INDIAN INSTITUTE OF TECHNOLOGY GUWAHATI
SHORT ABSTRACT OF THESIS

Name of the Student	:	Avinash Anand
Roll Number	:	206107101
Programme of Study	:	Ph.D.
Thesis Title:		Biohydrogen synthesis from food waste: Process development, optimization and intensification
Name of Thesis Supervisor(s)	:	Prof. V.S. Moholkar
Thesis Submitted to the Academic Division	:	Department of Chemical Engineering
Date of completion of Thesis Viva-Voce Exam	:	1 December 2025
Key words for description of Thesis Work	:	Biohydrogen, Ultrasound, Dark Fermentation, Intensification, Metabolic flux analysis

SHORT ABSTRACT

This thesis investigates the production of biohydrogen (bioH_2) from food waste through an integrated approach combining process optimization, ultrasound-assisted intensification, molecular simulations, and metabolic flux analysis. The research aims to enhance both the hydrolysis of food waste and the subsequent dark fermentation process to achieve higher bioH_2 yields and improved process efficiency. Food waste hydrolysis was first optimized using Box–Behnken design, achieving a total reducing sugar (TRS) yield of 263.4 mg/g biomass in 42 hours. Ultrasonic pretreatment (35 kHz, 20% duty cycle) significantly intensified hydrolysis, reducing the required time fourfold and increasing TRS yield by 22% (320 mg/g biomass). Fourier-transform infrared spectroscopy and molecular dynamics simulations revealed that sonication altered the glucoamylase enzyme's secondary structure by decreasing α-helix and increasing random coil content. These structural changes widened the enzyme's binding pocket, improving substrate transport and accelerating hydrolysis kinetics.Dark fermentation of the hydrolysate using *Clostridium pasteurianum* was optimized using response surface methodology (RSM-CCD) and an artificial neural network coupled with a genetic algorithm (ANN-GA). RSM-CCD predicted a bioH_2 yield of 1039 mL/L, while ANN-GA identified improved conditions that generated 1108 mL/L (1.73 mol/mol hexose). The modified Gompertz model showed a higher maximum production rate (185.34 mL/L·h) under ANN-GA conditions. Metabolite analysis indicated a metabolic shift toward the acetic acid pathway, reflected by an increase in the acetic-to-butyric acid ratio from 0.9 to 0.94, thereby enhancing hydrogen production.Further intensification through ultrasound-assisted fermentation was evaluated using a metabolic flux analysis model. Sonication increased hexose uptake by ~47% and substantially boosted acetate pathway fluxes, leading to a ~22% improvement in bioH_2 yield and a ~37% rise in the acetate-to-butyate ratio. Hypothetical flux simulations demonstrated the potential for further enhancement of hydrogen productivity through complete metabolic redirection or increased sugar uptake. To summarize, the net bioH_2 yield from 1 kg of food waste under statistically optimized conditions was 5.1 g per kg of food waste, which further improved to 5.73 g per kg of food waste under sonication. In essence, this thesis has addressed three Sustainability Development Goals of the United Nations, viz., SDG 7 (Affordable and clean energy), SDG 12 (Responsible consumption and production patterns or circular economy) and SDG 13 (Climate action).