ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Optimization of Media for Better Production of Biosurfactant by *Proteus vulgaris.*

Jaysree RC, Rajam C, and Rajendran N*.

Molecular and Microbiology Research Lab., Environmental Biotechnology Division, School of Bio Sciences and Technology, VIT University, Vellore-632014, Tamil Nadu, India.

ABSTRACT

To reduce the surface and interfacial tension of the pollutants in environment, biosurfactants which are produced by the microorganisms such as bacteria, yeast etc. are being applied in large quantity. These compounds are produced extracellularly by the microorganism into the environment and they are applied in different industries such as detergent, cosmetics etc. for different purposes. In the present study, *Proteus vulgaris* (2aH) was used to optimize different environmental parameters such as pH (6 to 9), NaCl concentration (1 to 4 %) and WSF concentration (1 to 4 %) for the good production of the biosurfactant. Culture broth was assayed at a regular interval for biomass, emulsification activity and biosurfactant production during the culture period of 5 days. Bacteria showed maximum emulsification activity at pH 9 and good yield was seen at pH 7. From the different tested NaCl concentrations *P. vulgaris* showed good emulsification activity at 2 % and 3 % NaCl concentration and maximum yield at 1 % NaCl concentration of the biosurfactant. The strain showed both good activity and yield of biosurfactant at 1 % WSF concentration. *Proteus vulgaris* showed good growth, activity and yield of the biosurfactant during the optimized environmental conditions of the bacteria.

Keywords: Biosurfactant, bioavailability, biodegradation, *Proteus vulgaris*, optimization, diesel, genbank, water soluble fraction.

Corresponding author

ISSN: 0975-8585

INTRODUCTION

Biosurfactants produced by the microorganisms such as bacteria, yeast, fungi etc. extracellularly, that can reduce surface and interfacial tensions. Biosurfactants are also known to solubilise or split-up hydrocarbon that makes it bioavailable to the microorganisms and be utilized as energy. These occur in different names such as rhamnolipid, lipopeptide, surfactin, polymeric biosurfactants etc. according to the sugar, amine or lipid group present with the compound [1]. They are effective at harsh temperatures, pH, salinity, and is specific in its action and causing less toxicity to the environment led to their popularity. There application in many industries such as detergent, cosmetics, food industry, clinics, chemical industry etc. and remediation of oil spills led to their vast significance in the large production of the biosurfactant [2, 3].

The potential of the bacterial strain to show surface active properties led to the optimization of environmental parameters for large production of the biosurfactant. The objectives of the study were (i) to optimize the different environmental parameters such as pH, salt concentration and water soluble fraction (WSF) of diesel in an effort for large production of biosurfactant and (ii) to compare the growth, activity and the yield of biosurfactant during the different environmental parameters.

MATERIALS AND METHODS

Media preparation

Nutrient broth medium which is composed of (g per 1000 ml of distilled water); peptone (10); meat extract (10), was used for this study. Medium pH was 7.2 ± 0.2 and the medium was autoclaved at 121^0C for 20 min at 15 lbs pressure.

Water soluble fraction (WSF) of diesel concentration was prepared by using 10% of diesel in the autoclaved double distilled water (v/v). It was kept in a rotary shaker at 100 rpm and the soluble part of the diesel fraction was used for the experiment.

Microorganism

The microorganism used in this study *Proteus vulgaris* (2ah) had already been isolated and the sequence data were submitted in Genbank with accession number KP289283. This strain has been qualitatively analyzed for surface active properties.

Inoculum

The strain was maintained in agar slants and *Proteus vulgaris* (2ah) was inoculated in a 10 ml of nutrient broth. After 24 hours of growth, 1 ml was transferred to the experimental flasks.

Optimization of environmental parameters

To determine the optimum environmental conditions for the maximum production of biosurfactant by the isolate, experiments were conducted. The different parameters such as pH (6.0 – 9.0), NaCl concentration (1-4 %), diesel concentration (1-4%) of the water soluble fraction of diesel were used. Nutrient broth was used as medium and water soluble fraction of diesel was used as carbon source. 1% of WSF was added in each of the experimental flask other than WSF experiment. 10 ml of the medium was withdrawn from the culture flask every day during the experimental period of 5 days and analyzed for biomass, emulsification activity and biosurfactant yield.

Biomass estimation

Growth of the culture was measured by turbidity method using calorimeter (Systronics 9130, Ahmedabad, India) at 600 nm.

Emulsification assay (E_{24})

The *P. vulgaris* was evaluated for emulsion forming capacity, according to the method proposed by Das et al. (10). 0.5 ml of the cell-free supernatant was added with 1 ml kerosene in a test tube. This mixture was homogenized and vortex at high speed for 2 min. After 24 hrs, relative emulsion volume (E_{24} %) was calculated using the following equation:

E_{24} (%) = Emulsion height (cm) / Total liquid volume x 100

Biosurfactant extraction

Bacterial culture broth was centrifuged at 10,000 rpm, for 20 min at 4°C. The cell-free broth or supernatant was adjusted to pH 2 using 1N H_2SO_4 [4]. It was kept at 4°C overnight, and then centrifuged at 10,000 rpm for 10 min. Thus formed precipitate was called as acid precipitate. After drying it, the weight was measured and expressed as mg/ml (w/v).

