Mahasagar–Bulletin of the National Institute of
Oceanography, *12*(2) 1979, 123–126.

STUDIES ON ESTUARINE YEASTS : II. LABORATORY EXPERIMENTS ON GROWTH AND PRODUCTION

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ABSTRACT

Five hydrocarbon degrading isolates : *Candida parapsilosis* (three isolates), *C. tropicalis* (one isolate), *Saccharomyces cerevisiae* (one isolate) were studied for their growth, biomass production and fermentation of diesel oil. Growth in Wickerham's medium and mineral base medium with diesel oil as the sole source of carbon were compared. The protein content in these yeasts ranged from 32·28-58·73% and the biomass production was optimum on the 20th day. The products of fermentation showed the presence of two fatty acids by TLC with Rf values 0·13 and 0·29 respectively.

Extensive research has been done on the utilization of yeasts for single cell protein (SCP) and for other metabolite production like amino acids and vitamins by hydrocarbon utilizers. *Candida tropicalis, C. intermedia, C. lipolytica, Rhodotorula* sp. and *Saccharomyces* sp. have been used for the above purposes (Yamada, Takahashi, Kaubata, Okade and Onhihara, 1968; Kasturi and Tamhane, 1969; Kilberg, 1972). Although considerable work has been carried out on the utilization of terrestrial yeasts for hydrocarbon degradations, the utility of their marine counterparts has not been fully exploited. In view of the potential for using yeasts in SCP production from the hydrocarbons and their hydrocarbon degrading ability in pollution abatement programmes, it was felt that the yeasts from the Goan estuaries could be studied keeping in view the above purposes. Such studies were carried out earlier by Pandya and Doctor (1977) who utilized *C. utilis* and bacteria for degrading refinery wastes for pollution control.

Of the 103 yeast isolates screened for hydrocarbon degrading ability, only 5 hydrocarbon degraders were studied further for their growth in Wickerham's medium of the composition: glucose 3·00 g; peptone 0·5 g; yeast extract 0·3 g; malt extract 0·3 g; sea water 100 ml and pH 5·5 and in mineral base medium (Chatterjee, Chatterjee and Banerjee, 1978) of the composition: KH_2PO_4 3·00 g, Na_2HPO_4 6·00 g; NaCl 5·00 g; $MgSO_4 \cdot 7H_2O$ 0·1 g; $CaCl_2 \cdot 2HO_2$,· $MnCl_2$, $(NH_4)_2MoO_4$ and $FeSO_4$ in traces; distilled water 1000 ml and pH 7·0. Diesel oil (source of energy) was sterilized separately. They were also studied for their biomass production and products of diesel oil fermentation.

Growth in Wickerham's broth

1% inoculum of O·D. 0·45 – 0·5 of the 5 isolates: *C. parapsilosis* (isolate nos. 40, 41, 44), *C. tropicalis* (isolate no. 46) and *Saccharomyces cerevisiae* (isolate

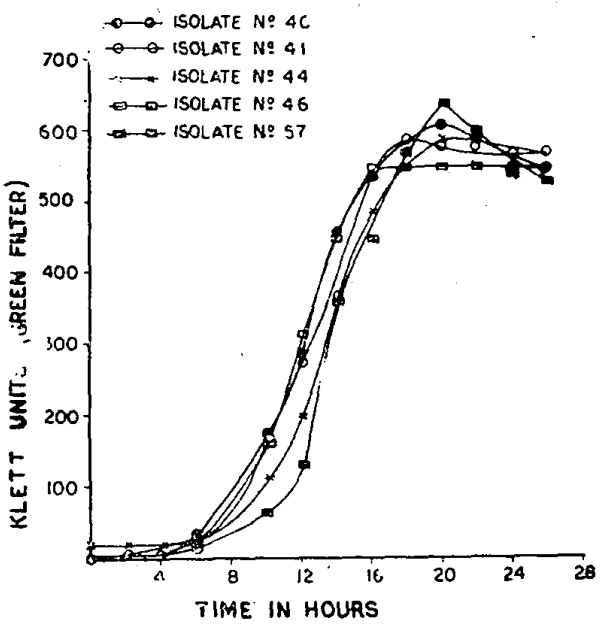

Fig. 1. Growth curve in Wickerham's medium.

