optimizing various growth and environmental factors a production of rhamnolipid was achieved.
Keywords: Pseudomonas aeruginosa, biosurfactant

INTRODUCTION

Bio-surfactants are amphiphilic living blends portrayed outside the cells or in place of portion of the cell membrane by a difference of filamentous fungi, bacteria and yeast[1] from uncountable constituents comprising wastes, oils and sugars. Nevertheless, for exploration of bio-surfactant manufacturing by the aeruginosa strains, vegetable oils and carbohydrates are mainly used substrates[1]. A widespread multiplicity of low and high molecular weight bio-surfactants, is the sole property of micro-organisms[2].

In order to effect in decreasing the surface and interfacial tension, low molecular weight bio-surfactants are chiefly the lipopeptides or glycolipids[2]. An effective stabilization, oil-in-water emulsions can be achieved by high molecular weight bio-surfactants, which are lipoproteins, lipopolysaccharides, protein and amphipathic polysaccharide in nature[3]. The cohesions related to molecular bio-surfactants mostly range from 500 to 1500 Daltons and 1-200mg/L is its serious micelle concentration range. Owing to its ability to produce rhamnolipid bio-surfactants by Pseudomonas species, numerous carbon sources are used, and it is clearly demonstrated by the studies that nature of carbon substrate is subjective to bio-surfactant production[4]. For being having potential to produce rhamnolipid, and contain very high amounts of sugars, the molasses were used as a substrate, and Sugar Beet and Sugar cane molasses are the two main types existing on trade scale.

Marked quantity of fructose and glucose along with 50% sucrose by dry weight mainly the constituents of sugar beet molasses, but also have non-sugar ingredients like chloride, oxalate, potassium and calcium. Sugar industry yield both these categories of molasses as a byproduct in large quantities[5]. Bio-surfactant production was previously studied using medium with varying concentration of molasses being used as the sole source of carbon[5]. With increasing demands and deficiency of such type of studies in our region, study was designed to produce rhamnolipid (A glycol-lipid biosurfactant composed of one rhamnose unit and a lipid tail) by Pseudomonas aeruginosa using agricultural resources i.e., molasses. The present work was

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designed to use 0.75g Molasses as a carbon source for the production of bio-surfactants and tried to develop a cheaper method to produce such useful molecules by fermentation process.

MATERIALS & METHODS

It was planned to determine the inoculum size for the production of rhamnolipid. The volume of sample taken are 1ml, of inoculum from developing culture of Pseudomonas aeruginosa was isolated from polluted soil collected from industrial area of District Kasur, Punjab and flasks were then engaged into an orbital shaker at speed of 120rpm. The samples were collected and saved in sterile screw capped container, by the use of aseptic measures and kept at 4^{o}C till auxiliary usage[6]. After every 24 hours the culture broth from each flask was taken to estimate bacterial cell mass. In our study chemicals we used like L-rhamnose, Molasses, Na2HPO4, K2HPO, MgSO4, Orcinol reagent, Diethyl ether, NaH2PO4, FeSO4, and Peptone were acquired from Sigma Aldrich from their local distributor in Lahore, Pakistan. Soil enrichment method was used to isolate the bacterial strains from tainted soil in industrial area. Briefly, 1g of soil blended with the mixture of 1g of molasses and 100ml sterile mineral salt media, kept on orbit shaker at 100RPM for 04 days at a constant temperature of 37^{o}C and after amelioration, taken cell suspension from the flask dispersed over nutrient agar plate and kept of 02 days at 30^{o}C. The apparent colonies were designated haphazardly and further cultured to obtain unpolluted isolates[6]. Phosphate with an organic nitrogen medium was arranged. The medium was composed of (gL-1): NaH2PO4 .H_2O, 4.0, Na_2HPO4.H2O, 1.0, MgSO4 .7H2O, 1.0, $CaCl_2$ .2H_2O, 0.005, Peptone, 1.38, 25ml of glycerol, used as basis of carbon substrate[5]. About 2.5L of distilled water was used in terms of measured volume and weighs of medium. The 211 micro-processor pH meter with 1.0M NaOH was used to maintain the pH of the medium at 7. During the experiments, sixteen Erlenmeyer flasks (250ml caliber) were used, and 200ml measuring cylinder to measure 150ml of already prepared medium. Cushion foam was used to clog the each flask and aluminum foil to cover and auto cleaved for 72 hours before being injected. Bacterial strains plus nutrient broth media (100ml) was injected and growth was checked at shaking incubator at 100rpm at 37^{o}C for 72 hours[7].

