Degradation of a simple lignin model substrate by microorganisms from different environments

N. Vasudevan and A. Mahadevan
Centre for Advanced Study in Botany, University of Madras, Madras 600 025, India

***Alcaligenes* sp. and *Bacillus subtilis* (i) utilized 3-(*o*-methoxyphenoxy)-1,2-propanediol as sole carbon source but *B. subtilis* (ii) and *Candida albicans* did not. Utilization of the compound by *B. subtilis* (i) and *Alcaligenes* sp. improved in the presence of glucose or sucrose.**

THE complex molecular structure of lignin, which has a variety of functional groups and different types of linkages between the monomer units, makes it recalcitrant to microbial attack[1]. Although fungi, especially basidiomycetes, have been mainly used to study lignin

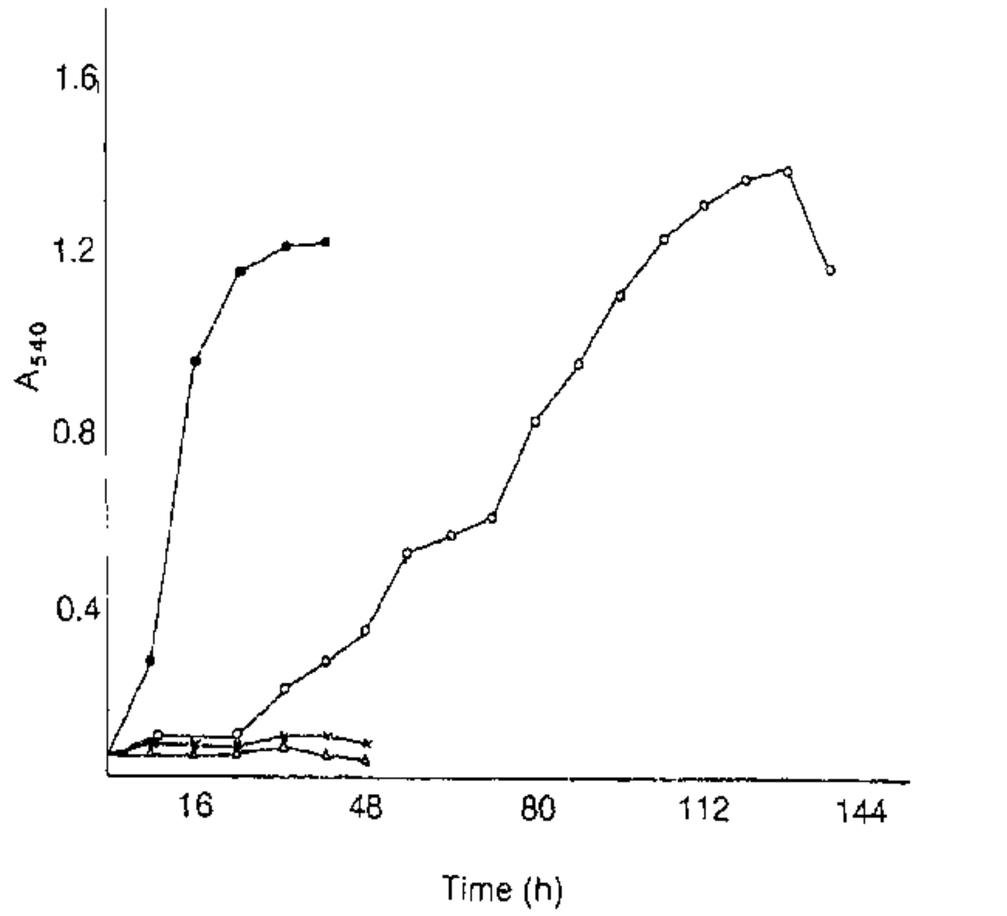

Figure 1. Utilization of guaiacol glyceryl ether by *Alcaligenes* sp. (○), *B. subtilis* (i) (●), *B. subtilis* (ii) (×) and *C. albicans* (△).

