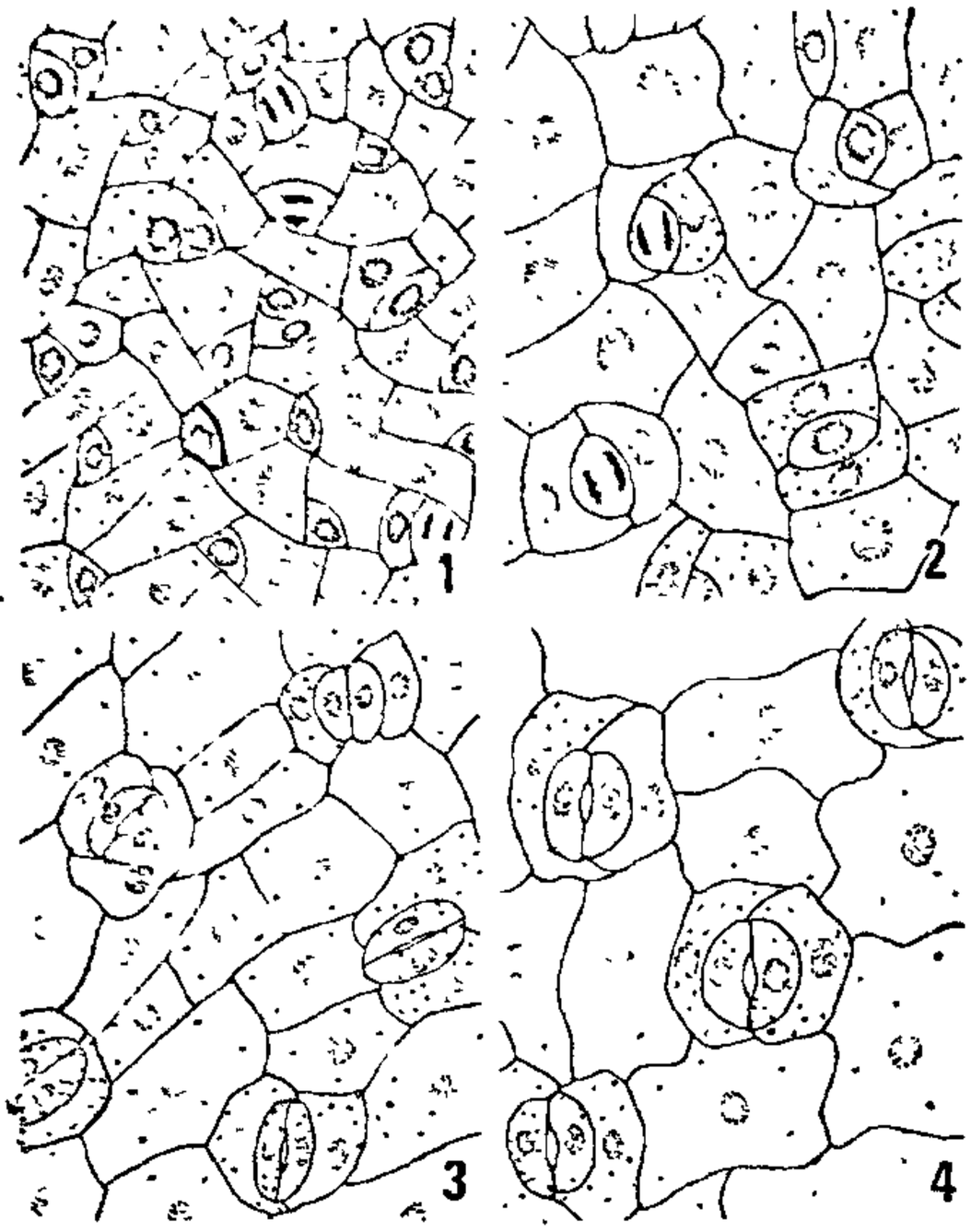


FIGS. 1–4. *Erythrina indica*. Portions of epidermis of leaves at different stages of development, showing almost simultaneous development of stomata (All × 450). Fig. 1. Young leaf showing meristemoids with only one mesogene subsidiary cell Fig. 2. Stage showing both subsidiary cells and guard cell mother cells, some of the latter showing division figures. Fig. 3. Young guard cells, some with pores. Fig. 4. Mature epidermis and fully differentiated stomata.

being cut short by the maturation of the leaf? This may be due to the fact that the leaves do not expand to such an extent as to make the "inhibitory zone" ineffective.

Clearly, there is need for an extensive study of the sequence of stomatal meristemoid formation in plants, both with large and small leaves and leaflets. Some studies are in progress and will be reported in detail elsewhere.

The author is grateful to Prof. D. D. Pant, Allahabad University, in whose laboratory this work was carried out.

Forest Research Centre,
Coimbatore 641 002,
January 29, 1979.