RESULTS

The results of the present study (Table 1) revealed that surface tension was 62.9, 53.8, 49.8 and 47.6 mN/m on day 1,2,3 and 4 respectively, constant temperature 37°C and molasses used 0.75g with 1ml of inoculation. The production of rhamnolipid was 0.25, 0.3, 0.28 and 0.3 g/L respectively. Similarly the mass of bacterial cell was 0.1, 0.2, 0.35 and 0.4 g/L respectively.

Fig. 1 shows that the mass of bacterial cell (g/L) increased as the time went on, as revealed in the fig that at zero time the bacterial cell mass was zero and it increased to 0.4 g/L bacterial cell mass when the time passage was 96 hours

The fig. 2 represents that rhamnolipid concentration (g/L) increased with the passage of time as revealed in the fig that at zero time the rhamnolipid concentration was zero and then increased to 0.3 g/L at day 4.

The fig. 3 represents that surface tension (mN/m) decreased with the passage of time as revealed in the fig that at zero time the surface tension was 70mN/m and it decreased to 47.6mN/m surface tension when the time passage was 96 hours.

Fig. 1: Bacterial cell mass estimation using 0.75g substrate

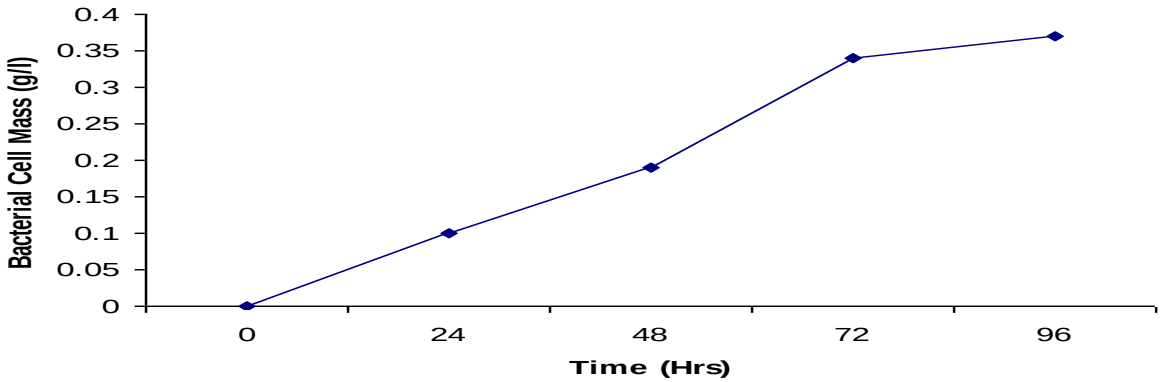


Fig. 2: Estimation of Rhamnolipid production using 0.75g substrate

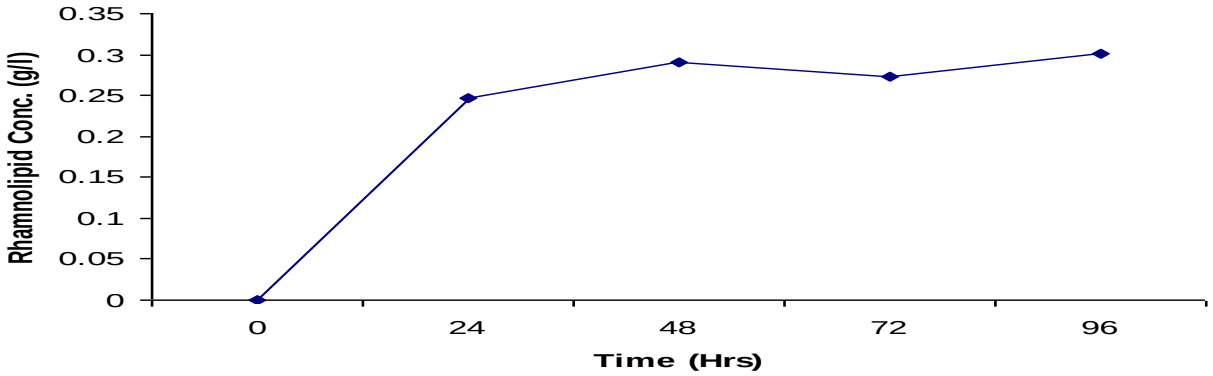


Fig. 3: Estimation of Surface tension reduction using 0.75g substarte

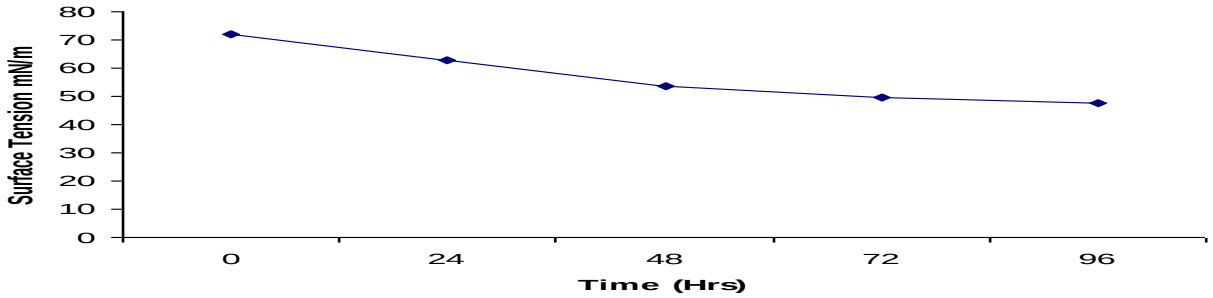
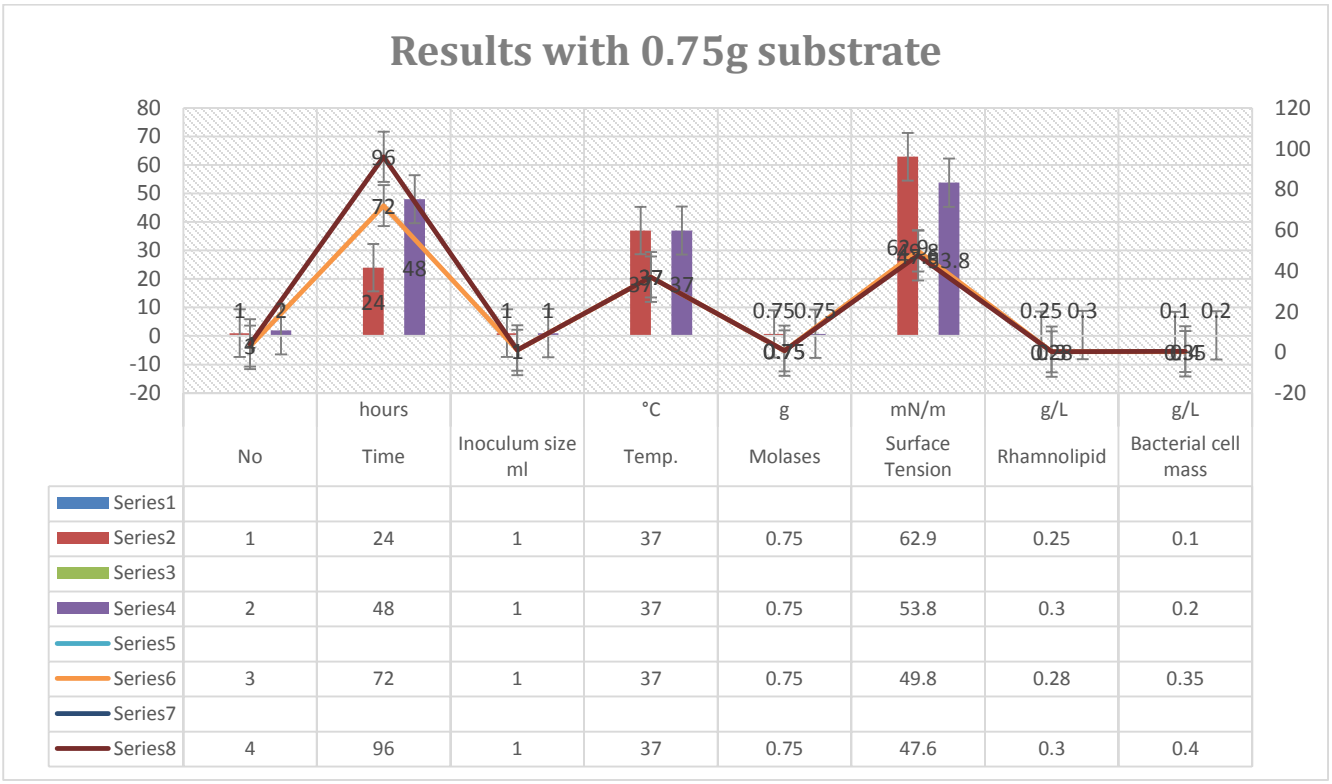


