Major Research Project Sponsored by University Grants Commission, New Delhi

Title of Major Research Project:	**Strategies for biosurfactant production by using combination of distillery waste with other industrial wastes**
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SUMMARY OF THE MAJOR RESEARCH PROJECT

Development of a cheaper process and use of low cost raw material for biosurfactant production is of prime importance if application of biosurfactants in different environmental remediation processes is to be realized. Therefore, the potential use of no-cost fermentative medium formulations using industrial wastes as substrate for biosurfactant production was evaluated. Our earlier studies have shown that distillery waste (1:3 diluted) and curd whey are the viable alternative sources for biosurfactant production and distillery waste cannot as such without dilution as it contains growth inhibitory concentrations of sulphate ions and potash. Therefore, aim of the present study wasto replace the use of precious water by suitable industrial waste/s, so that distillery waste after appropriate constitution can be used for biosurfactant production. To evaluate a suitable combination of distillery waste (DW) with other industrial wastes for biosurfactant production, curd whey (WW),sugar industry effluent (SIE), and fruit processing waste (FPW). Wastes characterized for various physico-chemical parameters highlighted that DW had high COD, sugar and nitrogen levels followed by WW, FPW and SIE.

Four microbial cultures designated as BS-A, BS-J, BS-K and BS-P were isolated from lube oil and DW contaminated soil effectively reduced the surface tension of the fermentedwastes, with emulsification indices (EI-24) comparable to known synthetic surfactants and biosurfactant productivity of 0.0043-1.631 g/l facilitated high reduction in COD, total nitrogen, sugars, and phosphatescontents in eachwastes. Among the four isolates, BS-J and BS-P were the biosurfactant high yielding strains and were therefore, selected for further study.

This is a first report on using combination of DW with WW and SIE/FPW biosurfactant production so that more nutritionally rich no-cost complete medium can be constituted which will replace the use of precious water reported earlier for diluting distillery waste in1:3 proportions. Among the two combinations evaluated, W+WW+FPW (1:1:1v/v) was better thanDW+WW+SIE for growth of biosurfactant producing isolate and for biosurfactant production without supplementation of costly chemicals for biosurfactant production. Benefits derived by using distillery waste along with other wastes were improved

production of biosurfactant (18.2-40.5%) and saving precious water with concomitant reduction in COD by 76-84.2%, and total sugars, nitrogenand phosphates in the range of 45-86%. This indicates a positive impact of waste combination on biosurfactant production capacities of the new microbial isolates and replacement of precious water with other wastes required for diluting distillery waste for biosurfactant production.

For further improving the process economics, best unsterile combination DW+WW+FPW was evaluated to study biosurfactant production profile of efficient isolates. Resultshave shown that unsterile combined wasteconstituted good substrate for the growth ofstrain BS-P and BS-J and resulted 1.6g/l and 1.3g/l of biosurfactant yields which were 19% and 34% lower than that obtained in sterilized condition, respectively. Use of unsterilized waste combination hasnot only provided total means of eliminating the use of costly chemicals for biosurfactant production but also minimized the cost of sterilization of combined waste thereby opening a new avenue for cost-effective biosurfactant production by the efficient isolates.

Based on morphological, biochemical, cultural, 16 S rRNA sequence and phylogenetic characteristics, the efficient biosurfactant producing isolates BS-J and BS-P were identified as *Kocuriaturfanesis*and *Pseudomonas aeruginosa*, respectively and were new strains.

