larger. However it is interesting to observe that a few volant young are still carried by their mothers.

At the time of breeding in several species of bats there is a high energy demand especially during the periods of advanced pregnancy and lactation. In addition to the cost of lactation the mother bats spend large additional amounts of energy soon after parturition in which mothers carry their infants continuously. The majority of bat species breed annually at energetically favourable periods when availability of insects for the nurture of the young and to meet their own demands of energy is maximum[4].

Since the newborn bats are hairless, sightless and flightless the mothers take care of their infants. The young never leave the nipple or pubic teats of the mothers during their early stage of life and may cling to the body of the mothers even while the latter forage[1]. Since the cave is a well-protected area, a few mothers of *H. speoris* leave their infants even at the newborn stage inside the cave to forage[5]. The mother–young relations progressively decline and thus more young bats are left behind in the cave as they become older (Figure 1). However it is of great interest to note that a few volant young (forearm length is about 40 mm) were still carried to the foraging areas. Even though this process increases the wing loading of mothers[3] the volant young must be highly benefitted by it. They practice the inborn and ingenious method of echolocation by following their mothers at foraging areas[6]. The volant young are able to emit the adult's type of CF–FM echolocation sounds of 135 kHz[7]. Brigham and Brigham[8] and Vaughan and Vaughan[9] have reported for temperate species of insectivorous bats evidence for association between mother bat and its young during and after foraging. In the case of the African yellow-winged bat *Lavia frons*[9] each young shared its parents foraging territory, synchronized its grooming and foraging periods with those of its parents and periodically huddled against its mother while roosting until at least 50 days after its first flight. Thus the relationship between mother and infant includes not only lactation but also training and practice the mother imparts to infants to navigate in the foraging areas dexterously using the echolocatory machinery. However we have no information as yet that the same infants get carried by the same mother bats on a daily basis. If that is not the case then one can account for volant subadults which are left behind. We can also then believe that being taken for the 'acquaintance flights' is a feature that applies to all subadults and is thus a part of their training and growing up.

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Studies on interrelationship between different anaerobic bacteria in biogas digester

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Studies on interrelationship between methanogenic sulphate-reducing and denitrifying bacteria were carried out in a anaerobic biogas digester. Most probable number of these bacteria, concentration of sulphate and nitrate in fermenting slurry, gas production and redox potential were compared for 52 days. It was observed that sulphate-reducing bacteria reduce initial sulphate content of the fermenting slurry to bring it to low level and denitrifying bacteria help to establish the anaerobiosis by reducing the redox potential through utilization of nitrate.

A large variety of microorganisms are involved in production of methane by anaerobic digestion of organic matter. Although only three types of microorganisms, viz. cellulolytic, acid-producing and methanogenic bacteria are directly involved in production of methane, there are some other species of bacteria also present in anaerobic ecosystem which affect methanogenesis.

Sulphate-reducing and denitrifying bacteria are of particular importance since they have been reported to inhibit methanogenesis when sulphate and nitrate respectively is available in the ecosystem. These bacteria have been reported to compete with methanogenic bacteria for their common substrates and thus inhibit methanogenesis[1–3]. It has also been reported[4,5] that when sulphate is depleted from the ecosystem sulphate-reducing bacteria help methanogenesis by interspecies hydrogen transfer.

All these studies have been carried out on marine sediment

and lake sediments where methane production occurs naturally. It is also necessary to study the exact role of these bacteria in anaerobic biogas digester.

Experiments were therefore carried out to study the interrelationship between sulphate-reducing, methanogenic and denitrifying bacteria in a anaerobic batch digester charged with cattle dung.

Fresh cattle dung slurry having 10% total solid contents was subjected for anaerobic digestion for 52 days in a laboratory model of biogas digester. Sulphate and nitrate were estimated by turbidometric and colorimetric methods respectively[6], on every 4th day. Enumeration of methanogenic sulphate-reducing and denitrifying bacteria was also carried out on every fourth day by most probable number (MPN) method. Total volume of gas produced, the percentage methane and redox potential of fermenting slurry were measured every day throughout the experiment. All the experiments were carried out in the Department of Microbiology, MACS Research Institute, Pune.

It can be seen from Figure 1 that the MPN of methanogenic bacteria reached the maximum level on day 12 and then declined to reach the lowest level on day 48. Biogas production was evident from day 4 but methane was first detected on day 8, methane percentage of biogas remained between 55 and 63.2% during the period of digestion. The MPN of sulphate-reducing bacteria reached the maximum level on day 12 and then declined to reach the lowest level on day 52. The initial concentration of sulphate of 21 mM was reduced rapidly to 6 mm on day 8 and then gradually to 1 mM on day 24 with no further decrease during the subsequent course of digestion.

