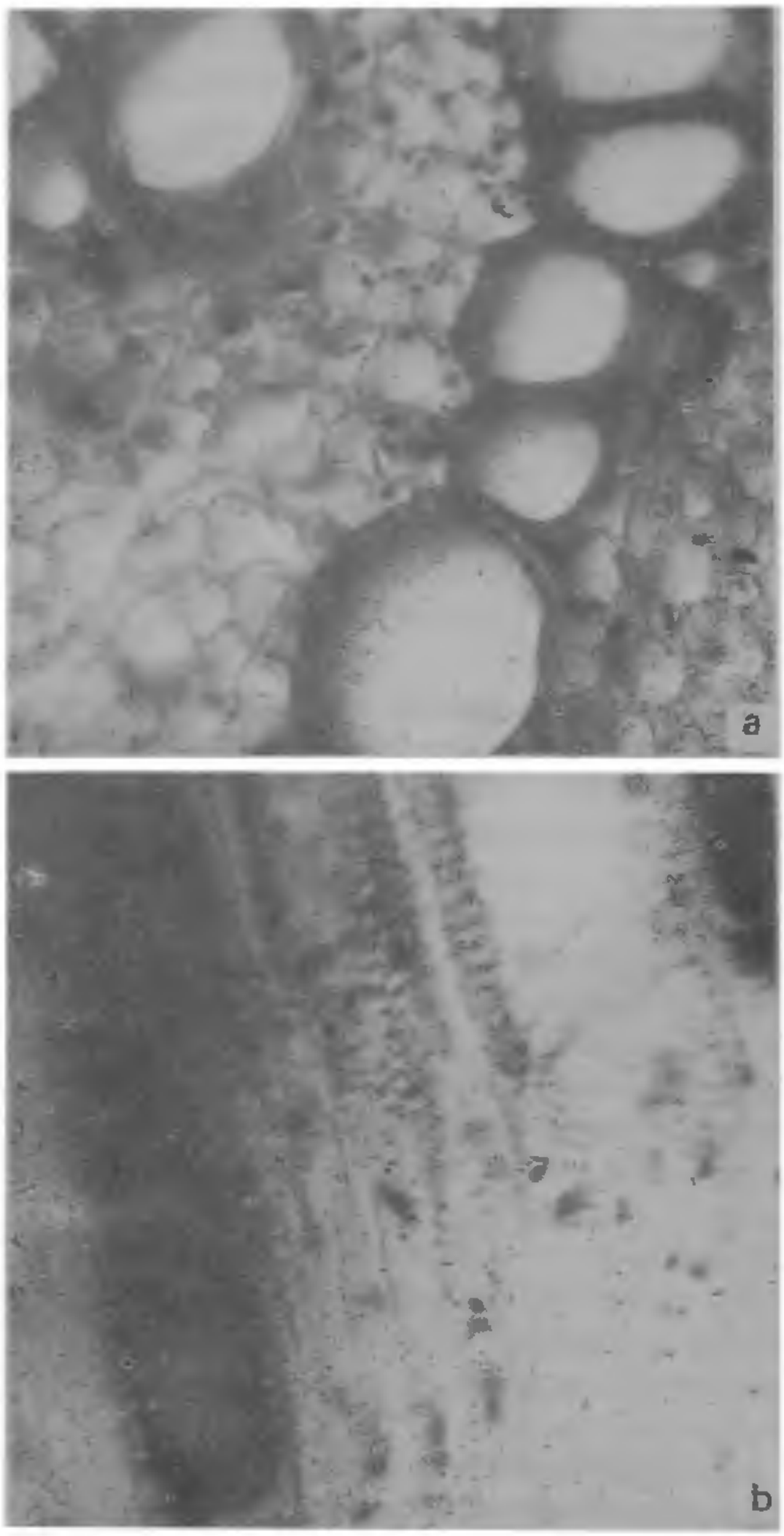

Figures 1a and b. Presence of microorganisms in stelar region of roots of mustard (×250). **a.** T. S. showing localization of microorganisms in vascular bundle. **b.** L. S. through secondary root.

indicate the presence of inherent microflora in the plant which may be enhanced by artificial bacterization. The entry of the introduced strains however could not be ascertained and requires further study.

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STUDIES ON FLOCCULATION OF *ZYMOMONAS MOBILIS**

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IN ethanol fermentations, flocculation facilitates removal of organisms from the product. In yeast, specific cell surface interactions under genetic control are responsible for flocculation[1], in which divalent and monovalent cations[2-4] play a key role. The present work describes the effect of divalent and monovalent ions on the flocculation of *Zymomonas mobilis*, an alternative organism for large scale ethanol production.

