first set received the nutrient solution of Hoagland and Arnon[4] which served as control. Phosphorus deficiency was created by replacing $KH_2PO_4$ with $KHSO_4$ in the second set. All the plants received the respective nutrient solutions once in 3 days.

$^{14}CO_2$ feeding technique as described by Berry *et al.*[5] was adapted with 10 and 20 min as feeding time. The incoporation studies were carried out in 20 day (10 days after treatment) and 30 day (20 days after treatment) old plants. The first compound leaf from the base was cut under water and used for feeding. After the selected periods of feeding the leaves were killed by plunging in boiling 80% ethanol, extracted and centrifuged. The supernatant was taken out and the residue was re-extracted with ethanol and centrifuged. The two alcohol soluble fractions were pooled and the alcohol insoluble residue was hydrolysed with 6N HCl. After hydrolysis, the acid was evaporated and the resulting residue was taken in 80% ethanol constituting the alcohol insoluble fraction. Both alcohol soluble and insoluble fractions were evaporated to a known volume and an aliquot was examined for radio activity using end window GM counter. Preparation of water soluble fraction and fractionation of alcohol soluble and insoluble fractions into amino acids, organic acids and sugar fractions were done according to Atkins and Canvin[6]. Total $^{14}CO_2$ incorporated into each of these fractions was determined as mentioned earlier. Triplicate samples were maintained for each experiment.

The results of the present study indicated a decreased $^{14}CO_2$ incorporation into alcohol soluble and insoluble fractions under P-deficient conditions. Incorporation rates were high 20 min after feeding both in the 20 day and 30 day old plants (Table I). A decrease of 23·88 and 31·16% incorporation over controls after 10 min and 20 min feeding time in 20 day old plants; and 19·95 and 35·30% decrease into alcohol soluble fraction in 30 day old plants was observed under P-deficiency. $^{14}C$ incorporation into alcohol insoluble fraction showed less decrease except in 20 day old plants after 10 min feeding; 53·39, 17·58% in 20 day old plants and 7·37 and 23·31% decrease in 30 day old plants was observed at 10 and 20 min feeding time respectively, under phosphorus deficiency.

Fractionation of alcohol soluble and insoluble fractions indicated higher rates of incorporation into amino acids and organic acids under phosphorus deficiency. However amino acids of alcohol insoluble fraction showed lowered incorporation in 20 day old P-deficient plants *initially, i.e., 10 min after feeding.* But $^{14}C$ incorporation into sugar fraction was found to decrease significantly both in 20 and 30 day old P-deficient plants (Table I).

Phosphorus is one of the essential elements that plays a *pivotol role in plant metabolism.* Deficiency of P in nutrient medium results in stunted growth of peanut plants and lowers the uptake of potassium[3]. The decreased potassium in turn reduces stomatal opening, which ultimately results in poor uptake of $^{14}CO_2$. The lowered $K^+$ under P-deficiency as observed in our previous studies[3] may even be responsible for the reduced photosynthesis and increased respiration, thereby lowering the amounts of carbohydrates. Our previous studies[3] also indicated low amounts of sugars in peanut leaves under P-deficiency. Thus the lowered *incorporation into alcohol insoluble fraction indicates* lowered synthesis of starch under P-deficiency. High rates of incorporation into amino acids under P-deficiency is explained in terms of their lowered utilization in protein synthesis. High rates of incorporation into organic acids and poor rates of incorporation into sugars indicate that the conversion of organic acids to carbohydrates may be slowed down under P-deficiency.

Phosphorus compounds have been shown to be essential for photosynthesis, the interconversion of carbohydrates and related compounds, fat metabolism and a host of other life processes. The reduced rates of $^{14}CO_2$ uptake in peanut leaves as observed in the present study indicates that the poor growth of plants might be due to lesser availability of carbohydrates for the vital growth processes.

GGR is grateful to CSIR (New Delhi) for providing financial assistance.

September 2, 1980.

