PRODUCTION OF BIOGAS FROM WATER HYACINTH: EFFECT OF SUBTRATE CONCENTRATION, PARTICLE SIZE AND INCUBATION PERIOD

JHY Katima
Department of Chemical and Process Engineering,
University of Dar es Salaam, P.O. Box 35131 Dar es Salaam, Tanzania

ABSTRACT

A study was conducted, at constant temperature, to determine the effect of substrate concentration, particle size and incubation time on biogas production from water hyacinth. Substrate concentration was varied between 5–30 g/l of water, particle size between <1 mm – 3 mm and incubation period between 1–6 days. The maximum methane prodction of 72.53% v/v at substrate concentration of 25 g/1 and particle size less than 1 mm for water hyacinth shoots was achieved after five days of incubation. However, for the whole water hyacinth plant, methane content was 65.67% v/v at the same conditions.

INTRODUCTION

Lake Victoria is the largest and most species-rich freshwater lake (Balirwa *et al.* 2000) in Africa and the second largest lake in the world with a surface area of about 80,000 km^2, a shoreline of 3450 km and a catchment of 193,000 km^2 (Crul 1993). However, the lake is facing three major problems, namely (i) loss of biodiversity (ii) eutrophication (iii) rapid growth of water hyacinths (van Horen 1996, Witte *et al.* 1992, Rutashobya 1996, Chege 1995, Balirwa *et al.* 2000).

The infestation of water hyacinth (WH), *Eichhornia crassipes*, plant in Lake Victoria has created serious social, economic and environmental problems (Van Horen 1996, Chege 1995, Rutashobya 1996). It has interfered with the use of water by causing direct obstruction to navigation and by degrading water quality for domestic use. It has been responsible for drastic changes in the plant and animal communities of the lake. Heckey *et al.* (2000) reported that since 1970s there has been a threefold increase of phosphorus, twofold of primary productivity, by a factor of 6-8 in algal biomass. Heckey *et al.* (2000) also reported an increase in deoxygenation of the deepwaters of the lake which is affecting the biodiversity of the lake. The water hyacinth has served as a

reservoir of pathogens and vectors responsible for spreading of several deadly diseases, such as dysentery, malaria, cholera and bilharzia (Gopal 1987).

Several methods of controlling the weed have been studied (Harley 1990, Harley & Wright 1984, Matagi 1998). These include mechanical harvesting of the plant, chemical control by using herbicides, biological control by using weevils. Uses of the water hyacinth have also been investigated (Rulangaranga 1993, Mshigeni 1995, Masende *et al.* 1999). However, utilisation of water hyacinth, for example in making compost, livestock feed supplement, upgrading wastewater treatment plants, recovery of metals (Rulangaranga 1993, Mshigeni 1995) has not been realized fully. Such use would aid the control of weed proliferation. Ecological control of nutrient loading into the lake and water hyacinth utilisation as a source of thermal energy and for production of ethanol, are being suggested as having potential for sustainable control of the plant (Masende *et al.* 1999 a, b and c). Conversion of biomass into biogas (methane) can be conceived as a viable option for controlling the weed. Masunzu (1994) reported that 92% of energy supply in Tanzania was coming from wood fuel, the largest consumer being in the domestic sector (Mwandosya & Luhanga 1983). It is therefore, hypothesised that if a small household biogas digester can be developed to utilise water hyacinth as a substrate for biogas production, some wood fuel users might switch to using the biogas.

Among the aquatic weeds, water hyacinth has been the most used as a substrate for methane production. Most of the anaerobic digestion systems used to convert the water hyacinth are the conventional single stage ones which use cowdung to initiate the digestion process. These include the continuously stirred tank reactors (CSTR) and the unstirred digesters (Ghosh & Klass 1981, Smith *et al.* 1988). Slow rates of cellulose hydrolysis have been limiting the performance of the conventional anaerobic digestion systems. Improving cellulose hydrolysis might significantly increase methane production. Cellulose hydrolysis can be improved by physical, chemical or biological pre-treatment or by operating at thermophilic temperatures, so as to maximise cellulase activity. Another biotechnological approach is that of applying anaerobic micro-organisms and their enzymes from some natural ecosystems where lignocellulolysis is efficient (Demeyer 1981, Hungate 1982, Gijzen 1987, Kivaisi & Mtila 1998).

