

Bio-surfactant Production from Waste Coconut Oil by Marine Isolate of *Rhodococcus sp.* RH-01

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Bio-surfactants (BS) production by microorganisms is given attention as a sustainable solution for chemically produced surfactants. Various BS-producing bacteria have been screened and optimized utilizing unconventional substrates. This study focused on the cost-effective production of BS using a marine isolate *Rhodococcus sp.* RH-01. The bacterium was identified using 16S rRNA gene sequencing. BS production was optimized under batch fermentation conditions. Two-liter scale batch fermentations were conducted using Minimal Salt Medium (MSM) with the following optimized conditions; 40% waste coconut oil, pH at 8.0, temperature at 37°C, shaking at 120 rpm. Acid precipitation was done to harvest crude BS and ethyl acetate extraction was used to obtain pure BS. The lipase activity of the culture supernatant was also determined. Surface active properties of BS were determined using oil displacement drop collapse and emulsification index assays. Cost analysis for the BS production at 2- L- scale was evaluated including cost for substrates, manpower, instrument, and overhead charges. NCBI Blast search showed 99% sequence similarity to *Rhodococcus sp.* Maximum BS production under optimum conations was 7691.3±42.25 mg/L. High lipase activity (23.58±2.18) was shown at 72 h fermentation. Scaling up was done to 2-L scale and the reproducibility was checked. Cost analysis showed LKR 22.5±6.12 is required for producing 1mg of BS which should be further optimized under large scale utilizing low-cost substrates in future studies. In conclusion, *Rhodococcus sp.* RH-01R could be an efficient strain to utilize for BS production at an industrial scale using low-cost substrates.

Keywords: *bio-surfactant, Rhodococcus sp., batch fermentation, waste coconut oil, cost analysis*