In the present study conditions were standardized for the maximum biosurfactant production by *B. subtilis* MTCC 2423 at thermophilic growth conditions. The strain was selected from 29 strains screened for their ability to grow and produce the biosurfactant at thermophilic conditions. The strain was gram positive capable of using many carbohydrate substrates supplied to it for growth. Biosurfactant produced by *B. subtilis* MTCC 2423 shows structurally the highest analogy with the surfactin produced by a strain of *Bacillus subtilis*. Preliminary chemical characterization of the biosurfactant by TLC reagents show it to be similar to surfactin. The similarity of the surfactant with standard surfactin was confirmed by IR analysis, ^{1}H NMR and mass spectroscopy.

The *Bacillus* strain could utilize glucose, sucrose, and sodium pyruvate for biosurfactant production. Biosurfactant could not be synthesized when sodium acetate was presented as the carbon source. Growth and biosurfactant production by *B. subtilis* was studied on minimal medium supplemented with sucrose as carbon source at a concentration of 2%. The strain could utilize n-hexadecane and pristane for biomass formation (growth), but could not form biosurfactant on either n-hexadecane or pristane. Sucrose (2%) was found to be a suitable carbon source for the maximum growth and biosurfactant production. Sodium nitrate and potassium nitrate were the preferred nitrogen sources. Nitrogen was required for the biosurfactant production as is evident from the observation that when there was no nitrogen in the medium, negligible reduction in surface tension and amount of biosurfactant was observed. Potassium nitrate (3g/l) was found to be optimal for biosurfactant production.

Effect of different concentration of individual metal cations on biosurfactant production was studied. Higher concentration however, inhibited biosurfactant production. The effect of metal cations was in conjunction with presence of other metal cations as indicated by net yield of biosurfactant when all the metal cations were supplemented in

the medium. Supplementation of amino acid in the medium resulted in higher yields of biosurfactant. *B. subtilis* MTCC 2423 was able to produce biosurfactant in pH range of 4.5 to 10.5 (considered to be extreme), although the maximum yield of the biosurfactant was obtained at pH 7.0. The biosurfactant was pH stable and retained its activity at pH values from 4.0 to 12.0. A slight loss of activity below pH 4.0 was due to precipitation of the biosurfactant.

The biosurfactant produced by *B. subtilis* MTCC 2423 was thermostable and retained its surface activity even after heating at 100°C for 2 h. Biosurfactant composition remain unaltered at thermophilic conditions (45°C). Increase in agitation speed resulted in increase in biosurfactant production with 200 rpm being optimum. The strain could utilize high salt concentration in the medium to grow and synthesize biosurfactant. Biosurfactant yield was reduced at 4% NaCl concentration. Fermention studies were done in 6.5 L fermentor. Biosurfactant yield could be enhanced to about 4.5g/l of biosurfactant in acid precipitates of the collected foam. This was approximately 4 fold higher than the surfactant that could be collected in shake flasks. Almost all the biosurfactant was recovered in foam.

The biosurfactant production in case of *Bacillus subtilis* MTCC 2423 was chromosomal mediated as is the case with other surfactin producing organisms. The appearance of cells grown at 45°C was elongated, with less dense cytoplasm. No remarkable or distinct change was observed. However, during growth on hydrocarbons it was interesting to note the appearance of distinct flagellum.

The biosurfactant exhibited a E_{24} value of 90 against diesel oil and sand pack recovery of 56%. These properties suggest the use of the biosurfactant in emulsification and MEOR. Moreover, the ability of organism to produce biosurfactant on molasses (a substrate added in oil wells as rich source of carbohydrate) makes the organism itself a

possible candidate for in situ oil recovery. The high stability (pH as well as temperature) of the biosurfactant and ability of the organism to grow at wide range of pH, salt concentration (upto 10%) and at thermophilic temperature (45°C) makes it very suitable for the extreme conditions encountered on application field.

The strain was able to produce the biosurfactant using molasses as carbon source and had good emulsification and sand pack value also. Conditions prevailing in the oil well are not congenial for the growth of mesophilic organisms or production of biosurfactants. Under these conditions the *B. subtilis* strain used in this study is a model organisms as it could utilize molasses at 45°C and produce biosurfactants. It can be concluded that biosurfactant production using molasses as growth substrate is relatively an inexpensive and economical process which can be easily adapted to field conditions for treating contaminated soil with hydrophobic pollutants and MEOR. The biosurfactant exhibited antibiotic activity against the Gram positive organism and against Actinomycetes. It did not show activity against the Gram negative strains tested.