RESULTS AND DISCUSSION

Optimization of environmental parameters

Environmental parameters such as pH, salt concentration, and concentration of water soluble fraction were optimized for the growth, emulsification activity and yield of the acid precipitate from *P. vulgaris* for a period of five days. The experimental medium for pH and salt was added with 1% water soluble fraction as additional carbon source.

pH evaluation

There was not much gradual increase in the growth of different pH when compared to control but at the end of the experimental period, the growth was high in pH 9. The growth of the organism reached up to a maximum O.D of 0.5 at pH 6, 7 and 8 and O.D of 0.7 at 119 hrs at pH 9 (Fig.1b). Since the organism was able to grow in a similar pattern in all the pH tested, it could be inferred that this bacterial strain could tolerate a range of pH from 6 to 9 and good growth was seen at pH 9. The emulsification activity was not detected on the first three days of incubation at pH 6 (Fig. 2a and b), which might be due to the fact that at pH 6, the biosurfactant might be not available for emulsification activity or pH 6 was not optimum for bacterium to produce biosurfactant.

The emulsification activity of *P. vulgaris* was observed from the first day in pH 7, 8 and 9 and maximum emulsification activity was noticed in pH 9 (Fig. 2b). Since biosurfactant was not required for the growth of the organism, it might be released into the ambient medium as opined by Agatha 1998 [5, 6]. This may be a plausible reason for the maximum biosurfactant activity that was seen during the end of the experiment. It has already been reported that stationary growth phase of the cells may contain high levels of biosurfactant [7]. In this study biosurfactant stability increases with increased pH as observed by Bernard et al., 2006 [8].

The biosurfactant yield was seen maximum on the 5th day having 2.8 mg/ml in control (Fig. 3a) and 4 mg/ml in test at pH 7 (Fig. 3b) having an O.D of 0.5 and 0.7 respectively as shown in figure 1 (a) and (b). This difference might be due to the addition of 1% WSF that had stimulated the production of biosurfactant to two times of the control. The biosurfactant produced at each consecutive day (Fig. 3) was fluctuating and was not correlating with (Fig. 2) the emulsification activity. Ana et al., 1997 [9] reported that when the concentration of biosurfactant was increased above the critical micellization concentration (CMC), micelles were formed, and thus emulsion became stable. The maximum production of biosurfactant was seen on 1st and 3rd day and then it showed a decreasing trend.

NaCl concentration

The growth of the organism at NaCl concentration of 1 % was found to be above 0.6 O.D but the growth at 2, 3 and 4% was lower than the 1 % NaCl concentration as shown in Fig.4b. There was an increase in

the growth of the organism compared to control that may be due to the addition of 1% WSF as carbon source and presence of salt. However at 3% and 4%, the growth was at a moderate level due to the high concentration of salt. The relation between growth and emulsification activity was not proportionate at different NaCl concentration, i.e. increase in biomass has not increased the emulsification activity (Fig. 5a and b). Maximum activity of control was seen having 23 % (Fig.5a) but a test with 32 % activity was noticed at 2 and 3 % NaCl concentration (Fig. 5b) on the 4th and 5th day of the experiment respectively.

The maximum yields of biosurfactant in control and test were 3.1 mg/ml and 5.1 mg/ml respectively as shown in Fig. 6a and b. It could be suggested that the high production of biosurfactant was obtained at 1 % NaCl concentration. However, the yield of biosurfactant was relatively higher at different salt concentrations than that were obtained for pH (4 mg/ml at pH 7).

Diesel concentration

The growth patterns of the organism at different water soluble fraction concentration were similar during the experiment. All the WSF concentration showed emulsification activity on 3rd and 4th day (Fig 8). The organism had produced biosurfactant to combat the increased concentration of the hydrocarbon (Fig. 9). The biosurfactant production was found to be higher at 1% of WSF concentration (3.1 mg/ml) compared to 4% WSF concentration (2.2 mg/ml) on the first day as shown in figure 7. These results indicate that the low concentration of WSF was able to induce the higher levels of biosurfactant production, even though the observed activity of all the four WSF concentrations were same as shown in figure 8.

The mean and standard deviation values were calculated during the experiment period and they were presented in Fig. 10-11. Good emulsification activity was noticed at pH 9 than the other pH levels (Fig. 10a) and showed significant difference between different pH values but the control did not show significant difference. This may be due to the absence of additional carbon source in control. Figure 11a showed the mean biosurfactant yields at different pH values. There was no significant difference among the pH values, but the maximum yield was (2.7 mg/ml) was observed at pH 8.

Figure 10b showed high emulsification activity at 2% NaCl concentration (20%) of the *P. vulgaris.* The average activity was less than 25% in all NaCl concentration but the activity was higher than the activity observed in pH (Fig 10a and Fig 10b). Figure 11b showed high biosurfactant yield in 1% NaCl concentration which was 3.2 mg/ml when compared to other NaCl concentrations. From the mean values, it could be inferred that this bacterium was capable of producing high levels of biosurfactant in salt concentration than at different pH (Fig. 11a and 11b).

