Petroleum based synthetic plastics have found various industrial and domestic applications worldwide for the past seventy years due to their versatility and durability (Ojumu et al., 2004). Preliminary estimates by European market research & statistics had put plastic production to more than 230 million tons in 2005 (www.dodyplast.net). However, these synthetic plastics possess several negative attributes mainly with regard to their disposal, one being that plastics are xenobiotics showing recalcitrance to biodegradation (Flechter, 1993), production of toxic substances during incineration (Atlas, 1993) and higher waste accumulation in the landfills and marine environments. It is reported that plastic production also causes high oil consumption, e.g. about 254 million barrels of oil consumed in the US is used for plastic and chemical production (http://seekingalpha.com/article/54648-metabolix-profitable-plastic-production). The crude oil prices and natural gas prices drive the cost of synthetic petroleum derived plastics. Recently global demand for an alternative to these non-degradable petroleum derived plastics has increased tremendously. Over the past few years, bio-based plastics have been developed rapidly owing to rising petroleum prices and due to the possibility that petroleum supplies will be exhausted in the near decades (Chen, 2010). As an alternative to non-biodegradable plastics, eco-friendly biodegradable plastics can help overcome pollution problems. Increasingly, reduction of carbon dioxide emissions has become another reason for promoting bio-based plastics.

According to ASTM D 6400-99 (1976), biodegradable plastic are those plastics in which the degradation results from the action of naturally occurring microorganisms such as bacteria, fungi and algae. Bacteria and fungi play a pivotal role in the process of biodegradation in the natural world. The breakdown of materials provide them with precursors for cell components and energy for
energy-requiring processes. Biodegradation is therefore nothing more than catabolism. Biodegradable materials are usually the products of life itself. Some synthetic polymers can be microbially degraded (Pramuda et al., 1995; Steinbucl, 1992), but the process is normally slow. Most have chemical compositions resisting enzymatic attack. This is not the case for polymers of biological origin, many of which have thermoplastic properties. Biodegradable plastics (bioplastics) are mainly categorized into three main types - photodegradable, semi-biodegradable and completely biodegradable plastics. Photodegradable plastics incorporate light sensitive groups into the backbone of the non degradable polymer as additives. Extensive UV radiation can cause disintegration of their polymeric structure and makes them susceptible for further bacterial degradation (Kalia et al., 2000). Semi-biodegradable plastics include starch based plastics, protein (soybean protein) based plastics, cellulose-based plastics etc. They can also be blended with conventional plastics like polyethylene (PE), polypropylene (PP) etc. making these bio-based plastics only partially biodegradable, while the residual petroleum-based plastics remain as broken pieces, creating additional pollution. In addition, these plastics have intrinsic thermal and mechanical weaknesses, and they are now discouraged for applications (Chen, 2010).

The completely biodegradable plastic is of recent origin and promising, because of its complete utilization by microbes in nature (Reddy et al., 2003). To produce biodegradable plastics resembling conventional plastics, bacteria are employed to make the building blocks for plastic polymers from renewable sources. Polyhydroxyalkanoates (PHAs), polylactic acid (PLA), polybutylenes succinate (PBS), polytrimethylene terephthalate and polyphenylene are the best studied polymers containing at least one monomer synthesized via bacterial transformation (Chen, 2010). Except for polymerization of hydroxyalkanoates which is conducted in vivo, all other monomers are polymerized in vitro by chemical reactions. Thus the PHA is completely natural in origin (Chen, 2010)
and have been reported to be truly biodegradable in both aerobic and anaerobic environments (Page, 1995) unlike many of the “so-called” biodegradable plastics made synthetically. In nature upon disposal, they are degraded completely into water and carbon dioxide under aerobic condition and into methane under anaerobic conditions by microorganisms in different environments (Santhanam and Sasidharan, 2010).