Zymomonas mobilis ATCC 10988 and ATCC 12526 were obtained from Oak Ridge National Laboratory, USA and maintained on YPS agar. The fermentation medium contained 1% yeast extract, 1% peptone and 15% (w/v) sucrose. The fermentation was carried out as reported earlier[5].

To measure flocculation, cells were harvested from 24 hr grown culture and washed twice with phosphate buffer (pH 7.0) and resuspended again in buffer. The standard procedure for seeded settling[6,7] was to mix the required quantity of seed with the suspension and allow it to settle. The initial absorbance was adjusted to 0.2 OD at 550 nm. After 10 min a sample of the supernatant was taken to determine the drop in absorbance of the samples at 550 nm using Klett-Summersion type colorimeter (Biochem Model M6).

The flocculation rate was calculated by the difference in the absorbance per initial absorbance multiplied by the time factor.

Figure 1 shows a comparison of the flocculation of

*Dedicated to Prof. S. Krishnaswamy, Vice-Chancellor, Madurai Kamaraj University in commemoration of his 60th birth anniversary, 1986.

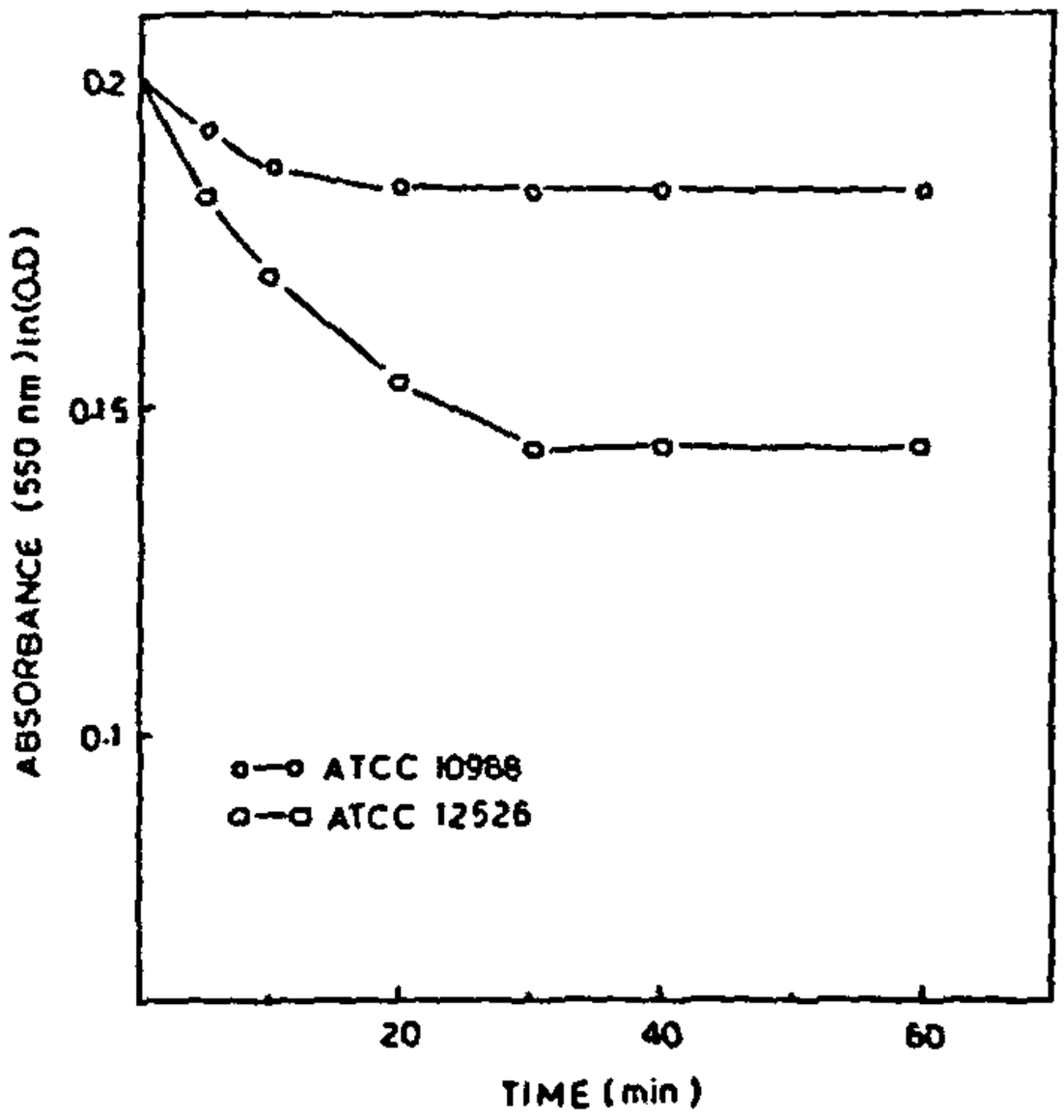

Figure 1. Comparison of flocculation pattern of *Z. mobilis* strains non-flocculent (ATCC 10988) and flocculent (ATCC 12526).

