

## Statistical Analysis of the Factors Affecting the Recovery of Microbial Oil

S.R. BRAHMANI PRIYADARSHINI\*, M.C. MISHRA,  
V. MURUGAN and THANGA ANGELIN

Department of Pharmaceutical Chemistry, Dayananda Sagar College of Pharmacy  
Kumaraswamy Layout, Bangalore-560 078, India  
E-mail: brahmani\_priya@yahoo.co.in

Production of microbial oil by fermentation process using oleaginous organisms is one of the potential alternative sources of collecting lipids. *Rhodotourula gracilis*, is one of the oleaginous yeast, explored for their oleaginicity. The degradation of the cell wall of the yeast is an absolute necessity in order to extract oil using organic solvents. Acid hydrolysis is one of the chemical methods commonly employed for this purpose. The concentration, contact time and temperature of acid hydrolysis are the three variable parameters observed during degradation of the cell wall. An analysis of variance of these three factors was carried out to analyze to know which of the three factors affected the extraction of oil significantly and also to know the interaction between these factors.

**Key Words:** Oleaginous organisms, *Rhodotourula gracilis*, Acid hydrolysis, Analysis of variance.

### INTRODUCTION

The potential of certain microorganisms to produce oils and fats similar to vegetable edible oils have been well documented<sup>1-3</sup>. Microorganisms, which have been reported to producing at least 20 % and above of their biomass as lipids, are termed as oleaginous organisms. In oleaginous yeast, the oil is accumulated intracellularly and is protected by rigid cell wall. The disruption of the cell wall is very much essential for effective penetration of the organic solvents for extraction. Various methods, like, chemical, mechanical and enzymatic are employed for degradation of the yeast cell wall<sup>4</sup>. In comparison to other treatments, acid hydrolysis was reported to be the most suitable economically<sup>5</sup>.

Analysis of variance<sup>6</sup>, an important statistical technique has been defined, as the separation of the variance ascribed to one group of causes from the variance ascribable to other group. In the analysis of variance for three factors, null hypothesis is that no one of the factors separately influences

the variance values. But it may be possible that any two of these factors together may do so. Therefore it is necessary to test for interaction between the factors analyzed. In this experiment, three factors, such as the concentration, contact time and temperature of acid hydrolysis were analyzed by carrying out the analysis of variance.

## EXPERIMENTAL

The oleaginous microorganism *Rhodotorula gracilis* CFR-1 was used in all the experiments. The organism was maintained on potato dextrose slants and subcultured every month.

**Fermenter medium and cultivation:** The seed inoculum medium consisted of molasses 270 mL, magnesium sulphate 0.8 g, calcium chloride 0.8 g, potassium dihydrogen phosphate 1.5 g and ammonium sulphate 1.63 g then pH was adjusted to 5.0 before the sterilization. The medium was sterilized at 10 psi for 15 min and inoculated with 2 d old culture slants and incubated for 24 h at 30 °C on a rotary shaker at 200 rpm.

The fermenter medium composed of molasses 1.3 L, magnesium sulphate 4 g, calcium chloride 4 g, potassium dihydrogen phosphate 7.5 g and ammonium sulphate 16.33 g. The pH was adjusted to 5.0 and then the medium was sterilized in an autoclave at 15 psi for 1 h. The organism was grown in a 15 L fermenter (New Brunswick, USA) with a working capacity of 10 L. The fermenter medium was inoculated with the inoculum. Operating conditions of the fermenter were temperature  $30 \pm 1$  °C, aeration 1 vvm, agitation 350 rpm and silicone oil was used as antifoaming agent.

**Harvesting of biomass:** The biomass was harvested after 96 h of fermentation and cells were separated by centrifugation.

**Extraction:** 8 g of the wet cell mass was taken on a filter paper and folded into packets. All the 9 samples were treated with 1.0 N HCl, but the time of hydrolysis were 0.5, 1.0 and 1.5 h. Similarly temperatures of hydrolysis were 30, 55 and 90 °C. The treated samples were washed until free from acid under running water and dried overnight at  $95 \pm 2$  °C.

The oil was extracted with petroleum ether (60-80) on a soxhlet apparatus for 4 h. The above procedure was adopted for 0.2 N and 0.6 N HCl.

**Estimation of percentage of free fatty acid content:** Percentage of free fatty acid (FFA) content was determined for the extracted oil. The oil samples were taken in 250 mL flask and dissolved in 50 mL petroleum ether by mixing. To this 50 mL of neutral alcohol was added and mixed. The mixture was titrated with 0.1 N NaOH using phenolphthalein as indicator. Acid value was calculated using the formula

$$\text{Acid value} = (\text{mL titrated} \times \text{normality} \times 50) / \text{weight of sample}$$
$$\% \text{ FFA} = \text{Acid value} \times 0.503$$

## RESULTS AND DISCUSSION

This experiment was done to determine the role of the critical factors, *viz.*, temperature, time and concentration acid of hydrolysis, on the yield of oil. The analysis of variance was carried out using the data obtained from the experiment.

