Production of Biobutanol from Cyanobacterial Biomass

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Doctor of Philosophy

By

Deepika Kushwaha

DEPARTMENT OF CHEMICAL ENGINEERING & TECHNOLOGY
INDIAN INSTITUTE OF TECHNOLOGY
(BANARAS HINDU UNIVERSITY)
VARANASI - 221005
INDIA

Roll No. : 13041002

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The detailed characterization of four local cyanobacterial/microalgal biomass revealed that out of four only two strains of cyanobacteria (*Lyngbya limnetica* and *Oscillatoria obscura*) meet the expected requirements for biobutanol production. Further studies were performed using only these two strains. These studies focused on the optimization of carbohydrate content and sugar release. Experiments in CSTR focused on the biobutanol production under optimized conditions. Based on the results of the present study it is possible to draw following significant conclusions:

- ✓ Initial growth optimization and two-stage growth studies have revelaed that both the strains possess high carbohydrate accumulation property under N-starvation condition. Yield of 0.375 and 0.373 g/g of increased carbohydrate have been found from *L. limnetica* and *O. obscura*, respectively, during the two-stage growth study at optimized conditions. This large increase in the carbohydrate yield for both the strains confirms their potency for use as efficient sugar source for biobutanol production.
- ✓ Optimization of pretreatment conditions using *L. limnetica* has resulted in maximum (0.33 g/g db) sugar yield with 1.63 M H_2SO_4 treatment for 60 min at 100°C. This has proved the efficiency of combinational (thermal and chemical) pretreatment that, even lower acid concentration is enough to release sufficient quantity of sugar available in the biomass in combination with appropriate temperature and treatment time.
- ✓ Efficient extraction of sugar content (~86%) after getting the lipid from both the biomass has proved the effectiveness of biomass for both biobutanol and biodiesel production.

- ✓ Further, enzyme extraction using cyanobacterial biomass as substrate has resulted in better βG activity in comparison to various commercially available enzymes. Presence of Fe_3O_4/Alg nanocomposites has improved the βG activity of *L. limnetica* extracted enzyme by 41%, this could be a potential source of βG for saccharification of biomass because other commercially available enzymes tested in the present work possessed very low βG activity.
- ✓ Butanol fermentation study has resulted in maximum butanol productivity of 1.565 g/L/d with *L. limnetica* hydrolysate supplemented with 10.0 g/L of glucose at the optimized conditions.
- ✓ Though the maximum (8.873 g/L) production has been found with glucose supplemented hydrolysate of *L. limnetica* while the highest yield (0.421 g/g sugar) has been obtained with pure cyanobacterial hydrolysate. This has indicated that the effect of glucose addition on butanol yield becomes insignificant under optimized conditions.
- ✓ Results of the CSTR studies are analysed by fitting the data in Mercier's kinetic model and the high regression coefficients (≥0.93) value shown excellent agreement between experimental values and model predictions.
- ✓ Potential of liquid-liquid extraction has been examined for separating butanol from fermentation broth at the lowest cost but due to high emulsion formation it has been found to be an inefficient technique. Amongst various membrane-solvent combinations, oleyl alcohol with polypropylene membrane has been found to be the best with nearly 54-56% butanol recovery from the fermentation broth.

- ✓ The findings of the present work clearly demonstrate the potential of *L. limnetica* and *O. obscura* biomass for synthesis and recovery of renewable and clean biobutanol. The results of this study may help in setting up a large fermentor for butanol production together with some other value added products such as biodiesel and biohydrogen.

Following aspects listed below require further exploration to make the process commercially viable:

- ✓ Use of large bioreactor (pilot-scale) for two-stage growth of cyanobacterial biomass to get better carbohydrate productivity.
- ✓ Sequential lipid/carbohydrate recovery from the two-stage grown biomass for biodiesel/biobutanol production.
- ✓ Large scale fermentation study in batch as well as continuous mode.
- ✓ Application of nanoparticles & nanocomposites (other than Fe_3O_4) to increase the butanol production rate.