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USE OF CULTURED BACTERIA IN ENHANCING BIOCONVERSION OF REFUSE TO COMPOST.

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Abstract

The microbial degradation of organic material was studied with a view to determining the terminal steps in the formation of biogas from the anaerobic decomposition of biomass in organic waste conversion.

Formulation of methanogenic bacteria that are cultured and particularly adopted to degrade refuse was formulated and mixed with macerated and chopped refuse with vigorous and regular turning. Early observation revealed that the bacteria obtained hydrogen, carbondioxide and acetate from complex organic substrates and complete bioconversion occurred under anaerobic conditions. However, substrates that are not readily metabolised as carbon and energy sources by pure cultures of anaerobic bacteria were metabolised by mixed cultures in anaerobic environments.

An increased concentration of nitrogen, phosphates, potash and presence of small amount of various metals was observed in the final material that was also found to increase the productivity of a virgin soil with increased crop yield.

Introduction

In developing countries, organic fertilizers have been widely accepted as a major source of improving and maintaining soil fertility (5). Basically, composting involves the conversion of heterogeneous mass of multiple particle size of the original organic materials to homogenous mass of suitable particle size and suitable moisture content. Moisture needs to be present during composting process to provide the correct humidity for the decomposition of the organic waste by microbiological decomposers (8). The optimum conditions occur when the water content is between 50 to 60% by wet weight (3). Conversely if too little moisture is present, the centre of the mass will reach very high temperatures and the rate of decomposition will be retarded.

Scientific study on the use of bacteria in improving the nutritive value of agricultural wastes as organic manure has received relatively little research. This research work therefore attempts to look at the mechanism, culture and use of formulated methanogenic bacteria in improving the nutritive value of organic material.

The works of Robert Bolton and Klein noted an inflammable gas 'methane' as the final product of mineralising the organic materials in anaerobic systems. Chemical energy in the substrates ends up in the methane released by these anaerobic bacteria(12). In direct contrast, aerobic bacterial metabolism releases most of the chemical energy in the substrates by oxidising them to carbon dioxide and water and bacterial cells are also produced (10). Anaerobic processes not only form an energy-rich product but also make less cell material and are consequently useful in the degradation of biomass (12).

Materials and Methods

All analytical procedure using titrimetry, infrared spectroscopy, flame photometry and microscopy proceeded in accordance with the methods of American Public Health Association (1). Specific research and experimental methods employed are defined below.

Media for the identification and isolation of methanogenic bacteria

The media used were the standard plate count agar, nutrient agar and potato dextrose agar. Other media used for identification included yeast extract agar, azide dextrose broth, MacConkey broth, glucose phosphate broth and nutrient broth. The media were prepared according to standard methods (1). The media were adjusted to pH 7 and sterilized at 121^{0}C for 15minutes.

Identification of methanogenic bacterial

Methanogenic bacterial identification was carried out by isolating the organisms and subjecting them to various microscopic, biochemical and sugar fermentation tests.

Bacterial isolation was carried out by preparing a 10 fold serial dilution of the test

sample. 1ml of each dilution was pipetted into petri-dishes. 15ml of standard plate count agar at 45^{0}C was then poured into the petri-dishes. The plates were allowed to cool and then inverted and incubated at 37^{0}C for 24 hours. After incubation, the different colonies on the standard plate count agar were subcultured by streaking on nutrient agar and MacConkey agar. Continous subculturing of the different colonies were carried out until pure and discrete isolates of each colony were obtained. These pure isolates were then preserved in nutrient agar slants at 4^{0}C. Microscopic, biochemical and sugar fermentation tests were then carried out on these bacterial isolates.

Formulation of bacterial degraders

Methanogenic bacteria were isolated from organic waste material. Pure culture of the various bacterial isolates was inoculated and sterilised after propagation in peptone water broth. The most effective waste degraders were then selected and further propagated in Nutrient broth agar.

These organisms were incubated singly in 15ml nutrient broth for 24hrs. They were then collectively used to inoculate sterilised refuse in big bowls. The bowls were incubated at 38^{0}C for seven days after which the pH was altered from 5.8 to 6.5 and dry cell powder was added to toughen the microbes. This process was repeated until a formulation of microbes that are genetically changed and adopted to degrade refuse was formulated (4).

Mechanics of bioconversion of refuse to compost

Collected refuse was taken to compost site and metal objects, stone, glass, broken crockery, large pieces of papers and cardboard are first removed. Further segregation and sorting was done by removal of digestable and non-digestable residues. The digestable materials were then macerated, chopped and granulated using a grinding machine before being transferred into large shallow pits.

The heap is built up in layers after proper mixing with virgin forest soils rich in plant nutrient and addition of potash base such as fly ash. Treated sewage was run on to the layer before the next layer is laid on. Adequate drainage system was provided. Some of the liquid draining from the lays was used to keep the material regularly moist.

