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Production of Polyhydroxybutyrate using agro-industrial waste by *Psuedomonas aeruginosa*

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ABSTRACT: Plastics have been an integral part of our life. However, disposal of these non-biodegradable (petrochemical derived) plastics poses a threat to our environment. Thus, much interest has been gained in developing biodegradable plastics. Polyhydroxybutyrates (PHBs) are polymers that accumulate as carbon/energy in microbial cells and provide an alternative to petrochemical plastic because of their biodegradability properties. However, major problems in commercializing PHB is the high production cost due to expensive carbon substrates. Therefore, the applications of cheap carbon sources have been explored. In this study*, P. aeruginosa* has ability to convert agro-industrial waste like Rice husk, Cotton seed husk, Walnut shell and Corn cob meal in to PHB. Highest cellular PHB content was obtained from Rice husk as source of carbon which was found to be 0.26 g/L. As Rice husk is agro-industrial waste its use in PHB production may prove beneficial, and hence may become an area of further research.

KEYWORDS: PHB, *Psuedomonas aeruginosa*, Agro-industrial waste, Rice husk, Crotonic acid.

INTRODUCTION

Today's world is almost unimaginable without plastics, but most of these materials have historically been derived from oil and face potential problems with increasing fossil fuel costs, potential scarcity and customer demands for alternatives. These concerns have helped generate new research and development of alternative raw materials for use in Bioplastics. In this experiment agro-industrial waste like Rice husk, Cotton seed husk, Walnut shell and Corn cob meal were used as a carbon source and PHB production were carry out. Moreover, the use of renewable sources in their manufacturing plays a key role in maintaining environment health. There are many advantages of bioplastics over conventional plastics such as reduced dependence on fossil fuels, non toxic, easier to recycle, require less energy to produce, renewable and eco-friendly. Earlier studies showed that Polyhydroxybutyrate (PHB) is bio-degradable material, which has physical properties similar to the synthetic plastic. PHB is synthesized by bacteria under unbalanced growth conditions. Some bacteria have been reported capable to produce PHA as much as 90%(w/w) of dry cells during depletion of essential nutrients such as nitrogen, phosphorus or magnesium(**Madison and Huisman, 1999)**.

MATERIALS AND METHODS

SCREENING FOR PHB PRODUCTION

PHB producing bacteria was detected using the lipophilic stain Sudan black staining. Sudan black stain was prepared as a 0.3% solution(w/v) in 60% ethanol. Smears of PHB producing bacteria were prepared on glass slides and heat fixed. Samples were stained for 10min with Sudan black solution, rinsed with water and counter-stained with 0.5% safranin for 5s. Stained samples were observed under oil immersion at 1000x magnification with direct bright-field illumination **(Burdon et al., 1942)**.

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USE OF DIFFERENT AGROINDUSTRIAL WASTE

Rice husk powder, Cotton seed husk powder, walnut shell powder and corn cob meal were obtained from surat city.

DILUTED ACID HYDROLYSIS OF SOLID AGRO-INDUSTRIAL WASTE

PHB production by *Psuedomonas aeruginosa* was tested using hydrolyzed wastes. Rice husk powder, Cotton seed husk powder, walnut shell powder and corn cob meal were hydrolyzed by 2.5% v/v sulphuric acid **(Buhner and Agblevor, 2004)** and autoclaved at 121°C and 15 psi for 30 min. The hydrolyzed samples were filtered and supernatants were neutralized using sodium hydroxide(6N). Reducing sugar content was determined using DNSA method **(Miller, 1959)**. The Basal media Broth (Yeast extract 1g/L, Peptone 5g/L, Di-Sodium hydrogen phosphate 1g/L, Magnesium sulfate 0.2g/L, Glucose 10gm/L) were prepared using this hydrolysate instead of glucose. 10%v/v and 24h pre-grown culture of *Psuedomonas aeruginosa* was inoculated and incubated at 37°C for 48-72h on shacking incubator **(Santimano et al., 2009)**.

EXTRACTION OF PHB

After incubation samples were centrifuge for 15min at 6000rpm. The pellet were washed twice with sterile deionized water and dried for 24h at 60°C. The total bacterial dry weight was determined. Sodium hypochlorite was added to dry cell biomass and was incubated at 37°C for 2h. to break the cell wall of bacteria. These samples were centrifuge at 6000rpm for 15min and supernatant was treated further. Using 96%v/v ethanol:acetone(1:1) cell lipid and other molecules except PHB were extracted from supernatant. The extraction of PHB done by hot chloroform (adding chloroform in waterbath). Crystals of PHB obtained after evaporation of chloroform **(Belma et al., 2002)**.

DETERMINATION OF PHB

PHB crystals undergo dehydration on treatment with sulphuric acid and heat to produced crotonic acid. The extracted PHB was converted to crotonic acid by adding 98% sulphuric acid and heating to 60°C for 1h. Crotonic acid shows maximum absorption at 235nm. The absorbance of the solution was measured at 235nm in a UV spectrophotometer against a sulphuric acid as blank. The amount of PHB per gram dry weight of bacterial cells was determined using standard curve of PHB using crotonic acid **(Belma et al., 2002)**.