Zymomonas mobilis strains ATCC 12526 (flocculent) and ATCC 10988 (non-flocculent). Evidently strain ATCC 12526 shows higher flocculation rate (4.6×10^{-3}) than the non-flocculent strain ATCC 10988 (10^{-3}).

Yeast flocculation is Ca^{++}dependent[2-4]. Taking this into consideration Ca^{++} ($CaCl_2$) was used from 10^{-3} M to 1.5×10^{-2} M concentration (data not shown) to study the effect of Ca^{++} on the flocculation of *Z. mobilis* ATCC 12526. This strain showed maximum flocculation rate at 10^{-2} M Ca^{++} (figure 2a and table 1). The same table and figure also show the effect of $MgSO_4$ (10^{-2} M), $MnCl_2$ (10^{-2} M) and $BaCl_2$ (10^{-2} M) on flocculation. Among the four divalent ions used, Ca^{++} remarkably increased the flocculation (figure 2a and table 1) and Mg^{++} had some effect, while Mn^{++} and Ba^{++} actually decreased flocculation. None of these ions increased the flocculation of *Z. mobilis* ATCC 10988.

In the presence of Ca^{++} flocculent cells may possess cell-cell interaction where the mannon layers might have involved as reported for yeast[3] and/or Ca^{++} may facilitate the flocculation by binding to cellulose fibrils more readily and firmly in flocculent cells. The results obtained by us also support our hypothesis that Ca^{++} significantly increases the flocculation in this organism. The decreased flocculation rate with Ba^{++}, compared to the control could be due to the inhibitory action on the formation of flocs[4]. Comparing the flocculation rate using

