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BIOSENSOR FOR THE ESTIMATION OF BIOLOGICAL OXYGEN DEMAND BASED ON *TORULOPSIS CANDIDA*

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***Torulopsis candida* has been used as the microbial biocatalyst for the estimation of biochemical oxygen demand [BOD]. A microbial sensor has been fabricated using a modified dissolved oxygen probe and coupling it with the microbe immobilised membrane. The analysis of various industrial effluents has been carried out using the probe and the results compared with the conventional method. The assimilation capacity of *Torulopsis candida* is evaluated and compared with that of *Trichosporon cutaneum* using the probe.**

Key words: Biosensor, microbe, *Torulopsis candida, Trichosporon cutaneum*, Dissolved oxygen probe, Biological Oxygen Demand

INTRODUCTION

Biochemical Oxygen Demand [BOD] is an important parameter in the estimation of organic content in industrial effluents, nutrient concentration in sea water and quality control in the waste water treatment [1,2]. The conventional method of BOD estimation takes five days. A biosensor using micro-organisms as the biocatalyst was developed by Karube et al [3]. This device is simple to operate and requires only 15 minutes to estimate the BOD. Since then, microbial sensors using *T Cutaneum* [4,5], *B Subtilis* [6], *H Anomala* [7, 8] as biocatalysts have been reported. Further, there are reports on sensors based on toxic resistant strains so as to avoid the problems of heavy metal interferences in the BOD estimation [9]. To have a better estimate of BOD value, the biocatalyst used should assimilate a wide spectrum of organic compounds in the sample. Attempts have been made, using microbes from activated sludge as biocatalyst and it was observed that the method gave erroneus results and the sensor's response could not be reproduced. In this paper, we have attempted to evaluate the assimilation capacity of the microbe, *Torlopsis Candida* as a biocatalyst and compared the data with that of *Trichosporon Cutaneum*.

EXPERIMENTAL

The microorganisms *T Candida* (NCIM 3234) and *T Cutaneum* (NCIM 3326) were procured from National Chemical Laboratory, Pune. They were grown in nutrient broth at 303 K for 48 hrs. The nutrient broth was having the ingredients of yeast extract 300 mg, glucose 1000 mg, malt extract 350 mg and peptone 500 mg in a total volume of 100 ml. The microorganism was harvested by centrifuging from the culture.

A dissolved oxygen (DO) probe was fabricated by us using a gold cathode (area 0.03 cm^2), a platinum counter electrode and a Ag/AgCl reference electrode. The gas permeable membrane used in the probe was purchased from *Century Instruments Co, Chandigarh*.

All the measurements were carried out using a Wenking Potentiostat Model POS 73 under stirred condition. All the reagents used were AR grade and used as received. The solutions were made using sterilised triple distilled water.

The conventional BOD estimation was carried out by incubation of the sample at 293K for a period of five days and subsequently the DO concentration was estimated using Winkler's method [1]. Reproducible data from duplicate experiments were used for the estimation of BOD.

Cellulose nitrate membrane (pore size 0.25 μ) procured from *Millipore* was used as the matrix for immobilisation. The microbe was immobilised by physisorption by filtration. After immobilisation, the membrane was washed thoroughly with buffer to remove the loosely bound organism on the membrane. The membranes were stored at 277K in phophate buffer when not in use.

RESULTS AND DISCUSSION

The configuration of the microbial sensor is given in Fig. 1. The sensor consists of the DO probe fabricated by us coupled to the immobilised membrane loaded with the microbe by sandwiching it with the aid of dialysis membrane and a 'O' ring.

The oxygen reduction current was monitored at −0.6 V against reference electrode. The performance of the DO probe was tested using oxygen saturated buffer, air saturated buffer and buffer containing sodium sulphite corresponding to 26 ppm, 7 ppm and 0 ppm of dissolved oxygen concentrations respectively. The steady state current (i_{ss}) showed linear dependancy on the concentration of DO and the reproducibility was good and has a response time of 10 s. In the two electrode assembly, the shortcoming of the probe arises due to the accumulation of Ag onto the cathode and drifting in the applied potential due to the formation of AgO on the Ag electrode. Both these problems are eliminated in the three electrode DO probe. This was evident from the fact that the sensor did not require routine clean up atleast for a month without any deterioration in the response for oxygen reduction. In the absence of the microbial membrane, the i_{ss} was about 0.8 μA wherein it decreased considerably in the presence of the microbe immobilised membrane due to the residual respiratory activity of the membrane and the barrier created by the additional membrane in the microbial sensor.

