Abstract

Natural surfactants are amphiphilic compounds derived from natural resources mainly plants and microorganism. Owing to their excellent physiochemical properties they are replacing synthetic surfactants in verity of commercial applications. In present study natural surfactants obtained from bacteria and two plants were studied for their possible role in rehabilitation of crude oil contaminated soils. In first phase of this study a biosurfactant producing bacterial strain was isolated from crude oil contaminated soil samples of *Missa Kaswal* oil field. Out of 51 bacterial strains only seven were found to be surfactant producing. The most efficient biosurfactant producing strains were including; M8, M9 and M10 based upon surface tension reduction, emulsification index, oil displacement and drop collapse test. The strain M9 showed highest reduction of surface tension of the culture medium i.e. 66.7 to 26.6 mN/m and 6.2 cm of oil displacement zone considered as the most efficient biosurfactant producing bacteria. The isolates M8, M9, and M10 identified by using morphological, biochemical and molecular techniques as different strains of *Pseudomonas aeruginosa*. Out of four different media, medium 4 proved to be the best in term of yielding highest amount of biosurfactants with all the three strains of *Pseudomonas aeruginosa*. Biosurfactant production was 2.31 g/L in medium 4, after 96 hrs by strain M9, whereas strain M8 and M10 produced relatively less biosurfactants. The strain M9 was the most efficient and selected for further studies. Optimization of different carbon sources revealed glycerol as the best in medium for the highest bacterial growth 1.37 g/L and biosurfactants production 2.890 g/L. The rhamnolipid production reached up to 4.44 g/L at optimum conditions i.e., pH 7, temperature 34 °C, agitation speed (rpm) 155, and 2.8 % inoculum. The media components were also optimized by using a combination of response surface and central composite design. The optimized medium composition pertaining to maximum rhamnolipids production of 5.67 g/L was obtained by using $NaNO_3$ 3.92 g/L, KH_2PO_4 2.3 g/L , $MgSO_4$ 0.26 g/L and $FeSO_4$ 0.0028 g/L.

The chemical composition of biosurfactant produced by *P. aeruginosa* M9 was determined using HPTLC, FTIR and MALDI-Tof techniques. Results indicated that strain M9 produced a mixture of RL-1 and RL-2 during its growth on glycerol. The rhamnolipid produced by *P. aeruginosa* M9 were studies under effect of varying pH, salt concentration and temperatures. Results suggested that rhamnolipids retained their activity between pH 4-10, 1-21 % NaCl and 121 °C. In

addition, 0.2 % of the crude rhamnolipid was sufficient to decrease the surface tension of the waster to 26.6 mN/m.

The *n*-butanolic extract of S. *mukorossi* and *A. concinna* were analyzed for the presence of saponins using TLC and FTIR spectroscopy. The results indicated presence of saponins in both the plants. Results indicated that saponins from *S. mukorossi* and *A. concinna* were stable at pH 4-9, 25-121 °C, and 1-21 % NaCl. The crude saponin extracted from *S. mukorossi* reduced the surface tension of the water to 39.1 mN/m at a concentration of 0.2 % (w/v). On the other hand, surface tension *Acacia* saponin reduced the surface tension to 42.0 nM/m.

The role of natural surfactants obtained from microbial and plants resources were studied in surfactant enhanced soil remediation by using *Taguchi's* Orthogonal Array Design. Removal of crude oil from the soil collected from *Missa Keswal* oil field was determined under the effect of different temperatures, shaking speed, surfactant concentration and time. Results indicated 94 % reduction in crude oil from the soil at 55°C, 200 rpm, 1% rhamnolipid concentration after 15 hrs. In case of *Sapindus* saponins, maximum removal was 87 % at optimum conditions of; 65 °C, 200 rpm, 0.8 % saponins and 15 hrs of process time. The results of soil washing using *Acacia* saponins revealed that 65°C, 200 rpm, 0.8 % saponin and 15 hours were the optimum soil washing conditions pertaining to a maximum removal of 78 % crude oil from the soil. The soil obtained from GCU garden was washed at optimized condition obtained for each natural surfactant. Maximum removal of 81 % was carried out by *Sapindus* saponins, followed by 78 % by rhamnolipids and 67 % using *Acacia* saponins. These findings suggested that natural surfactants have excellent potential to be used in soil remediation of complex hydrocarbons such as crude oil.