Biosurfactants produced by strain BS-J and BS-Pin combination of DW+WW+FPW has the potential to reduced the surface tension of liquid and had low critical micelle concentrations well within the range reported for effective biosurfactants and synthetic surfactants with high emulsifying activity (EI-24) of more than 60% indicating that biosurfactants were of emulsifier-type. Detailed chemical analysis of biosurfactants performed using various analytical techniques have shown that biosurfactants produced were complex of lipids, carbohydrates and proteins,with differences in their percentages. Biosurfactant produced by strain BS-J was glycolipid and that of strain BS-P was a complex mixture of both lipoprotein and glycolipidssuggesting that the chemical compositions and natureof the two biosurfactants are widely divergent. Thin layer chromatographic analysis revealed protein conjugated glycolipid type of biosurfactants with differences in $R_f$values their separated constituents. FTIR analysis revealed biosurfactant produced by strain BS-J wasof glycolipid type and that by strain BS-P contains carbohydrate, aliphatic and peptide-like moieties.LC-MS/Mass spectroscopic analysis of carbohydrate moieties present in bisourfactants haveshown variations in types and the percentage of total sugars in the two biosurfactants. Biosurfactants produced by the two isolates also differ in their amino acids composition and their concentrations. LCMS/MS of lipid fraction has shown thatfatty acids present in crude biosurfactants were generally similar and they differ greatly only in theirconcentrations.

Kinetics of biosurfactant production in combinations of DW i.e.DW+WW+SIE and DW+WW+FPW in 1:1:1v/v ratio was studied to generatedata useful in designing and developing future process for large scale cost-effective biosurfactant production by using waste as substrate. Results have shown that in both the combinations, biosurfactant production by *Kocuriaturfanesis* strain BS-J took place under growth-limiting conditions and it was growth associated production in *Pseudomonas aeruginosa* strain BS-P. Specific

growth rates evaluated from the Monod's kinetic constants (μ_{max} and K_s) has shown that specific growth rates of the isolates BS-J and BS-P and specific product formation rates ($V_{max}$and Km) were maximum inDW+WW+FPW combination and minimum in DW+WW+SIE.This could probably due to better availability of the high levels of nutrients in DW+WW+FPW as compared to DW+WW+SIE indicating DW+WW+FPW combination can serve as potential combination of distillery waste as no-cost fermentation medium for cost-effective production of biosurfactant.

A novel cost-effective enhanced biosurfactant producing mutant screening technique wasdeveloped using whey as principal component of mutant isolation protocolreplacing the costly synthetic medium. Growth of UV- irradiated cells of *P. aeruginosa* BS-Pon curd whey cetyl tri-methyl ammonium bromide – Methylene blue agar demonstrated formation of varying sizes of green halos due to differences in biosurfactant production capacities of mutant strains as compared to wild cells. Isolated mutants have shown 28-60 % improved biosurfactant yield in curd whey than 9-37% increase observed in combination of DW+CW+FPW due to rich source of nutrients available incurd whey.Mutant screening technique developed issimultaneously applicable for both standardization of mutagen dose and isolation of biosurfactant hyper producing mutants on the basis of the size of the halo developed around the colony which can also be used for screening of other types of anionic biosurfactant producing mutants.

Novelprocess for cost-effective recovery of biosurfactants produced by strains BS-P andBS-Jfrom fermented combination of DW+WW+FPW was developed based on adsorption–desorption technique using egg shell as new adsorbent no-cost material. As adsorption is a surface phenomenon, optimum 2.5% v/v HCl treatment was used to modify the surface topography of the egg shell which resulted efficient removal of biosurfactant from fermented waste combination at lower dose of egg shell (3 % w/v). Adsorption isotherm studies has shown that biosurfactants adsorption follows Langmuir's and Freundlich models in the same way as that on wood activated carbon reported earlier for biosurfactant recovery. An equilibrium time of 5 h and 6 h resulted in 100% adsorption of biosurfactant produced by strains BS-J and BS-P, respectively and acetone resulted in 92.88-98.60 % recovery of adsorbed biosurfactants from the egg shell. Acetone treatment regenerated the adsorption potential of the egg shell surface topography thereby facilitating regeneration reuse potential for recovery of both the types biosurfactant maximum until five consecutive cycles.

The outcome of the present study will not only help to solve the problem of industrial wastes by their reuse but also can recover value added ecofriendly surfactants which has tremendous use in various industries. Moreover, data generated through such studies can be used for up gradation of biosurfactant production at pilot scale level.