BIOGAS PRODUCTION FROM CASSAVA TUBERS

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วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
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แก๊สชีวภาพซึ่งเป็นแก๊สที่ผลิตจากจุลินทรีย์ภายใต้สภาวะไร้ออกซิเจน เป็นแหล่งพลังงานอีกแหล่งหนึ่งนอกเหนือจากแหล่งพลังงานจากธรรมชาติซึ่งมีการใช้กันมากและมีมูลค่าสูงขึ้นในปัจจุบัน โดยปกติมีการผลิตแก๊สชีวภาพอยู่แล้วด้วยวัตถุประสงค์เพื่อบำบัดของเสียแต่งานวิจัยนี้ผลิตแก๊สชีวภาพเพื่อเป็นแหล่งพลังงานทดแทนโดยใช้หัวมันสำปะหลังดิบซึ่งเป็นวัตถุดิบที่หาได้ง่ายและมีมูลค่าต่ำ จากการวิเคราะห์ส่วนประกอบทางกายภาพและทางเคมีของหัวมันสำปะหลังซึ่งเก็บมาจากแหล่งปลูกในพื้นที่จังหวัดนครราชสีมา พบว่าส่วนประกอบหลักของหัวมันสำปะหลังสดโดยเฉลี่ยมีความชื้น 65% แป้ง 18% ปริมาณคาร์บอน 17% ไนโตรเจน 0.20% และของแข็งทั้งหมด 35% ในการทดลองผลิตแก๊สชีวภาพโดยใช้กระบวนการหมักแบบขั้นตอนเดียวนี้ได้เตรียมมันสำปะหลังดิบในลักษณะแห้ง (ความชื้น 18.65% ปริมาณคาร์บอน 39.56% ไนโตรเจน 0.46% และของแข็งทั้งหมด 81.35%) และเริ่มหมักด้วยปริมาตร 5 ลิตร ที่อุณหภูมิห้อง (โดยเฉลี่ย 30 องศาเซลเซียส) เป็นเวลา 30 วัน โดยใช้ปริมาณหัวมันแห้งดิบที่ความเข้มข้นของของแข็งทั้งหมดต่างกันพร้อมทั้งทดลองเติมแหล่งไนโตรเจน (ยูเรีย) ในปริมาณต่างกัน และเติมหัวเชื้อที่เตรียมจากมูลสัตว์และน้ำทิ้งจากโรงงานผลิตแป้งมันสำปะหลัง พบว่าที่ 1.00% (น้ำหนักต่อปริมาตร) ของของแข็งทั้งหมด และการเติมยูเรีย 0.04% (น้ำหนักต่อปริมาตร) (สัดส่วนคาร์บอนต่อไนโตรเจนเท่ากับ 20 ต่อ 1) ให้ผลผลิตของแก๊ส 1.95 ลิตรต่อวัน ที่มีปริมาณมีเทนสูงสุดคือ 67.92% ที่ระยะเวลาการหมัก 10 วัน โดยกระบวนการหมักสิ้นสุดเมื่อหมักได้ 16 วัน และมีปริมาณแก๊สและมีเทนที่เกิดขึ้นทั้งหมด 530 และ 259 ลิตรต่อกิโลกรัมของของแข็งทั้งหมดที่เติมลงไป ตามลำดับ ซึ่งเมื่อเพิ่มปริมาตรของวัสดุหมักเป็น 20 และ 50 ลิตร ตามลำดับ เพื่อเป็นแนวทางของการขยายกำลังการผลิต พบว่าทั้งในถังหมักที่มีวัสดุหมัก 20 และ 50 ลิตร นั้นเมื่อหมักได้ 16 วัน มีปริมาณแก๊สเกิดขึ้นทั้งหมด 517 และ 546 ลิตรต่อกิโลกรัมของของแข็งทั้งหมด ซึ่งมีปริมาณมีเทน 252 และ 299 ลิตรต่อกิโลกรัมของของแข็งทั้งหมด แต่กระบวนการหมักสิ้นสุดที่ 21 และ 24 วัน และได้แก๊สที่เกิดขึ้นทั้งหมดคือ 580 และ 564 ลิตรต่อกิโลกรัมของของแข็งทั้งหมด ที่มีมีเทน 334 และ 322 ลิตรต่อกิโลกรัมของของแข็งทั้งหมด ตามลำดับ กล่าวโดยสรุปได้ว่าหัวมันสำปะหลังดิบ (ความชื้น 18.65%) 1 กิโลกรัม ใช้ผลิตแก๊สชีวภาพได้ 443 ลิตร ที่มีมีเทน 242 ลิตร ซึ่งคิดเป็นค่าพลังงานได้ 9765 กิโลจูล

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BIOGAS/METHANE /CASSAVA TUBER/SINGLE-STATE DIGESTER

Biogas, a gas produced by microorganisms under anaerobic conditions, is another source of energy apart from natural energy which is being reduced by human activities and becoming expensive. The gas has usually been produced from organic waste treatment but this research aims to produce biogas for applying as an alternative source of energy using raw cassava tubers, the cheap and abundant agriculture product. From the analyses of physical and chemical compositions of cassava tubers collected from their plantation areas in Nakhon Ratchasima Province, the fresh tuber has the average contents of 65% of moisture, 18% of starch, 17% of total carbon, 0.20% of total nitrogen, and 35% of total solids. The dry cassava tuber (18.65% of moisture, 39.56% of total carbon, 0.46% of total nitrogen, and 81.35% of total solids) was then prepared for biogas production using the simple single-state digester of 5-L working volume. The fermentation was performed at room temperature (approximately 30°C) for 30 days. Various concentrations of total solids as well as nitrogen source (urea) were fed into the digester with the addition of seed cultures prepared by mixing animal manure and wastewater from cassava starch production factory. It was found that at 1.00% (w/v) total solids and 0.04% (w/v) urea (carbon-to-nitrogen ratio of 20:1) gave the gas yield of 1.95 L/day containing the maximum methane content of 67.92% at 10-day retention time. The fermentation reactions were ceased after operating for 16 days. The total biogas and total methane yields were 530 and 259 L/kg TS fed, respectively. The fermentation volumes were then scaled up to 20 and 50 L, the total biogas yields of 517 and 546 L/kg Ts fed containing the total methane yields of 252 and 299 L/kg TS fed were obtained at 16-day retention time. The fermentation reactions were ceased after operating for 24 and 21 days, with the total biogas yields of 580 and 564 L/kg TS fed containing the total methane yields of 334 and 322 L/kg TS fed, respectively. These results reveal that 1 kg of dry cassava tuber (18.65% of moisture) could be biologically converted to 443 L of biogas containing 242 L of methane which could be calculated to energy value of 9765 kJ.