The MPN of denitrifying bacteria (Figure 2) reached the maximum level on day 12 followed by gradual decrease on day 40. It then remained constant during the subsequent course of digestion. The concentration of nitrate declined and reached the lowest level of 14 mM on day 20 and then remained constant throughout. The initial redox potential (E_h) was gradually reduced till day 24.

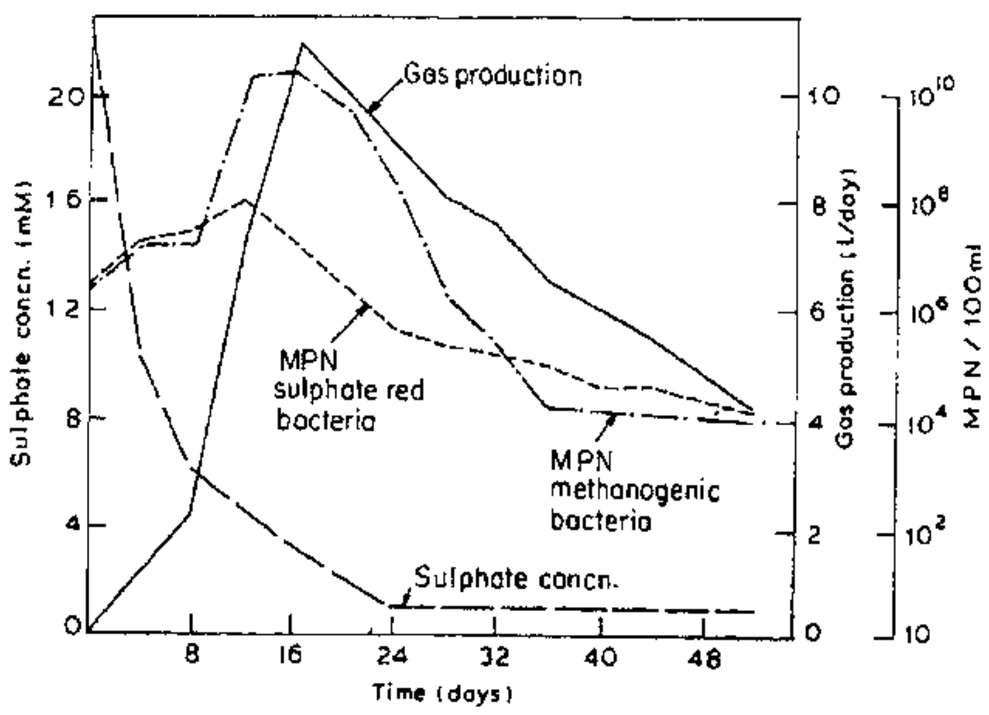

Figure 1. Ecology of batch anaerobic digestion of cattle dung with special reference to sulphate reduction.