Assimilation capability of the microbes

The assimilation capacity of the two microbes has been studied using the fabricated sensor by the method described by Hikuma [10] and Riedel [11]. Typically 20 ppm of the organic substrate was taken and the oxygen reduction monitored in its presence (i_2) and in its absence (i_1). The change in the current ($\Delta i, i_1 - i_2$) is

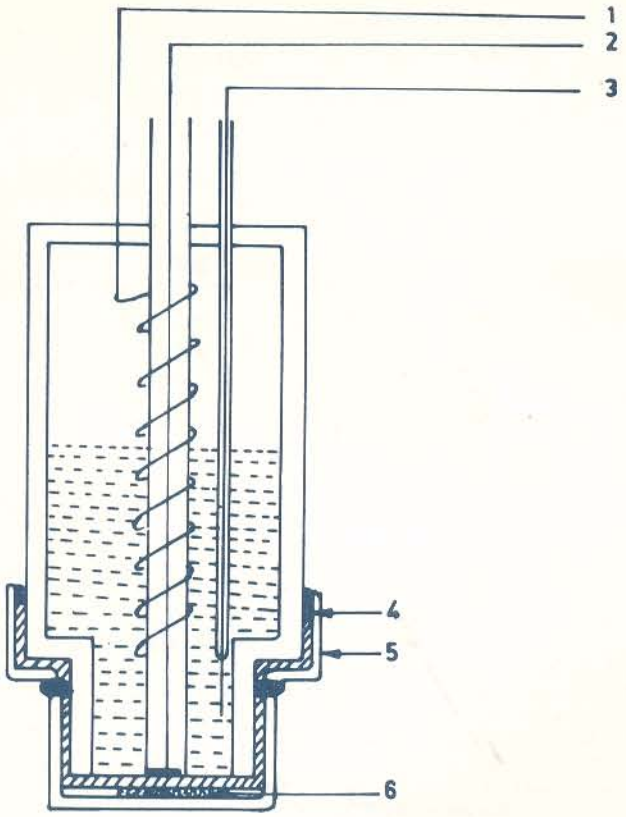

Fig. 1: Configuration of the microbial sensor. (1) Platinum counter electrode (2) Au working electrode (3) Ag/AgCl reference electrode (4) Oxygen permeable teflon membrane (5) Dialysis membrane and (6) Microbe immobilised membrane

seen to be a function of the assimilation capacity of the microbe. The decrease in the oxygen current in presence of 20 ppm of different substrates is given in Table I. From the Table, one can see that both the microbes assimilate a wide spectrum of organic substances. Further, it is clear from the Table that the assimilation by *T. Candida* is better as seen by the increment in the Δi values when compared to that of *T. Cutaneum*. Both the microbes did not respond to lactose and urea.

Glucose and glutamic acid were used as a standard BOD mixture. The BOD value of the standard mixture was estimated by conventional method. It is found that 20 ppm each of glucose and glutamic acid corresponds to 33 ppm of BOD value and is in agreement with the reported value [12]. The microbial sensor having the immobilised membrane with *T. Candida* was evaluated using a mixture of glucose and glutamic acid. The decrement in the oxygen reduction current was monitored with the increase in the addition of the BOD mixture. From the typical response curve (Fig. 2), it can be seen that the response time of the sensor is about 5 minutes. From the calibration graph (Fig. 3), it can be seen that the sensor is having a linear range upto 100 ppm. The life time of the microbe membrane was evaluated and found to be about 45 days without any drastic decrement in the activity as observed with the standard sample.

The microbial sensor with the biocatalyst *T. Cutaneum* as the catalyst was evaluated as mentioned above. From the calibration graph (Fig. 3), one can see that the linearity window for this sensor is only upto 80 ppm of BOD. However, there is no significant change in the response time of the probe.

A number of industrial samples have been analysed using both the BOD probes and the values estimated by the probe along with the values obtained by conventional method are listed. It can be

TABLE-I: Decrease in oxygen reduction current in the presence of 20 ppm of the organic compound using the microbe

Organic compound	Decrease in oxygen reduction current (μA)	
	T. candida(μA)	T. cutaneum
1. Glucose	0.122	0.085
2. Glutamic acid	0.122	0.085
3. Maltose	0.030	0.010
4. Mannose	0.015	0.018
5. Sucrose	0.033	0.010
6. Arabinose	(a)	0.020
7. Gluconic acid	0.066	0.020
8. Manitol	0.006	0.001
9. Glycine	0.066	0.066
10. Alanine	0.314	0.211
11. Glycerol	0.066	0.069
12. Ascorbic acid	0.085	0.085
13. Acetate	0.224	0.135
14. Ethanol[b]	0.023	0.014
15. Lactose	(a)	(a)
16. Starch	(a)	(a)
17. Urea	(a)	(a)