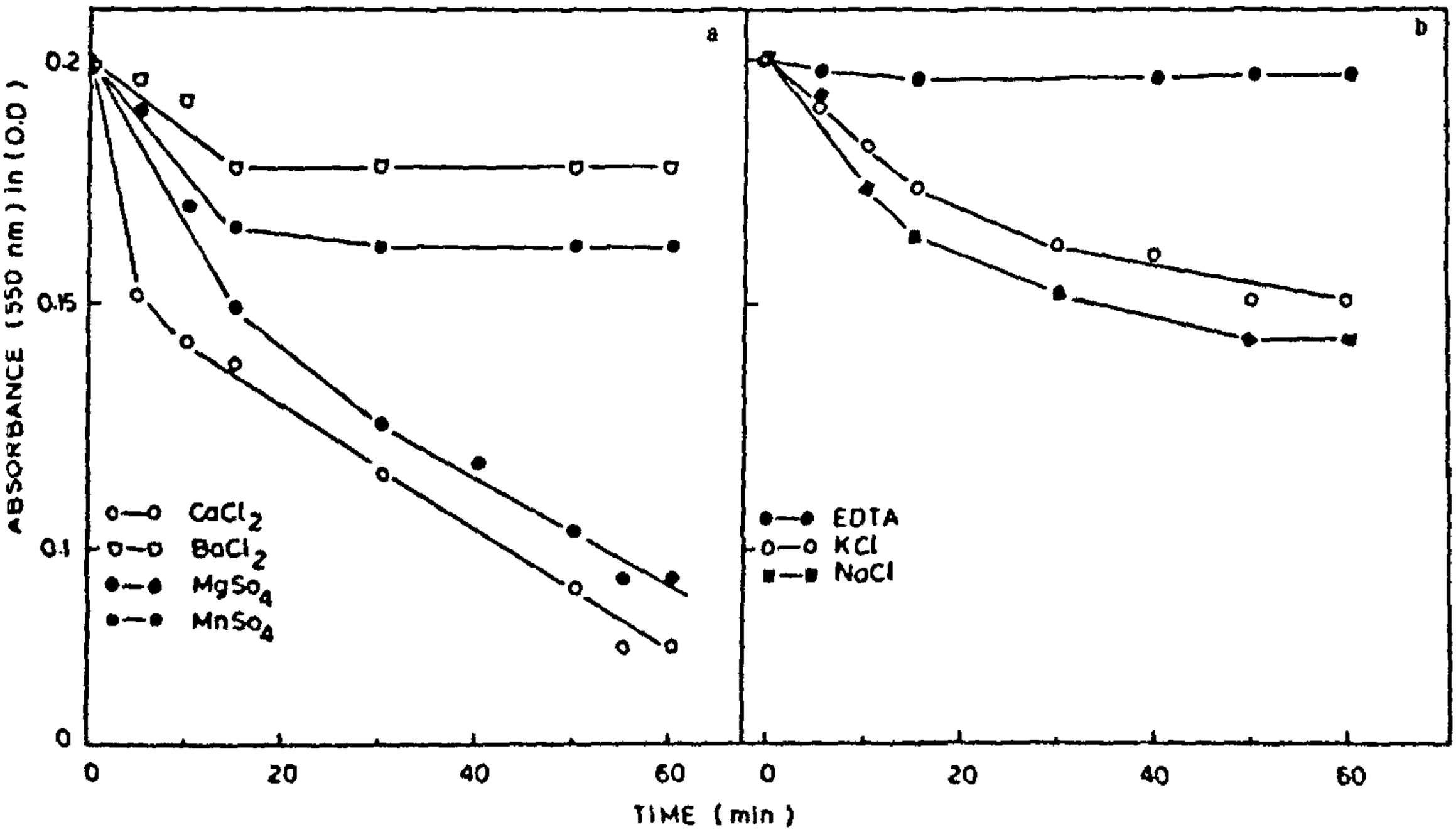

Figure 2. Effect of divalent and monovalent ions and EDTA on the flocculation pattern of *Z. mobilis* ATCC 12526.

Table 1 *Effect of divalent and monovalent ions and EDTA on the flocculation pattern of Zymomonas mobilis ATCC 12526*

Ions used (10^{-2} M)	Flocculation rate ($\times 10^{-3}$)
Control	4.6
Ba^{++}	1.6
Mn^{++}	2.8
Mg^{++}	9.2
Ca^{++}	10.0
K^{+}	4.2
Na^{+}	5.0
EDTA (5×10^{-3} M)	Nil

Table 2 *Effect of divalent ions on flocculation and fermentation of Zymomonas mobilis ATCC 12526*

Divalent ions used (10^{-2} M)	Ethanol production % (w/v)[a]	Flocculation rate
Control	6.6	4.6×10^{-3}
Mg^{++}	6.6	9.2×10^{-3}
Ca^{++}	2.5	1.0×10^{-2}

[a]Ethanol produced from 15% (w/v) initial sugar concentration at 72 hr.

various divalent ions (table 1) next to Ca^{++}, Mg^{++} facilitate the flocculation.

Monovalent ions KCl (10^{-2} M) and NaCl (10^{-2} M) had no effect, while EDTA (5×10^{-3} M) strongly inhibited the flocculation of *Z. mobilis* ATCC 12526, presumably by chelating divalent ions as in the flocculation of yeast[2-4,6].

From all the above experiments, it is clear that Ca^{++} and Mg^{++} could be used to enhance flocculation. In order to find out whether these divalent ions could be useful for flocculation in cell-recycling systems, we conducted batch fermentation of *Z. mobilis* ATCC 12526 with these ions. Mg^{++} (10^{-2} M) did not affect the ethanol production at 15% (w/v) initial sugar concentration[8] but Ca^{++} (10^{-2} M) drastically reduced the ethanol production (table 2). From these it is evident that although Ca^{++} and Mg^{++} enhance the flocculation of *Z. mobilis* ATCC 12526, Mg^{++} alone could act as the efficient flocculating agent in cell-recycling systems.

16 June 1986; Revised 25 August 1986

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A PRELIMINARY REPORT ON VENOM APPARATUS IN *CONUS AMADIS* (GMELIN) FROM PORTO NOVO COAST

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There are about 400 species recorded so far in the family: Conidae (Gastropoda: Mollusca), mostly tropical and characteristically associated with coral reefs. The cones are nocturnal carnivores[1]. All have a venom bulb and a harpoon-shaped radula. Some important studies on the cones were made earlier[1-5]. In Indian waters, there is no information on the structure and function of the venom apparatus of cones. Habermeh[6] described the effects of venom in the human body; the sting is painful, and the site of the sting swells and the pain gradually spreads over the whole body especially the lungs and mouth leading to visual disturbance, vomiting and death may ensue in human beings[4]. The present study deals with the structure of venom apparatus in *Conus amadis*.

C. amadis were collected from a depth of 3–13 fathoms in the Porto Novo Coast (Lat. 11 29'N; Long 79°46'E) and dissected to examine the structure of venom bulb and associated structures. The venom apparatus as a whole has one cucumber-shaped venom bulb, about 1.6 cm in length and 0.5 cm in diameter with a blunt tip of 0.2 cm diameter (figure 1).

The venom bulb is embedded in the muscles near the anterior oesophagus connected with the pharynx through the venom duct. The coiled venom duct originates at the right end of the venom bulb and passes under the bulb. The portion of the duct just after