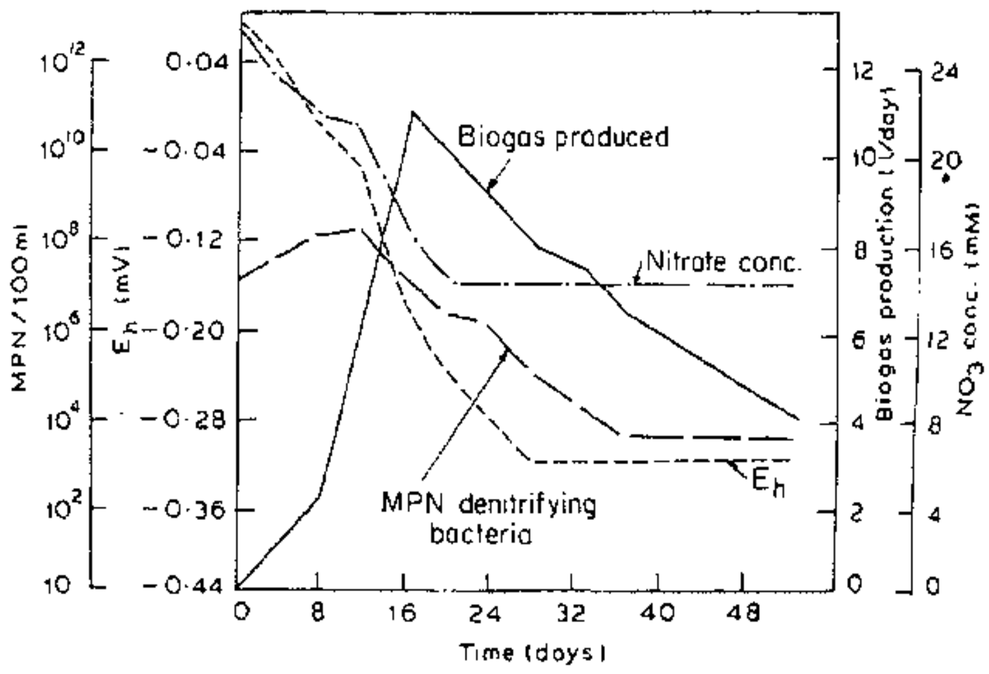

Figure 2. Ecology of batch anaerobic digestion of cattle dung with special reference to denitrification.

It has been reported earlier[3] that 10 mM concentration of sulphate inhibited methanogenesis in lake sediments. In the present studies it was observed that initial concentration of sulphate in cattle dung slurry was 21 mM.

It was seen that methane was detected only when sulphate concentration dropped to 6 mM on day 8. The quantity of biogas produced during the first seven days was relatively small and did not contain any methane. These results supported the findings of Cappenberg[2], and Martens and Berner[7], who have reported that methane was not produced in marine sediments until after the depletion of sulphate.

During the period of digestion initially there was rise in MPN of methanogenic and sulphate-reducing bacteria. Methane production increased to its peak when sulphate level was minimum. These findings indicate that during the initial non-methanogenic phase of anaerobic digestion sulphate-reducing bacteria play an important role in reducing the sulphate content of the fermenting slurry to bring it to a minimal level. The increase in gas production after six days might be due to interspecies hydrogen transfer between sulphate-reducing and methanogenic bacteria as reported by Mah *et al.*[4]

When the data on the denitrifying population, the nitrate content of the fermenting slurry, and the redox potential (E_h) during the course of digestion are compared, it becomes evident that a rise in MPN of denitrifying bacteria was concomitant with a drop in redox potential of the fermenting slurry. Since the methanogenic bacteria require very low redox potential for their growth it can be said that denitrifying bacteria help methanogenesis by reducing redox potential.

The present results thus establish the exact role of sulphate-reducing and denitrifying bacteria in the non-methanogenic phase. The sulphate-reducing bacteria reduce the sulphate content of the fermenting slurry, and the denitrifying bacteria help to establish the anaerobiosis by reducing the E_h through utilization of nitrate.

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The *ortho* cleavage of catechol by *Azotobacter chroococcum*

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Azotobacter chroococcum **utilized catechol as sole carbon source. Maximum growth was supported by 2 mM. Catechol was cleaved by the *ortho* pathway mediated by catechol 1, 2-dioxygenase.**

In an effort to isolate efficient strains of *Azotobacter* that would rapidly cleave phenolic waste, we isolated a strain of *Azotobacter chroococcum* from Kalakad Reserve Forest, Tamil Nadu. Among the dihydric phenols which are substrates for ring cleavage, catechol is the most prominent one[1]. Azotobacters are known to cleave catechol by the *meta* pathway[2]. A study[3] of benzoate catabolism in *Azotobacter* sp. established the oxalocrotonate branch for *meta* cleavage of catechol.

A. chroococcum strain MSB1 was grown in nitrogen-free growth medium (mannitol, 5 g; K_2HPO_4, 0.03 g; $MgSO_4 2H_2O$, 0.2 g; $Na_2SO_4 10H_2O$, 0.2 g; $CaCl_2$, 0.01 g; $Na_2MoO_4 2H_2O$, 0.005 g; $FeSO_4 7H_2O$, 0.002 g; distilled water 1000 ml; pH 7.0) to which filter sterilized catechol at different concentrations was added. The cells were grown at 30°C at 125 rpm in an orbital shaker. After 3 days of incubation the mode of ring cleavage was determined by Rothera's reaction[4] and by the assay of catechol 1,2-dioxygenase[5].