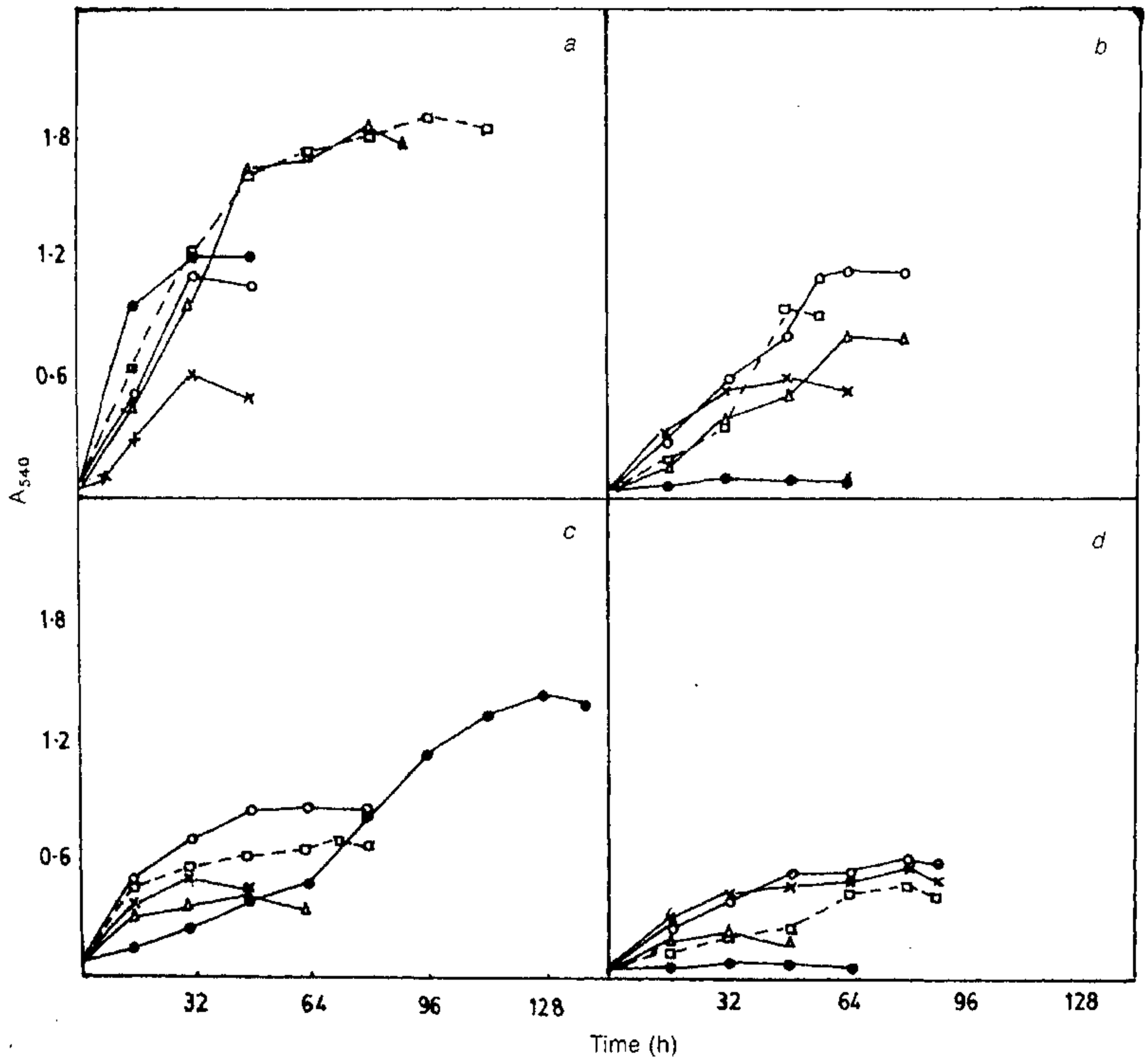

Figure 2. Effect of glucose on utilization of GGE by *a*, *B. subtilis* (i); *b*, *B. subtilis* (ii); *c*, *Alcaligenes* sp.; *d*, *C. albicans*. ×2.5 mM Glucose; ○, 5.0 mM glucose; ●, 2.5 mM GGE; △, 2.5 mM GGE + 2.5 mM glucose; □, 2.5 mM GGE + 5.0 mM glucose.

cleavage, bacterial degradation of lignin and lignin model compounds has not received much attention[2]. Of the many types of linkages, *β-O*-4 and phenylcoumaran type of linkages containing ether linkages are important[2]. We tested *Bacillus subtilis* (i), *B. subtilis* (ii) and *Alcaligenes* sp. isolated from forest soil containing decaying wood for their capacity to degrade 3-(*o*-methoxyphenoxy)-1,2-propanediol (guaiacol glyceryl ether, GGE), a lignin model compound. The influence of sugars on utilization of the model compound by the organisms was also studied.

