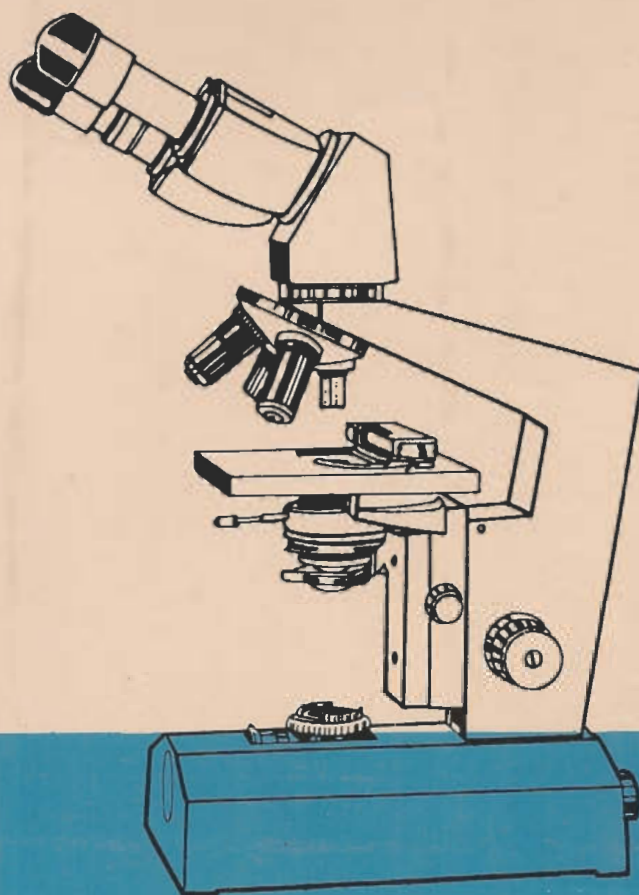


**ASSOCIATION OF
MICROBIOLOGISTS
OF INDIA**

XXVIII ANNUAL CONFERENCE, PUNE

**16-18 January, 1988
GOLDEN JUBILEE YEAR**

ABSTRACTS



Kunhi, A.A.M. and Joseph, R. 1988. A rapid semi quantitative method for estimation of fat in *Rhodotorula gracilis*. XXVIII Annual Conference and Golden Jubilee Celebration of Association of Microbiologists of India. 16-18 January, 1988, Pune, India.

GEN-30

A RAPID SEMI-QUANTITATIVE METHOD FOR ESTIMATION OF FAT IN *RHODOTORULA GRACILIS* BY EMPLOYING FAT STAINING DYES

A.A.M.Kunhi and R.Joseph

Discipline of Microbiology & Sanitation
Central Food Technological Research Institute
Mysore-570 013

A number of coal tar dyes such as Sudan IV, Sudan Fat red 7B have long been employed in histochemistry for visualization of fat in plant and animal tissues. In the present studies, the possibility to use these dyes to determine fat content in the oleagenous red yeast *Rhodotorula gracilis* was examined. Since whole cells were employed, the ability of the dye to penetrate cell wall/membrane was of primary concern. This was facilitated by pre-treatment of cells with ethanol which also removes considerable amount of water. The principle of the present method lies in the estimation of unreacted dye (residual dye) after treatment with the cells by measuring absorption at 520 nm.

By using pure groundnut oil, the residual Sudan IV was measured as a function of quantity of oil. A parabolic relationship was seen with the two extremities of the curve not considered suitable for fat determination. A similar parabolic relationship for the same dye with cells of *Rhodotorula gracilis* containing approximately 60-70% of fat (determined by Soxhlet method) was obtained. The yeast cells containing different levels of fat (i.e. those harvested at different ages of the culture e.g. 24, 48, 72 hrs. etc.) were subjected to fat determination employing Sudan IV and compared with corresponding values of fat by Soxhlet method. The results indicated that under the conditions of the procedure adopted, if the fat content of the cells are below or above the range of 60-70% the error by the dye procedure is as high as 100%. Within this range of the fat content in cells, however, the error was not more than 5%. Among different dyes tested for suitability Sudan IV was found to be more effective in view of better uptake by fat in the cells. Sudan Black-B and Fat red 7B were found to bind to a lesser degree. It was, therefore, concluded that the procedure, despite, being accurate only for a narrow range of 60-70% fat content in the cell, is extremely useful for rapid determination of fat in yeasts particularly those that make high levels of fat. Another advantage of this procedure is that the relatively small quantity of cells required for fat estimation (300-500 mg of wet cells, which is about a tenth required for Soxhlet method). In view of the ease, low cost and rapidity the present procedure would prove to be more practicable under certain conditions than the traditional method employing solvent extraction by the Soxhlet procedure. The procedure could well be advocated for monitoring fat production by oleagenous yeasts during the process of fermentation.