INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	Jayakrishnan U
Roll Number	:	156152005
Programme of Study	:	Ph.D.

Thesis Title: Novel bioprocessing strategy for polyhydroxybutyrate production from agro-industrial effluents: A waste biorefinery

Name of Thesis Supervisor(s)	:	Prof. Gopal Das and Dr. Deepmoni Deka
Thesis Submitted to the Department/ Center	:	Centre for the Environment
Date of completion of Thesis Viva-Voce Exam	:	31/01/2022
Key words for description of Thesis Work	:	Waste biorefinery, Volatile fatty acid, Polyhydroxybutyrate, Activated sludge, Acidogenic fermentation, Aerobic feeding strategy, Rice mill effluent, Brewery effluent.

SHORT ABSTRACT

Effective waste management is still a burning issue in the modern world due to the generation of more waste than that can be properly disposed off. In this scenario, waste biorefinery has shown tremendous potential to drive improved waste disposal through income generation. The research work presented in the thesis investigates bioprocessing strategies to recover a value-added product, polyhydroxybutyrate (PHB), from agro-industrial effluents under the waste biorefinery approach. Initially, the volatile fatty acid (VFA) production from rice mill effluent (RME) and brewery effluent (BE) was influenced by gobar gas plant sludge (GPS) and brewery anaerobic sludge (BAS) in an untreated state and pretreated under cyclic heat and acid shock regime to convert the effluent streams into media suitable for PHB production using acidogenic fermentation. The acidification of RME with pretreated gobar gas plant sludge at the optimized feed to microbe ratio led to enhanced total VFA content (TVFA) and degree of acidification of 2437±0.03 mg/L and 86±0.13 % compared to all other combinations. Acetate and butyrate were the major VFAs produced after the pretreatment of GPS, along with stable COD and acidic pH profiles.

However, the fermentation of RME with pretreated BAS had an enhanced even to odd ratio of 20.97±0.08 mg/mg along with the highest acetate and butyrate yield compared to all other combinations. The improvement can positively affect polymer composition and property. Meanwhile, the untreated system had the upper hand regarding TVFA concentration compared to the pretreated system due to the negative effect of the pretreatment regime on different microbial communities in BAS. Therefore, pretreatment of anaerobic inoculum inhibited carbon sinks and enhanced even-numbered VFA production from RME, indicating its potential implications as a fermentation media for improved PHB production.

The study also evaluated the cumulative effect of the interaction between the origin of two activated sludge, petroleum refinery sludge (PRS) and brewery sludge (BS), and nitrogen availability on the PHB production, its characteristics, and microbial community distribution. Nitrogen excess and limited enrichment of both the sludges

produced mix microbial culture with adequate PHB storage of 7.8±0.05%, 14.4±0.04%, 14.4±0.04%, 13.4±0.02%, respectively. In contrast, batch accumulation revealed higher PHB accumulation of 76.1±0.03% and 71.7±0.05% under nitrogen limitation for PRS and BS enriched under nitrogen excess condition compared to any other combination. Long-term exposure to growth-limiting conditions might have drained cell machinery resulting in reduced accumulation during the limited condition. The physiochemical characterization confirmed PHB production from both enriched inoculums having higher degradation/lower melting temperature and high molecular weight. The overlapping microbial community distribution in PRS and BS after accumulation also indicates no significant sludge origin impact on PHB production under an extended feast-famine enrichment regime.

Finally, the bioconversion of RME to PHB was carried out using different accumulation strategies with brewery sludge enriched under the influence of sludge retention time. Brewery sludge enriched under an SRT of 10 d had a higher storage response of 10 ±0.003 % than an SRT of 5 d (3.9±0.05 %). Fed-batch accumulation with pulses-wise feeding resulted in the highest storage response. PHB accumulation of 60 ±0.004 % resulted from enriched BS and acetate as carbon sources. The PHB accumulation was lower for raw RME at 12 ±0.1 %, while double was the storage response with fermented RME at 29.5 ±0.01%. Similar was the case of PHB yield. The higher biomass concentration in 10 d SRT and faster growth requirement in 5 d SRT results in faster substrate consumption and lower feast to famine ratio, implementing a strong selective force. However, washout of initially available accumulator takes place with 5 d than 10 d SRT. Also, microbial communities have extensive exposure to selective pressure under 10 d than 5 d SRT. Hence, shorter SRTs require an extended enrichment period than longer SRTs to increase PHB production. Also, pulse-wise feeding exposes the enriched culture to excess carbon that saturates the cells with PHB compared to batch or continuous feeding. In addition to this, acidogenic fermentation can further enhance PHB production from waste feedstocks as substrates. Therefore, the waste biorefinery approach has the potential for treating RME with concomitant PHB production in a cost-effective manner.