School of Biology Student's Signature ________________

Academic Year 2004 Advisor's Signature ________________

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Wantanee Anunputtikul

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LIST OF ABBREVIATIONS

%	percent
µL	micro litre
A	alkalinity
ABR	anaerobic baffled reactor
APS	anaerobic-phase solid digester system
C	carbon
°C	degree Celsius
CH_4	methane gas
cm^3	cubic centimetre
cm/sec	centimetre per second
CO_2	carbon dioxide gas
COD	chemical oxygen demand
CSTR	completely stirred tank reactor
dm^3	cubic decimetre
DO	dissolved oxygen
et al.	et alia (and others)
g	gram
g/L	gram per litre
H_2	hydrogen gas
H_2O	water

LIST OF ABBREVIATIONS (Continued)

H_2S	hydrogen sulfide
H_2SO_4	sulfuric acid
$HClO_4$	perchloric acid
HRT	hydraulic retention time
J	joule
kg	kilogram
KH_2PO_4	monobasic potassium phosphorus
kJ	kilo joule
kJ/cm^2	kilo joule per square centimetre
kWh	kilowatt-hour
L	litre
LPG	liquefied petroleum gas
m^3	cubic metre
mg as acetate/L	milligram per litre of equivalent acetate
mg as $CaCO_3$/L	milligram per litre of equivalent calcium carbonate
min	minute
mL	millilitre
mL/min	millilitre per minute
MW	megawatt
N	Normal
N	nitrogen
N_2	nitrogen gas

LIST OF ABBREVIATIONS (Continued)

$NaHCO_3$	sodium hydrogen carbonate
$(NH_4)_6 MO_7 O_{24} .4H_2O$	ammonium molybdate
NHO_3	nitric acid
NH_4VO_3	ammonium vanadate
nm	nano metre
P	phosphorus
pp	page
ppm	part per million
rpm	round per minute
SRT	sludge retention time
TS	total solids
TVS	total volatile solids
UASB	upflow anaerobic sludge blanket
v/v	volume by volume
VS	volatile solids
VSS	volatile suspended solids
w/v	weight by volume

CHAPTER I

INTRODUCTION

1.1 Significance of the study

Natural energy has been the most important source of energy for human being for several decades. Examples of natural energy sources are liquefied petroleum gas (LPG), benzene fuel, diesel fuel, and fire wood. At present, the natural source is being reduced by human activities, and becoming expensive. Therefore, some other sources of energy have to be investigated. Biogas, the gas generated from organic digestion under anaerobic conditions by mixed population of microorganisms, is an alternative energy source which has been commenced to be utilized both in rural and industrial areas at least since 1958 (Acharya, 1958). Biogas generally composes of methane (55-65%), carbon dioxide (35-45%), nitrogen (0-3%), hydrogen (0-1%), and hydrogen sulphide (0-1%) (Chomchat *et al*., 1984; Milono *et al*., 1981). The quantity and quality of biogas depend on characteristics of feed materials (Calzada *et al*., 1984; Cuzin *et al*., 1992; Kalia *et al*., 2000; Prema *et al*., 1992; Zhang and Zhang, 1999).

Biogas production technology has been introduced to Thailand around 1978 for various applications such as the production of energy from organic waste treatments and organic fertilizers, and the improvement of the hygienic condition by reducing pollution (Bhumiratana *et al*., 1984). The application for organic fertilizers reduces the need of chemical fertilizers. Organic wastes including domestic, industry, and agriculture wastes can be treated using the biogas production process. The process could potentially reduce plant, animal, and human pathogens. Biogas is also a clean-burning fuel that can reduce the incidence of eye and lung problems (Stuckey, 1984).

In Thailand, animal manure and crop residues have been widely used for biogas production and investigation (Chomchat *et al*., 1984; Kunawanakit, 1986; Punyawattoe, 1986; Supajunya *et al*., 1984; Tanticharoen *et al*., 1984). Another available raw material, which could be one of the suitable materials for biogas production in Thailand, is cassava tubers. The starchy tubers are cheap and abundant agriculture product (Office of Agricultural Economics, 2003). Cassava production in Thailand ranks as one of five world's cassava production (Pandey *et al.,* 2000). The plant is one of the main cultivars in the Northeast region of Thailand. Nakhon Ratchasima Province, where Suranaree University of Technology is located, also ranks first for both planted areas and production yields of cassava in Thailand (Office of Agricultural Economics, 2003). Cassava tubers contain several organic nutrients which can efficiently support the anaerobic digestion of microorganisms (Pandey *et al*., 2000). Thus, this study aims to investigate the potential production of biogas for applying as an alternative source of energy from raw cassava tubers.

1.2 Research objectives

Objectives of this study are as follows:

1) To investigate the potential production of biogas from raw cassava tubers using single-state digester,

2) To study some factors affecting the biogas production from raw cassava tubers, and

3) To investigate the potential production of biogas when the digestion volumes are increased.

1.3 Research hypothesis

Cassava tubers compose of ingredients, which would be suitable for using as a potential raw material for microorganisms involved in the biogas production. The gas product could be served as an alternative source of energy.

1.4 Scope and limitations of the study

The potential production of biogas, a source of energy, from raw cassava tubers was investigated. Fresh cassava tuber was collected from their plantation areas in Nakhon Ratchasima Province. Microbial inocula (seed cultures) were prepared by mixing animal manure (chicken dung), molasses, and liquid waste collected from the open-anaerobic pond of cassava starch production factory in Nakhon Ratchasima Province. The anaerobic digestion process was performed using the simple single-state digester at ambient temperature for 30 days with working volume of 5 liters (L). In this study, various total solids (TS) concentrations were applied to the 5-L reaction volume to obtain the optimum TS concentrations. Then the addition of urea (46% of nitrogen) as a nitrogen source was investigated. Change in biogas yield, gas composition, pH, alkalinity, volatile fatty acids (VFA), temperature, TS, volatile solids (VS), and starch content were monitored during the raw cassava slurry fermentation. Finally, the optimal concentrations of both TS and urea addition were applied to produce biogas in the scaled-up digesters, 20-L and 50-L working volumes.

1.5 Expected results

This research involves the utilization of raw cassava tubers, a cheap and abundant agricultural product in Thailand, for biogas production. The expected outcome is the evaluation of potential biogas production from raw cassava tubers. Data concerning biogas quantity and quality, biogas production process, and some factors affecting the gas production from cassava tubers will be obtained. These data will be useful for the production of energy source, the increase in cassava tuber utilization, and also plantation and improving the economic value of raw cassava tubers.

CHAPTER II

LITERATURE REVIEW

2.1 Biogas production

Biogas refers to the gas that is generated from organic digestion under anaerobic conditions by mixed population of microorganisms. Usually this anaerobic conversion of organic matter is thought to occur in three steps: fermentative, acid-forming, and methanogenic stages (Aiman *et al.,* 1981; Bitton, 1994; Milono *et al.,* 1981). The gas generally composes of methane (CH_4, 55-65%), carbon dioxide (CO_2, 35-45%), nitrogen (N_2, 0-3%), hydrogen (H_2, 0-1%), and hydrogen sulphide (H_2S, 0-1%) (Chomchat *et al.,* 1984; Milono *et al*., 1981). The methane contents of 32-50%, 74-79%, 30-40%, 57%, and 51-53% were obtained from the anaerobic fermentation of tapioca-processing solid waste, coffee pulp juice, chinese cabbage, cassava peel, and fruit and vegetable wastes, respectively (Aiman *et al*., 1981; Calzada *et al*., 1984; Chomchat *et al*., 1984; Cuzin *et al*., 1992; Viswanath *et al*., 1992). Organic wastes including domestic, industry, and agriculture wastes can be treated using the biogas production process. Since the anaerobic digestion reduces the amount of organic wastes and produces methane, a valuable fuel, it is becoming more and more attractive as a waste treatment alternative. The process could potentially reduce plant, animal, and human pathogens (Stuckey, 1984).