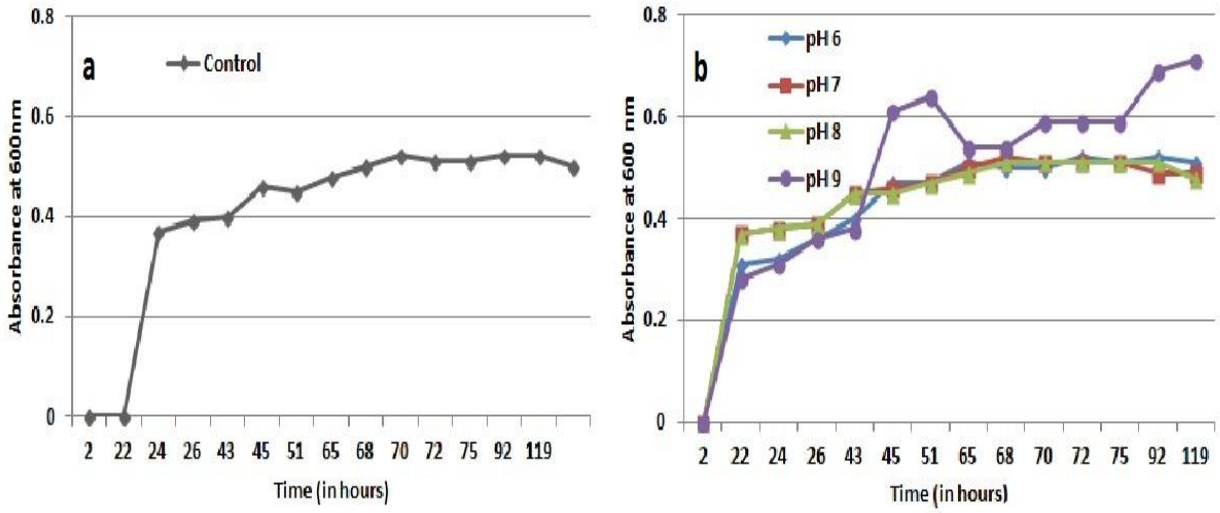


Figure 1: Growth of *Proteus sp*. at control (a) and different pH (b).

ISSN: 0975-8585

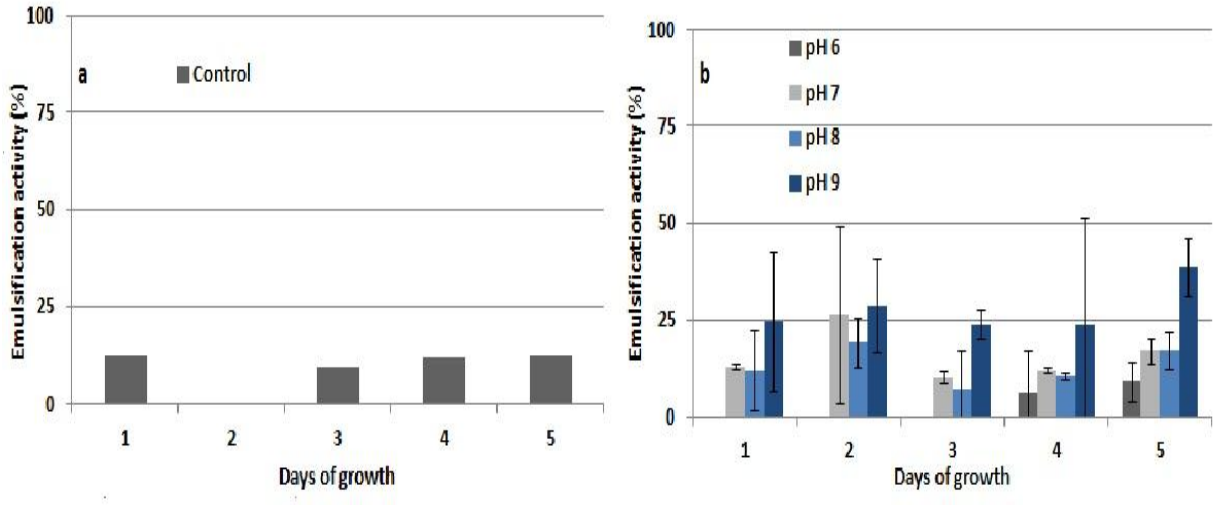


Figure 2: Emulsification activity of *Proteus sp.* at control (a) and at different pH (b).

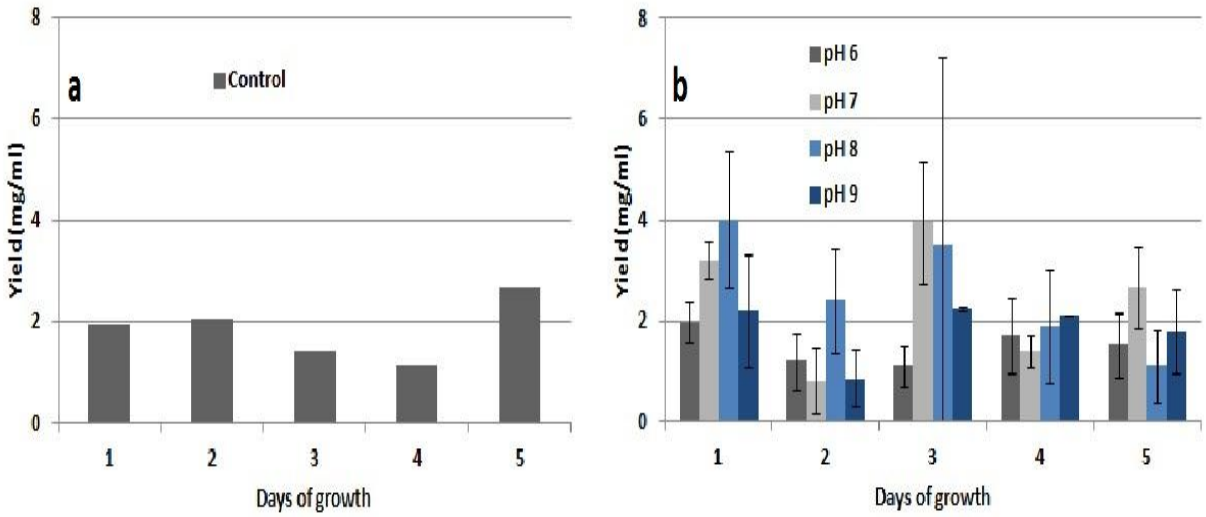


Figure 3: Biosurfactant yield of *Proteus sp.* at control (a) and different pH (b).