The rumen micro-organisms have been found to be efficient in cellulolysis and thus their employment in anaerobic digesters is expected to enhance hydrolysis, which is generally considered to be the rate-limiting step in the overall anaerobic digestion of cellulosic materials (Hungate 1982, Kivaisi & Mtila 1998).

Several studies have concluded that methane production is affected by digestion temperature, toxicity, feeding mode, retention time, nutrients, pH,

composition of the feed and mixing (Delgado *et al.* 1992, Smith *et al.* 1988). With such diversity of factors it is not easy to superimpose results obtained in different environmental setting without validating their suitability for the environment in question. This paper focuses on substrate concentration, particle size and incubation time on the fermentation of water hyacinth found in Tanzanian waters.

METHODS

Water hyacinth plants were harvested during April to August 1998, at a distance of about 10 metres from the Victorial lakeshore. Plants were then cleaned to remove soil and dead plant materials, put in one heap and mixed thoroughly. Sampling was done in two ways: (i) water hyacinth shoots (WHS) on which the roots were removed by cutting and the shoots were collected. (ii) whole water hyacinth plant (WWH) where the intact plants without cutting the roots, were collected. The plants in both groups were chopped, blended by using a food blender (about 5-10 mm length) and sun-dried for 5-6 days for ease of transportation.

Two batches of experiments were done, one in Dar es Salaam and the other in Eindhoven, the Netherlands. For the Dar es Salaam experiments the inocular for batch cultures were prepared from the rumen content of slaughtered cows collected from Kimara slaughterhouse, in Dar es Salaam. The rumen liquor was strained through the cheesecloth as described by Mtila (1994). For the experiments done in the Netherlands, the inocular for batch and semi-batch experiments were collected from a live cow from Wageningen Agricultural University in the Netherlands.

The experiments were performed in 100 ml serum bottles. Each experiment was duplicated. A desired substrate load was introduced into the serum bottles followed by 40 ml of water. The inoculations of 10 ml of rumen fluid were done in the serum bottles making the total volume of the digestion mixture 50 ml.

The bottles were closed with n-butyl rubber stoppers and aluminium caps immediately after inoculation. The bottles were flushed with nitrogen gas for about 6 minutes to create anaerobic conditions and then incubated at 39°C in an incubator shaker which ensured mixing of the digestion mixture during the entire incubation period.

Samples for methane and volatile fatty acids (VFA) were taken at specified time in the respective experiment. Samples for gaseous mixture were drawn from bottles by means of 1 ml gas syringes. Samples for VFA and pH determination were taken from the bottles after thoroughly mixing of the digestion mixture and put in eppendorf vials. The pH of digestion mixtures was measured using a digital sentron 1001 pH meter. All sample bottles were stored in a freezer below 0°C until VFA analysis.

Effect of substrate concentration
The effect of substrate concentration on anaerobic digestion of dry water hyacinth shoots (WHS) and the whole water hyacinth (WWH) plants were tested at substrate concentrations of 5, 10, 15, 20, 25 and 30 grams per litre of water. The incubation period for all experiments was six days.

The concentration of VFA was determined on the third day, fifth day and at the end of the fermentation. The total amount of methane produced over the six days was the sum of methane produced after every day.

Effect of particle size
The effect of particle size on anaerobic degradation of dry ground WHS and WWH was tested for particle sizes below 1mm, 1-2 mm and 2-3 mm. The analyses of each group were done at different concentrations, at the incubation time of six days. Sampling for gas and VFA was done as described earlier.

Effect of incubation time
The effect of incubation time on anaerobic degradation of the dry ground WHS and WWH was tested at a substrate concentration of 5, 10, 15, 20, 25 and 30 grams per litre of water. In these experiments sampling times were 0, 1, 2, 3, 4, 5 and 6 days after incubation.

A gas chromatography (GC) technique was used for analysing methane and VFA. The GC (Hewlett Parkad 5980) was equipped with flame ionisation detector and capillary porous polymer poraPLOT Q column (25 m x 0.53 mm; cat. no: 7574, column no: 434670) fused with silica, for methane analysis. The flame ionisation detector detected methane separated by the column while a Penelson model No. 1020 was used for the integration of the signals obtained from the detector. Helium was used as a carrier gas. A column of a Wall Coated Tubular (WCDOT) (25 x 0.2 μm) fused with silica was used for the VFA analysis.