2.1.1 Raw materials for biogas production

Raw materials for biogas production are mostly organic wastes from domestics, industry, and agriculture.

2.1.1.1 Domestic wastes

An example of the biogas production from domestic waste is reported by Alaa El-Din *et al.* (1984). The two-stage digester of 190 m^3 total capacity (150 m^3 digesting volume) was used for the biogas production from kitchen refuses of army camps in Egypt. The plant included a fixed film compartment (20 m^3), and was operated for 422 days consuming 203 tons of camp refuses with the average feeding rate of 480.9 kg of fresh garbage per day. The fresh garbage containing 248.5 kg TS/day. The plant produced 84,668 m^3 of biogas with the average of 200.6 m^3/day or 1.337 m^3/m^3 of digesting slurry/day. Based on international fuel prices, preliminary economic evaluation indicated a pay-back period of less than six months.

Sosonowski *et al.* (2003) presented the results of investigation of methane fermentation of sewage sludge and organic fraction of municipal solid wastes as well as the co-fermentation of both substrates under thermophilic and mesophilic conditions. Five experiments were conducted in two types of experimental arrangement; thermophilic batch wise and two-stage quasi-continuous, acidogenic digestion under thermophilic condition (56°C) and mesophilic methane fermentation (36°C). Three different kinds of feedstock were filled into the bioreactors: sewage sludge, organic fraction of municipal solid wastes, and the mixture of sewage sludge (75%, v/v) and organic fraction of municipal solid wastes (25%, v/v). The addition of the organic fraction of municipal solid wastes increased the carbon-to-nitrogen ratio from 9:1 to 14:1. The results showed that methane concentration in the biogas was above 60% in all cases. Biogas productivity varied between 0.4 and 0.6 dm^3/g of volatile suspended solids (VSS) added depending on substrate added to the digester.

2.1.1.2 Industry wastes

Several organic wastes from agro-industry have been reported to be used for biogas production. For example, tapioca-processing solid was used for biogas production with the addition of ammonia as nitrogen source and monosodium phosphate as phosphorous source depending on the carbon to nitrogen to phosphorus ratio of raw material (Aiman *et al.,* 1981). This mixture was inoculated with anaerobic fermented cow dunk in the amount of 0.1, 1.5, 4.0, 6.5, and 9.0% (w/w). This inoculum contained about 10% (w/w) solid. The feed contained 4.8, 7.5, 9.2, and 12% TS. Digestion process was carried out in the batch digester of 3, 4, and 30 L that contained 1.5, 2.0, and 20-L digesting materials, respectively. The retention time of these experiments was 30-44 days, and digestion temperature was 22 to 28°C. The best carbon to nitrogen to phosphorus ratio was 100:2.68:0.6 with 9.2% TS and 1.5% (w/w) inoculum size. The volume of gas obtained was about 265 L/kg of dried materials. The composition of gas was 32-50% carbon dioxide or 50-68% methane.

Bunchueydee (1984) studied the biogas production from different kinds of wastes from factories: tapioca, noodle, sodium monoglutamate, canning food, and packing milk. Tapioca waste was found to be suitable for biogas production. The ratio of methane to carbon dioxide yield was 85:15, which was a very high ratio compared to other sources.

Supajunya *et al. (*1984) described the biogas production from pineapple cannery waste using 3.8-L glass bottle with 3-L working volume, and operating at mesophilic and thermophilic temperature ranges of 32, 37, 45, 50, 55, and 60°C. The increase in the feed concentration from 12.5 to 17.5 g of wet weight/L of reactor volume/day at 50-day retention time increased methane production from 1.27 to 1.79 L of reactor volume/day at 55°C, and 1.22 to 1.40 L at 37°C.

Methane fermentation from pineapple cannery's solid waste using single-stage and two-stage digesters was also reported (Punyawattoe, 1986). The single-stage digester was maintained at ambient temperature (32°C) in the 38-L PVC reactor with 18-L working volume. The maximum loading rate for the single-stage digester was 19.4 g/L/day at 17 days of hydraulic retention time (HRT). The average gas yield was 20.6 L/day or 1.147 L/L of digester volume/day or 1.065 L/g of loading material/day. For the two-stage digester, the acidogenic stage was maintained at 32°C in a 2-L open tank. The pH of this stage was 3.5-4.5. The methanogenic stage was operated in 4-L reactor with 3-L working volume. The average gas yield was 1.9 L/L of digester volume/day or 1.1 L/L of digester volume/day at organic loading rate of 6 g of chemical oxygen demand (COD)/L and 7.5 days of HRT. The methane content of the gas produced from the second stage was 60%.

Calzada *et al*. (1984) used coffee pulp juice for biogas production by both one-phase and two-phase systems. Loading rate for one-phase system ranged from 0.5 to 3 g of volatile solids (VS)/L/day with 10 days of HRT. The gas product was 1.55 L/L/day, with loading rate of 0.97 g VS/L/day and methane content of 79%. Two-phase system consisting of acidogenic and methanogenic stages with 0.5 days of HRT for acidogenic stage and 0.55 g VS/L/day, and 8 days of HRT and 1.8 g VS/L/day for methanogenic stage gave the maximum methane content of 74%. The two-phase anaerobic digestion system was better for treating coffee pulp juice than a one-phase unit.

Two-stage anaerobic co-digestion of a 10% (w/v) TS waste activated sludge/fruit and vegetable mixture with approximately 25% (w/v) of the VS arising from the fruit and vegetable was investigated. Acidogenic completely stirred tank reactors (CSTR) and methanogenic inclined tubular digesters operated at 30°C achieved stable anaerobic digestion at an overall system loading rate of 5.7 kg VS/m^3/day, 13 days of overall HRT (3 days of acidogenic HRT and 10 days of methanogenic HRT), with 40% VS reduction and a system biogas yield of 0.37 m^3/kg VS added with methane content of 65% (Dinsdale *et al.,* 2000).

Rani *et al*. (2003) described ensilaging of pineapple processing waste and its effects on physico-chemical characteristics and methane production. Pineapple peel was found to be a potential substrate for methane generation by anaerobic digestion because there was rich in cellulose, hemicellulose, and carbohydrates. Results of the anaerobic digestion from ensilaging of pineapple peel were the conversion of 55% carbohydrates into volatile fatty acids (VFA) and reduction of 91% the biological oxygen demand (BOD). The biogas yield from biogas digester fed with ensilaged pineapple peel was 0.67 m^3/kg VS added (65% methane content) whereas fresh and dried pineapple peels gave biogas yields of 0.55 and 0.41 m^3/kg VS added with methane contents of 51% and 41%, respectively.

2.1.1.3 Agriculture wastes

A. Animal manure

Animal manure from cattle, swine, horse, elephant, chicken, and duck, is widely used for biogas production (Bhumiratana *et al.,* 1984; Energy Policy and Planning Office, 2002 and 2003; Krishna *et al.,* 2000; Mackie and Bryant, 1995). The manure is often readily applied after mixing with water. Large quantities of biogas and low production cost are usually obtained (Milono *et al.,* 1981).