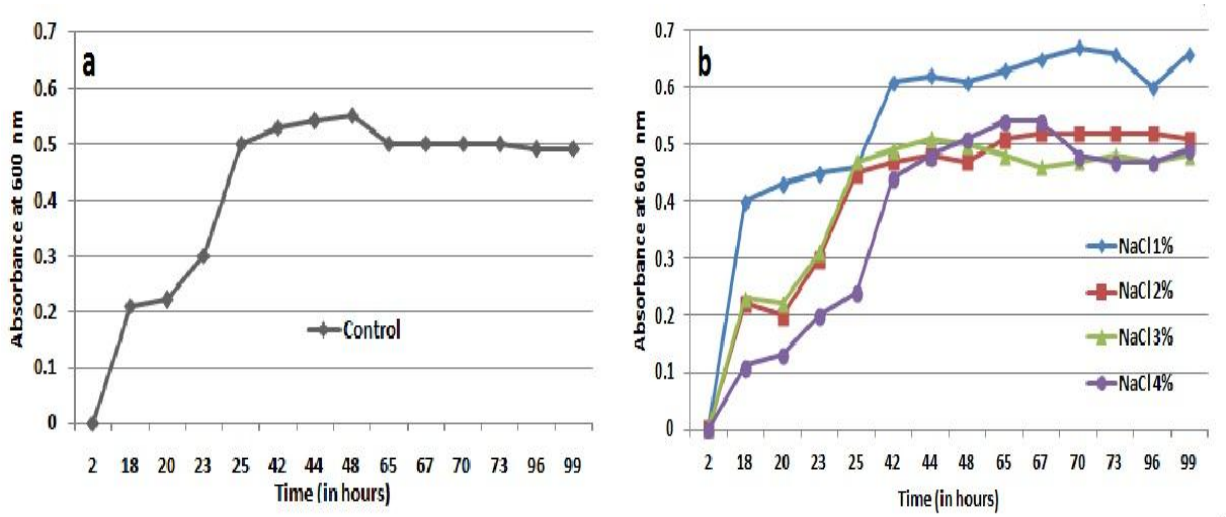


Figure 4: Growth of *Proteus sp.* at control (a) and different NaCl concentration (b).

ISSN: 0975-8585

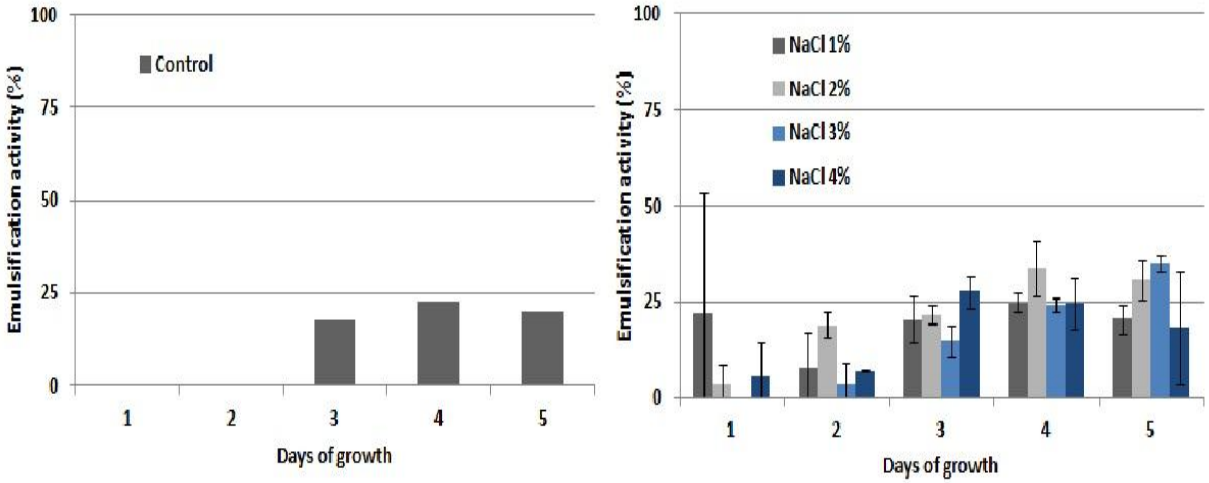


Figure 5: Emulsification activity of *Proteus sp.* at control (a) and different NaCl concentration (b).