(a) No change in the oxygen reduction current

(b) 0.0001% of ethanol

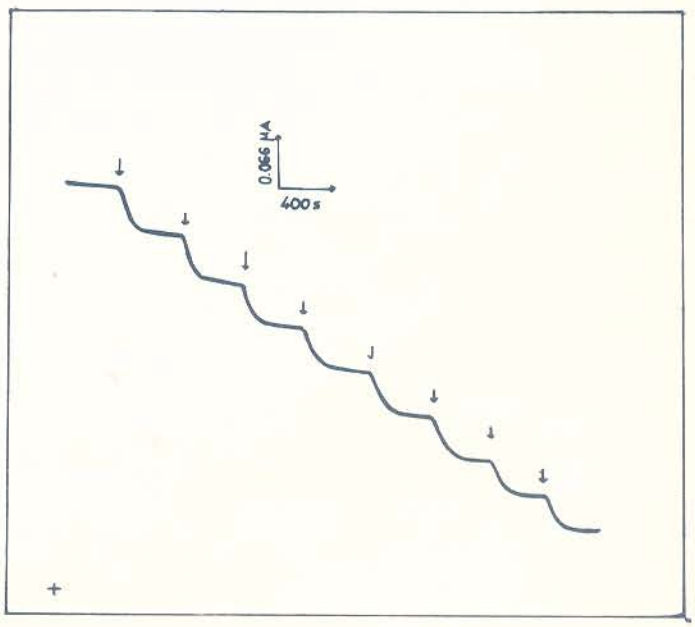

Fig. 2: Typical response curve of the biosensor for the successive addition of 0.2 ml of BOD solution (500 ppm of glucose + 500 ppm of glutamic acid) in 5 ml of phosphate buffer (pH 7.0). Arrow mark indicates the time of addition

seen that the values agree fairly well for 1 to 3 in Table II. A perusal of Table I indicates that both the microbes did not assimilate lactose within the time of response, which constitutes a major portion in the dairy waste. This may be the cause of lower estimation of BOD value in this case. Presently attempts are being made to condition

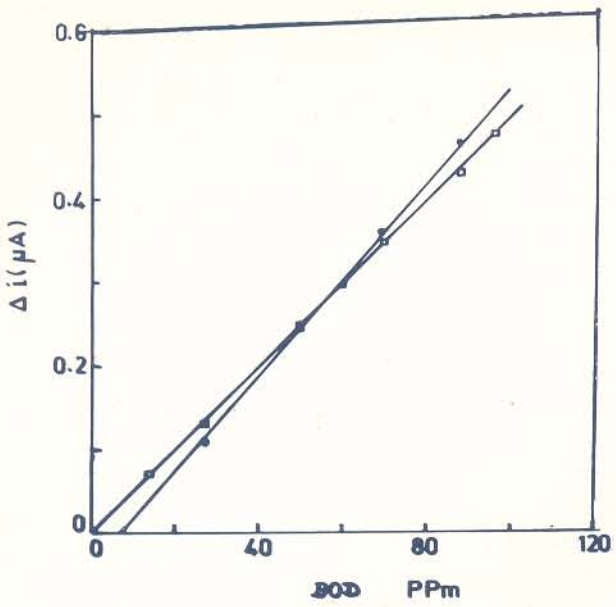

Fig. 3: The plot of BOD value in ppm vs change in oxygen reduction current (i in μA) for the fabricated BOD sensor based on ----■---- ■---- T. Candida; ----●----●---- T. Cutaneum

the microbes for the estimation of BOD of the dairy wastes.

CONCLUSION

The results clearly indicate that *T Candida* is a promising candidate for its potential use as biocatalyst for the estimation of BOD in paper and pulp and distillery effluents. The assimilable capacity of *T Candida* is seen to be better than that of the *T. Cutaneum*.

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TABLE-II: Estimated BOD values

Sample	BOD (ppm)		
	Conventional method	Using the sensor based on	
		T. Candida (ppm)	T. Cutaneum (ppm)
Paper and Pulp	61, 000	59,000	62,400
Paper and Boards	270	260	240
Distilleries	3,375	3,900	3,300
Sugars & Chemicals	1,326	1,657	1,152
Dairy waste	1,673	147	130

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