Waste obtained from cattle fed with a high grain, was reported to be subjected to anaerobic digestion at mesophilic and thermophilic temperatures (40 and 60°C) using stirred, bench-top fermentors of 3-L working volume fed on a semi-continuous basis (Mackie and Bryant, 1995). The methane production was 11.8, 18.3, 61.9, and 84.5% higher in the thermophilic than the mesophilic digesters at the 3, 6, 9, and 12 g VS/L reactor volume loading rates, respectively. Energy values of methane production were 7.3, 18.3, 72.9, and 107.3 kJ/day higher in the thermophilic than the mesophilic digesters. Methane production decreased more rapidly with each increase in VS loading rate and decrease in retention time in the mesophilic than the thermophilic digesters.

Krishna *et al.* (2000) investigated two-stage aerobic thermophilic and anaerobic mesophilic treatments of swine waste. The two-stage system included a 1-day sludge retention time (SRT) of the aerobic thermophilic reactor, which was operated at 62°C, and with 1.0 mg dissolved oxygen (DO)/L, followed by a 5, 9, and 14-day of SRT of the anaerobic mesophilic digester operated at 37°C. Whereas a single-stage anaerobic mesophilic digester operated at 6, 10, and 15-day of SRT and 37°C was used as the control. The two-stage system of anaerobic digester produced

0.56-0.64 m^3 of methane/kg VS destroyed compared to lower levels of 0.47-0.51 m^3 of methane/kg VS destroyed by the control, both operating at 6, 10, and 15 days of SRT. The methane gas produced by the two-stage system, of 0.26, 0.32, and 0.39 m^3/kg VS fed at 6, 10, and 15-day SRT, respectively, was significantly higher than that by the control system (0.17, 0.22, and 0.25 m^3/ kg VS fed at 6, 10, and 15-days SRT), respectively.

Biogas has been produced and applied in pig farms in Thailand (Energy Policy and Planning Office, 2003). Average yields of 95 L of biogas/pig/day (equal 44 g of LPG/pig/day) and 350 g of organic fertilizer/pig/day could be obtained by the anaerobic wastewater treatment process. It can be estimated that 0.1188 million kg/day of LPG and 0.95 million kg/day of organic fertilizer are obtained from the biogas production process in the pig farm having 207 million animals. The biogas obtained can generate electricity of 12.4 MW (24.8 MW Peak Demand Period). Biogas production in pig farms provides 1.7-2.3 million Baht/day of gross value.

B. Plant residues

Plant residues including cassava peel, pineapple peel, banana peel, banana stem, chinese cabbage, water hyacinth, and rice straw, have been used for biogas production. One problem of the utilization of plant residues is the composition of lignin, cellulose, and hemicellulose resulting in the longer period of fermentation than animal manure. Pretreatment of plant by chopping and grinding, as well as using hydrolytic enzymes such as amylase, cellulose, pectinase, lipase, and protease, has been reported (Hobson and Shaw, 1971).

Cuzin *et al.* (1992) studied the methanogenic fermentation of cassava peel using the pilot plug flow digester (128 L). The average methane content of 57% was obtained from $0.217 m^3/kg$ of fresh cassava peel. Energy-saving calculations were considered and concluded that the amount of 1.5 tons of cassava peel obtained from 5 tons of cassava roots would be enough to produce methane providing sufficient energy for drying one ton of cassava meal. The volume of the digester needed would be 88 m^3 with a loading rate of 3.6 kg $VS/m^3/day$.

Vicenta *et al.* (1984) reported some factors affecting biogas production of fresh and dried pineapple peelings. Dried samples gave 3 times higher yields of gas than fresh pineapple peelings. The addition of urea or chicken manure did not enhance the production of biogas. Finally, they explained that a low pH value deactivated the methane microorganisms. The successful operation of a biogas digester depended on the correct equilibrium between the acid-forming bacteria and methanogenic bacteria. The maximum microbial activity normally occurred at pH ranges of 6.8-7.2.

Tanticharoen *et al.* (1984) investigated the biogas production from solid pineapple waste. The production was carried out using 4-30-L vessels without mixing, 200-L plug flow reactor, and 5 m^3 stirred tank. Their results indicated that pineapple waste could be used for biogas production but the plug flow reactor might not be suitable. The loading rate was as low as 2.5 g of dry solid added/L/day because of the high acidity of the substrate. The average gas yield of 0.3-0.5 L/g of dry substrate was obtained from all feasible bioreactors. The pretreatment of solid waste with sludge effluent prior to loading the digester resulted in the stability of the digester than without pretreatment.

The anaerobic digestion of solid waste from chinese cabbage (*Brassica junceavar*) could generated 48.35 mL of biogas/g of chinese cabbage by batch operation at 50 g-loading rate. When the experiment was done on continuous basis, the gas production decreased to 25.38 mL biogas/g of waste. The methane content was 30-40%. The pretreatment of raw material by drying at 70°C for 2 hours provided better yield than the fresh material (Chomchat *et al*., 1984).

Kunawanakit (1986) reported the biogas production from anaerobic digestion of water hyacinth at three different hydraulic retention time (10, 15 and 25 days) with organic loading rates of 0.5 and 0.8 kg of total volatile solids (TVS)/m^3/day. The total of methane gas volume yields was in the range of 0.15-0.33 L/g VS destroyed. The organic loading rates of 0.8 kg TVS/m^3/day and 0.5 kg TVS/m^3/day confered on maximum values of 0.33 and 0.27 L of methane gas/g VS destroyed at 15 days of HRT.

The anaerobic digestion of fruit and vegetable wastes could generated 0.5-0.6 m^3 of biogas/kg VS added at 16 days of HRT with a methane content of 51-53% (Viswanath *et al.,* 1992).

Bardiya *et al.* (1996) investigated the biogas production from banana peel by different HRT and pretreatment methods (chopping and grinding). The gas production rate from chopped banana peel with 25 days of HRT was higher than other treatments.

Kalia *et al.* (2000) studied the potential production of biogas from banana stem wastes using a two-stage anaerobic digestion at mesophilic temperature (37°C) and thermophilic temperature (55°C). The mesophilic temperature gave higher amount of biogas than thermophilic temperature, but the thermophilic digestion rate was 2.4 times faster than mesophilic digestion rate.

The biogas production from rice straw by anaerobic-phased solid digester system (APS) at 35°C was also carried out (Zhang and Zhang, 1999). The biogas yield of untreated whole rice straw with 24 days of retention time was 0.38 L/g/VS fed. The pretreatment of rice straw using one or more methods, such as mechanical grinding or chopping, heating, and ammonia treatment, could increase the biogas yield. Ammonia was used as a supplemental nitrogen source for rice straw digestion. A combination of grinding (10 mm length), heating (110°C), and ammonia treatment (2%) resulted in the highest biogas yield, 0.47 L/g/VS fed, which was 17.5% higher than the biogas yield of untreated whole straw.

2.1.2 Microbiological processes for biogas production

A series of microbiological processes involves in the conversion of organic matters to biogas under anaerobic conditions (Bitton, 1994; Nebel and Wright, 2000). The same reactions can occur in nature, rumens, soils and streams, and in the oceans (Hobson and Wheatley, 1993). The microbiological processes for biogas production can be divided into three stages (Figure 1).

In the first stage, which is the fermentative stage, organic materials (protein, cellulose, lipid, starch) are broken down by external enzymes produced by fermentative microorganisms to lower molecular weight molecules (Bitton, 1994; Milono *et al.,* 1981).