Catechol was utilized by *A. chroococcum* after 8 h. Catechol at 2 mM was a better carbon substrate than at 5 mM; 10 mM was inhibitory, 20 mM was toxic (Figure 1). The generation time of the bacterium in 2 mM catechol was 4.7 h. Rothera reaction with catechol-grown cells yielded deep purple colour indicating the *ortho* cleavage of catechol and the presence of keto compounds. Catechol-grown cells displayed catechol 1,2-dioxygenase activity as evidenced by decrease in O.D. at 278 nm, the absorption maxima of catechol and increase in 258 nm, indicating *cis*,-*cis* muconic acid formation (Figure 2, *a*). The specific activity of the

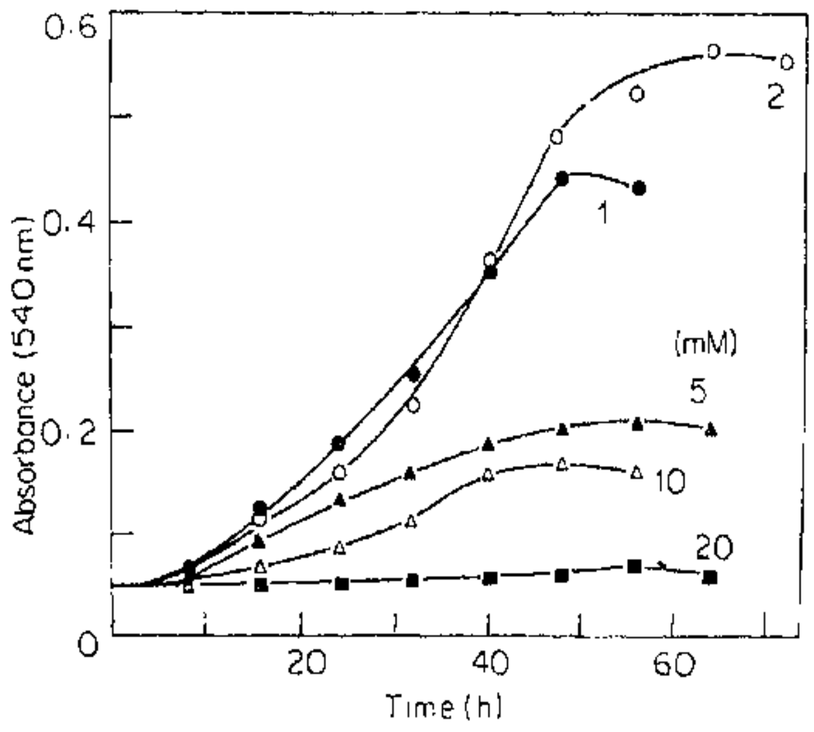

Figure 1. Growth of *A. chroococcum* in catechol.

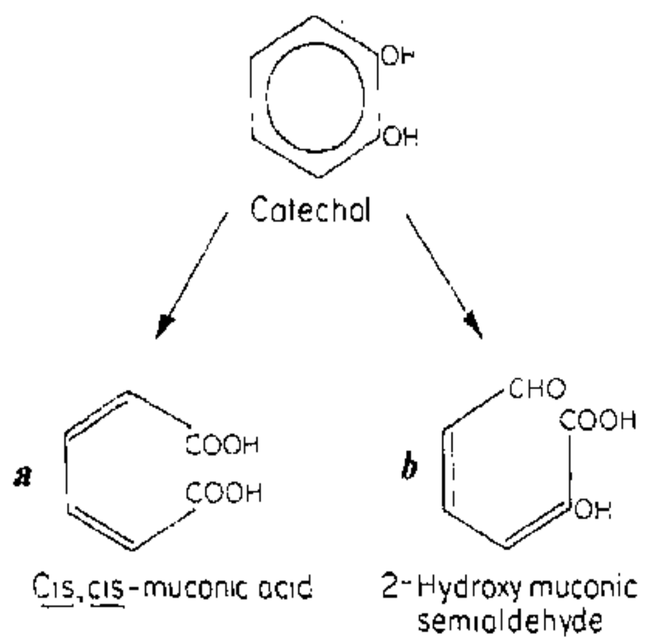

Figure 2. Ring cleavage of catechol. *a*, *Ortho* cleavage. *b*, *meta* cleavage.

enzyme was 1.8. No catechol 2,3-dioxygenase activity was detected.

This is the first report on the *ortho* cleavage of catechol by *A. chroococcum* in contrast to the existing reports on the *meta* cleavage (Figure 2, *b*). Not surprisingly this bacterium also efficiently cleaved 2,4-D (ref 6), which could be attributed to the presence of the *ortho* enzyme, since *meta* enzyme is irreversibly inactivated[7].

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