PARVEEN FAROOQUI
(NEE KIDWAI).

1. Ziegenspeck, H., *Protoplasma*, 1944, **38**, 197.
2. Pant, D. D., *Pl. Sci. Ser.*, 1965, **1**, 1.
3. Bünning, E., In *Growth of Leaves*, Ed. F. L. Milthorpe, Butterworths Sci. Pub., London, 1956, p. 18.

USE OF WATER HYACINTH AS AN ADDITIVE IN BIO-GAS PRODUCTION

DIFFERENT organic wastes, including aquatic vegetative growths, can be used as supplements in the production of bio-gas through anaerobic fermentation of cattle dung[1,2]. Guha *et al.*[2] further reported that the rate of gas production is greater when water hyacinth is allowed to ferment anaerobically, either mixed with cattle dung or without it; the gas production starts earlier as compared to cattle dung alone. However, there is no mention of the variation in the pH level of the fermenting slurry and the nature of volatile fatty acids produced during the course of fermentation. It was thought, therefore, worthwhile to conduct an experiment with water hyacinth as an additive, mixed with cattle dung with a view to study the pattern of volatile fatty acid production, the pH levels and the gas produced during the above fermentation.

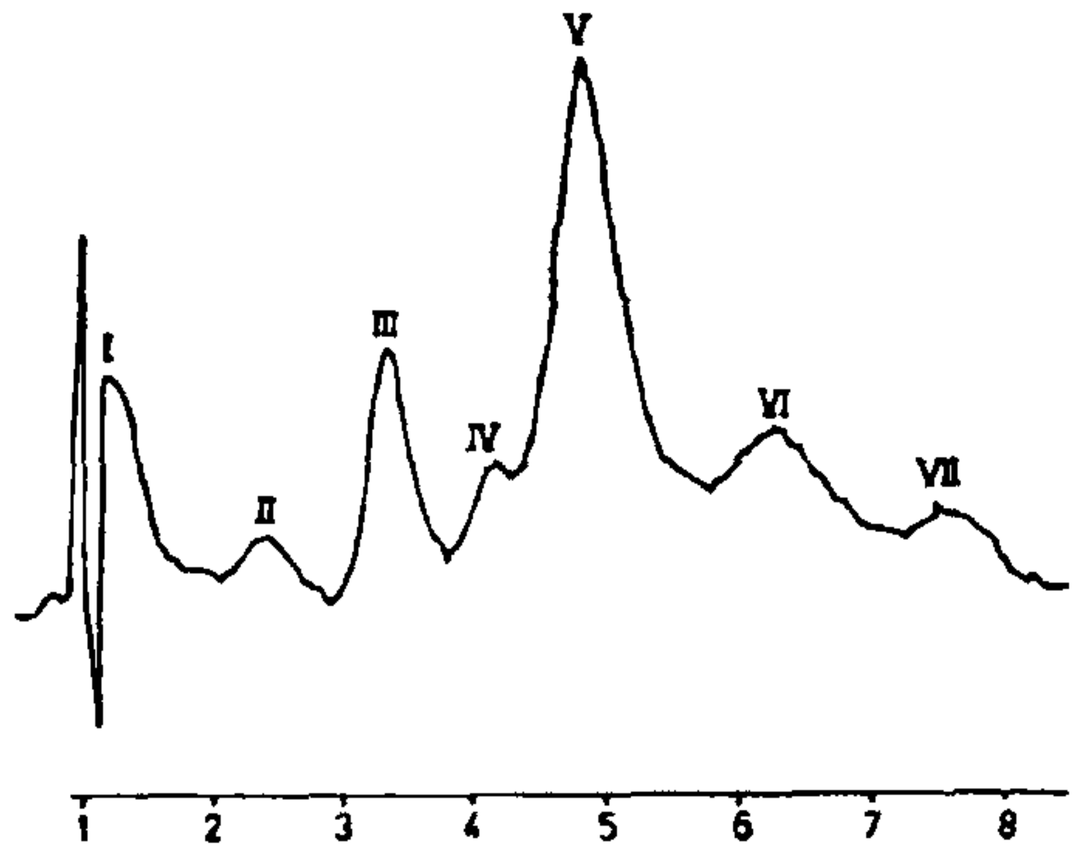


FIG. 1. VFA pattern on the 25th day in the fermenting slurry containing cattle dung and water hyacinth.

I, Disturbance due to water; II, Acetic acid; III, Propionic acid; IV, Isobutyric acid; V, Butyric acid; VI, Isovaleric acid; VII, Valeric acid.