From the results presented in Tables 1a and 1b, it can be concluded that temperature, time and concentration of acid of hydrolysis have significant effect on the oil yield. It is also clear that while time factor have some significant effect, the temperature and concentration of acid factors have highly significant effect. Thereby it is indicated that the yield varies with the variation in these three factors.

TABLE-1a  
EFFECT OF DIFFERENT PARAMETERS ON  
THE YIELD OF OIL FROM *Rhodotourula gracilis*

Time (h)	Concentration acid (N)	Temperature (°C)		
		30	55	90
0.5	1.0	52.08	89.11	90.76
1.0		58.23	96.66	100.00
1.5		84.18	112.74	107.25
0.5	0.6	2.41	6.58	60.05
1.0		2.75	24.82	89.72
1.5		5.01	43.89	90.06
0.5	0.2	3.76	2.54	24.79
1.0		2.03	2.68	62.53
1.5		2.65	3.09	91.54

It can also be concluded that the interaction between the two factors, acid concentration and temperature have significant effect on the hydrolysis, *i.e.*, no significant interaction between time and temperature or time and treatment factors. Therefore, it is shown that temperature and acid concentration have significant effect on the hydrolysis of the cell wall of *R. gracilis* and thereby on the yield of oil obtained.

From the Table-1a, it is seen that at lower acid concentration (0.2 N) maximum oil was extracted at 90 °C with hydrolysis time of 1.5 h. With an increase in acid amount the maximum oil extracted was at 90 °C with a hydrolysis time of 1 h. The optimum factors were found to be 1.0 N of acid concentration at 55 °C for 1 h of hydrolysis. The increase in oil yield can be studied by increasing the acid concentration of hydrolysis, but the percentage of free fatty acid concentration also increases, which is undesirable.

TABLE-1b  
ANALYSIS OF VARIANCE OF THE FACTORS  
AFFECTING THE YIELD OF LIPID

Source of variance	Sum of square	Degree of freedom	Estimate of variance	F observed	F Tabular
Between temperature	14597.5270	2	7298.7635	42.293‡	4.46
Between acid concentration	21791.4020	2	10889.7010	63.159‡	4.46
Between time of contact	2413.4060	2	1206.7032	6.992†	4.46
Interaction between temperature and acid concentration	3596.1900	4	899.0475	5.209†	3.84
Interaction between acid concentration and time of contact	80.4156	4	20.1039	0.116*	3.84
Interaction between temperature and time of contact	670.4890	4	167.6224	0.971*	3.84
Residual	1380.6100	8	-	-	-
Total	44530.0410	26	-	-	-

†Significant; ‡Highly significant; \*Not significant at 5 % confidence.

Table-2 gives the effect of hydrolysis parameters (temperature, concentration of acid and time) on the free fatty acid concentration. It is clear that the percentage of free fatty acid of the oil obtained by hydrolyzing the cells with 1.0 N HCl, increases with both increase in temperature and time of hydrolysis. Free fatty acid is minimum at 30 °C at 0.5 h of hydrolysis and maximum at 90 °C at 1.5 h of hydrolysis.

TABLE-2  
EFFECT OF ACID HYDROLYSIS ON THE % FREE  
FATTY ACID CONTENT OF THE OIL

Temperature (°C)	Time (h)		
	0.5	1.0	1.5
30	5.16 %	5.65 %	8.03 %
55	6.97 %	7.32 %	7.94 %
90	8.31 %	8.48 %	9.21 %

### Conclusion

The analysis of variance done with respect to three factors affecting the oil yield showed that acid concentration and temperature of hydrolysis have significant effect on the yield of oil.

It is a known fact that acid treatment or alkali treatment would lead to some hydrolysis of the lipid classes (triglycerides and phospholipids) and thereby increases the free fatty acid percentage. From the above experimental results, it is indicated that the percentage of free fatty acid content depends on the time and temperature of hydrolysis.

### ACKNOWLEDGEMENTS

The authors are thankful to school of Biochemical Engineering, I.T, Banaras Hindu University, Varanasi and CFTRI Mysore for providing all facilities to pursue the research work. Thanks are also due to Dayananda Sagar College of Pharmacy for their encouragement and support.

### REFERENCES

1. S. Papanikolaou, I. Chevalot, M. Komaitis, I. Marc and G. Aggellis, *Appl. Microbiol. Biotechnol.*, **58**, 308 (2002).
2. C. Ratlege, *Biochimie*, **86**, 807 (2004).
3. Z. Cohen and C. Ratlidge, *Single Cell Oils*, AOCS (2005).
4. A. Azeem, F. Neelagundy and V. Rathod, *Plant Foods Human Nutr.*, **53**, 381 (1999).
5. Food Technology Overview, International Food Convention, Association of Food Scientists and Technologists (India), CFTRI, Mysore, India, p. 105 (1988).
6. T.W. Anderson, *Introduction To Multivariate Statistical Analysis*, Wiley Eastern Private Ltd. (1974).

(Received: 24 February 2007;

Accepted: 1 January 2008)

AJC-6158