RESULTS AND DISCUSSION

Effect of substrate concentration
The effect of increasing substrate concentration, up to 25 g/l, was a corresponding increase in methane and VFA productions for both (WHS and WWH) types of substrates (Figs 1-6). The increasing amount of both methane and VFA with increasing substrate may be attributed to the abundant availability of readily degradable materials in the substrate. However, production of methane decreased when the substrate concentration was increased beyond 25 g/l. This could be attributed to poor microbe/enzymatic substrate contact caused by increased substrate amount (Archer 1983).

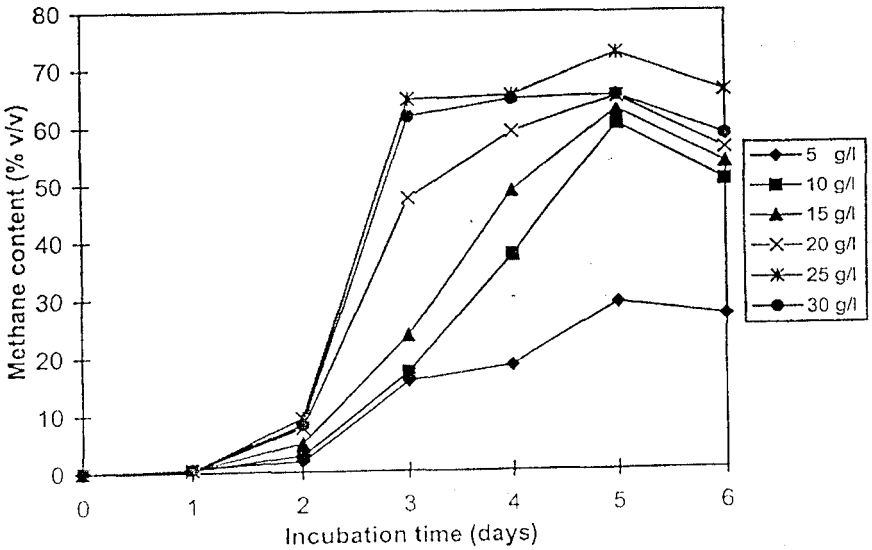

Fig. 1: Variation of methane content with substrate concentration and incubation time for water hyacinth shoots (WHS) from lake Victoria, Tanzania, 1998. (WHS: <1mm)

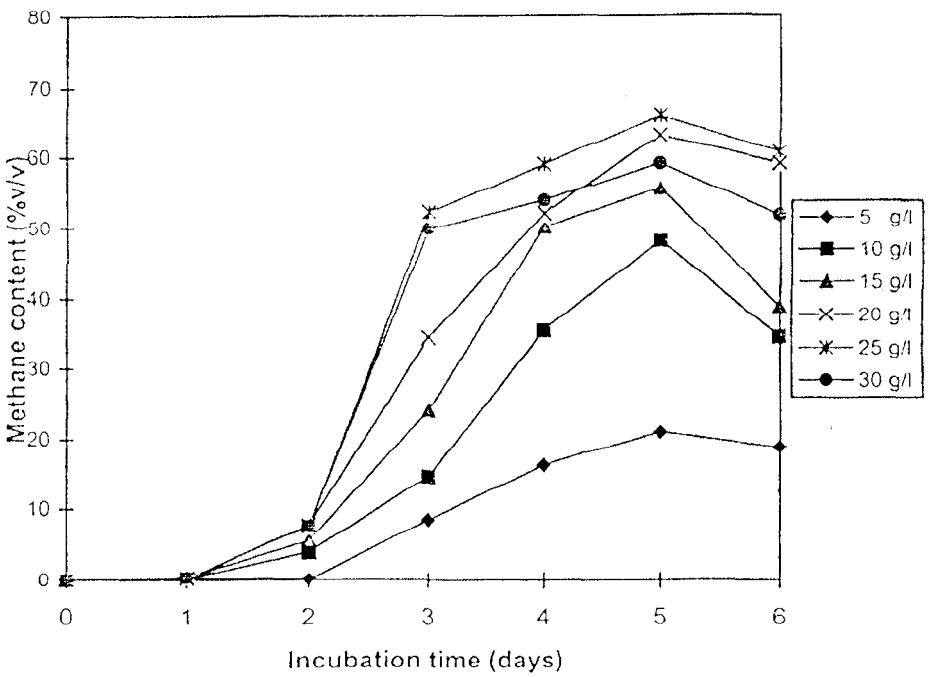

Fig. 2: Variation of methane content with substrate concentration and incubation time for water hyacinth shoots (WHS) from Lake Victoria, Tanzania, 1998. (WHS: <1-2 mm)