Alcaligenes sp., *B. subtilis* (i) and *B. subtilis* (ii) were isolated by enrichment culture technique from paper mill sediments and forest soils containing decaying wood using black liquor lignin (0.05% w/v) as sole carbon source. They were identified by standard tests[3]. Cultures were maintained on Dye's medium[4] containing glycerol as carbon source. The medium (pH 7.2) contained (in g/l) $(NH_4)_2HPO_4$, 1.0; KH_2PO_4, 2.0; KCl, 2.0; $MgSO_4 \cdot 7H_2O$, 0.2; $Fe(NH_4)_2SO_4$, 0.01; glycerol 2.15 ml. *Candida albicans* was from our culture collection and maintained on malt extract medium.

Growth of the organisms on GGE was studied by aseptically adding filter-sterilized GGE (Sigma) to medium (pH 7.0) containing[5] (in g/l) K_2HPO_4, 1.6; KH_2PO_4, 0.5; $(NH_4)_2SO_4$, 1.25; $MgSO_4 \cdot 7H_2O$, 0.5; NaCl, 0.25; $FeCl_3 \cdot 6H_2O$, 0.025; $CaCl_2$, 0.010. Cells grown (24 h; 8×10^4/ml) on glycerol or malt extract medium were inoculated into 20 ml of medium and incubated at 30°C in static culture. Growth rate was monitored by measuring turbidity at 540 nm in a Spectronic 710 colorimeter.

GGE served as a good substrate for *B. subtilis* (i) isolated from forest soil. But another strain, *B. subtilis* (ii), did not utilize GGE (Figure 1). *Alcaligenes* sp. isolated from paper mill effluents utilized GGE after a prolonged lag; *C. albicans* did not.

Nutritionally simple carbon sources such as glucose and sucrose may influence the dissimilation of GGE. Therefore we tested the utilization of the compound in the presence of glucose or sucrose. Addition of 2.5 mM glucose to GGE markedly enhanced the growth of *B. subtilis* (i) compared to glucose alone at 5 mM (Figure 2*a*). In the case of *B. subtilis* (ii) growth was

