INTRODUCTION

Biodiesel production has increased exponentially over the years; leading to the large crude glycerol generation obtained by the transesterification of vegetable oils. There are wide range of applications of pure glycerol in food, pharmaceuticals, cosmetics and many other industries. It is very cost effective to refine crude glycerol to a high purity especially for the small and medium biodiesel producers. Also the increasing amount of crude glycerol is causing storage problem and environmental hazard. Many research studies have been taken up with innovative ideas finding alternative utilization of crude glycerol. One such alternative is to use it as carbon source for PHA/PHB/PHB production.

Accumulation of nondegradable plastic in the environment is one of the major causes of pollution nowadays. Indian Supreme court made a statement stating “Plastic bags threat is more serious than the atom bomb. Plastic bags photo-degrade; over time they breakdown into smaller, more toxic petropolymere which eventually contaminate soils and waterways. As a consequence microscopic particles can enter the food chain (National Geographic news Sept 2, 2003). The effect on wildlife can be catastrophic, birds become terminally entangled, and nearly 200 different species of sea life including whales, dolphins, seals and turtles die due to feeding on plastic accumulated in the aquatic habitat which is mistaken for food (World life fund report 2005).

Taking into consideration the tremendous threats caused by the plastic, there is need to search for the alternative which can replace this plastic. One alternative is to use the bioplastic (polyhydroxyalkanoates/Polyhydroxybuterates) produced using renewable substrates and which are ecofriendly. Bioplastics are biobased biodegradable plastics with almost similar properties to synthetic plastics made from variety of sources like polysaccharides, lipids and also proteins (Averous, 2014; Hernandez and Krochta, 2008; Siracusa et al, 2008; Gonzalez et al, 2009). The available literatures shows number of biodegradable substrates such as Fruit wastes (Preethi et al, 2012), molasses & corn steep liquor (Chaivanmus & Udpuay, 2008) and Edible oil (Darshan & Nishith, 2011) etc. are available and can be used for the Polyhydroxyalkanoates/ Polyhydroxybuterates (PHA/PHB) production out of which the crude glycerol obtained from the biodiesel production process is been particularly focused.
Recently, much work has been done using biodiesel-derived waste glycerol for PHA/PHB production by Cupriavidus necator JMP134, Paracoccus denitrificans (Mothes et al., 2007), Cupriavidus necator DSM.545 (Cavalheiro et al., 2009), Bacillus sonorensis, Halomonas hydrothermalis (Shrivastav et al., 2010), Halomonas sp. KM-1 (Kawata and Aiba, 2010), osmophilic organism (Koller et al., 2005), Pseudomonas oleovorans NRRL B-14682 and Pseudomonas corrugate 388 (Ashby et al., 2014) from different sources of biodiesel feedstock (Jantima TEEKA et al 2010).

Looking into the problems associated with synthetic plastics and biodiesel derived crude glycerol, advantages of biodegradable plastics/ bioplastics over synthetic plastics and the potentiality of microorganisms in utilizing biodiesel derived crude glycerol and producing the bioplastic, the present work was undertaken with following objectives.

**Objectives**
1. Collection of marine samples.
2. Isolation of glycerol utilizing organisms.
3. Screening for PHA/PHB producers using biodiesel derived crude glycerol as carbon source.
4. Quantification of PHA/PHB from screened isolates.

**Materials and Methodology**

**Isolation of marine organisms utilizing biodiesel derived crude glycerol:**
Isolation of marine organisms was done by using standard microbiological techniques like serial dilution on sea water agar medium supplemented with 1% (V/V) crude glycerol as a carbon source, the crude glycerol was pretreated prior to its usage. The isolates were screened based on the morphological characters and axenic cultures were obtained by subsequent sub culturing the isolates.

**Screening of PHA/PHB producing isolates:**

**Primary screening:**
The obtained isolates were further screened for their potentiality to produce PHA/PHB using staining techniques like Sudan Black B and Nile blue a staining (Ostle and Holt, 1982). The Sudan Black B stain, a lipophilic stain used to stain the lipid granules, helping in differentiating the PHA/PHB producers and non producers. The Nile Blue, which particularly stains the PHA/PHB granules within the cell, illuminating bright orange fluorescence under UV light of 460nm wavelength.

**Secondary screening:**
The isolates showing potentiality are being further subjected to secondary screening which are being done using fluorescence microscopy, the specimen preparation for the microscopy are prepared as per the protocol prescribed by (Ostle and Holt, 1982) and further confirmation of PHA/PHB are being done using higher end technique like FTIR.

**Quantification of PHA/PHB from screened isolates:**
Quantification of PHA/PHB are being done using spectroscopic method using chrotonic acid as internal standard as per protocol prescribed by Giin- Yu Amy Tan et al, (2014).
Results

Collection of marine samples
The marine soil samples were collected from coastal areas of Maharashtra, Goa & Karnataka. The biodiesel derived crude glycerol was obtained from Biofuel Information and Demonstration Centre, Gulbarga University, Kalaburagi.

Isolation of Marine organisms utilizing biodiesel derived crude glycerol
Based on colony morphology 35 different isolates were obtained on Sea Water Agar medium supplemented with 1% (V/V) crude glycerol. These were sub cultured and maintained as axenic cultures.

Screening of PHA/PHB producing isolates:
Primary screening:
In primary screening by Sudan Black B all 35 isolates showed positive results. In Nile Blue A staining, among 35 isolates only 10 isolates showed bright Orange fluorescence under UV light of 460nm.

Summary and Conclusion
Global interest in biodiesel as an alternative have increased during past years, which is usually produced by transesterification of tree borne oil in presence of an alcohol and a strong base, generating crude glycerol as a primary by product. As the biodiesel production has increased over the years has led to increased production of crude glycerol, which is in impure form containing large amount of methanol and sodium hydroxide which causes environmental hazard. Hence, narrowing its commercial applications.

The present work was taken up with an objective to find out an alternative usage of crude glycerol by using it as a carbon source for isolating the marine organisms and further converting it to the PHA/PHB. Thus providing a value added market to the crude glycerol. Successfully isolated 35 different isolates which could utilize the crude glycerol as carbon source, further screening with sudan black and nile blue staining revealed 10 isolates to be potent in converting the crude glycerol to the PHA/PHB.

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