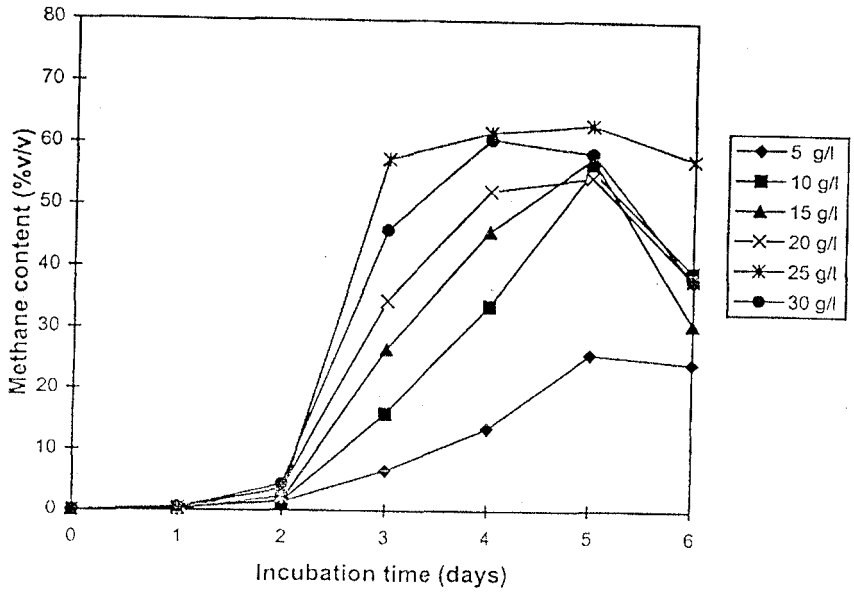

Fig. 3: Variation of methane content with substrate concentration and incubation time for water hyacinth shoots (WHS) from Lake Victoria, Tanzania, 1998. (WHS: 2-3 mm)

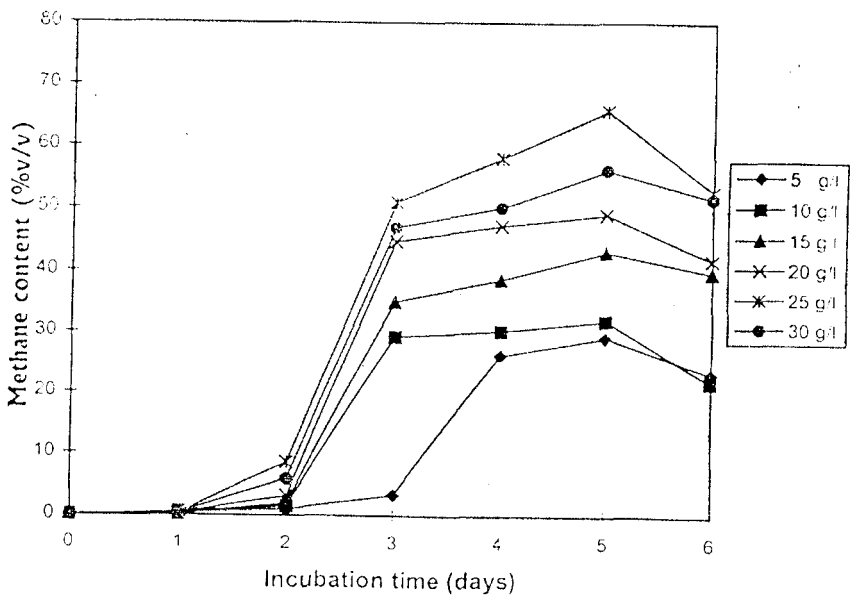

Fig. 4: Variation of methane content with substrate concentration and incubation time for whole water hyacinth plants (WWH) rom Lake Victoria, Tanzania. (WWH: <1 mm)

