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Full Length Research Paper

Utilization of various industrial wastes for the production of poly-β-hydroxy butyrate (PHB) by *Alcaligenes eutrophus*

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Considering the industrial interest of poly-β-hydroxy butyrate (PHB) and its high production cost, work has been undertaken for the production of PHB by *Alcaligenes eutrophus*. Different industrial wastes (malt, soya, sesame, molasses, bagasse and pharmaceutical waste) were used as a cheap substrate to minimize the production of cost and nitrogen limited minimal agar synthetic medium was also used for comparison. Accumulation of PHB granules in the organism was analyzed by sudan black method. The PHB production in various industrial waste based medium and nitrogen limited minimal agar synthetic medium was studied by crotonic acid method. The pure form of PHB was collected and qualitatively analyzed by infrared and nuclear magnetic resonance methods. Highest PHB production was found in nitrogen limited minimal agar synthetic medium. Among the various industrial wastes based media, highest yield was obtained with sesame oil waste as carbon source.

Key words: Poly- β- hydroxy butyrate, PHB, *Alcaligenes eutrophus*.

INTRODUCTION

Plastics, due to their versatile qualities of strength, lightness, durability and low production cost, have now become the essential ingredients to enhance the comfort and quality of our life. Plastic materials have become an integral part of contemporary life because of their many desirable properties including durability and resistance to degradation. The current global plastic industry turn over is about $1 trillion/year and represent 3.7% of the world GDP. These non degradable plastics accumulate in the environment at a rate of millions of ton per year causing several problems. Recently, issues concerning the global environment and solid waste management have created much interest in the development of biodegradable plastics (Anderson and Dawes, 1990). Poly-β-hydroxyl butyrate (PHB) is the best-known poly hydroxy alkanoate.

PHB is the alternative source of the plastics which has similar physical properties like polypropylene and it can be easily biodegradable aerobically and anaerobically (Hankarymer and Jieerde, 1998; Luzier, 1992). PHB is the storage polymer separated from cytoplasm. β-Hydroxyl butyrate is connected by ester linkage and form PHB. *Alcaligenes eutrophus* is the prime PHB producer that accumulates PHB up to 80% of its dry weight (Doi et al., 1987). This bioplastic has many obvious applications in bone plates, nails, screws (Azehar et al., 2003) and in the treatment of osteomyelitis (Fusun and Zeynep, 2000)

The current cost of the PHB production is considerably more than that of the synthetic plastics (Byrom, 1987). In biotechnological aspects, cheap substrate and genetically modified high PHB yielding bacteria (or) plants can be used in this biopolymer production technique. The present study attempts the production of PHB using various

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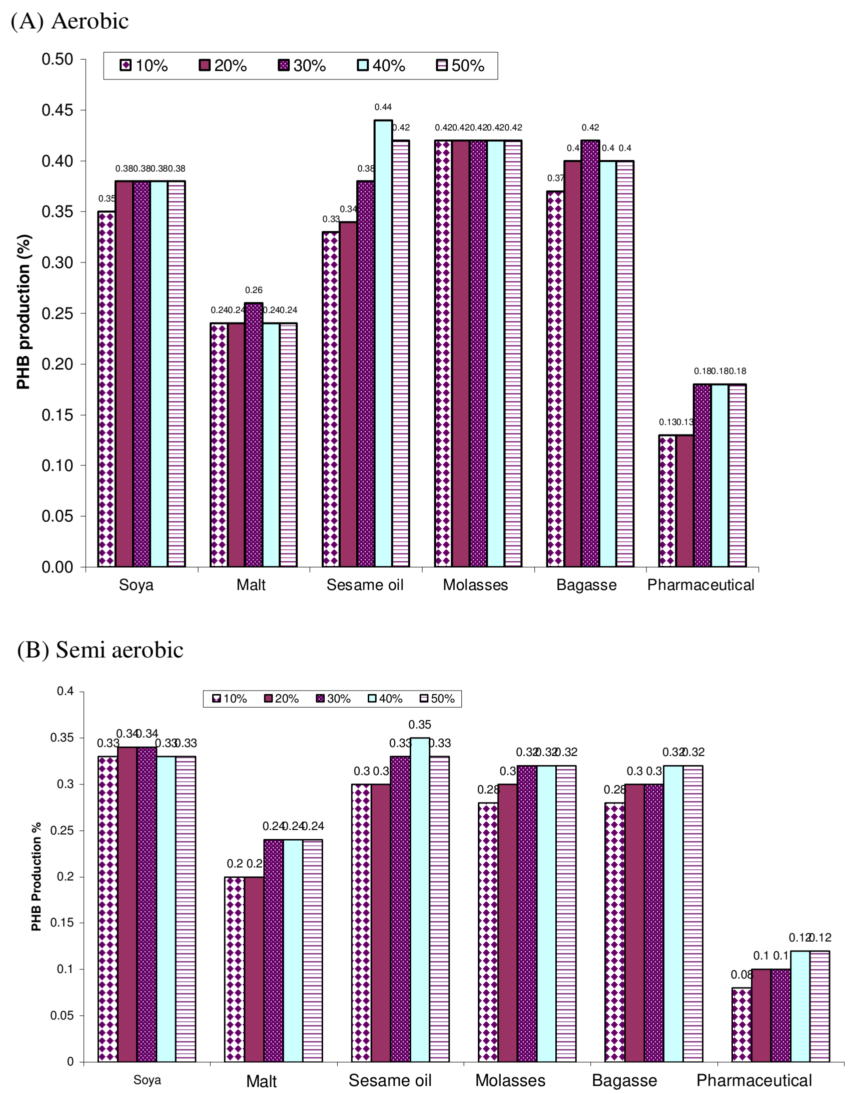