1. Salisbury, F. B. and Ross, C., *Plant Physiology*, Prentice-Hall of India Pvt. Ltd., New Delhi, 1977.
2. Tisdale, S. L. and Nelson, W. L., *Soil Fertility and Fertilizers*, Macmillan Pub. Co., Inc., New York, 1975.
3. Mahaboob Basha, S. K. and Rajeswara Rao, G., "Physiological changes in peanut (*Arachis hypogaea* L.) under phosphorus deficiency; *Indian J. Pl. Physiol.*, 1981 (in press).
4. Hoagland, D. R. and Arnon, D. I., "Water and sand culture methods for growing plants without soil; *Cir.* 347, *Calif. Agric. Expt. Stn.*, 1950.
5. Berry, J. A., Downton, W. J. S. and Tregunna, E. B., *Can. J. Bot.*, 1970, 48, 777.
6. Atkins, C. A. and Canvin, D. T., *Ibid.*, 1971, 49, 1225.

## PRESS MUD AS ADDITIVE TO INCREASE BIOGAS PRODUCTION FROM CATTLE WASTE

RAJENDRA SINGH, M. K. JAIN AND P. TAURO
Department of Microbiology
Haryana Agricultural University
Hissar 125 004, India

ANAEROBIC digestion depends on several different microbial activities functioning concurrently and at

balanced rates. Complex waste components, including polysaccharides, fats and proteins, are first hydrolysed to their component subunits which are subjected to fermentations, $\beta$-oxidations and other metabolic processes leading to the formation of simple organic compounds, especially lower fatty acids and alcohols. In turn, these simple compounds are converted to gases, chiefly methane and carbon dioxide, by the strict anaerobic, acid sensitive, highly specialized methane forming bacteria[1,8]. For successful anaerobic digestor operation it is essential to maintain a balance among the three major categories of microbial functions. In India, the biogas plants are charged with cattle waste for methane production; however, for a variety of reasons, the gas production has been found to be low. As a part of improving gas production from cattle waste we present here the results obtained on addition of press mud from sugar factory as an additive to cattle waste.

The press mud, obtained from a sugar factory at Panipat (Haryana), was added to the cattle waste @ 10% (on dry weight basis). Experiment was conducted in 5 litre aspirator bottles (working volume of 3·75 litres) after the slurry was made with tap water in a 1 : 1 ratio (W/V). The bottles were sealed with paraffin wax and the waste was allowed to ferment anaerobically at room temperature (30–33° C) for a period of eight weeks. Thoroughly mixed samples were drawn at weekly intervals from each fermentor to analyse for pH, total volatile fatty acids (VFA), carbon, nitrogen, cellulose, lignin and volatile solids. The analysis was done as per the methods described elsewhere[5]. The gas production was measured by water displacement method while the analysis was done using Orsat gas analyser. The component acids of VFA mixture were determined using gas chromatographic technique[2].

The data presented in Table I indicate the fall in pH within the first week of fermentation of cattle waste (CW) as well as that of CW and press mud (PM) mixture. However, due to the utilization of acids, pH of the fermenting material gradually increases to neutral range. Further the pH during the advanced stage of fermentation stabilizes in the vicinity of 7·0 due to the high buffering capacity of the system although the acid production as well as utilization take place simultaneously. The reduction in acid concentration coincided with the gas production suggesting that utilization of VFA in the digestion process was generally for methanogenesis[5]. The pH in the CW fermentor increased above 7·0 after 4 weeks while it happened so in CW+PM fermentor

