Material and Method

Sample collection:-

Soil samples shall be collect from two different oil contaminated sites

(A) Popular service garage, station road, Parbhani. 431401(Maharastra)

(B) R. R. Petrol pump, vasmat road, Parbhani. 431401(Maharastra)

Soil had collect randomly 5-10 cm beneath the surface using spatula and packed in sterile polybags will transfer to the laboratories [Okoh, 2003; Oja 2006].

Isolation of bacteria from soil sample:-

Bacterial species shall be isolate from the collect soil sample by serial dilution and agar plating method.

The purify cultures will be cross check by gram staining procedure [Khan et.al. 2011].

Staining and biochemical activities of purified cultures:-

In order to identify the purify culture tentatively on the basis of Bergye’s Manual [Aneja 2003] various staining and biochemical tests shall be performe namely Gram staining, Endosperm staining, Catalase test, Mannitol fermentation, Glucose fermentation, fructose fermentation, and lactose fermentation. IMViC Test [Khan et.al. 2011].

Identification of biosurfactant-producing bacterium:-

Morphological, Biochemical, and physiological characteristics will be analyse to identify the isolate, according to the method propose by [Buehanan
and Gibbons 1974]. To identify the isolate more accurately will be analyse partial 16S-rRNA gene nucleotide sequencing [Tong liu et.al. 2011]

**Production and extraction of Biosurfactant:-**

Production of biosurfactant will be done with *Bacillus weinhenstephanesis* KBAB4 in a liquid mineral salt medium containing 2% (v/v) Olive oil at. 27°C, 140 rpm, and pH – 7. After 7 days incubation, cell free supernatant will be taken for partial purification of the supernatant.

The biosurfactant will be extract by cold acetone method [Wang et.al. 2007]. The organic phase will be separate and evaporate to dryness using a rotary vacuum evaporator to give the crude biosurfactant at 45°C [Sahoo et. al. 2010].

**Characterization of biosurfactants:-**

Preliminary characterization of the biosurfactant shall be done thin layer chromatography [TLC]. The biosurfactant separate on the plate using chloroform: methanol: water [4:1:1] Ninhydrin reagent will be spray to detect lipo peptide biosurfactant as red spots. Throne reagent will be spray to detect glycolipid biosurfactant as yellow spots. [Anandray et.al. 2010].

**Analysis of molecular Weight:-**

The extract crude biosurfactant will be obtain as sediment. The sediment will be mixed with chloroform and given for GC-MS analysis [Raj et. al. 2010].