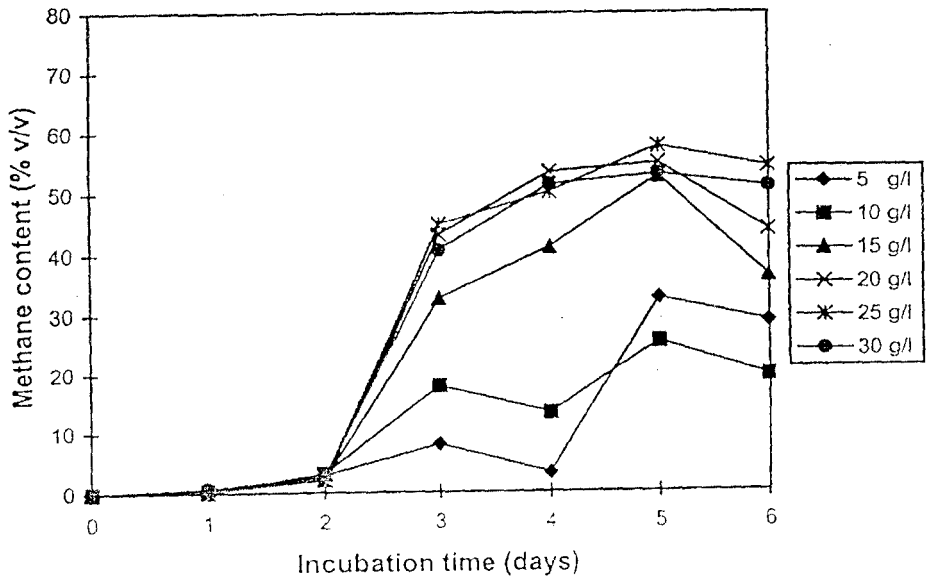

Fig. 5: Variation of methane content with substrate concentration and incubation time for whole water hyacinth plants (WWH) from Lake Victoria, Tanzania 1998. (WWH: 1-2 mm)

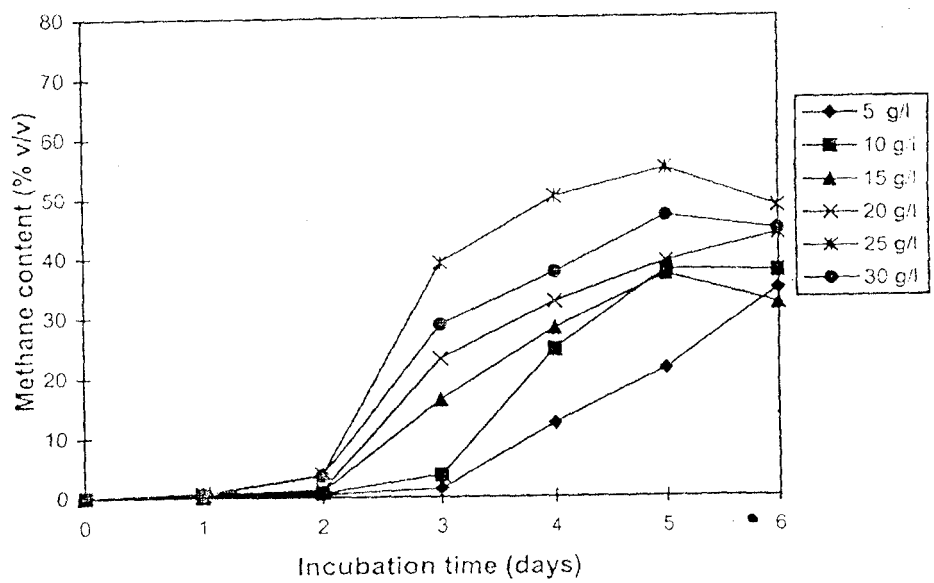

Fig. 6: Variation of methane content with substrate concentration and incubation time for whole water hycianth plants from Lake Victoria, Tanzania, 1998. (WWH: 2-3 mm)

At each concentration WHS gave relatively higher concentrations of both methane and VFA than WWH. Although the substrates used in this study were prepared from the same species of plant, they exhibit considerable variation in composition (Table 1), which influenced the biodegradability and the corresponding fermentation products. This was attributed to high lignin content in the WWH. The highest methane content for WHS was 72.53% at the substrate concentration of 25 g/l (particle size < 1 mm) while for WWH the maximum methane content of 65.67% was obtained after 5 days of incubation.

Total VFA concentrations ranged from 58.52-78.20 mmol/1 for WHS while it ranged from 29.30 - 66.80 mmol/1 for WWH (particle size <l mm). The pH for WHS ranged from 6.86-7.45 while for WWH is ranged from 6.22-7.28 (at particle size < 1 mm) (Table 2). This was attributed to the variations in VFA production in the two types of substrates.

