STUDIES ON THE BIOCONVERSION OF RENEWABLE FEEDSTOCK TO 1,3-PROPANEDIOL

by

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Consecrated to God

CERTIFICATE

This is to certify that the thesis entitled "**Studies on the bioconversion of renewable feedstock to 1,3-propanediol**" being submitted by **Ms. Guneet Kaur** 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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ABSTRACT

1,3-propanediol (1,3-PD) is a versatile organic chemical of immense importance particularly for polycondensation reactions to synthesize polyesters, polyethers and polyurethanes. It has been a matter of increasing interest in the recent past due to its usage as a monomer for a new polyester polytrimethylene terephthalate (PTT) which has many interesting properties superior to the conventional polyesters such as PET and nylon. PTT has myriad applications such as clothing, fibers, automobile lining and carpeting. It is also used in the production of engineering thermoplastics, biodegradable films and coatings. Besides this, 1,3-PD can be formulated into solvents, adhesives, laminates, moulds, resins, cosmetics, detergents and medicines. Production of this valuable metabolite by fermentation is particularly important as it utilizes a renewable resource e.g. glycerol, surplus amounts of which are being generated as a 'waste' by-product by the burgeoning biodiesel industry throughout the globe. Valorization of waste glycerol provides a renewable, clean and environmentally favorable route to 1,3-PD while increasing the economic viability and sustainability of the biodiesel plant.

Inhibition from both substrate and product reduces the overall growth and product formation rates in 1,3-PD fermentation and represents the major bottleneck in the biotechnological production of 1,3-PD. Thus the objective of the present study was to optimize glycerol bioconversion to 1,3-PD using *Clostridium diolis*. *Clostridium diolis* is a native 1,3-PD producer of particular interest as it offers high 1,3-PD yield and titres and is non-pathogenic.

Nutrient medium optimization by statistical design methodology for high growth and 1,3-PD production by *C. diolis* resulted in a high biomass concentration of 1.5 g/L and 1,3-PD accumulation of 3.5 g/L in shake flask. Experiments on growth inhibition by glycerol and 1,3-PD demonstrated a decrease in specific growth rate of *C. diolis* in medium containing

high initial glycerol and 1,3-PD concentrations with complete growth inhibition at glycerol concentration of 100 g/L and 1,3-PD concentration > 60 g/L. Batch cultivation in a 3.7 L bioreactor using statistically optimized medium (glycerol 54.15 g/L, K_2HPO_4 3.21 g/L; KH_2PO_4 2.75 g/L; $(NH_4)_2SO_4$ 2 g/L; $MgSO_4.7H_2O$ 0.2 g/L; $CaCl_2.2H_2O$ 0.02 g/L; $FeSO_4.7H_2O$ 5 mg/L; Yeast extract 2 g/L; Trace element solution (TES) 2 mL) yielded significantly high biomass growth (3.4 g/L), 1,3-PD concentration (25.8 g/L) and enhanced 1,3-PD yield (0.65 mol/mol), which indeed is the highest 1,3-PD yield reported so far in literature using this strain.

Further improvement in 1,3-PD concentration and/or productivity was achieved by repeated-cycle batch fermentation which featured the removal of 20% (v/v) of culture broth and its consequent replacement by an equal volume of fresh nutrient medium when residual glycerol concentration in the bioreactor declined below a limiting value (15 g/L). This time of fresh nutrient feeding was decided on the basis of *off-line* glycerol estimation by HPLC and thus a constant reactor volume was maintained throughout the experiment. A total 1,3-PD concentration of 67.8 g/L and a 1,3-PD productivity of 1.08 g/L was obtained in repeated-cycle batch 1,3-PD fermentation.

Batch cultivation was then carried out in an 8 L bioreactor equipped with NADH probe for *on-line* $NADH+H^+$ fluorescence measurement in order to develop a method for *on-line*, *in-situ* measurement and monitoring of cell concentration and metabolic activity during 1,3-PD fermentation. Linear correlation between net fluorescence and biomass concentration during both initial and final phases of fermentation obtained in this study can be used as an *on-line*, *in-situ* indicator for biomass concentration and; dip in the signal towards the end of fermentation is an important indicator of substrate depletion in bioreactor which can be used for guiding fresh nutrient feeding strategies for enhanced 1,3-PD production.

Average batch kinetic data was used for developing a mathematical model for 1,3-PD production. Inhibition data (substrate/product) was also used to identify the key inhibition parameters in model equations. Statistical validity of the model to graduate the batch experimental kinetics was demonstrated with an accuracy of 99% using 'F' test. The model was extrapolated to fed-batch cultivation by taking the mass balance around the bioreactor and various simulations were done *off-line* using the model to design suitable fresh nutrient feeding strategies in fed-batch cultivation which were then experimentally implemented. These included fed-batch cultivation(s) with constant feed rate, decreasing feed rate and pseudosteady state of substrate. Among these, the highest 1,3-PD concentration and productivity of 63.5 g/L and 1.35 g/L/h respectively was obtained in fed-batch cultivation with pseudo-steady state of substrate.

Thereafter, high cell density continuous cultivation with *in-situ* cell retention was designed using the model and experimentally implemented. Steady state concentrations of 9.43 g/L and 45.5 g/L of biomass and 1,3-PD respectively were obtained with an overall 1,3-PD productivity of 2.73 g/L/h. This represented an enhancement in 1,3-PD productivity by 4-fold as compared to the result obtained in batch 1,3-PD fermentation.

In order to address the problem of product (1,3-PD) inhibition and further improve 1,3-PD productivity, *in-situ* removal of 1,3-PD by liquid-liquid extraction was attempted in bioreactor. Prior to bioreactor studies, preliminary experiments on phase separation and biocompatibility were conducted for various solvents to determine the best extractant for use in *in-situ* 1,3-PD fermentation. Ethyl acetate was found to be the best in terms of extraction capacity of 1,3-PD and biocompatibility with *C. diolis*. *In-situ* batch extractive fermentation was carried out in a 3.7 L bioreactor using ethyl acetate. It resulted in a biomass and 1,3-PD concentration of 4.02 g/L and 35 g/L respectively. Thereafter extractive fed-batch

fermentation with constant feed rate was carried out which yielded a high 1,3-PD concentration of 74.5 g/L and a productivity of 1.38 g/L/h in 54 h.

An innovative integrated fermentation-extraction system was developed which involved programmed addition of nutrients into the bioreactor and simultaneous *in-situ* product removal along with cell retention. A 1,3-PD concentration of 58 g/L translating into a 1,3-PD productivity of 3.5 g/L/h was obtained thus representing a 5-fold improvement in 1,3-PD productivity as compared to batch cultivation.

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