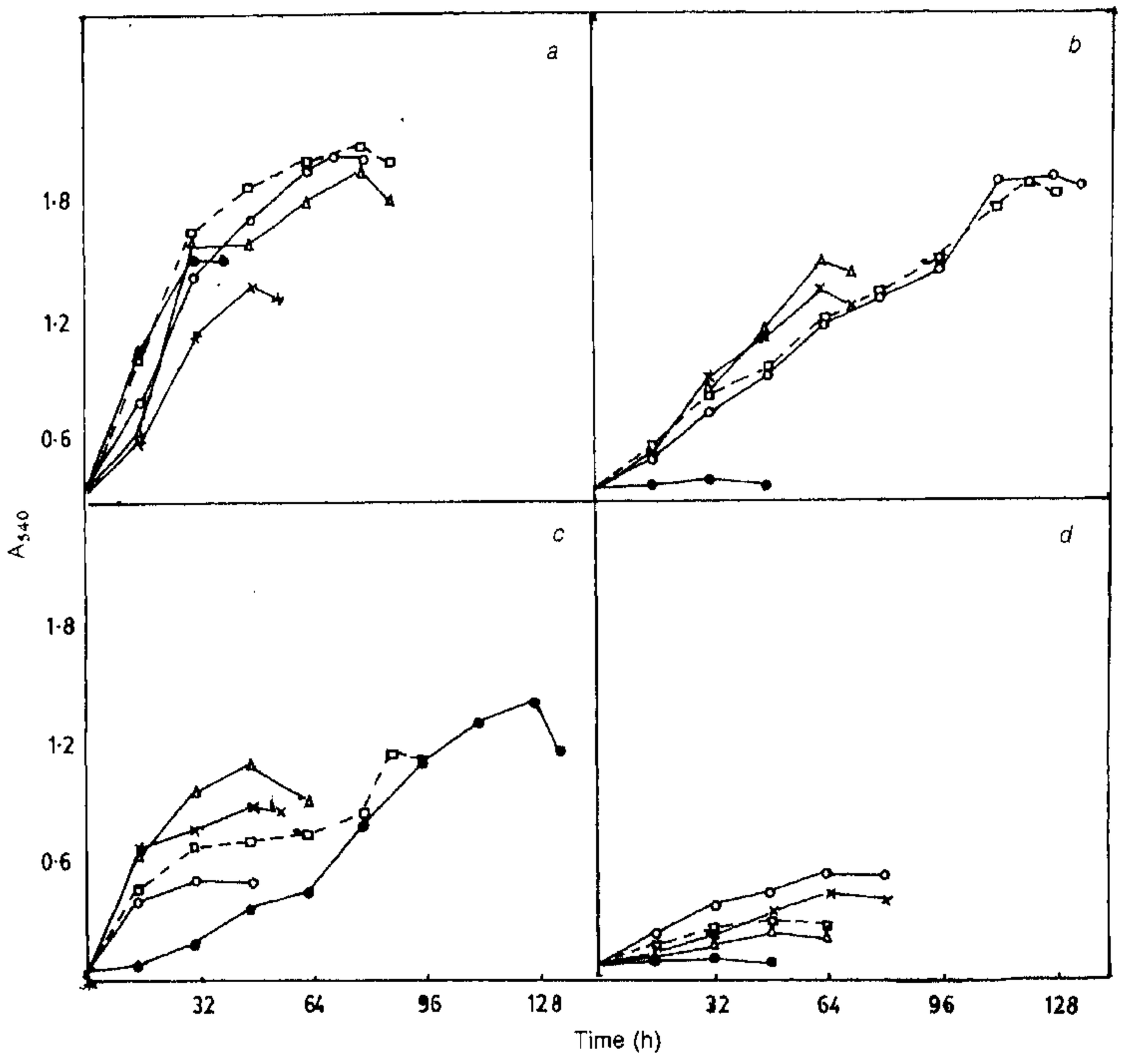

Figure 3. Effect of sucrose on utilization of GGE by *a*, *B. subtilis* (i); *b*, *B. subtilis* (ii); *c*, *Alcaligenes* sp.; *d*, *C. albicans*. ×, 2.5 mM Sucrose; ○, 5.0 mM sucrose; ●, 2.5 mM GGE; △, 2.5 mM GGE + 2.5 mM sucrose; □, 2.5 mM GGE + 5.0 mM sucrose.

diauxic with GGE + 5 mM glucose (Figure 2*b*). Addition of glucose to GGE significantly suppressed growth of *Alcaligenes* sp. (Figure 2*c*).

Growth of *B. subtilis* (*i*) on GGE was high in presence of GGE + sucrose (Figure 3*a*) and was diauxic in GGE + 2.5 mM sucrose. In GGE + 5 mM sucrose *Alcaligenes* sp. showed diauxic growth (Figure 3*c*). *C. albicans* did not utilize GGE even in the presence of glucose or sucrose (Figure 2*d*, 3*d*).

It is of interest to record that two strains of *B. subtilis* from the same forest soil had contrasting characteristics with respect to utilization of GGE. The inability of *B. subtilis* (ii) and *C. albicans* to utilize GGE suggests that they lack the capacity to produce the enzymes required for utilization of the compound.

Attempts have been made to enhance the degradation of lignin model substances by using additives such as succinate, glucose and cellobiose[6]. Muranaka *et al.*[7] reported that glycerol increased the degradation of 3-(2-methoxy-4-formylphenoxy)-1,2-propanediol by *Flavobacterium ferungium* and *F. ringense*. In contrast, cell yield of *Alcaligenes* sp. and *B. subtilis* (i) was higher when grown on GGE alone than when grown on glucose.

Low levels of glucose and high levels of nitrogen allowed rapid release of $^{14}CO_2$ from methoxy groups of vanillic and syringic acids[8]. *Pseudomonas tabaci*[9], *P. putida*[10] and *Rhodotorula mucilaginosa*[11] cells grown on glycerol or glucose oxidized aromatic substances only after a prolonged lag because of enzyme repression. The diauxic pattern we have observed must be due to utilization of the easily metabolizable substrate glucose or sucrose initially, followed by utilization of GGE.