Table 1: Composition of Lake Victoria water hyacinth

Parameter	WHS	WWH
Wet Basis		
Moisture content	88.6±0.6	89.6 ±0.3
Total Solids	11.4 ±0.2	10.4 ±0.1
Dry Basis		
Dry weight Total Solids	93.2 ±0.5	96.0 ±0.1
Volatile Solids	80.8 ±0.2	76.5 ±0.3
Ash	19.2 ±0.5	23.5 ±0.1
Total Fibre (NDF)	68.1 ±0.1	63.1 ±0.1
Cellulose and Lignin (ADF)	41.7 ±0.1	44.7 ±0.2
Hemicellulose	26.3 ±0.2	18.4 ±0.2
Lignin	18.2 ±0.1	25.6 ±0.1
Cellulose	23.5 ±0.3	19.1 ±0.1
Cellulose solubles	22.7 ±0.2	17.0 ±0.1
Crude protein	15.4 ±0.2	21.1 ±0.2
C:N Ratio	24:1	18:1

Data are presented as mean (≠Standard deviation)

Apart from lignin there are other factors which are known to influence digestibility of the plant material. For maximum biogas production and substrate degradation the C:N ratio should be approximately 30:1 (Delgado *et al.* 1992). The low C:N ratios of the WWH substrates were probably a contributing factor to the low degradation efficiencies. The effect of toxic substances such as heavy metals that might have interfered with the microbial

activities during fermentation was not significant. This is probably due to the fact that most of the elements were found in small concentrations.

Effect of incubation time

Generally, the relative methane concentration (% v/v) increased with the increasing incubation period (Figs. 1-6). Beyond the 5th day, methane content started to fall and at the incubation time of day six the methane content decreased sharply for both types of substrates. The methane content of WHS (particle size < 1mm) was 10.45% higher than for WWH (Fig.7). The production of VFA in both types of substrates increased with the increase of incubation time (Table 2). The highest VFA production obtained was 78.20 mmol/l of WHS at 30 g/l, and was 4.5% higher compared to that from WWH, probably this low difference may not have practical significance. The initial rapid production of methane and VFA was probably due to the fast degradation of readily degradable materials (cellulose solubles), such as starch, pectin and soluble sugars. The rates of production decreased considerably because the remaining material might have been more resistant to hydrolysis. Mtila (1994) had similar observations in the fermentation of water hyacinths–cow-dung mixture. The increase and decrease in the rates of formation of fermentation products can be associated with high and diminished microbial activity respectively. The microbial population might have decreased with time, because the remaining organic matter was almost unavailable to them. Therefore, the few micro-organisms that survived were those capable of degrading the hard materials (Wood *et al.* 1988).

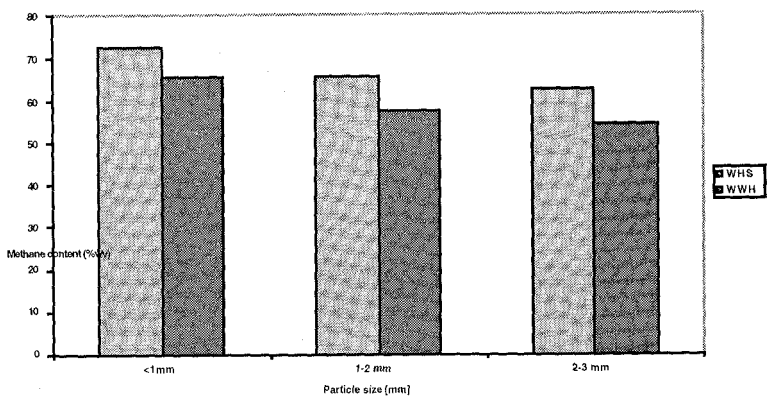

Fig. 7: Effect of Particle Size on Methane Production after 5 days of incubation time at substrate concentration of 25 g/l. Water hyacinth plants from Lake Victoria, Tanzania

The pH values changed gradually during the first five days of incubation; during the last day of incubation there was a decreased of about 1% and 3% for WHS and WWH respectively (Table 2).

The observed decrease in methane content in the last day of incubation was attributed to the acid build-up and subsequent lowering of pH of the contents (see Table 2) thus leading to inhibition of methanogenesis. Mtila (1994) observed an increase in methane concentration at substrate concentration between 10-30 g TS/litre fermentor volume for the incubation of 132 hours, in the fermentation of WH-cow-dung. In this study the methane production was inhibited in day six because of the increased incubation time (14 hours). This give rise to need of increasing the incubation period in the batch cultures to get a clear picture of its effect on degradation.