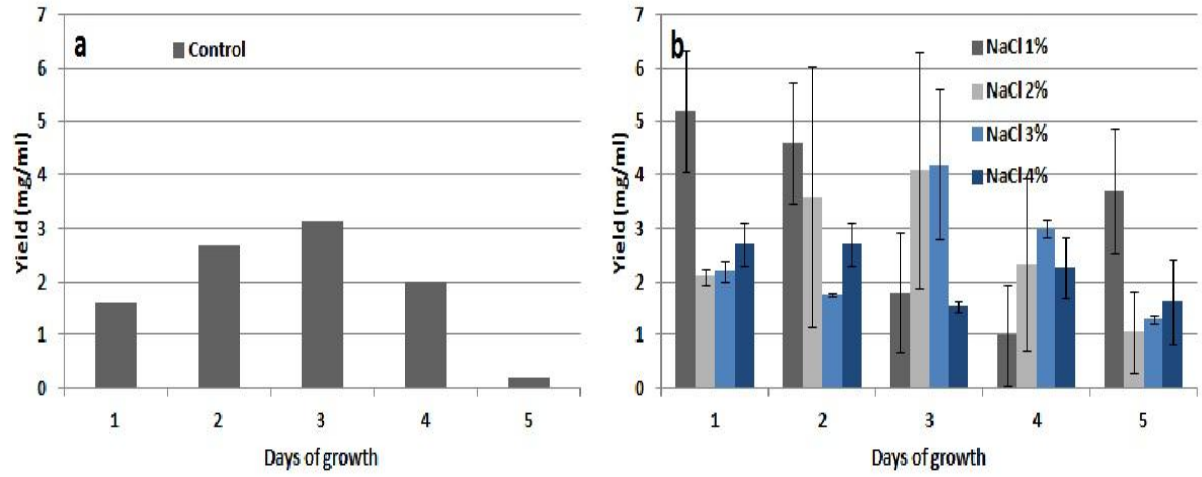


Figure 6: Biosurfactant yield of *Proteus sp.* at control (a) and different NaCl concentration (b).

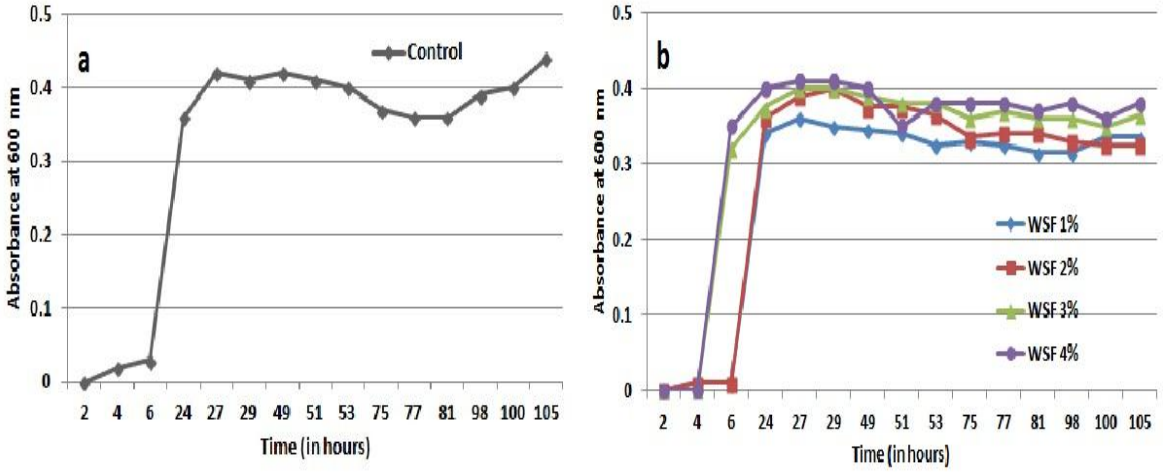


Figure 7: Growth of *Proteus sp.* at control (a) and different WSF concentration (b).

ISSN: 0975-8585

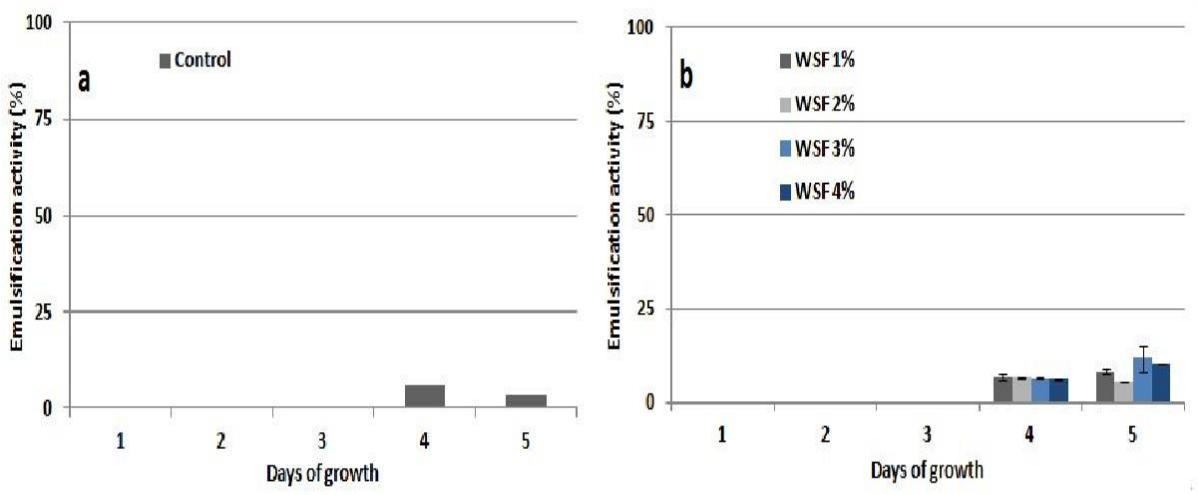


Figure 8: Emulsification activity of *Proteus sp.* at control (a) and different WSF concentration (b).