TABLE I

*Gas production in relation to changes in pH and total volatile fatty acids*

| Time (weeks) | Cattle waste | | | CW + Press mud | | |
|---|---|---|---|---|---|---|
| | pH | TVFA (ppm) | Gas production (l/kg waste) | pH | TVFA (ppm) | Gas production (l/kg waste) |
| 0 | 7·5 | 1725 | .. | 7·65 | 1425 | .. |
| I | 6·05 | 3250 | 1·37 | 6·35 | 5385 | 0·99 |
| II | 6·15 | 6000 | 0·45 | 6·50 | 5865 | 1·35 |
| III | 6·15 | 6630 | 0·41 | 7·05 | 3150 | 6·59 |
| IV | 6·20 | 6300 | 1·51 | 7·20 | 2160 | 7·29 |
| V | 7·25 | 4180 | 6·25 | 7·35 | 735 | 8·00 |
| VI | 7·15 | 2475 | 6·88 | 7·25 | 555 | 5·05 |
| VII | 6·90 | 1770 | 4·81 | 7·25 | 270 | 4·00 |
| VIII | 7·25 | 1290 | 4·79 | 7·25 | 255 | 2·04 |
| Total gas production (l/kg/8 weeks) | .. | .. | 26·476 | .. | .. | 35·30 |
| % increase over CW | .. | .. | .. | .. | .. | 33·0 |

ust after 2 weeks. In CW+PM fermentor the level of acids decreased and gas production commenced after 2nd week suggesting that addition of press mud helped in the development of methanogenesis 2 weeks earlier as compared to CW in which no press mud was added. This could probably happen because

TABLE II

*Volatile fatty acids at various stages of fermentations (ppm)*

| Volatile acids | Weeks | | | | |
|---|---|---|---|---|---|
| | III | IV | V | VII | VIII |
| | | | *Cattle waste* | | |
| Acetic acid | 4191 | 3920 | 483 | 552 | 474 |
| Propionic acid | 130 | 1478 | 2198 | 3002 | 1158 |
| Iso-butyric acid | .. | 106 | 202 | .. | .. |
| Butyric acid | 1467 | 1643 | 1173 | 704 | .. |
| Valeric acid | 397 | 374 | 459 | 567 | .. |
| | | | *CW + Press mud* | | |
| Acetic acid | 1416 | 741 | 305 | 180 | 79 |
| Propionic acid | 1566 | 1712 | 389 | 322 | 154 |
| Iso-butyric acid | 105 | 88 | .. | .. | .. |
| Butyric acid | 677 | 391 | .. | .. | .. |
| Valeric acid | 306 | 561 | .. | .. | .. |

TABLE III

*Changes during anaerobic digestion of cattle waste and its mixture with press mud*

| Parameter | Cattle waste | | | CW + Press mud | | |
|---|---|---|---|---|---|---|
| | Initial (%) | Final (%) | % Degradation | Initial (%) | Final (%) | % Degradation |
| Total solids | 9·61 | 7·70 | 19·88 | 9·25 | 7·15 | 22·70 |
| Volatile solids | 79·40 | 77·10 | 22·78 | 76·50 | 70·60 | 28·70 |
| Carbon | 46·08 | 44·72 | | 44·38 | 40·95 | |
| Nitrogen | 1·89 | 1·93 | | 1·81 | 2·07 | |
| Cellulose | 22·81 | 18·04 | 33·00 | 21·34 | 18·41 | 35·32 |
| Lignin | 20·12 | 22·20 | .. | 18·66 | 21·95 | .. |
| C/N ratio | 24·4 | 23·1 | | 24·6 | 19·8 | |
| pH | 7·50 | 7·25 | | 7·65 | 7·25 | |

the press mud contains some sugars which were utilized immediately by the bacteria to produce the desired substrates (*e.g.*, acetic acid, $H_2$ and $CO_2$) for methanogenesis. The other reason could probably be the availability of some trace elements and growth substances from press mud for microbial community. Besides this, addition of some desired bacterial community (*e.g.*, propionate catabolizing) due to addition of press mud cannot be ruled out. The last reason is substantiated on the basis of the results presented in Table II. Significant level of propionate, iso-butyrate, butyrate and valerate was built up in CW fermentor while in the case of CW+PM fermentor the build up of these acids was not significant. Utilization of propionate has been reported to be slow and does not allow methanogenesis to proceed faster[6].