1. Kirk, T. K. and Farrell, R. L., *Annu. Rev. Microbiol.*, 1987, **41**, 465.
2. William, F., Boominathan, K., Vasudevan, N., Gurujeyalashmi, G. and Mahadevan, A., *J. Sci. Ind. Res.*, 1986, **45**, 232.
3. Buchanan, R. E. and Gibbons, N. E., *Bergey's Manual of Determinative Bacteriology*, 8th edn, Williams and Wilkins, Baltimore, 1974.
4. Dye, D. W., *Nature*, 1958, **182**, 1813.
5. Janshekar, H., Haltmeier, T. and Brown, C., *Eur. J. Appl. Microbiol. Biotechnol.*, 1982, **14**, 174.
6. Kirk, T. K., Connors, W. J. and Zeikus, J. G., *Appl. Environ. Microbiol.*, 1976, **32**, 192.
7. Muranaka, M., Kinoshita, S., Yamada, Y. and Okade, H., *J. Ferment. Technol.*, 1976, **54**, 635.
8. Ander, P., Eriksson, K. E. and Yu, H. S., *Arch. Microbiol.*, 1983, **136**, 1.
9. Nagarajan, M. and Mahadevan, A., *Indian J. Exp. Biol.*, 1979, **17**, 757.
10. Ornston, L. N., *J. Biol. Chem.*, 1966, **241**, 3800.
11. Huber, J. J., Street, J. R., Bull, A. T., Cook, K. A. and Cain, R. B. *Arch. Microbiol.*, 1975, **102**, 139.

ACKNOWLEDGEMENT. NV thanks the Department of Environment, New Delhi, for financial assistance.

21 January 1989

Ethanol injection into the suprachiasmatic nuclei disrupts the day night feeding rhythm in the Wistar rat

V. Reghunandanan, R. K. Marya, B. K. Maini and R. Reghunandanan
Department of Physiology, Medical College, Rohtak 124 001, India

Effect of ethanol injection on the circadian rhythm o food intake in Wistar rats was investigated. Ethanol wa injected into the bilateral suprachiasmatic nuclei (SCN through a chronically implanted cannula. The circadia rhythm of food intake was disrupted by injections durin the beginning of light and dark (LD) phases of the LI cycle, with no significant change in total daily foo intake. Increase or decrease in concentration of ethano did not produce any change in the extent of disruption Saline injection or injection of ethanol into the area nea SCN had no effect on the circadian rhythm of foo intake. It is suggested that, by inhibiting vasopressi function, ethanol altered the circadian rhythm of foo intake.

UNDER normal environmental conditions the light dark (LD) cycle acting as a *zeitgeber* or entraining stimulu brings about the entrainment of various circadia rhythms. In the absence of the environmental time cues these endogenously generated circadian rhythms free run with a period of approximately 24 h (ref. 1) Entrainment by periodic stimuli in addition to the LI cycle is also possible. Certain chemicals can be a source of such periodic stimuli and act as a non-photic *zeitgeber*, though their ability to modify the circadian rhythms varies, from mimicking the effect of light by carbachol[2] to slowing or dissociation of circadian rhythms[3].

Ethanol, which is one of the inhibitors of vasopressin (VP) function[4], has been found to lengthen the endogenous tidal rhythms in isopods[5] and to lengthen the period of locomotor activity rhythm in hamsters[6]. VP has been identified as a putative neurotransmitter at the suprachiasmatic nuclei (SCN)[1] and it is likely to take part in the control of various circadian rhythms. The objective of the present study was to investigate the effects of ethanol injection into SCN on the circadian rhythm of food intake in the rat.

Wistar rats weighing 200 300 g were housed in individual cages. The temperature of the animal room was kept within 22 ± 2°C. The lighting regime consisted of 12 : 12 LD cycle with lights on from 0900 h to 2100 h. All animals had a minimum habituation period of 7 days prior to the start of the experiment. Food and water were given *ad libitum*.

Under nembutal anaesthesia (50 mg/kg), intraperitoneally 22-gauge stainless steel guide cannulae were chronically implanted in the midline to deliver injections into SCN bilaterally. The stereotaxic co-