The second stage is the acid-forming stage. In this stage, products from the first stage are converted by acetogenic bacteria (acetate and H_2-producing bacteria) into acetate, hydrogen gas, carbon dioxide, and a few other VFA such as propionic and butyric acids (Aiman *et al.,* 1981; Milono *et al.,* 1981).

The third stage is the methanogenic stage. The methanogenic bacteria or methane-forming bacteria take over the job of the acid-forming bacteria to what extent the methane producer can utilize the end products of the acid-forming bacteria. Products of the methanogenic stage are methane, carbon dioxide, trace gases (e.g., H_2S), and water. It is almost certain that 70% of methane is formed from acetate, and the rest is formed from carbon dioxide and hydrogen (Aiman *et al.,* 1981; Bitton, 1994; Milono *et al.,* 1981).

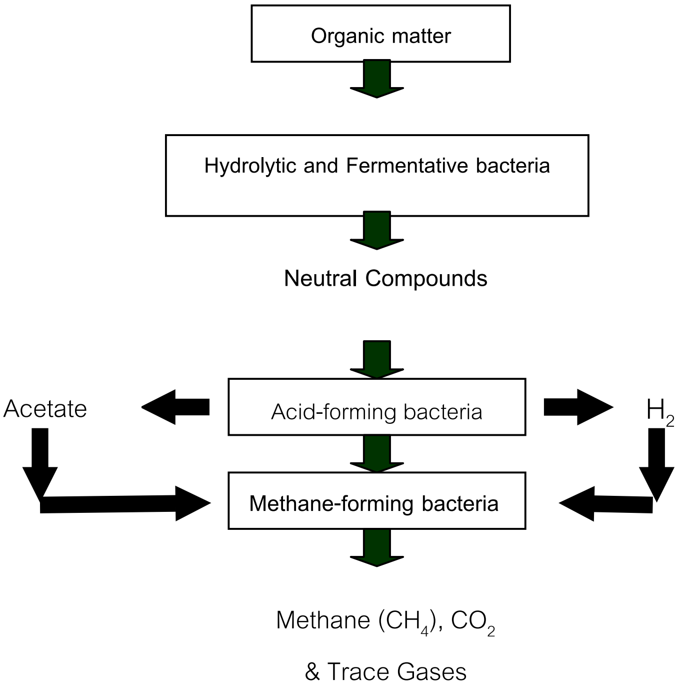


Figure 1. Partway in anaerobic digestion of complex wastes.

Source: Bitton (1994).

Microorganisms in the anaerobic digestion are separated into 3 groups. The first group is responsible for hydrolyzing organic materials to lower molecular weight molecules. The second group of anaerobic bacteria ferments the breakdown products to H_2, CO_2, and simple organic acids. This group of microorganisms, described as non-methanogenic bacteria, consists of facultative and obligate anaerobic bacteria. Collectively, these microorganisms are often identified in the literature as acid-forming bacteria. Among non-methanogenic bacteria that have been isolated from anaerobic digesters are *Clostridium* spp., *Peptococcus anaerobus, Bifidobacterium* spp., *Desulphovibrio* spp., *Corynebacterium* spp., *Lactobacillus, Actinomyces, Staphylococcus, and Escherichia coli* (Higgins and Burns, 1975; Holland *et al.*, 1987). The third group of microorganisms converts H_2, CO_2, and organic acids especially acetic acid formed by acid-forming bacteria to CH_4 and CO_2. Third bacterial group is called methanogenic bacteria. Non-methanogenic bacteria can grow in a wide range of pH, and their growth rates are higher than methanogenic bacteria. Methanogenic bacteria or methanogens are the important group of bacteria to produce methane in the biogas production process.

Taxonomic level higher than kingdom was proposed, with the methanogens classified into the urkingdom *Archaeobacteria* (Archae) (Woese *et al.*, 1990), and subdivided into two subcategories (Bitton, 1994) as follows:

1) *Hydrogenotrophic methanogens* converting H_2 and CO_2 to CH_4

$$CO_2 + 4H_2 \longrightarrow CH_4 + 2H_2O$$

2) *Acetotrophic methanogens* converting acetate to CH_4 and CO_2

$$CH_3COOH \longrightarrow CH_4 + CO_2$$

Methanogenic bacteria are also grouped in five orders; *Methanobacteriales, Methanococcales, Methanomicrobiales, Methanosarcinales,* and *Methanopyrales* (Boone *et al.*, 1993; Bitton, 1994). The bacteria are highly sensitive to oxygen, even traces of oxygen (Milono *et al.*, 1981), and they are much slower growing and more sensitive to environmental changes than non-methanogenic bacteria (Bunchueydee, 1984).

2.1.3 Digester systems for biogas production

The biogas production can be operated in either batch, semi-continuous, or continuous fermentation system (George and Franklin, 1991; University of Florida, 2002). Organic matters are filled in the reactor at one time, and allowed digesting in batch fermentation system. Organic matters are refilled in reactor again when reactor emptied. Efficiency of batch fermentation is low, and the rate of gas production is variable. In semi-continuous fermentation, organic matters are regularly added to the reactor in some stages. The gas production is rather constant in the semi-continuous process. In the process of continuous fermentation, organic matters are fed into the reactor continuously. This process could be more efficient for the production of biogas than batch and semi-continuous fermentation.

Fermentation systems for the biogas production are performed in anaerobic digesters, which can be categorized as follows:

2.1.3.1 Single-state digester

Two types of single-state digester are identified as standard-rate and high-rate digestion processes. In the standard-rate digestion process, the contents of the digester are usually unheated and unmixed. In the high-rate digestion process, the contents of the digester are heated and mixed completely (George and Franklin, 1991). Sludge is digested in a fermentation tank and settling occurs simultaneously in the tank. Non-methanogenic and methanogenic bacteria grow in the same digester. Sludge stratifies and forms the following layers from the bottom to the top of the tank: digested sludge, actively digesting sludge, supernatant, a scum layer, and gas (Figure 2) (Bitton, 1994).

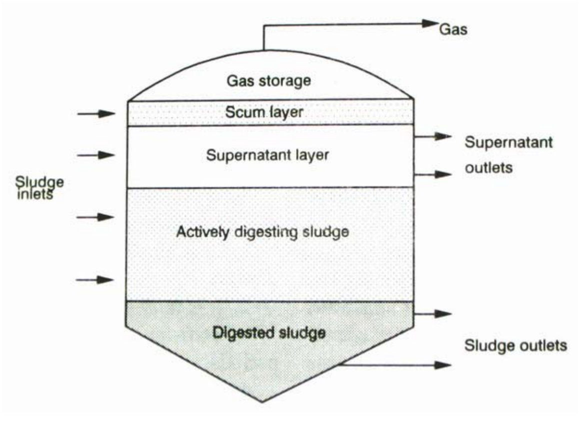


Figure 2. Conventional single-state anaerobic digester.

Source: Bitton (1994).

