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KINETICS OF ACETATE DISSIMILATION DURING METHANOGENESIS FROM CATTLE WASTE

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ACETATE is a key intermediate in the anaerobic production of methane from organic wastes[1]. However, so far only two species of methanogenic bacteria, namely *Methanosarcina barkeri*[2] and *Methanothrix soehngenii*[3] have been reported to favour this reaction at mesophilic temperature. The kinetics of this reaction have been examined using either enrichment cultures[5,6] or sewage sludge[7–9]. Although a key reaction in the production of methane from cattle waste, very little information is available regarding the rate of acetate utilization in cattle waste digesters[10,11]. In this paper, we report the kinetics of acetate dissimilation using effluent slurry from an Indian cattle waste digester.

To exhaust the residual acetate, 10 ml of the slurry was transferred to 20 ml CO_2 flushed tubes and incubated under CO_2 for 2 weeks at 37°C with regular discharge of gas produced. Acetate was injected into the tubes at different concentrations and the tubes were incubated further for 3 hr. The gas phase was analysed for methane and the slurry for the residual acetate using gas chromatographic techniques[12]. The rate of methane production was calculated from the increase in methane content of the gas in the tube at the end of incubation.

Table 1 shows the rate of acetate dissimilation and methane production at different concentrations of the acetate. It appears that the rate of acetate dissimilation of about 3 mmol/hr represents 75–85 % of the rate of methane production. This indicates that acetate is the major route in the methane production in anaerobic cattle waste digester. Mountfort and Asher[10] reported the rate of acetate dissimilation under steady state conditions to be the 0.6 mmol/l/hr. The difference between the values reported[10] and the present data

Table 1 *Kinetics of acetate dissimilation and methane formation.*

Acetate concentration (mmoles/l)	Rate of acetate dissimilation (mmolwa/l/hr)	Rate of methane formation (mmoles/l/hr)
1.42		1.57
7.01		3.20
9 25		3 64
11.83		3.33
17 04		3.60
22.24	3.08	3.67
27.45		3 86
32.65		4.00
43.06		3.41
53.47	3.00	3.26

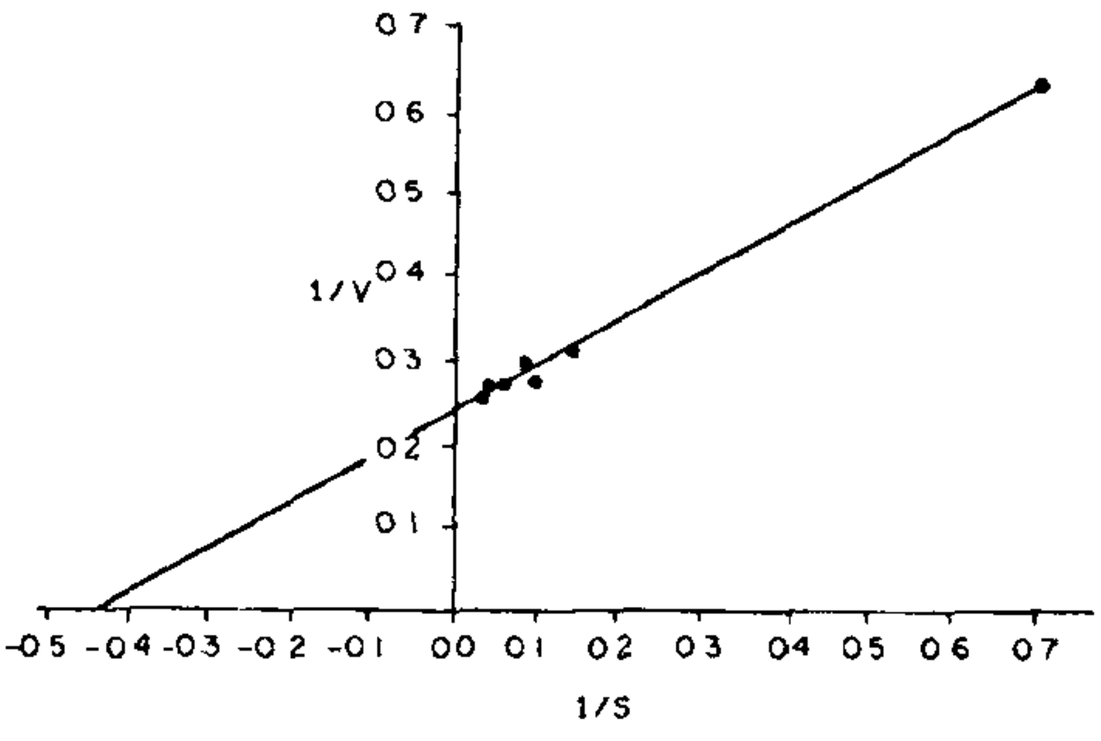

Figure 1. Double reciprocal plot between substrate concentration and rate of gas production.

appears mainly due to differences in the composition of two substrates (2.5 *vs* 8 % volatile solids) and the retention periods (10 *vs* 50 days). The use of spent slurry to determine the rate of methane production from acetate is a better approach to estimate the potential of this reaction in Indian cattle waste digesters. The kinetic parameters, saturation constant (K_s) and the maximum rate of methane production (V_{max}) were determined from the graphical relationship between reciprocals of substrate concentrations and rates of methane production (figure 1). The estimated values of K_s and V_{max} in Indian cattle Waste digesters are 2.22 mM and 4.17 mmol/l/h, respectively. Divergent values for these parameters (K_s, 0.17 to 10 mmol/l) from other systems have appeared in literature[13] and the value of K_s (2.2 mM) presented here falls within this range.

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containing 50 ml tap water and subjected to the following treatments: (i) High relative humidity (94 to 96 %) during day (9.00–20.00 hr) alternating with low RH (63 to 74 %) during night (8 pm to 9 am), (ii) High RH during night alternating with low RH during day, (iii) Continuous high RH, (iv) Continuous low RH. High RH was provided by keeping the inoculated earheads under bell jar lined with moist cottonwool. Two conical flasks containing four earheads were kept under each bell jar (figure 1B). To provide low RH each conical flask with two earheads was covered with pollination bag (figure 1A). Each treatment was replicated thrice with 12 earheads/replication. Proper controls were maintained.

The earheads were examined daily for 8 days for recording incubation period (day taken to develop first symptoms of the disease) and incidence. (No. of infected ears/No. of inoculated ears × 100). During this investigation the maximum and minimum temperatures and RH observed were as follows.

	Average temperature (°C)		
	maximum	minimum	RH range (%)
Inside bell jar	25.0	21.1	94–96
Outside bell jar	24 3	20.0	63–74

DETACHED EARHEAD CULTURE OF PEARL MILLET ERGOT

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THE ergot (*Claviceps fusiformis* Lov) is an important disease of pearl millet (*Pennisetum americanum* (L) Leeke) in Asia[1] and Africa[2]. This note deals with a technique to develop the ergot disease in detached earheads of pearl millet.

Boots of pearl millet hybrid BJ-104 raised under controlled conditions were covered with pollination bags and allowed to develop. When earheads developed up to stigma bifid stage, they were clipped from the plant (in evening) along with flag leaf and stalk of 10 cm length, brought to the laboratory and inoculated. The individual earheads were dipped in conidial suspension (6×10^5 conidia/ml) prepared by suspending fresh honey dew in sterile water. Two inoculated earheads were kept in 150 ml conical flasks

Figure 1. Low RH (A) and high RH (B) treatments of detached earheads of pearl millet for development of ergot.