Figure 1. PHB Production by *Alcaligenes eutrophus* in different industrial waste as sole carbon source with nitrogen limited minimal broth synthetic medium (NLMM).

industrial wastes (malt, soya, sesame, molasses, bagasse, and pharmaceutical waste) as the carbon source by using *A. eutrophus* to minimize the production cost of the PHB.

MATERIALS AND METHOD

Organism

Alcaligenes eutrophus MTCC1285 was obtained from the Microbial type culture collection, Chandigarh, India. The PHB producing capability of the organism was confirmed by Sudan black staining method (Kitamara and Doi, 1994).

PHB production and extraction

Six different industrial waste substrates such as (malt, soya, sesame, molasses, bagasse and pharmaceutical waste) were collected from industries and were used for the PHB production in different percentage (10, 20, 30, 40 and 50%). The PHB production by *A. eutrophus* on different industrial wastes [as sole carbon source in nitrogen limited minimal broth synthetic medium (NLMM)] were compared with PHB produced in nitrogen limited minimal broth synthetic medium (Lee and Choi, 1999) under aerobic and semi aerobic conditions. *A. eutrophus* was grown at 37°C for 72 h. For aerobic condition, it was incubated in shaker and for semi aerobic condition they were incubated without shaking. PHB produced were extracted as described in the method of Ramsay et al. (1994).

Estimation and qualitative analysis of PHB

The amount of PHB in the extracted samples was determined spectrophotomertrically at 235 nm (Lee et al., 1995; Law and Slepecky, 1960). The pure form PHB was collected (Lee, 1996) and qualitatively analyzed by infrared method (Silversteine et al., 1981) and by NMR method (Bernard et al., 1989).

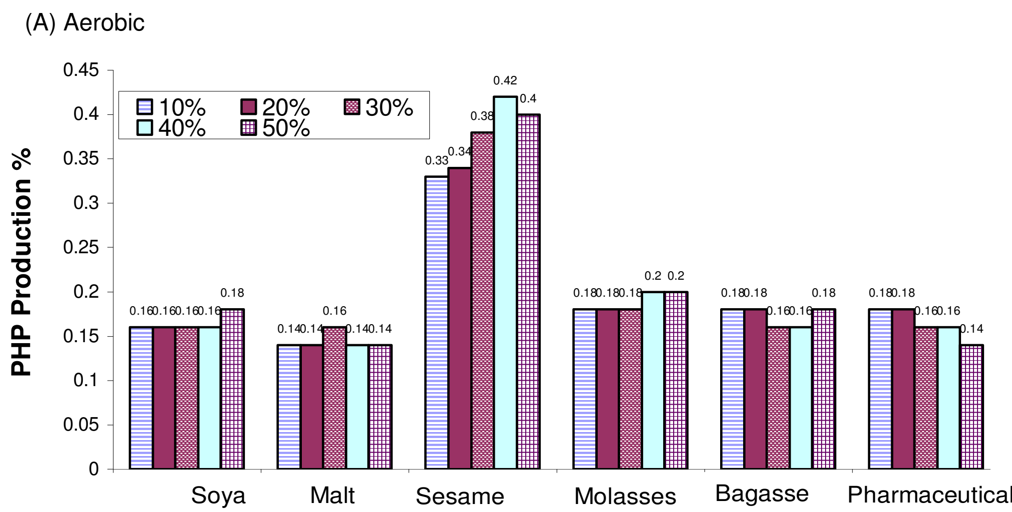

Figure 2a. PHB Production in different industrial waste as such(A) Aerobic.

