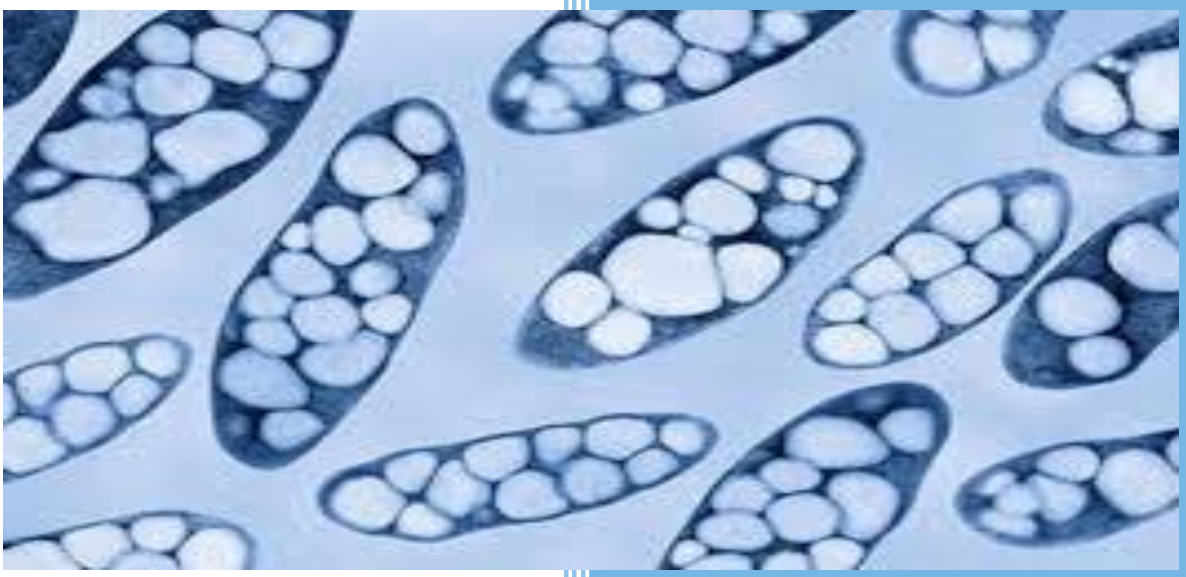
Optimal Medium Composition to Enhance Poly-βhydroxybutyrate Production by *Ralstonia eutropha* Using Cane Molasses as Sole Carbon Source



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JOURNAL: APPLIED FOOD BIOTECHNOLOGY, 2015, 2(3):39-47

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RATIONALE OF THE WORK

The main aim of this experiment was to reduce the costs associated with poly-B-hydroxybutyrate production and growth and poly-β-hydroxybutyrate production of *Ralstonia eutropha* studied in batch culture on different carbon sources. Instead of using expensive pure chemicals in this experiment - low cost, abundant carbon sources, including cane molasses, beet molasses, soya bean, and corn steep liquor were used. Results revealed that cane molasses with production of poly-β-hydroxybutyrate was selected as the most efficient carbon source. Various different chemicals were used to pre- treat the cane molasses in order to improve the bacterial growth and poly-B-hydroxybutyrate production. Highest bacterial growth and PHB concentration were recorded when the molasses was pre-treated with sulphuric acid. To make the process more efficient and effective urea and corn steep liquor were used. Using the response surface methodology and through a 2n factorial central composite design, the medium composition was then optimized, and maximum biomass concentration and poly-β-hydroxybutyrate concentration were obtained.

METHODS ADOPTED

*Ralstonia eutropha,*the PHB accumulating bacteria were maintained as stock culture on LB Agar slants at 4^{o}C. These bacteria were then tested for PHB production using Sudan black staining method and then inoculated at 30^{o}C for 24 hr. The four carbon sources used were- **cane molasses, beet molasses, soya bean** and **CSL** along with urea and CSL as, nitrogen and vitamin sources. pH of which was maintained at 7, all steps being carried out in sterile environment. Soya bean extract prepared by drying, grounding and oil extraction. Cane molasses was pre-treated by dilution with distilled water, sulphuric acid for maintaining pH, tricalcium phosphate and EDTA for precipitation of heavy metals. **Analytical methods** used were- **Dry cell weight**, for analysis of total biomass via filtration and **PHB Measurement** by spectrophotometry and chemical methods followed by development of a standard curve using pure PHB. Also, **RSM (Response Surface Methodology**) was used for calculating nutrient concentrations as independent variables along with **CCD** and **MINITAB software**. Experiment was done in triplicate, results were imported to software followed by generation of surface plots to understand interactions of the carbon sources and determine optimal composition for PHB production.