Table 2: Changes in VFA and pH of the fermentation mixture during incubation of WHS and WWH at particle sizes of less than 1 mm and concentration of 25 g/1. Plants collected from Lake Victoria, Tanzania

Parameter tested	Substrate	Incubation time (day)						
		0	1	2	3	4	5	6
PH [-]	WHS	7.45 ±0.1	7.23 ±0.2	7.15 ±0.2	7.02 ±0.1	7.00±0.3	6.96±0.3	6.86±0.2
	WWH	7.28 ±0.2	7.01 ±0.05	7.00 ±0.1	6.95±0.2	6.87±0.2	6.22±0.2	6.52±0.3
VFA [mmol/l]	WHS	0	ND	ND	58.52±0.2	ND	68.7±0.1	78.2±0.1
	WWH	0	ND	ND	29.3±0.1	ND	64.09±0.1	66.8±0.1

ND= not determined. Data is presented as mean (≠Standard deviation)

The accumulation of the VFA can also be attributed to a probable failure of the hydrogen-utilisers and syntropic species, which would result in increased acidity within the system. The concentrations of acids in the fermentor (i.e. 72.2 and 69.10 mmol/l for WHS and WWH respectively) were still far below inhibitory levels. It has been found that provided that the pH is maintained at around neutral, VFA concentrations could be tolerated to values as high as 260 mmol/l(Playne 1984).

Effect of particle size

Generally the cumulative methane concentration decreased with the increase in the particle sizes (Fig. 8). However, for each particle size class, WHS gave relatively higher concentrations of methane than WWH.

The VFA production ranged from 12.09-72.20 mmol/litre for WHS while for WWH the production was 12.73-69.10 mmol/litre Table 3. Methane content in WHS for smallest particle size was 10.45% higher than that obtained for the largest size, while for WWH it was 15.33% higher than that for the largest size .

The big increase of the fermentation products with the decrease in particle sizes complied with the increased microbe/enzyme-substrate contact. In the

substrates with small particle sizes the micro-organisms and their enzymes were in more contact with the substrate material, hence higher utilisation of the material was achieved. Also agitation (mixing) of the fermentor content enhanced the contact.

However, the percentage increase in methane production (15.33%) obtained by using smallest particle size, have to be weighed against the additional grinding energy input and the ease of handling or pumping the ground hyacinths.

It should be noted that, at 2-3 mm WWH, the effect of changing the substrate concentrations was insignificant. This could be due to the fact that large particles have relatively lower surface area and as such the microbial/enzymes-substrate contact was reduced, possibly leading to the observed low methane production rates.

The observed relation between the cumulative amounts of methane and lignin contents of the substrate shows that lignin indirectly hinders methane production, because of its cross-linked polymers structure (Delgado *et al.* 1992).

CONCLUSION

The methane and VFA yield increases with substrate concentration up to 25 g/l. Above that concentration the methane yield decreases with increasing substrate concentration. The incubation period of five days gave maximum cumulative methane concentration after which it starts falling. This was attributed to acid build up leading to inhibition of methanogenesis. Small particle sizes enhance methane yield, likely because the smaller the particle size the higher the contact area between the enzymes and the substrate. However, the optimum particle size has to be balanced with energy requirement for the grinding.

ACKNOWLEDGEMENT

The Author would like to acknowledge the contribution made by E. Edward. The Jan Tinbergen Scholarship for financing part of the study.

REFERENCES

Archer DB 1983 The microbiological basis of process control in methanogenic fermentation of solid wastes. *Enzyme Microbiol. Technol.* 5: 162-169

Balirwa J, Witte F, Welocomme RL, Chapman L and McConnel RH 2000 *The role of conservation in biodiversity and fisheries biodiversity.* Proceedings of Lake Victoria 2000, (in press)

Chege M 1995 Lake Victoria: a sick giant. *People and Planet* 4(2)

Crul RCM 1993 *Monographs of great African lakes: Limnology and hydrology of Lake Victoria.* UNESCO / IHP – IV Project M – 5.1

Delgado M, Guariola E and Bigeriego M 1992 Methane generation from water hyacinth biomass: *J. Enviro. Sci. Health* 27(2): 347-367

Demeyer DL 1981 Rumen microbes and digestion of plant cell walls. *Agric. Environ.* 6: 121-143

Ghosh S and Klass DL 1981 Advanced process development for methane production from biomass-waste blends. *ACS Symposium S.* 144: 251-278