2.1.3.2 Two- stage digester

The two-stage digester is developed from the single-state digester. The processes composed of two linked digesters (Figure 3) (George and Franklin, 1991), one tank continuously mixed and heated for sludge stabilization and the second tank for thickening and storage prior to withdrawal and ultimate disposal (Bitton, 1994). The running of the digesters is arranged so that the initial hydrolysis and fermentative steps in digestion can take place in the first tank and the products from the first tank (acidogenic reactor) are converted to methane by methanogenic bacteria in the second tank (methanogenic reactor) (Hobson and Wheatly, 1993). The efficiency of two-stage digester is higher than single-stage digester because the environmental conditions can be proper controlled for non-methanogenic and methanogenic bacteria. Two-stage digester is suitable for biogas production from acid and solid materials (Carbone *et al.,* 2002; Punyawattoe, 1986).

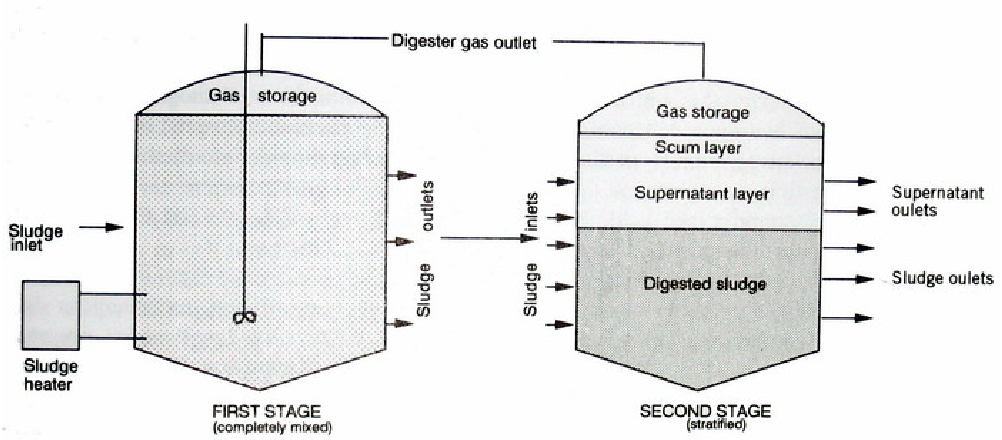


Figure 3. Two-state anaerobic digester.

Source: Bitton (1994).

2.1.4 Types of digesters used for biogas production

2.1.4.1 Fixed dome digester

The fixed dome digester is the familiar digester type in developing countries (Figure 4). It is a closed, dome-shaped digester with an immovable, rigid gas-holder, and a displacement pit, also name compensation tank. The digester is usually constructed underground to protect it from physical damage and to save area. The pressure of gas increases with the volume of gas stored. The fixed dome digester is abundantly found in China for biogas production from agriculture residues, which can produce biogas of 0.1-0.2 v/v/day with 60 days of retention time at 25°C. The construction and operation cost of fixed dome digester is low (Stuckey, 1984).

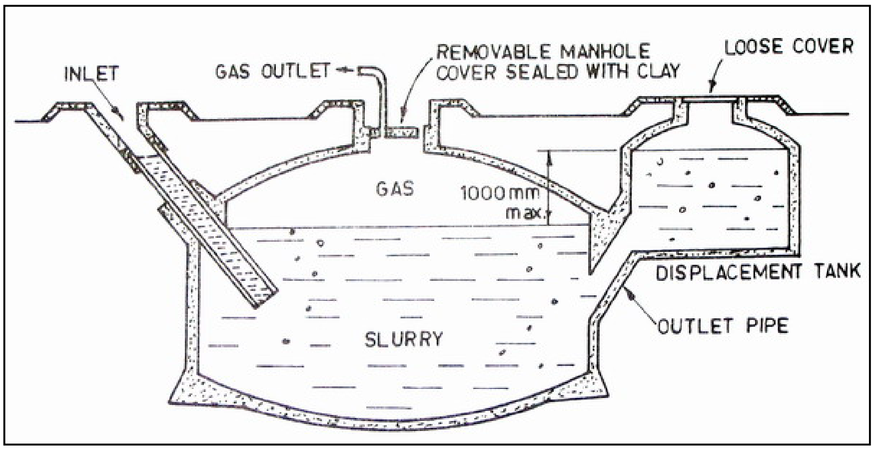


Figure 4. Fixed dome digester.

Source: Stuckey (1984).

2.1.4.2 Floating cover digester

In the past, the floating cover digester could be found mainly in India for digesting animal and human feces (Moulik and Vyas, 1984). The digester consists of a cylindrical or dome-shaped digester, and floating gas-holder (Figure 5), and has been applied in small to middle size farms and larger agro-industry. The digester is easy to operate, and it provides gas at a constant pressure. The retention times vary from 30 days in warm season to 50 days in cold season. The biogas production yields of 0.2 to 0.3 v/v with cattle manure at 9% TS have been reported (Stuckey, 1984).

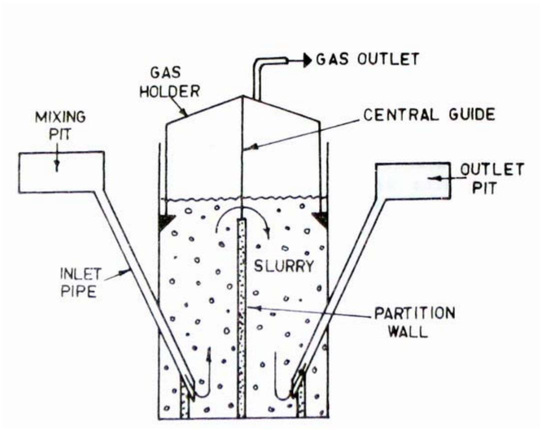


Figure 5. Floating cover digester.

Source: Stuckey (1984).

2.1.4.3 Bag digester

The bag digester is a long cylinder comprising either PVC or a neoprene-coated fabric (nylon) (Figure 6). The bag is packed in a trench that measures approximately half the diameter of the bag (Stuckey, 1984). The gas produced is stored in the digester under the flexible membrane, as well as in a separate gas bag (Park and Park, 1981). In Korea, biogas could be produced at the amounts varying from 0.14 in winter (8°C) to 0.7 v/v/day in summer (32°C) for pig manure (Park and Park, 1981).

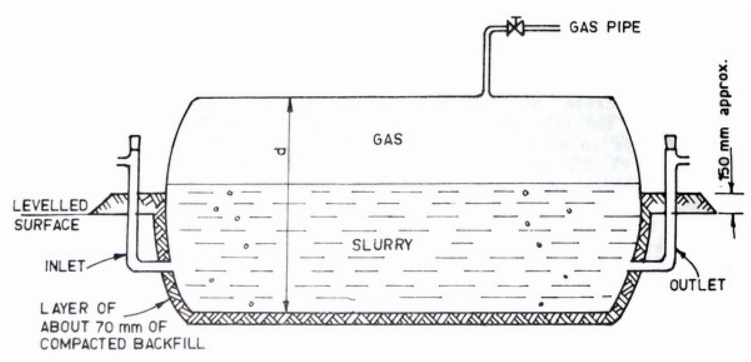


Figure 6. Bag digester.

Source: Stuckey (1984).