RESULTS OBTAINED

Poly-b-hydroxybutyrate production by *Ralstonia eutropha* using Cane Molasses as Sole carbon source was studied. Experiments were done not only to lower the cost of PBH by using inexpensive substrates but also to increase PBH production by optimizing the culture medium composition. The preliminary studies state that media with carbon sources the maximum PBH concentration could be achieved by 120h.The maximum biomass concentration and PBH content were obtained with cane molasses as the sole carbon source. The soya beans turned to be second best compound. PBH content was much lower as compared to cane molasses. Minimum content was obtained in CSL. Therefore, cane molasses with maximum PBH and biomass yield was used in rest experiment.

DISCUSSION

In this research article, production of poly-ß-hydroxybutyrate (PHB) by *Ralstonia eutropha* under various carbon sources were studied. Several experiments were carried out to enhance the production of PHB. Expensive pure chemicals were replaced with domestic low-cost and renewable resources (carbon sources) like beet molasses, cane molasses, soya bean and corn steep liquor. Urea and corn steep liquor were used as nitrogen, vitamin and mineral sources. Experiments revealed that the highest production/ increased production of PHB was achieved by using cane molasses as the sole carbon source. Further improvements were done with cane molasses so as to improve bacterial growth and PHB production. After several trial and errors, sulphuric acid treatment was seen to be as the most efficient method in eradicating interruptive heavy metals and other suspended impurities from cane molasses. Response surface methodology was used to optimize the medium composition that was associated with enhanced PHB production. Comparison of new composition with the previous one revealed that the new ones were not capable of stimulating PHB synthesis in *Ralstonia eutropha* indicating that the improved PHB concentration was only due to increased number of cells in the optimal medium. This article concluded with the need for further studies and investigations to assess how PHB synthesis can be stimulated in *Ralstonia eutropha* with cane molasses as the sole carbon source.

CONCLUSIONS

Poly hydroxy butyrate production costs can be effectively reduced by replacing the use of pure expensive chemicals with other low cost naturally available renewable resources such as cane molasses, beet molasses, and soya bean and corn steep liquor. These resources are carbon based and as a result help in the production of PHB by *R.eutropha.* Also, urea and corn steep liquor are nitrogen, vitamin and mineral based sources. Through preliminary tests it has been seen that the highest biomass and PHB concentrations could mainly be obtained by cane molasses. Prior to addition of cane molasses to the culture media it was being treated differently to improve bacterial growth and PHB production. The results showed that using sulphuric acid treatment was selected as most efficient method to remove the undesired particles and impurities. Alongside RSM was used to optimise the medium composition thus enhancing the PHB production. About 58% and 41% improvements were observed by the use of optimal media, in biomass and PHB concentrations respectively. The new composition was not able to stimulate PHB synthesis in *R.eutropha*, rather enhanced PHB production is related to higher the number of cells in the optimal media. Although industrial scale biotransformation of low cost renewable resources is viable, further studies are required to assess the production of PHB using cane molasses as the sole carbon source.

SCOPE

Due to the expensive raw materials available, agroindustrial wastes are already being used. Response surface methodology (RSM) is used to assess the optimal concentration of nutrient sources to enhance PHB production in shake flask cultures. RSM is used to find a statistical model to determine the optimum concentration of nutrient sources in culture media independent variables. A 2n factorial Central Composite Design (CCD) is established and MINITAB software (Minitab®Inc. v17) is used to develop a model to optimize the concentration of the components. Improved media composition and cultivation methods, as well as more efficient downstream processes have been developed to make PBHB production economically viable and commercially acceptable

OPINION ABOUT THE PAPER

The research article emphasized upon low cost, poly-β-hydroxybutyrate production of *Ralstonia eutropha* and how the production could be increased by optimizing the culture medium composition. Their study based on different carbon sources used; provided an idea on using naturally renewable carbon sources such as cane molasses, beet molasses, soya bean, and corn steep liquor against other chemical sources. It also provided an overview on how the efficiency of production could also be increased by using a variety of treatments throughout the production course. Overall, the research article was very resourceful as efficient production of compounds like poly-β-hydroxybutyrate being the need of the hour, as sources of biodegradable plastics.