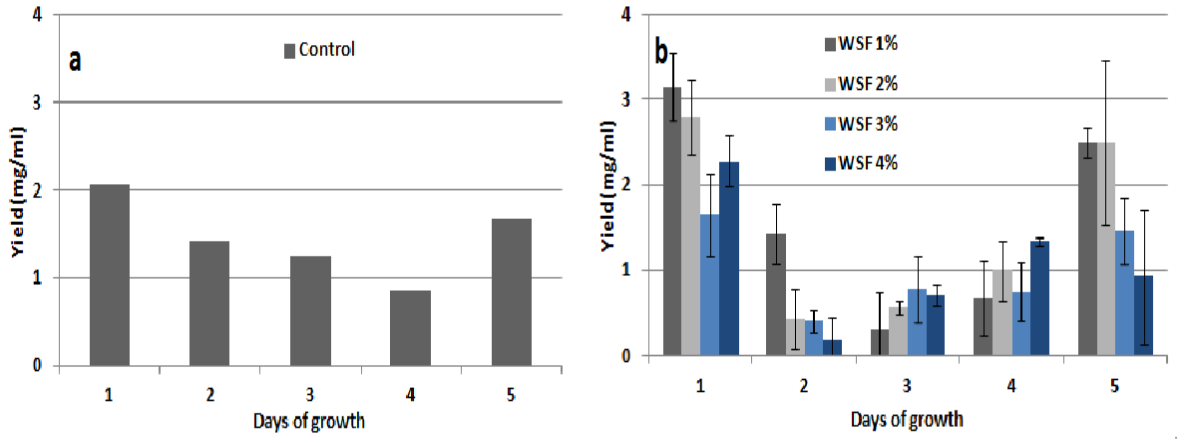
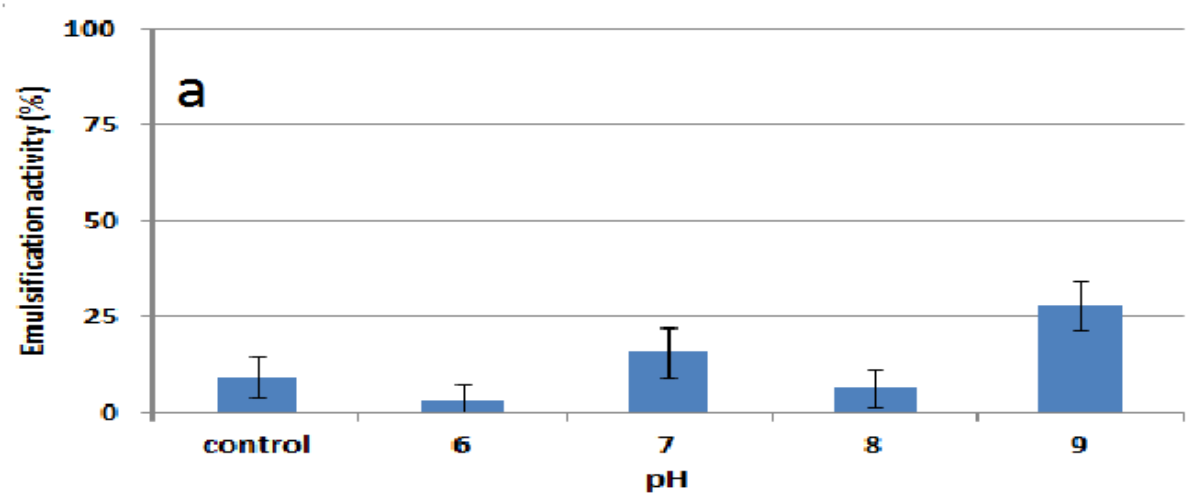


Figure 9: Biosurfactant yield of *Proteus sp.* at control (a) and different WSF concentration (b).