Water hyacinth was sundried and 1 kg of the powder containing 9% moisture was mixed with 6·25 kg of wet cattle dung containing 80% moisture. This mixture was diluted with 17 litres of water and then charged into a laboratory scale model of gobar gas plant of KVIC design and allowed to ferment anaerobically for 60 days. The pH and temperature of the fermenting slurry was recorded daily. The bio-gas generated from this fermentation was measured and analysed daily. In order to find out the volatile fatty acid contents in the fermenting slurry, the sulphuric acid distillate was analysed daily. The analysis of bio-gas and that of distillate was done chromatographically on Toshniwal gas chromatograph using porapack Q column, with hydrogen as a carrier gas and thermal conductivity

detector for gas analysis and nitrogen as a carrier gas, hydrogen for flame and flame ionisation detector for distillate analysis. The data are given in Table I and Fig. 1.

Another experiment was done with cattle dung as exclusive charge. The data are given in Table II.

each other, it becomes evident that during the first stage of fermentation of 1 to 13 days there is an accumulation of volatile fatty acids liberated through the degradation of organic matter, resulting in a decrease in the pH of the fermenting slurry. As the methanogenic bacteria are pH sensitive and show their

TABLE I

Volatile fatty acid and bio-gas from cattle dung + water hyacinth (1 : 1) fermentation

Duration Days	pH	Temp. °C	Volatile fatty acids	Average gas produced l/day	Methane %
1–4	7 to 7–5	30	Acetic acid, propionic acid, butyric acid	0·95	0
5–13	6·5	27–30	Acetic acid, propionic acid, butyric acid (ethyl alcohol on 7th day)	1·22	3–8
14–28	6·5 to 7	26–29	Acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid	0·81	10–60
29–49	7	27–28	Propionic acid, isovaleric acid intermitently	3·01	57–62
50–60	8	26–28	—	4·31	60–64

TABLE II

Volatile fatty acids and bio-gas from cattle dung fermentation

Duration Days	pH	Temp. °C	Volatile fatty acids	Average gas produced l/day	Methane %
1–60	8 to 8·5	26–28	Acetic acid, propionic acid, butyric acid	3·84	50–60

The data show that while volatile fatty acids in only cattle dung fermentation consist of acetic, propionic and butyric acids, water hyacinth and cattle dung mixture gave isobutyric, valeric, isovaleric acids and ethyl alcohol in addition to acetic, propionic and butyric acids. These extra fatty acids have, therefore, been contributed by water hyacinth. When the pH range, volatile fatty acid pattern, rate of gas production and methane percentage during cattle dung and water hyacinth fermentation are compared with

activity above pH 6·5[3] gas evolved has lower methane content (3–8%) during this period. In the second stage of fermentation, *i.e.*, after 13 to 28 days, the pH of the fermenting slurry rose to 7, still showing the presence of various acids up to valeric. This may be due to the fact that during this second stage of fermentation hydroxyl ions were produced because of the deamination of bio-degradable protein which helped in the neutralization of the volatile fatty acids produced[1,4], resulting in a rise in pH from

6·5 to 7. According to Sathianathan[4] once the pH is adjusted between 7 and 8, the fermenting mixture becomes well buffered and there is no change in pH even after the addition of acids or alkalies. This effect is clearly seen from the fermentation between 29 and 49 days where even though propionic and isovaleric acids are detected intermittently, the pH is steady at 7 when the environment became more congenial for methanogenesis.

The data thus show that the aquatic vegetative growth such as water hyacinth enhances the volatile fatty acids in the fermenting slurry which is a precursor of methane in the anaerobic fermentation and thus can profitably be used as additives in the gobar gas plant.

The authors are thankful to Dr. G. B. Deodikar, Director, Maharashtra Association for the Cultivation of Science, Pune 4, for providing necessary laboratory facilities.

Department of Microbiology,
M.A.C.S. Research Institute,
Pune 411 004,
February 9, 1979.

PRADNYA DESHPANDE.
SEEMA SARNAIK.
S. H. GODBOLE.
P. M. WAGLE.