Gijzen HJ 19^^ *Anaerobic digestion of cellulosic waste by a rumen-driven process.* Ph.D. thesis, University of Nijmegen, The Netherlands

Gopal B 1987 *Water hyacinth: aquatic plant studies-1.* Eisevier Science B.V., The Netherlands

Harley KLS 1990 The role of biological control in the management of water hyacinth, *Eichhornia crassipes. Biocontrol News and Information* 10(1): 11-22

Harley KLS and Wright AD 1984 Implementing a program for biological control of water hyacinth, *Eichhornia crassipes.* In: Thyagarajan G (ed) *Proceedings Int. Conf. On Water Hyacinths, Hyderabad (India).* UNEP, Nairobi

Hecky RE, Mugidde R, Twongo T, Ndawula L, Balirwa J and Mavuti K 2000 Ecosystem change in Lake Victoria. *Proceeding of Lake Victoria 2000,* (in press)

Hungate RE 1966 *The rumen and its microbes.* Academic Press, New York

Hungate RE 1976 The Rumen Fermentation In: Schlegel HG, Gottschalk G and Pfenning N (eds) *Microbial production and utilisation of gases*

Hungate RE 1982 Methane formation and cellulose digestion: biochemical ecology and microbiology of rumen ecology. *Experincia* 38: 189-192

Kivaisi AK and Mtila M 1998 Production of methane from water hyacinth (*Eichornia crassipes*) (Mart) (Solms) in a two stage bioreactor. *World Journal of Microbiology and Biotechnology* 14:125-131

Masende Z, Katima JHY and Masanja E 1999a Ethanol from water hyacinth: fermentability of water hyacinth hydrolysates from acid-catalyzed hydrolysis process. *The Tanzania Engineer* 6(5): 27 – 39

Masende Z, Katima JHY and Masanja E 1999b Production of fermentable sugars from water hyacinth by low-temperature concentrated-acid hydrolysis method. *UHANDISI Journal (in Press)*

Masende Z, Katima JHY and Masanja E 1999c Production of fermentable sugars from water hyacinth by high-temperature dilute-acid hydrolysis method. *UHANDISI Journal (in Press)*

Masunzu EM 1994 Energy and Sustainable Environment in Tanzania. *CEEST Publications:* 192 - 203

Matagi SV 1998 *A review of herbicide use in the chemical control of water hyacinth in Uganda.* A Paper Presented Under the Auspices of

the Lake Victoria Environmental Management Programme, Mwanza, Tanzania

Mshigeni KE 1995 The water hyacinth weed in Africa: a problem or an opportunity. *Discov. Innov.* (*editorial*) 7(2)

Mtila M 1994 *Utilisation of water hyacinth (Eichhornia crassipes) as a substrate for biogas production employing rumen microorganism. M.Sc. thesis,* University of Dar-es-Salaam

Mwandosya MJ and Luhanga MLP 1983 *Energy resources flow and uses in Tanzania.* Dar es Salaam University Press

Playne MJ 1984 Increased digestibility of bagasse by preatment with alkalis and steam explosion. *Biotechnol Bioeng.* 26: 426-433

Rulangaranga ZK 1993 *Water hyacinth (Eichhornia crassipes (Mad) Solms): Tanzania's untapped resource.* A paper presented at the National Wetlands Technical Committee (NAWETCO) Meeting of floating water weeds, Dar-es-Salaam

Rutashobya DG 1996 *Lake Victoria water quality problems, including pollution.* Regional workshop on lake victoria environmental management program, Dar-es-Salaam

Smith PH, Bordeaux FM, Shiralipour A, Walkie A, Andrews JF, Ide S and Barnet MW 1988 Biological production of methane from Biomass. In: Smith W and Frank JR (eds) *Methane from biomass a systems approach* pp: 391-334

Van Horen JJM 1996 *The eutrophication of lake Victoria, East Africa.* M.Sc. thesis, Eindhoven University of Technology, The Netherlands.

Witte F, Goldsmith T, Ligtvoet PC, Oijen MJP and Van Wanink 1992 Species extinction and concomitant ecological changes in Lake Victoria. *Netherlands Journal of Zoology* 42(2-3): 214-232

Wood TM, McCrae SI, Wilson CA, Bhat KM and Gow L 1988 Aerobic and anaerobic fungal cellulases with special reference to their mode of attack on crystalline cellulose. In: Aubert JP, Beguin P and Millet J (eds) *Biochemstry and genetics of cellulose degredation.* Academic Press, London