As can be seen from the data presented in Table III, comparatively, there was more destruction of volatile substances in CW+PM mixture than in CW alone. Although there was not much difference in cellulose degradation between the two wastes (Table III), yet there was about 33% more gas production from CW+PM mixture than from CW alone (Table I). Higher cellulose degradation would not necessarily result in increased gas production unless the products of hydrolysis are fermented only to acetate, $CO_2$ and $H_2$. This is, however, dependent upon the partial pressure of $H_2$ in the system[3,4,7]. The methane content varied between 62–66% in both the fermentors, the remaining 34–38% being the $CO_2$. There was no degradation of lignin. The increase in lignin per cent, however, was due to the reduction in total solids.

These results suggest that gas production can be increased by supplementing cattle waste with press mud @ 10% to start with as this would help in an early establishment of the system.

*November* 20, 1980.

1. Bryant, M. P., In: *Proc. Seminar on Microbial Energy Conversion* (H. G. Schlegel and J. Barnia, eds.), E. Coltze, K. G., Gottingen, *1976*.
2. Carlsson, J., *Appl. Microbiol.*, 1973, 25, 287.
3. Chen, M. and Wolin, M. J., *Appl. Environ. Microbiol.*, 1977, 34, 756.
4. Ianotti, E. L., Kafkewits, D., Wolin, M. J. and Bryant, M. P., *J. Bacteriol.*, 1973, 114, 1231.
5. Jain, M. K., Singh, R. and Tauro, P., *Agricultural Wastes*, 1981, 3, 65.
6. Jeffries, T. W., Onstead, D. R., Cardenas, R. R. and Gregor, H. P., *Biotech. and Bioengg.*, 1978, Symp. No. 8, 37.
7. Latham, M. J. and Wolin, M. J., *Appl. Environ. Microbiol.*, 1977, 34, 297.
8. Zeikus, J. G., *Bacteriol. Rev.*, 1977, 41, 514.

## ROLE OF SOLUBLE SUGARS IN THE RESISTANCE OR SUSCEPTIBILITY OF GREENGRAM VARIETIES TO BACTERIAL LEAF BLIGHT CAUSED BY *XANTHOMONAS PHASEOLI*

T. MARIMUTHU
National Pulses Research Centre
TS. 1484, West III Street
Pudukkottai 622 001, India
AND
T. K. KANDASWAMY
Professor and Head
Agricultural College and Research Institute
Madurai 625 104, India

GREENGRAM (*Vigna radiata* var. *aureus*) varieties differ in their reaction to *X. phaseoli* inoculation[1]. The present study was aimed at understanding the role of soluble sugars in imparting tissue resistance/susceptibility to the selected cv. of greengram, *viz.*, Co 2, Co 3 and Co 1 which are moderately resistant, susceptible and highly susceptible to *X. phaseoli*, the incitant of bacterial leaf blight, respectively. Extensive studies have been made on the role of sugars in host parasite interaction of fungal pathogens[2,3]. The information on the bacterial pathogens are scanty[4] and no information is available on the role of soluble sugars in the greengram—*X. phaseoli* interaction.

Seeds of all the three cultivars, *viz.*, Co 2, Co 3 and Co 1 were sown in mud pots (30 cm dia) containing standard pot mixture. Five plants were maintained in each pot. Thirty-five days old plants were spray inoculated with the cell suspension of the pathogen (ca. $10^8$ cells/ml of dist water) as per the method of Kiraly[5]. Leaf samples were collected from all the varieties at 0, 12 hr, 1, 3, 5 and 10 days after inoculation.

Five g of fresh leaf tissues were extracted in boiling 80% ethanol[6] for the estimation of sugars.

The non-reducing sugars of the alcohol extract was converted into reducing sugars[7] and then the total sugars estimated[8].

The qualitative nature of sugars in the extract was determined by unidimensional ascending chromatography following the methods of Barton[9]. The solvent system consisted of *n* butanol : acetic acid : water : 4 : 1 : 1 (v/v). Aniline hydrogen phthalate served as detection reagent. Co-chromatography with the authentic samples of sugars helped in the identification of unknowns in the extract. Spot areas from the corresponding unsprayed chromatogram were cut and eluted with deionized water and quantified using anthrone reagent[10].

The data on the sugar content reveal that the variety Co 2 contained the least amount of total soluble