

Production of citric acid by *Yarrowia lipolytica* using biodiesel derived glycerol

M. INIYAKUMAR¹, A. RAMALAKSHMI² AND P. SANTHANAKRISHNAN²

¹Department of Civil Engineering, National University of Ireland, GALWAY (IRELAND)

²Department of Plant Molecular Biology and Biotechnology, Tamil Nadu Agricultural University, COIMBATORE (T.N.) INDIA

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Crude glycerol, a by product of Jatropha biodiesel was used as carbon source for production of citric acid by *Yarrowia lipolytica* MTCC 35. Concentrations of crude glycerol ranging from 1 to 4 per cent were taken in buffered and unbuffered broth for the optimized production of citric acid by *Yarrowia lipolytica* MTCC 35. The amount of citric acid produced in buffered broth (11.2 gl⁻¹) was doubled when compared to unbuffered broth (5.2 gl⁻¹). Quantitative estimation through HPLC also confirmed the same.

Key words : Citric acid, *Yarrowia lipolytica*, Crude glycerol-biodiesel by product

INTRODUCTION

Citric acid is the most important organic acid produced in tonnage by fermentation. Global production of citric acid in 2004 was about 1.4 million tones (Soccol *et al.*, 2003.) Citric acid is widely used to impart a pleasant, tart flavour to foods and beverages. It also finds applications as a function of additive detergents, pharmaceuticals, cosmetics and toiletries. Raw glycerol is an important feed stock when bio-diesel is applied on a large scale production. With the production of 10 kg of biodiesel from rape seed oil, one kg of glycerol becomes available. Numerous carbon sources, such as molasses, n-paraffins, hexadecanes, edible oils, glucose, starch hydrolysates etc., have been used to produce citric acid from *Yarrowia lipolytica*. Although glycerol has been widely used as a carbon source in the production of 1,3 propane diol. (Biebl and Marten, 1995). The aim of present study was to investigate utilization of crude glycerol by *Yarrowia lipolytica* for citric acid production.

MATERIALS AND METHODS

The standard strain, *Yarrowia lipolytica* was obtained from Microbial type culture collection (MTCC 35), IMTECH, Chandigarh. The Raw material of crude glycerol, a by product of Jatropha biodiesel, obtained from bio energy department, Tamil Nadu Agricultural University (TNAU), India.

Production of citric acid on crude glycerol:

To test the ability of *Yarrowia lipolytica* MTCC 35

for citric acid production on crude glycerol, the glucose in the liquid broth (Papanikolaou *et al.*, 2002) was replaced with crude glycerol. This broth was named as unbuffered broth (UBM). The buffered broth (BM) was prepared by increasing the concentration of K₂HPO₄ and Na₂HPO₄ to 12 gl⁻¹ individually. The citric acid production was analysed by inoculating loop full cells into 250 ml Erlenmeyer flasks containing 50 ml sterile broth and incubated in a rotary shaker at 28°C with 200 rpm agitation for seven days. After incubation cells were harvested by centrifugation and dried at 80°C until the constant weight was reached. Filtered aliquots were used to determine quantitative estimation of citric acid. The internal standard with glucose as carbon source also maintained to compare the citric acid production.

Determination of citric acid:

Incubated flasks containing yeast cells were removed and harvested by centrifugation. After centrifugation, the filtered aliquots of the culture medium were analysed for the presence of citric acid. 10 ml of the supernatant samples were taken and volume was made up to 20 ml by adding distilled water. Then a few drops of phenolphthalein indicator were added and the material was titrated against 0.1 N NaOH to a light pink colour end point. The per cent acidity was calculated based on the following formula:

$$\text{Per cent acidity} = \frac{\text{Vol. of 1N NaOH consumed}}{\text{Wt. of the sample}} \times 100$$

From the total acidity, citric acid content was