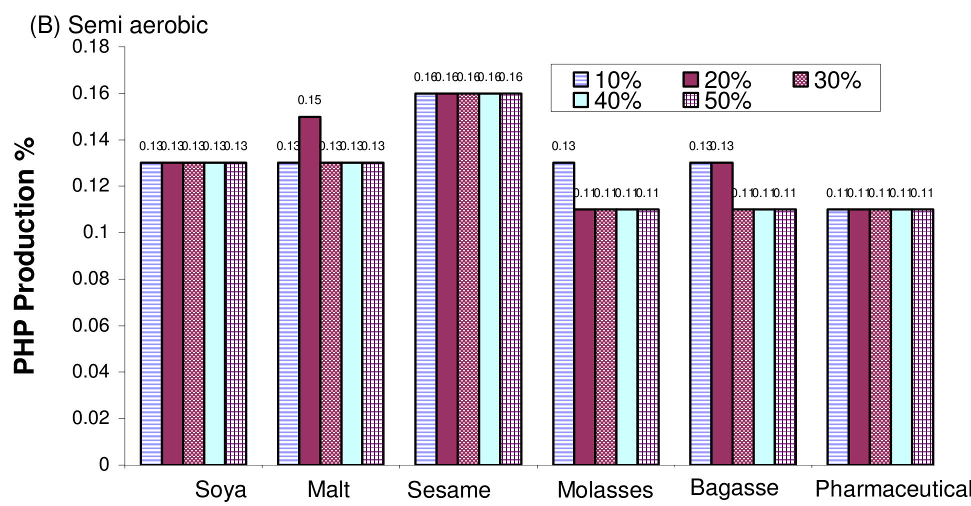

Figure 2b. PHB production in different industrial waste as such (B) Semi aerobic

RESULT AND DISCUSSION

The use of PHB as a substitute for non biodegradable petroleum based plastics cost substantially more than its fossil fuel based counterparts and offer a no performance advantage other than biodegradability. To minimize the cost of PHB production, different industrial wastes were used in the present study. Maximum PHB production by *A. eutrophus* observed was in the synthetic medium (0.44%) and also in 40% sesame oil medium under aerobic condition. Haywood et al., 1989 also reported PHB production by *Pseudomonas oleovorans* grown in two phase medium containing 50% (v/v) octane n-alkanoic acids and C_8 to C_{10} alcohols. In the present study PHB production was also found to be influenced by the dilutions of the industrial wastes. Of the different dilutions of the industrial wastes used (as sole carbon source with NLMM), high level of production of PHB was observed under aerobic condition using 40% sesame oil (0.44%) followed by 10% molasses waste (0.42%), 30% bagasse (0.42%), 50% soya (0.38%) and 30% malt (0.26%). Lowest PHB production was observed with pharmaceutical waste (0.13 - 0.18% with different dilutions). Under semi aerobic condition the high level of PHB production was observed in 40% of sesame oil (0. 35 %) followed by 30% soya waste (0. 34%), 50% molasses (0.32%), 10% bagasses (0.28%) and 30% malt (0.24%). Lowest PHB production was also observed with pharmaceutical waste (0.08 - 0.12% with different dilutions) (Figure 1).

The PHB production in industrial waste without NLMM is recorded in Figures 2a and 2b. In that, under aerobic condition the maximum level PHB production was observed in 40% of sesame oil (0.42%) followed by 50%

of molasses waste (0.20%). Lowest PHB production was observed with malt waste (0.14 - 0.16% at various dilutions). In semi aerobic condition the maximum PHB production was observed in 40% of sesame oil (0.16%) followed by 20% of malt waste (0.15%). While lowest PHB production was observed with pharmaceutical waste (0.11% with different dilution).

The IR spectrum analysis of the PHB product clearly reveals its purity. The IR absorption peaks of the product ranges from 2924 to 1057 cm^{-1}. The absorbance peak values obtained were compared with the available literature values and they confirm the product as PHB. The peak values obtained in this study coincides with previous results (Ramsay et al., 1994). The product obtained in this study was further subjected to NMR spectrum analysis. The NMR spectral data (not shown) of the product obtained indicates that the six protons as –CH, $-CH_2$ and $-CH_3$ respectively, which also confirm the product obtained as PHB.

In conclusion, this study not only gives information on PHB production by *A. eutrophus* but also demonstrate the utilization of industrial wastes as an alternative for an efficient production of PHB.

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