The advantages of PHAs and other bioplastics over petroleum-based polymers are manifold. PHAs and other bioplastics are natural polymers, showing diverse characteristics ranging from thermoplastic to elastic properties. They undergo degradation in presence of microbes within 5-6 weeks and aerobic degradation process results in production of carbon dioxide and water (Brandl et al., 1990), which are environmentally friendly byproducts. The released carbon dioxide and water are absorbed during photosynthesis in nature. The synthesis and biodegradation of biopolymers are totally compatible with the carbon-cycle. The production of PHAs can be from renewable carbon resources, whereby it is unaffected by the depleting fossil fuels, or rise in crude oil prices, in turn resulting in their neutrality with regard to CO$_2$ emission, leading to conservation of finite fossil resources like mineral oil and coal. The wider use of bioplastics in daily life will solve the increasing problem of organic wastes and decrease the country’s dependence for fossil fuels (Ceyhan and Ozdemir, 2011).

Polyhydroxyalkanoates (PHAs) are considered to be strong candidates for biodegradable plastics as their material properties are similar to various synthetic plastics currently in use and also because they show complete degradation in nature (Lee, 1996). PHAs are ubiquitous in nature as they are found in bacteria, several eukaryotic cells including plant and animal tissues. PHAs are polyesters accumulated by various bacteria under unbalanced growth conditions when the carbon substrate is in excess of other nutrients such as nitrogen, sulfur, phosphorus or oxygen (Madison and Huisman, 1999; Kim and Lenz, 2001; Reddy et al., 2003). There have been reports of PHAs and their derivatives produced by and
Introduction

derived from a variety of microorganisms, over 300 different bacteria, including Gram-negative and Gram-positive species. Until recently, there were only few reports on marine PHAs producing microorganisms (Arun et al., 2009; Ayub et al., 2004; Berlanga et al., 2006; Chien et al., 2007; Lopez et al., 2008; Odham et al., 1986; Rawte and Mavinkurve, 2004; Sun et al., 1994; Weiner, 1997)

Biopolymers from marine prokaryotes, both bacteria and archaea, offer a number of novel material properties and commercial opportunities. Accumulation of PHA enhances the survival ability of microorganisms under adverse environmental conditions and the relation between PHA accumulation and stress were discussed by many researchers (Ayub et al., 2004; Kadouri et al., 2005; Lopez et al., 1995, 1998; Lopez-Cortes et al., 2008; Wang and Bakken, 1998; Zhao et al., 2007). Microbial mats being a highly diverse and challenging environment, are considered important sources for isolation of novel PHA accumulating strains (Berlanga et al., 2006; Lopez-Cortes et al., 2008; Rothermich et al., 2000).

When nutrient supplies are imbalanced, PHA accumulate as discrete granules in bacteria and act as carbon and reducing equivalents sink in microbes. This property helps bacteria to store excess nutrients invivo and the polymerization of these soluble intermediates into insoluble molecules prevents the leakage of this valuable nutrients out of bacterial cell (Peters and Rehm, 2005). For PHA accumulating bacteria, the “self-digestion” of PHAs occurs via mobilization which involves enzymes which are different from those responsible for extracellular degradation of PHAs.

PHAs and other bioplastics are biocompatible and hence suitable for medical applications. Some possible applications of PHAs and bioplastics include biodegradable carriers that demonstrate the ability to deliver drugs for a given time within the individual's body and can be used for surgical needles, suture materials, bone tissue replacement, etc. The major advantage of using biodegradable plastics is that it does not require surgical removal. Their
biocompatibility and low oxygen permeability allows for applications, like in the production of films and coatings, besides other special biomedicals like patch materials, stents, bone implants, drug delivery systems and scaffolds for tissue engineering (Ceyhan and Ozdemir, 2011). The other applications in medicine and pharmacy include osteosynthetic materials in the stimulation of bone growth, in bone plates, surgical sutures, blood vessel replacements and cardiovascular products (Chen and Wu, 2005; Galego et al., 2000; Oeding and Schlegel, 1973; Philip et al., 2007; Reddy et al., 2003; Senior and Dawes, 1973; Williams and Martin, 2002). Due to their wider range of property i.e., as thermoplastics to elastomers, they find several applications in the domestic (Glazer and Nikaido, 1994), agriculture (Hocking and Marchessault, 1994; Holmes, 1985; Dobbelaeere et al., 2001), marine (Asrar and Gruys, 2002) and industrial (Bucci and Tavares, 2005; Chen et al., 2000) fields. The biodegradability of the PHAs can be modified with the incorporation of different monomers and also by structural modifications and these modified properties can be exploited for their application in various fields.