The material was turned regularly with incorporation of formulated bacterial degraders. The composting was allowed to stand for four weeks with regular turning and sprinkling of treated sewage. Proximate and mineral contents of the final product was analysed to check for the nutritive value of the product. The final product was also used as organic manure on a farmland.

Results and Discussion

Scientific interest in the gases produced by natural decompostion of organic matter was first reported in the sixteen century by Robert Boyle and Stephen Hale, who noted that an inflammable gas was released by disturbing the sediment of natural stream and lakes (12). This inflammable air was associated with decomposing organic material in the sediments. Similar study by Berryman led to the conclusion that fermentation intermediates produced from cellulose and not cellulose itself were the substrate for methanogenesis. A simple mechanism of methanogens is shown below: In a typical anaerobic digestion, two major methanogenic precursors are acetate and hydrogen-carbondioxide. This relationship holds for both mesophilic or thermophilic anaerobic digestion processes (11).

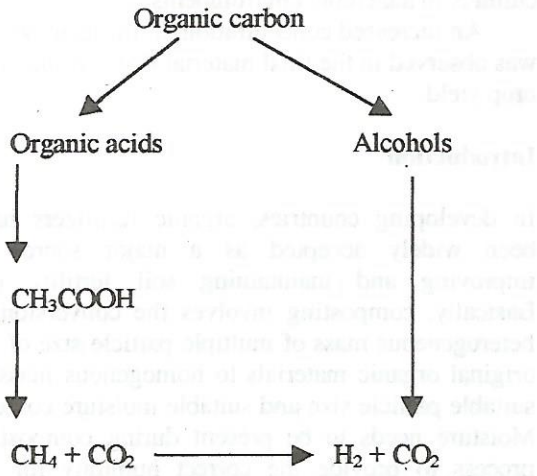

Figure 1: Mechanism of methanogenesis under anaerobic processes.

Methanogens exhibit organic growth factor requirements and stimulated by growth factors such as vitamins, fatty acid, unusual trace minerals such as Co and Ni or specific cofactors unique to the methanogenic bacteria. Identified methanogenic bacteria in the organic waste include *Methanogenium thermophilicum, Methanobacterium ruminantium, Methanococcus voltae, Methanomicrobium mobile.* *Methanobacterium ruminantium* requires coenzyme M, the fatty acids 2-methyl-butyrate and acetate as well as certain amino acids, *Methanococcus voltae* requires the amino acids leucine and iso-leucine for growth. *Methanomicrobium mobile* requires the vitamins thiamine, pyridoxine and p-aminobenzoic acid for growth. Because of the importance of acetate splitting methanogens in anaerobic digestion, attention is given to identification of specific growth requirements for this group of methanogens. Identification of growth requirements could be of major importance to the

understanding and stimulation of anaerobic digestion processes (15).

The energy liberated during bioconversion caused a rise in temperature (7) and organic degradation was rapid at this point and it was during this phase that pathogenic organism, fly larvae and weed seeds were destroyed. At this stage, a 20 to 25cm deep grey layer was formed at about 7cm below the outer surface. This is where the conditions are at their optimum and turning was required to ensure that all the material passed through this layer. The final material that was applied on farmland and increased productivity of a virgin soil with increased crop yield was observed.

Table 1: Proximate and mineral analyses of compost

Nutrients %	Fresh Compost
Crude fibre	40.2
Carbohydrate	62.0
Kjeldahl nitrogen	9.1
Crude protein	41.0
Sulphur	1.82
Phosphorous	1.3
Magnesium	14.5
Sodium	1.35
P_2O_5	0.85%
K_2O	0.40%
Humus	58%

Values are means of 3 experiments drawn from field studies.

There is considerable variation in the Carbohydrate, crude fibre, protein, nitrogen(N), phosphorous(P), and potassium(K) content of compost (table 1). During digestion or composition, much of the organic matter was used up; however, most of the plant nutrients are retained. For this reason, the nutrient content of agricultural wastes may be four times higher than in the original plant material (6).

Compost provides a good source of those trace elements that are necessary for satisfactory plant growth (9). The plant nutrient composition of refuse usually has some relationship to the nutrient content of plant materials. This is understandable, as most refuse wastes originate directly or indirectly from plant materials (10).

While the foregoing may appear complex, it is a naturally achieved process, as the waste naturally contain the spores, eggs and propagules of the decomposers (13). It should be noted that methanogens are limited to a few substrates including H-CO_2, formate, methanol, acetate, mono-, di- and trimethylamines.

Wolfe and his colleagues at the University of Illinois isolated and characterised a number of interesting enzymes and cofactors from methanogenic bacteria. Coenzyme M (CoM), the first cofactor unique to methanogens was identified and serves as the terminal methyl carrier in methane formation (14). The genetics of methane-producing bacteria is in its infancy. The results of preliminary studies have attracted a great deal of interest because of the potential methanogens capable of more effective substrate use and rapid increases in biogas generation (2). Much work need to be done on isolation of new methanogens and basic studies on the biology and physiology of these organisms. This information would also provide foundation for further genetic and molecular studies.

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