Table 1:



	No	hours Time	Inoculum size ml	°C Temp.	g Molases	mN/m Surface Tension	g/L Rhamnolipid	g/L Bacterial cell mass
Series1								
Series2	1	24	1	37	0.75	62.9	0.25	0.1
Series3								
Series4	2	48	1	37	0.75	53.8	0.3	0.2
Series5								
Series6	3	72	1	37	0.75	49.8	0.28	0.35
Series7								
Series8	4	96	1	37	0.75	47.6	0.3	0.4

DISCUSSION

The essential properties of biosurfactants, like, foaming, cleansing quality or power, emulsifying, demulsifying, wetting, solubilizing, metal sequestering, condensing, vesicle creating and phase dispersion properties, among others, can be displayed by RLs[8,9]. These properties are related to the amphiphilic character of the RL molecules, and give them the ability to build between different phases of fluid, therefore, reducing interfacial tensions and surface[8]. Currently, the environmental uses of RLs are taken as the major field for the potential application of RLs[10-15], but as the numbers of patents are increasing day by day, this indicates the successful application of RLs in different industries like, food, cosmetics, agriculture, nanotechnology and pharmacology[16]. For microbial production, substrate optimization is very important factor. It is very important to disclose the wanted molasses concentration in which the bacteria can sustain, as high sugar and salt concentrations causes the loss of water from the cell and in the end bacterial cell dies[4]. Various substrate sizes 0.75g /100ml of broth were tested and rhamnolipid production, surface tension and bacterial cell mass was estimated. The production achieved with this research in which surface tension was 62.9, 53.8, 49.8 and 47.6mN/m at day 1,2,3 and 4 respectively and the temperature was kept 37°C (constant) and molasses used 0.75g with 1ml inoculum size. The production of rhamnolipids by Pseudomonas aeruginosa using different quantities of substrate as n-hexadecane, molasses and glycerol was 6g/L[6]. Rhamnolipid production is a growth associated process[4]. The substrate optimization in media was very important parameter as stated above that high sugar concentration can kill bacterial cells.

CONCLUSION

After optimization of molasses a production of rhamnolipid was achieved at different time intervals.

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