Despite the common practice of exploiting the diversity of bacteria in the environment for the industrial production of novel compounds, there are very few reports that have explored the potential of industrial production of PHAs by bacteria (Chen et al., 2000; Reddy et al., 2003). Steinbuchel (2005) reported more than 140 different monomeric units as constituents of PHA in bacteria that contribute to the differences in the physical and chemical characteristics of PHAs, which in turn is influenced by type of microorganisms, media ingredients, fermentation conditions, modes of fermentation and the recovery process (Keshavarz and Roy, 2010). Hence, there is a need for screening large number of organisms that accumulate PHA with varied combinations of monomers, which are high yielding and with the desirable trait. Isolation of diverse PHA producing bacteria via different enrichment techniques can help to identify novel and more efficient PHA producers.
**Objectives of the study**

Vibrios are a group of gram negative, curved or straight motile rods that normally inhabit the aquatic environments. They can be found in their natural habitat as free living bacterium or in association with phyto or zoo plankton (Lipp \textit{et al.}, 2003). Being ubiquitous in the aquatic environment, free living or in association with aquatic organisms, they occur in a wide range of aquatic environments, including estuaries, marine coastal waters and sediments, and aquaculture settings worldwide (Barbieri \textit{et al.}, 1999; Heidelberg \textit{et al.}, 2002; Thompson \textit{et al.}, 2001; Urakawa \textit{et al.}, 2000; Vandenberghe \textit{et al.}, 2003; Venter \textit{et al.}, 2004). Halophilic vibrios represent as much as 40\% of the total microbiota of subtropical coastal water (Chan \textit{et al.}, 1986). Currently the genus \textit{Vibrio} consists of 51 species of which at least 12 are known to be associated with human diseases.

Vibrios were among the first reported strains of PHAs producers from marine sediments (Baumann \textit{et al.}, 1971; Oliver and Colwell, 1973) and were reported to be a dominant flora among the commensally heterotrophic bacteria in Cochin backwaters and near-shore areas of west coast of India (Chandrasekaran \textit{et al.}, 1984). Boyandin \textit{et al.} (2008) identified the ability of luminous bacteria of different taxa including \textit{Vibrio} sp. to synthesize PHAs as storage macromolecules. PHA accumulation in \textit{V. harveyi} (Boyandin \textit{et al.}, 2008; Sun \textit{et al.}, 1994), \textit{Vibrio} sp. strain MK4 (Arun \textit{et al.}, 2009), \textit{Vibrio} sp. BM-1 (Wei \textit{et al.}, 2011b), \textit{V. natriegens} (Chien \textit{et al.}, 2007), \textit{V. fischeri} (Boyandin \textit{et al.}, 2008) and \textit{V. neries} (Rawte and Mavinkurve, 2004) were reported. Optimisation of different bioprocess variables influencing PHA production in \textit{Vibrio} sp. were discussed by several workers (Arun \textit{et al.}, 2009; Chien \textit{et al.}, 2007; Rawte and Mavinkurve, 2004; Wei \textit{et al.}, 2011b). Sun \textit{et al.} (1994) discussed the role of lux autoinducer, N-(3-hydroxybutanoyl) homoserine lactone, in the regulation of PHB synthesis in
**Objectives of the study**

V. *harveyi*. It was interesting that majority of the reported PHA accumulating vibrios were sampled from marine environments (Arun *et al.*, 2009; Chien *et al.*, 2007; Rawte and Mavinkurve, 2004; Wei *et al.*, 2011b).

The present study therefore aimed at evaluating the occurrence of PHA accumulating vibrios inhabiting marine benthic environments; characterizing the potential PHA accumulators employing phenotypic and genotypic approaches and molecular characterization of the PHA synthase gene. The study also evaluated the PHA production in *Vibrio* sp. strain BTKB33, through submerged fermentation using statistical optimization and characterized the purified biopolymer.

**The specific objectives are**

1. **Screening for PHA producing vibrios from marine benthic environments.**
2. **Characterization of PHA producers employing phenotypic and genotypic approaches.**
3. **Optimization of bioprocess variables for PHA production by strain BTKB33 by submerged fermentation (SmF).**
4. **Characterization of the PHA produced by strain BTKB33.**