2.1.4.4 The plug flow digester

The plug flow digester is similar to the bag digester but it is usually constructed in the ground with membrane cover on the top portion (Figure 7). The feed concentration in plug flow digester can be higher than fixed dome (Stuckey, 1984). Hayes *et al.* (1979) reported the application of cattle manure at 12.9% TS as a raw material for biogas production. The rate of gas was 1.26 v/v at 30 days of retention time at 35°C. This design has considerable potential in developing countries because of its low capital cost and relatively high biogas production rates (Stuckey, 1984).

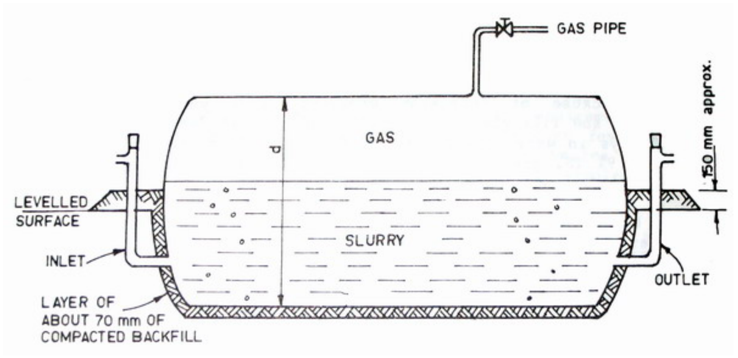


Figure 7. Plug flow digester.

Source: Stuckey (1984).

2.1.4.5 Anaerobic filter

The anaerobic filter is one of the earliest types of anaerobic digesters (Figure 8). It was introduced at the beginning of the century, and developed in 1969 (Bitton, 1994). The anaerobic filter is a column filled with various types of solid media (Metcalf and Eddy, 1991). The packing materials are rock, gravel, and plastic with a void space of about 50% or more (Frostell, 1981; Jewell, 1987). Anaerobic bacteria grow and attach to the filter medium, but some flocs become trapped inside the filter medium. The upflow of wastewater through the reactor helps to retain suspended solid in the column (Bitton, 1994). The anaerobic filter is proper for wastewater rich in carbohydrates (Sahm, 1984). Waste strengths from 480 mg/L COD up to 90,000 mg/L COD can be successfully treated in the anaerobic filter (Young and McCarty, 1969). Retention times as low as 9 hours (base on void volume) are possible with the COD removal of 80%. However, more usual retention times are the order of 1-2 days with the achievement of over 90% COD removal. Loading rates as high as 7 kg COD/m^3 are possible, and the biogas production rate of 4 v/v/day could be obtained under these conditions (Xinsheng *et al*., 1980). A diluted animal manure can be treated successfully with 2.0% of TS content (Stuckey, 1984).

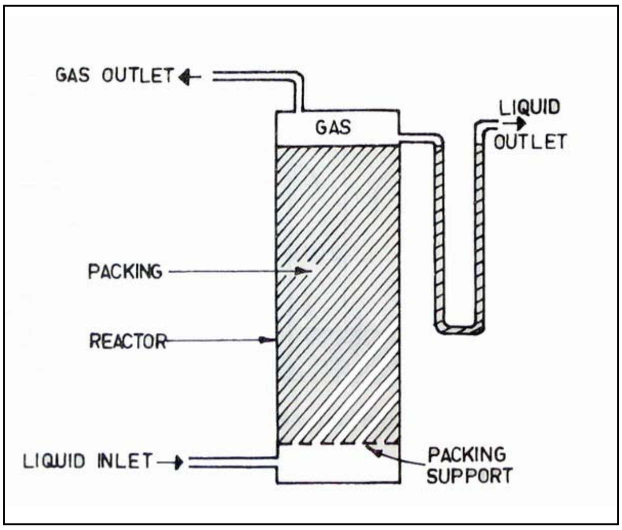


Figure 8. Anaerobic digester.

Source: Stuckey (1984).

2.1.4.6 The anaerobic baffled reactor (ABR)

The anaerobic baffled reactor (ABR) consists of a simple rectangular tank with dimensions similar to a septic tank, and divided into 5 or 6 equal-volume compartments by means of walls from the roof and bottom of the tank (Figure 9). The soluble waste flows upward and downward between the walls, and on its upward passage the soluble waste flows through an anaerobic sludge blanket, of which there are five or six. So, the soluble waste in the tank contacts with the active bacteria (Stuckey, 1984). Bachmann *et al.* (1982) treated the soluble waste containing 7.1 g/L of COD in the ABR with 1 day of retention time at 36°C. Eighty percent removal efficiency of COD could be achieved. The volume of biogas production was 2.9%. The ABR can be applied to treat waste with high solid contents (Stuckey, 1984).

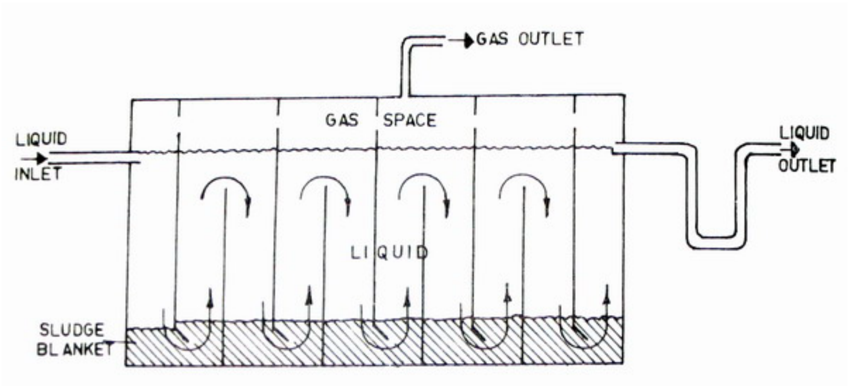


Figure 9. Anaerobic baffled reactor.

Source: Stuckey (1984).

2.1.4.7 Upflow anaerobic sludge blanket (UASB)

The Upflow anaerobic sludge blanket digester was introduced at the beginning of the century (Bitton, 1994). The digester composes of a bottom layer of packed sludge, a sludge blanket, and an upper liquid layer (Figure 10) (Lettinga *et al.*, 1980). Wastewater flows from the bottom to the top, through a sludge bed that is covered by a floating blanket of active bacteria flocs. Active bacteria are required for the UASB digester in the form of a high-density granular sludge. Settle screens separate the sludge flocs from the treated water, and gas is collected at the top of the reactor (Schink, 1988). Problems of operating the UASB digesters are in the start-up and maintenance of the flocculated granules (Hobson and Wheatly, 1993). The advantages of UASB in biogas production are the small size of reactor, high loading rates, high gas yields, and minimal effect of temperature (Stuckey, 1984).

Gavala *et al*. (1999) investigated the wastewater treatment using the UASB. Wastewater from cheese-producing industries in Greece was high in organic matter (about 40-60 g/L COD) and had a relatively low content in suspended solid (1-5 g/L) was treated by UASB. A upflow anaerobic sludge blanket reactor of 10-L working volume was constructed and inoculated with anaerobic mixed liquor from daily wastewater and glucose fed digesters. The operation was performed at an organic loading rate of 6.2 g of COD/L/day. The methane content in biogas obtained was 68-74%.

