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under light vacuum and stored at 4°C.

For immobilization of the DAAO, 10 ml of a solution of 30 mg of DAAO/ml, was mixed with 94 ml of 0.2 M sodium bicarbonate, pH 9.5. This solution was gently mixed and added to the activated Sepharose and allowed to react for 48 hours at 4°C with gentle shaking. The aqueous layer was then checked to determine the percentage of the protein that had been immobilized.

Effect of pH on DAAO Immobilized by Polyaziridine: Dehydrated cellulose beads (0.5 g) were added to an appropriate buffer containing 200 μl of DAAO and 200 mg of HTA in 800 μl of buffer and allowed to cure overnight at 4°C. The buffers used were MES, pH 5.5, MOPS, pH 6.5, EPPS, pH 7.5, TAPS, pH 8.5, and CAPS, pH 10.0.After immobilization, the catalyst was washed in 10 ml of 0.1 M potassium phosphate buffer, pH 8.3 and assayed.

Effect of temperature on DAAO Immobilized by Polyaziridine: DAAO immobilized at pH 7.5 was added to 3.0 ml of 5 mM D-phe at pH 8.0, at temperatures between 26 and 49°C and then assayed.

Immobilized Enzyme Assay: A jacketed glass column (1.5 X 30 cm) was filled with 30 ml of catalyst from the purified DAAO polyaziridine immobilization and a second column of the same dimensions was filled with 30 ml of CNBr immobilized catalyst. A solution of 1 mM D-Phe containing 1 mM sodium phosphate pH 8.3 and 100 μl of catalase (24,000 units/ml)/L was passed over the column at 20.5 ml/hr. The 1 mM D-phe solution was also passed over the CNBr Sepharose column at 22 ml/hr. The substrate solution was stored at 4°C to prevent the breakdown of the catalase and D-phe before passage over the column.

The activity was monitored by determining the absorbance at 320 nm (A_{320}) of a 1:10 dilution of the column effluent in 1 N NaOH and comparison with a standard curve. The columns were maintained at 20°C by a circulating H_2O bath. The samples were obtained over a 30 minute period daily, while simultaneously determining the flow rate.

Removal of 5% D-phe from L-phe: A solution of 190 mM L-phe and 10 mM D-phe (100 ml) was added to immobilized crude DAAO (30 ml of polyaziridine immobilized DAAO), stirred gently for 2 hr after which the solution was assayed.

RESULTS AND DISCUSSION

DAAO has previously been immobilized by a variety of methods as shown in Table 1. While CNBr-Sepharose immobilized DAAO retained 15-42% of its activity in the hands of Carrea et al., (1983) and 50% of its activity in the hands of Naoi et al., (1978), we obtained only a 14% yield of free enzyme activity. It is clear from Table 1, that CNBr Sepharose is superior in terms of retained activity when compared to the other methods reported.

To compare the polyaziridine immobilization system with these previously reported methods of immobilization, DAAO was immobilized on cellulose beads using polyaziridine and on CNBr activated Sepharose 4B as described in materials and methods. In contrast to the results previously obtained by various methods of immobilization, the polyaziridine immobilized DAAO retained 62% of the free enzyme

activity. The effect of pH on the polyaziridine immobilization of DAAO is shown in Fig. 1. The catalyst retained 46 to 53% of its initial activity. The optimum pH for DAAO immobilization was pH 7.5.

The stability of immobilized DAAO was studied in fixed bed columns containing 25 ml of catalyst. The catalyst column was operated continuously at 20°C and a flow rate of 22 ml/hr. CNBr immobilized DAAO was also analyzed in a continuous column at the same time to compare the stability of the two immobilization systems (Fig. 2). The half-life of DAAO activity for the CNBr immobilized catalyst was approximately 3 days, which is consistent with the literature. After 7.5 days of continuous operation, the HTA immobilized DAAO catalyst retained 91.6% of its initial activity. A projected half-life of 69 days was calculated.

