

# Biodiesel Production from Dairy Wastewater using *Rhodococcus opacus*: Batch, fed-batch and Continuous Bioreactor Study

Nikhil Gupta<sup>1</sup> and Kannan Pakshirajan<sup>2\*</sup>

<sup>1,2</sup>Department of Biosciences and Bioengineering, Indian Institute of Technology Guwahati, Guwahati 781039, Assam, India

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**Abstract**—This study focused on biodiesel production from *Rhodococcus opacus* PD630 grown using dairy wastewater using a bioreactor operated under batch, fed-batch and continuous condition. In all these experiments, the dairy wastewater was supplemented with mineral salt medium (1:3 ratio) and subsequently used with or without autoclaving. Initial experiments were performed using the batch operated bioreactor with uncontrolled or controlled temperature and pH conditions. The *R. opacus* cell density reached 1.5 and 2.64 ( $OD_{600}$ ) when the bioreactor was operated with uncontrolled and controlled conditions, respectively, and using the autoclaved dairy wastewater. These values were high (2.12 and 2.73, respectively) when raw dairy wastewater was used. Lipid content (g/L) reached 0.95 and 1.5 under uncontrolled and controlled conditions, respectively, using the autoclaved dairy wastewater. The lipid values obtained using the raw dairy wastewater was 1.25 and 1.8 g/L, respectively. Besides, the wastewater COD removal was higher in the batch bioreactor with uncontrolled condition compared with that obtained under controlled condition using either the raw or autoclaved wastewater. Under fed-batch mode of operation, the bacteria could treat the wastewater more quickly and efficiently than under the batch operated condition despite the low biomass content obtained in the fed-batch operated bioreactor, probably due to a low amount of organics in the feed stream. In situ trans-esterification of the bacterial lipid was carried out using  $H_2SO_4$  as the catalyst and 6:1 molar ratio of methanol to acid. A maximum biodiesel yield of 20% (w/v) was obtained from the bacterial biomass grown using raw dairy wastewater in the batch operated bioreactor. Further investigation on continuous lipid production from dairy wastewater using *R. opacus* for biodiesel application is currently underway. Overall, this study proved a very good potential of *R. opacus* in simultaneous dairy wastewater treatment and lipid production for bioenergy application.

**Keywords:** Biodiesel; *R. opacus*; Lipids; Dairy wastewater; Bioreactor; Fed-batch.