RESULTS AND DISCUSSION

The Sudan black B stained slide was observed under compound microscope with oil immersion lens. Then dark black condensed molecules of PHB were observed inside cell surrounding pink cytoplasm **[Figure : 1]**. The crystal of PHB obtained after evaporation of chloroform. The PHB crystal was dried in oven and measured the dry weight of PHB production. The sensitivity quantification of PHB content was determined by crotonic acid assay. The PHB yield and biomass concentration increased during 48h and highest PHB content obtained from Rice husk **[Table : 1] [Graph : 1]**.

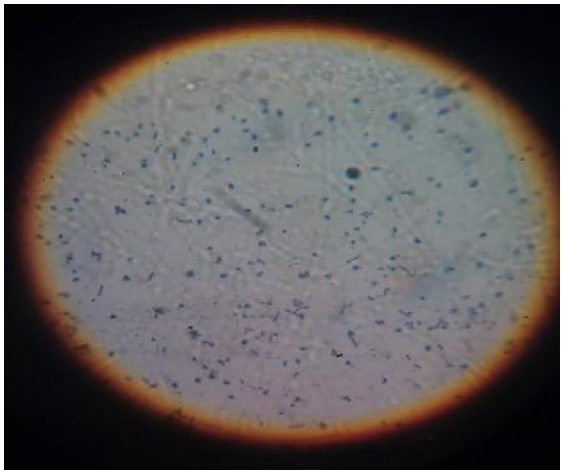
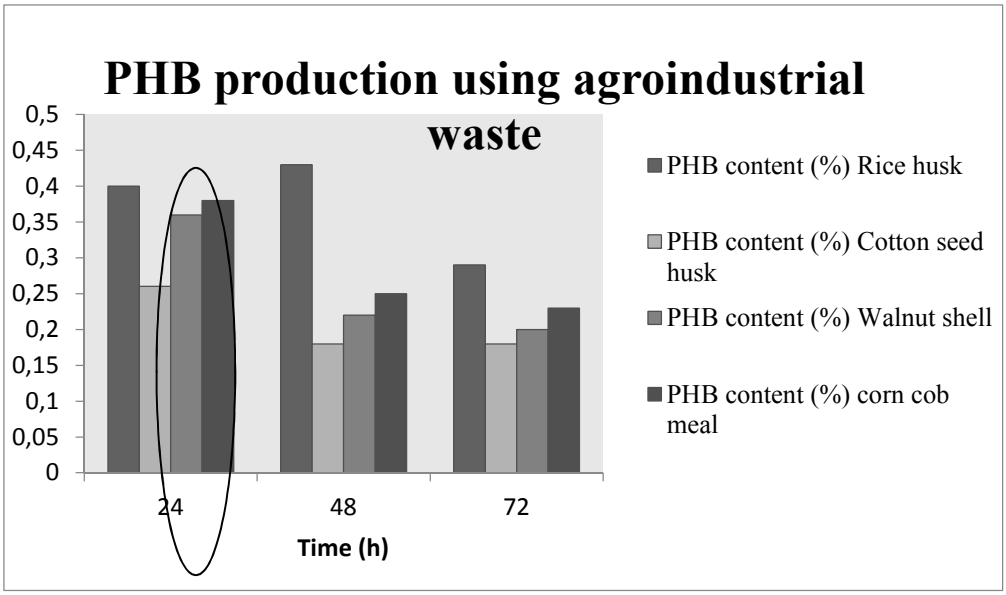


Figure : 1 Sudan black staining of PHB crystal

Table : 1 PHB production using agro-industrial waste

Agro-industrial waste	Time (h)	Cell biomass (dry weight) [g/10mL]	Dry weight PHB production [g/10mL]	PHB concentration by crotonic acid test [g/10mL]	PHB content (%)
Rice husk	24	0.50	0.1479	0.0020	0.40
	48	0.83	0.2003	0.0026	0.43
	72	0.79	0.1731	0.0023	0.29
Cotton seed husk	24	0.49	0.1135	0.0013	0.26
	48	0.95	0.1880	0.0018	0.18
	72	0.92	0.2536	0.0017	0.18
Walnut shell	24	0.50	0.1845	0.0018	0.36
	48	0.83	0.1942	0.0022	0.22
	72	0.70	0.0347	0.0014	0.20
Corn cob meal	24	0.68	0.2048	0.0022	0.38
	48	0.89	0.2290	0.0023	0.25
	72	0.84	0.2070	0.0020	0.23



Graph : 1 PHB production using agro-industrial waste

CONCLUSIONS

It was observed that Rice husk was best feedstock among the other crude source used in this experiment. But also other agro-industrial waste like cotton seed husk, walnut shell and corn cob meal can also produce PHB in significant amount. This can be exploited for the production of PHB at commercial level. Utilisation of agroi-ndustrial materials in production of biodegradable plastic (PHB) will not only ensure the low production cost but also solve the problem of management of waste material to a certain level.

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