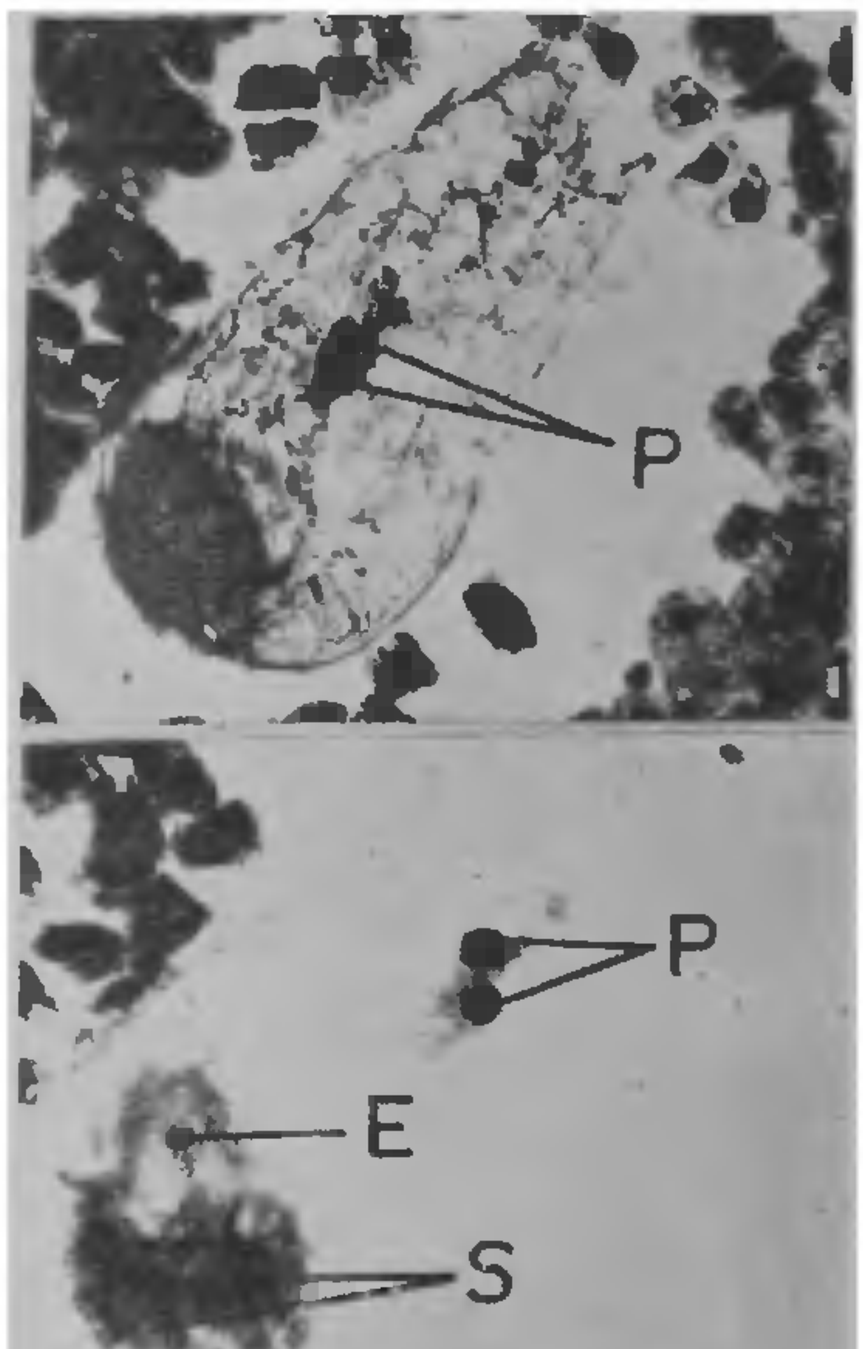
1. *Methane Generation from Human, Animal and Agricultural Wastes*, National Academy of Science, Washington, D.C., 1977.
2. Guha, B. R., Bandopadhyay, T. K. and Chaudhary, K. K., *Khadigramodyog*, 1976, 22, 483.
3. Barker, H. A., *Bacterial Fermentation*, John Wiley and Sons, New York, 1956.
4. Sathianathan, M. A., *Bio-gas—Achievement and Challenges*. Association of Voluntary Agencies for Rural Development, New Delhi, 1975.

A RAPID METHOD FOR THE STUDY OF FERTILIZATION IN GROUNDNUT, *ARACHIS HYPOGAEA* L.

A STUDY of pre- and post-fertilization events in groundnut is of significance in attempts of interspecific gene transfer. Earlier studies on the details of fertilization in groundnut were based on conventional techniques using serial sections[1–4]. An attempt was made in the present study to find out the possibility of studying the pre- and post-fertilization events in the embryo sacs in iron acetocarmine squashes and the results are given in this report.

Flowers were emasculated on the evening preceding anthesis and pollinated at the time of anthesis. Ovaries were fixed at 2 h intervals in Carnoy's fluid for 48 h. The ovaries were mordanted in saturated ferric ammonium sulphate solution at 75° C for 5 minutes, washed twice in water at 75° C for 6 minutes, macerated in 50% HCl for 10 minutes and washed for 20 minutes in running water. Ovules were dissected in a drop of water and squashed in acetocarmine.

Embryo sacs got popped out of the ovules by a little pressure on the coverglass. The structure of the embryo sacs was brought out very clearly in the squashes (Figs. 1 and 2). The embryo sacs were of the normal



FIGS. 1–3. Fig. 1. Embryo sac before fertilization. Fig. 2. The polar nuclei and the egg apparatus enlarged from Fig. 1, showing details. Fig. 3. Embryo sac after fertilization showing the primary endosperm nucleus and the zygote. (S, synergids; E, egg apparatus; P, polar nuclei; En, primary endosperm nucleus; Z, zygote.)