STRATEGIES FOR OPTIMIZATION AND PRODUCTION OF BIODEGRADABLE POLYMER POLY (β-HYDROXYBUTYRATE / VALERATE) BY WAUTERSIA EUTROPHA

by

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to the

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'degradable polymers (2) Hydroxybutyrate

Dedicated to my father

Late Mr. O. R. Khanna

He would have been proud……

CERTIFICATE

This is to certify that the thesis entitled "**Strategies for optimization and production of biodegradable polymer Poly (β-hydroxybutyrate) by *Wautersia eutropha***" being submitted by **Ms. Shilpi Khanna** to the Indian Institute of Technology, Delhi, for the award of degree of "Doctor of Philosophy", is a record of the bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with the rules and regulations of Indian Institute of Technology, Delhi. The results contained in it have not been submitted in part or full to any other university or institute for award of any degree / diploma.

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Only when I started with my research, I realized the hard work that goes into making of a Ph. D. thesis. The subject was new; the work seemed to be never ending. The reactors looked like big monsters. The hours that were needed in running of the reactors were long and tiring..., ... but the results made me work harder. The hard work paid off and now finally this day has arrived when I am writing the last part of my thesis.

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Shilpi Khanna

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ABSTRACT

Increasing concern about environmental pollution caused by waste petrochemical plastics has led to extensive research on biodegradable polymers such as poly(hydroxyalkanoates) (PHAs). These PHAs (represented mostly by poly(3-hydroxybutyric acid) (PHB) and poly(3-hydroxybutyric-co-3-hydroxyvaleric acid) [P(3HB-co-3HV)]), are accumulated as energy reserve materials by a variety of microorganisms under unbalanced cultivation conditions i.e. limitation of some essential nutrient and excess carbon source. An additional carbon source (mostly organic acid) is needed for the production of derivatives of PHB e.g. P(3HB-co-3HV). However, PHB is a homopolymer of (*R*)-3-hydroxybutyric acid (3HB) whereas P(3HB-co-3HV) is a random copolyester of (*R*)-3-hydroxybutyric acid (3HB) and (*R*)-3-hydroxyvaleric acid (3HV). These polymers are considered to be a strong candidate as a replacement to conventional plastics because they possess material properties similar to various synthetic thermoplastics currently in use (polypropylene, synthetic rubber) and can be completely degraded upon disposal, by microorganisms in various environments such as soil, sea, lake water and sewage. The industrial production of these polymers requires an improvement of yield and productivity so as to make them economically comparable with the production cost of conventional plastic material.

In order to improve the growth and PHB production, *Wautersia eutropha* was used. This strain has been reported to accumulate PHB upto 50-70% of its weight.

Growth of *W. eutropha* NRRL B14690 with media reported in literature resulted in a maximum biomass of 3.3 g/L with a PHB concentration and productivity of 1.4 g/L and 0.03 g/L.h, respectively. To establish the nutrient uptake capacity of *W. eutropha*, it was

grown on different carbon, nitrogen and mineral sources. Fructose, urea and corn steep liquor were found to be most suitable carbon, nitrogen and mineral sources for the growth of *W. eutropha* and PHB production. Statistical media optimization design was then used to optimize the composition of culture medium for maximizing the productivity of PHB. A significantly higher maximum biomass of 19.7 g/L with a PHB concentration and productivity of 10.9 g/L and 0.18 g/L.h, respectively was obtained in a 7 L lab scale bioreactor using the optimized media composition (fructose: 40 g/L, KH_2PO_4: 1.5 g/L, Na_2HPO_4: 4 g/L, $MgSO_4.7H_2O$: 0.51 g/L, urea: 1 g/L, CSL: 0.5 g/L, trace metal solution: 10 mL/L, $CaCl_2$: 0.02 g/L).

The batch kinetic data was eventually used for developing a mathematical model. The kinetic parameters of the model were identified by original batch kinetic data. The statistical validity of the model to graduate the batch experimental kinetics was demonstrated with an accuracy of 95% using 'F' test. The model was extrapolated for fed-batch cultivation and offline computer simulation of the model was carried out to develop suitable nutrient feeding strategies. Various nutrient feeding strategies like constant feed rate of carbon and nitrogen, varying (decreasing) feed rate of nitrogen and fructose and alternate feeding of fructose and nitrogen were adapted. Of these, alternate feeding of the nutrients was the best and easiest to implement giving a total biomass of 39.2 g/L with a PHB content of 18.46 g/L in time span of 40 h. The volumetric productivity obtained was 0.5 g/L.h.

In order to overcome the problem of volume limitation encountered in fed-batch cultivation, the production of PHB was carried out by repeated batch cultivation in which 20% (v/v) of the fermenter broth was removed and supplemented with an equal volume

of fresh media at different cultivation times. The time of fresh feeding was decided on the basis of off-line residual fructose analysis. Thus, the reactor volume was maintained constant throughout the experiment. A total of 49 g/L biomass with a PHB concentration of 25 g/L was obtained in 67 h cultivation.

Thereafter, high cell density cultivation of the culture was carried out under continuous mode of operation. The reactor was converted to fed-batch mode of cultivation towards the end of the batch cultivation. The fed-batch cultivation conditions were simulated offline by the model developed earlier. The fed-batch cultivation was carried out in order to obtain a high biomass and PHB concentration. Then it was switched to continuous mode so that the biomass and PHB obtained can be maintained at that concentration continuously. The reactor was operated at a dilution rate of 0.1 h^{-1} by feeding a media containing 90 g/L fructose and 2.5 g/L nitrogen. After a transient period, steady state biomass of 27.7 g/L with a PHB concentration of 5.5 g/L was obtained. The overall productivity of the system was 0.55 g/L.h.

Shake flask cultivation for the production of P(3HB-co-3HV) was investigated by addition of different organic acids. Of all the organic acids used, high production of this copolymer of PHB was obtained with propionic acid and valeric acid. Of the two acids, addition of valeric acid yielded a higher HV content. Thus, fed-batch cultivation with the addition of valeric acid was conducted in a 7 L bioreactor. The feeding of valeric acid was started after 20 h of batch growth. As soon as valeric acid feeding was started, accumulation of HV units started in the polymer chain. Maximum %HV content of 54% was obtained after 40 h of fermentation. A total biomass of 15 g/L containing 7.5 g/L PHAs was obtained after 72 h of cultivation.

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