Exogenous FAD, the cofactor for DAAO, was not added to the substrate stream in these assays. Lemainque et al., (1988) showed that DAAO immobilized on chitosan did not lose its prosthetic group whereas the free enzyme required FAD addition to maintain

Table 1 Comparison of D-amino Acid Oxidase Activity and Stability after Immobilization by Different Methods

Support	Enzyme binding Ligand	% Protein Bound	% Activity Retained	Stability $T_{1/2}$	Reference
Sepharose	CNBr	98-100	15-42	3 days[1]	Carrea, 1983
Aminoethyl-Sepharose	glutaraldehyde	79-98	16-29	-	Carrea, 1983
Sepharose	CNBr	80	50	-	Naoi et al., 1978
Aminoalkyl Sepharose	1-ethyl-3-(3-dimethyl aminopropyl)	36	19	-	Naoi et al., 1978
Carboxyalkyl Sepharose	carbodiimide	29	10	-	Naoi et al., 1978
Attapulgite	glutaraldehyde	-	9-19	3-5 days[2]	Parkin, 1979
Chitosan	glutaraldehyde	80	17	20-30 hrs	Lemainque, et al.,1988
Controlled-pore glass	1-ethyl-3-[3(dimethylamine) propyl] carbodiimide	-	-	5-6days[1]	Chung, 1985
Sepharose	CNBr	91-97	14	3 days	Present Work
DP-1 resin	HTA	ND	62	69 days[3]	Present Work
DP-1 resin	HTA	ND	89[4]	ND	Present Work

1 FAD present in assay
2 Stability of immobilized DAAO stored at 4°C
3 Projected
4 Crude enzyme preparation
ND not determined

Table 1 summarizes the average temperature, HRT, loading rate, and the reductions of TOC and BOD_5. Reactor 1 reduced the BOD_5 from 1062 mg/l to 149 mg/l (86% reduction) at an average temperature of 24°C, HRT of 3.8 hrs and MLVSS level of 699 mg/l. Since there was no sludge recycled to Reactor 1, it could thus be calculated that 0.77 gram of sludge was produced for each gram of BOD_5 removed.

Table 1. Summary of the Activated Sludge Treatment

Characteristics	Reactor 1	Reactor 2	Reactor 3	Overall
Temperature (°C)	24.1	20.9	20.6	---
Volume (m^3)	0.87	182	1.82	4.51
HRT (hrs)	3.8	8.0	8.0	19.8
BOD_5 Loading Rate (g/m^3d)	6707	493	75	---
TOC reduction (%)	57	73	38	93
BOD_5 reduction (%)	86	82	64	99
TKN reduction (%)	---	---	---	91
NH_4-N reduction (%)	---	---	---	92

On the other hand, both Reactors 2 and 3 were operated at about 21°C, 8 hrs of HRT, and 2500 mg/l of MLVSS by controlling the amount of sludge recycled. Effluents from Reactors 2 and 3 respectively contained 29 mg/l and 9 mg/l of BOD_5, corresponding to overall reductions of 97% and 99%, whereas the overall TOC reductions were 57%, 83% and 93% in Reactors 1, 2 and 3, respectively, as shown in Figure 2. The respective overall reductions for TKN and NH_4-N were 91% and 92% after three stages of activated sludge treatment.

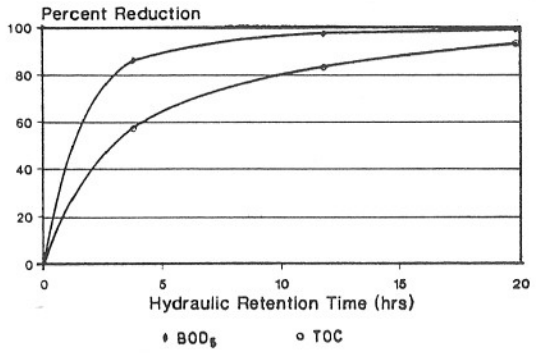

Figure 2 : Overall BOD_5 and TOC Reductions

Figure 3 shows that TOC/BOD5 ratios steadily increased from 0.59 in the plant wastewater to 1.81 in Reactor 1, 2.76 in Reactor 2, and 4.78 in Reactor 3. This clearly indicated that, as the treatment progressed, the remaining organics became less and less biodegradable, as what would be expected.

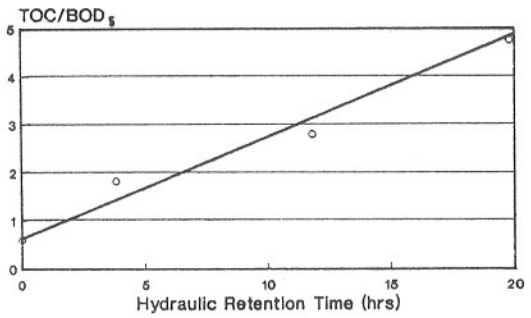

Figure 3 : TOC/BOD_5 in Wastewater and Reactors

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