ISSN: 0975-8585

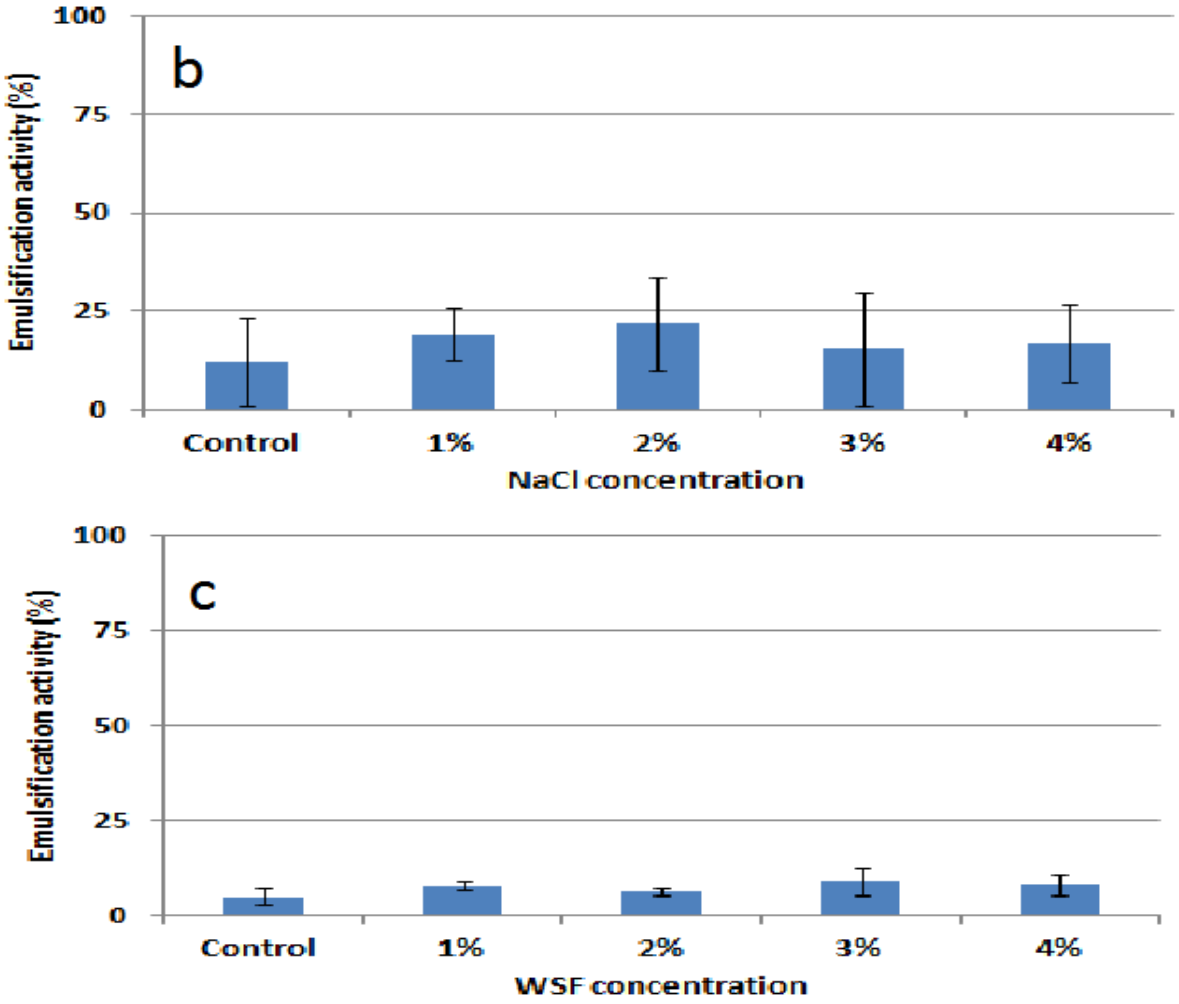
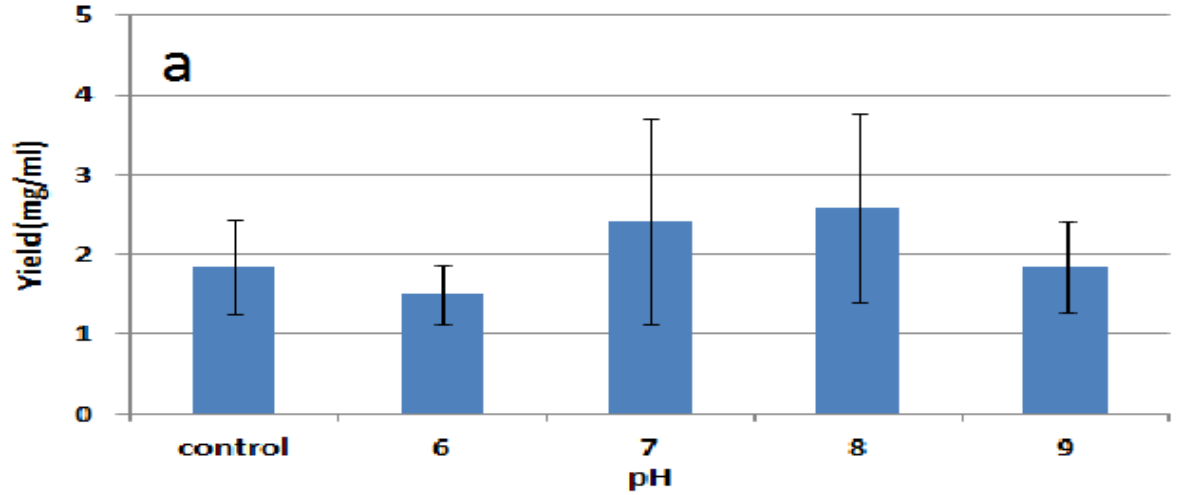


Figure 10: Mean and standard deviation of emulsification activity of different (a) pH (b) NaCl (c) WSF concentration and control during the experimental period.