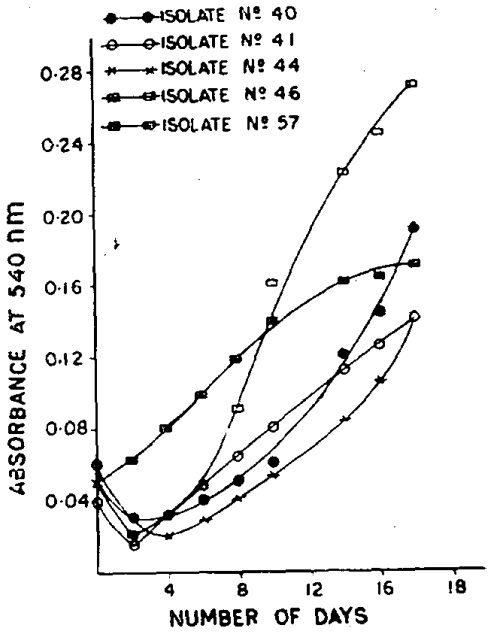

Fig. 2. Growth curve in mineral medium with 5% inoculum.

no. 57) was added to 100 ml sterilized Wickerham's broth and incubated on a rotary shaker at 200 rpm till the stationary phase was reached and O.D. was read every hour on Klett Summerson's Colorimeter at 540 nm.

Growth on mineral base medium (M. B. M.)

1% and 5% inoculum of the 5 isolates were added to 100 ml of the sterilized mineral base medium with 10% sterile diesel oil and incubated on a rotary shaker at 200 rpm for 10 and 18 days respectively. The O.D. was read every 24 hours on the Specktrokol at 540 nm till the 10th and 18th day respectively.

Biomass, protein production and fermentation of diesel oil products

The five isolates noted above were inoculated into 100 ml of above mineral medium with 10% diesel oil and incubated for a period of one month on a rotary shaker at 200 rpm.

The growth of the organism was obtained at the interphase of diesel oil and aqueous medium. The cells harvested on the 10th, 22nd and 30th day according to the techniques given by Godinho, D'Souza and Freitas (1978) and their dry mass was determined. The nitrogen present in the cells was estimated by a modification of Kjeldahl – Nesslerization method. The protein present in the cell mass was then calculated by multiplying the nitrogen value by 6·25 and then percentage determined. The fatty acids in the fermentation broth were concentrated by extracting with ether twice (Pandya, 1975). Then the concentrated extract was checked for the presence of fatty acids, by thin layer chromatography using a solvent system consisting of petroleum ether: ether (90: 10) with standard references. Iodine fumes were used as detecting agent.

In the present investigation, it was found that the yeasts grow rapidly in Wickerham's medium and reach their stationary phase in about 18 hours (Fig. 1) but the growth in mineral based medium depended much on the size of the inoculum and the stationary phased reached in about 18 days (Fig. 2). It was found that 1% inoculum did not support considerable hydrocarbon utilization, whereas 5% inoculum and above supported faster degradation and SCP production with considerable oil emulsification at the interphase. The protein content in these yeasts ranged from 32·28 – 58·73% and the biomass production was optimum on the 20th day (Table I). The work done on SCP production on n-Paraffin oil produced 60% protein (Sheckladý, 1975), thus indicating that the present isolates could serve as a potential protein source from the diesel oil which is a relatively cheaper substrate. The products of diesel oil fermentation showed the presence of two fatty acids on the tenth and twenty-second day by thin layer chromatography with Rf values of 0·13 and 0·29 respectively. The spots, however, disappeared after one month's growth on diesel oil. This could be due to the usual oxidation pathway of hydrocarbon where diesel oil is oxidized to fatty acids which are later metabolized over a period of one month. It was observed that these isolates were able to utilize 40–50% diesel oil within one month's incubation. This result is of much significance as these organisms could probably be used as inoculants to curb oil pollution which has already affected the beaches of Goa in the form of tar balls (Nair, Devassy, Dwivedi and Selvakumar, 1972; Dwivedi and Parulekar, 1974). It is also well known that oil pollution is somewhat toxic to marine biota. Thus such yeasts can be utilized to disintegrate the oil spilled.

Table I. Biomass and protein production by yeasts on diesel in 100 ml mineral medium on incubation for different periods.

Isolate No.	Dry mass of cells (mg)	Total nitrogen in dry mass of cells (mg)	% of protein in dry mass of cells
	1 month incubation		
40	22·209	13·5	37·98
41	8·827	6·462	35·75
44	11·957	10·52	53·98
46	9·812	6·714	42·77
57	39·952	23·83	37·29
	22 days incubation		
40	4·3	0·3113	45·23
41	7·0	0·3616	32·28
44	9·3	0·8656	58·73
46	19·1	1·088	35·60
57	21·0	1·431	42·58
	10 days incubation		
40	3·2	0·2033	39·77
41	6·2	0·4545	39·77
44	5·2	0·3710	44·61
46	14·1	0·8615	38·19
57	16·1	1·1206	42·59

ACKNOWLEDGEMENTS

The authors express their gratitude to Dr. S. Z. Qasim, for his valuable suggestions on the manuscript and to Shri C. V. G. Reddy, Dr. A. B. C. Fernandes and Shri Albert Araujo for the help rendered.

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