ISSN: 0975-8585

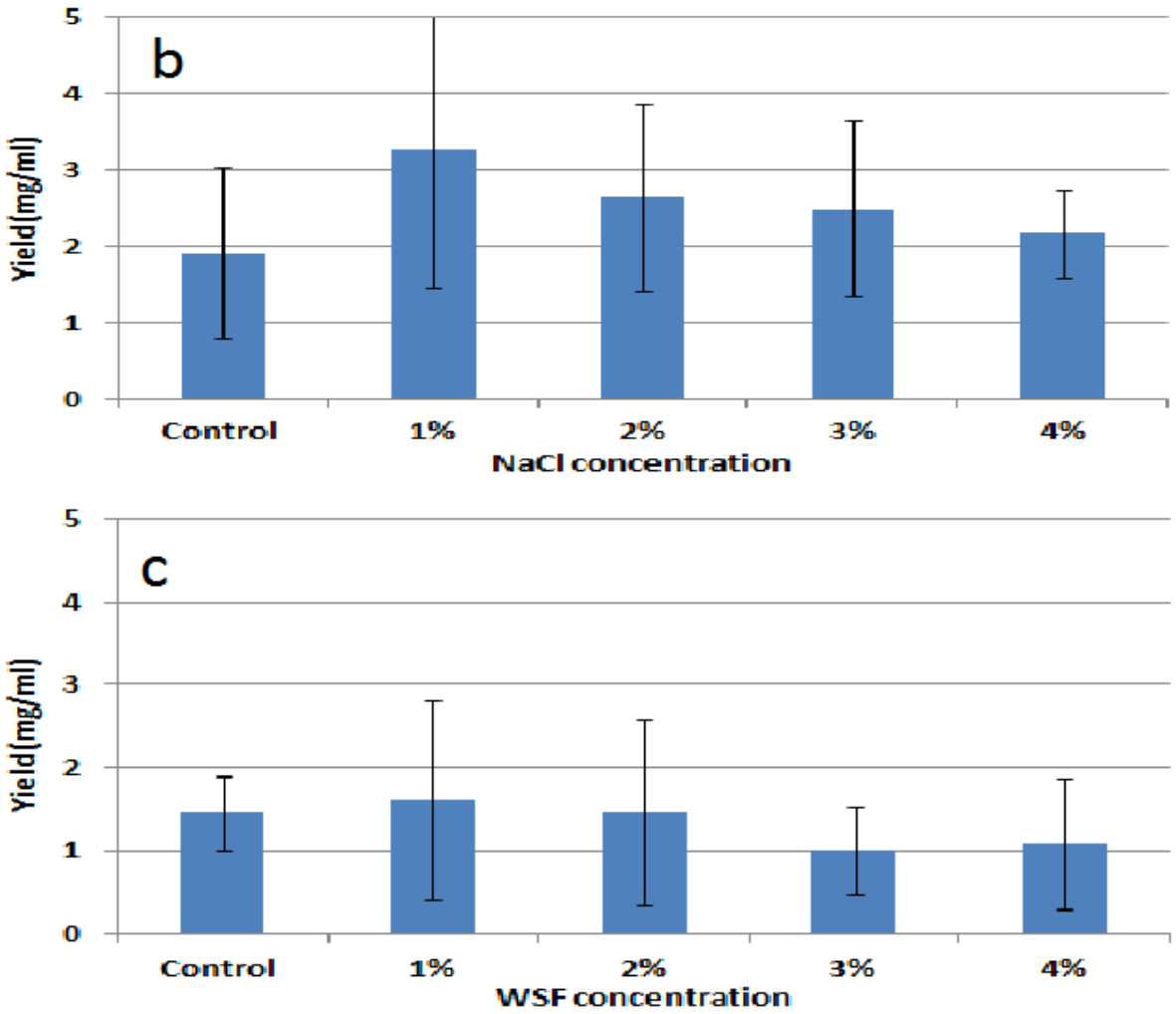


Figure 11: Mean and standard deviation of biosurfactant yield of different (a) pH (b) NaCl (c) WSF concentration and control during the experimental period.

Mean emulsification activity of the WSF concentration showed less than 25% activity at different WSF concentrations (Fig. 10c). When WSF concentration activity was compared with pH and NaCl, WSF gave very less emulsification activity. In fig. 11c showed the mean biosurfactant yield in different WSF concentration was always equal to 1.5 mg/ml having the maximum at 1% but these yields were less than the values of pH (2.7 mg/ml) and were higher than NaCl concentration (3.2 mg/ml).

This bacterium showed good activity at different pH and NaCl concentrations but comparatively low activity in WSF concentration. It should also be noticed that this bacterium showed slow growth in NaCl concentration.

CONCLUSION

To understand the potential of *P. vulgaris,* it has been subjected to the different environmental parameters such as pH, NaCl concentrations and WSF diesel concentrations. From the activity and biosurfactant yield of this bacterium, it could be inferred that this bacterium can be a potential candidate for future application in the presence of salt concentration.

ACKNOWLEDGEMENTS

We wish to thank the management and staff of VIT University, Vellore for providing financial support and facilities to carry out the research work.

ISSN: 0975-8585

REFERENCES

[1] Desai JD and Banat IM. Microbiol Mol Biol Rev 1997; 61: 47–64.
[2] Fiedler HP and Umbach W. Cosmetic and toiletries. In Surfactants in Consumer Products: Theory, Technology and Applications ed. Falbe, J. 1987 pp. 350–398.
[3] Desai JD and Desai AJ Biosurfactants: production, property, application. In Surfactant Science Series ed. Kosaric, N. vol. 48. New York: Marcel Dekker 1993.
[4] Cooper DG, Macdonald CR, Duff SJB, and Kosaric N. Appl Environ Microbiol 1981; 42: 408-412.
[5] Agata M. Fallon Study of Hydrocarbon Waste Biodegradation and the Role of Biosurfactants in the Process. Virginia Polytechnic Institute, Blacksburg, Virginia, 1998.
[6] Dilsad Onbasli and Belma Aslim. J Environ Biol 2009; 30: 161-163.
[7] Bernard P. Binks, Ryo Murakami, Steven P. Armes, and Syuji Fujii. Langmuir 2006; 22: 2050–2057.
[8] Ana Domínguez, Aurora Fernández, Noemí González, Emilia Iglesias, and Luis Montenegro. J Chem Edu 1997; 74: 1227-1231.
[9] Das M, Das SK, Mukherjee RK. Biores Tech 1998 63: 231-235.