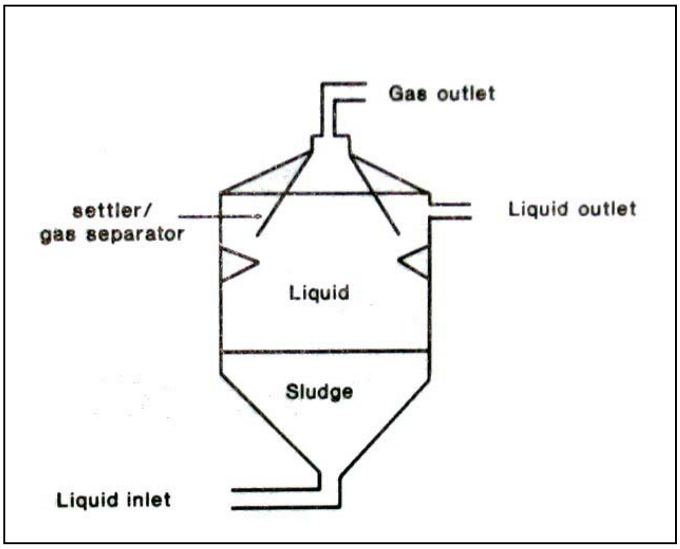


Figure 10. Upflow anaerobic sludge blanket.

Source: Bitton (1994).

2.1.5 Factors affecting biogas production

There are several factors affecting the biogas production. These factors are both chemical factors (organic nutrient concentration, pH, alkalinity (A), VFA, and toxic substances) and physical factors (mixing, temperature, and digestion time) (Bunchueydee, 1984; Kunawanakit, 1986).

2.1.5.1 Chemical factors

A. Nutrients

Organic matters, which are broken down by microorganisms without oxygen, will produce significant quantities of methane. All biological system requires sufficient supply of nutrients particularly carbon and nitrogen as well as other elements are also required in trace quantities. Animal manure contains large quantities of well-balanced nutrient supply, but crop residues and some food processing wastes may lack some of the nutritional requirements (Vicentra *et al*., 1984; Viswanath *et at*., 1992: Bardiya *et al*., 1996; Zhang and Zhang, 1999). The lack of specific elements required for microbial growth will limit the production of biogas. Organic matters are nutrients for microorganisms in the biogas production process. Nutrients are assigned by the ratio of carbon and nitrogen (approximate 20-30:1) (Polprasert, 1989; Sanders and Bloodgood, 1965). Furthermore, other elements such as iron, sodium, potassium, calcium, and magnesium, can stimulate the production of biogas at low concentrations of these elements and inhibit at high concentrations (McCarty, 1964b).

B. pH

Basicity or acidity of the fermenting slurry is indicated by pH. Most methanogenic bacteria have pH optima near neutrality (Jones *et al*., 1987). The optimum pH for the biogas production is in the range of 6.8-7.2 with the limitation of the range for operation without significant inhibition being 6.5 to 7.6 (Anglo *et al.,* 1978; Bunchueydee, 1984; Haga *et al*., 1979). Methanogenic bacteria could occasionally grow at pH ranges which defined as 6.5-8.2 (Buyukkamaci and Filibeli, 2004).

C. Alkalinity and volatile fatty acids

Alkalinity is a measure of the amount of carbonate in slurry that is expressed in mg per L of equivalent calcium carbonate (mg as $CaCO_3$/L). It is important because as acid is added to slurry, carbonates will contribute hydroxide ions that tend to neutralize the acid. This is known as the buffering effect of alkalinity (มั่นสิน ตัณฑุล

เวศน์, 2542; University of Florida, 2002). The alkalinity concentrations of 2500-5000 mg as $CaCO_3$/L have been reported to be suitable for biogas production from animal manure (Graef and Andrews, 1974). The monitoring of VFA concentrations is very important for the operation performance of an anaerobic digester. Volatile fatty acids are expressed in mg per L of equivalent acetate (mg as acetate/L). For a normal anaerobic fermentation process, the concentration of VFA in term of acetate should not exceed 2000-3000 mg/L (Buswell and Mueller, 1952). Whereas McCarty and McKinney (1961) discovered that at higher VFA concentrations (10000 mg as acetate/L) can not inhibit methanogenic bacteria whenever the digesters have adequate buffering capacity. The VFA-to-alkalinity ratio should not exceed 0.8. If the ratio of VFA-to-alkalinity exceeds 0.8, pH depression and inhibition of methane production occur and the process is failing (Water Pollution Control Federation, 1987). Increases in VFA-to-alkalinity above 0.3-0.4 indicate upset and the need for corrective action of the fermentation process. A proper ratio for VFA-to-alkalinity is between 0.1 and 0.2 (Water Pollution Control Federation, 1987).

D. Toxicants

Toxicants have affected the production of biogas if they are at high concentrations (Graef and Andrew, 1974; Bunchueydee, 1984). The toxicants include the alkalinity and alkaline cations (sodium, potassium, calcium, and magnesium), heavy metals (copper, nickel, zinc, ammonia and ammonium ion), sulfide, some organic compounds, oxygen (O_2), cyanide, tannin, salinity, benzene-ring compounds, long-chain fatty acids, and volatile acids (Bitton, 1994; Bunchueydee, 1984).

2.1.5.1 Physical factors

A. Temperature and digestion time

A delicately balanced population of various microorganisms carries out the anaerobic digestion process. These microorganisms can be very sensitive to changes in their environment. Temperature is an important operation factor in the anaerobic digestion process. Methane production has been documented under a wide range of temperatures ranging between 0 and 97°C (Bitton, 1994). Two optimum temperature levels have been established the mesophilic level (25-40°C) and the thermophilic level (50-65°C) (Bitton, 1994; Busby *et al*., 1977; Mackie and Bryant, 1995). The best anaerobic digestion is obtained when operating at temperatures near 35°C for mesophilic digester (McCarty, 1964b). Methanogenic bacteria are very sensitive to temperature changes, and slower growth as compared with acid-forming bacteria (Bitton, 1994). As the temperature falls, microbial activity decreases and the biogas production decreases. As the temperature increases some microorganisms begin to die, once again the production of biogas decreases. Thermophilic digestion requires energy for maintaining a temperature in the biogas production. Time required for sludge digestion or stabilization depends on the temperature of digestion (Bunchueydee, 1984). Ranges of time of mesophilic and thermophilic digesters vary from 25 to 30 days but can be shorter (Sterritt and Lester, 1988).

B. Mixing

Mixing is considered as an important factor in the anaerobic digester. It can help the contact of substrate to microorganisms, and increases the system efficiency. Methods of mixing of liquid in the digester are recycling of sludge by pump, pumping of compressed gas to the bottom of digester, mechanical mixing, and pumping draft tube (Kunawanakit, 1986).

2.1.6 Biogas utilization

Methane is the main component of biogas. Pure methane gas has no color, no odor, and provides heat energy. Physical and chemical characteristics of methane are shown in Table 1. One cubic metre of biogas equals to 0.46 kg of liquefied petroleum gas (LPG), 0.67 L of benzene, 0.60 L of diesel fuel, or 1.50 kg of fire wood (Energy Policy and Planning Office, 2002). The calorific values of biogas, coal gas, pure methane, propane, and butane are 20.0-26.0, 16.7-18.5, 33.2-39.6, 38.9-81.4, and 107.3- 125.8 J/cm^3, respectively (Visuthirungsriurai, 1983).

Biogas has been used for cooking, lighting, and generation of electricity for household as a substitute for gasoline and diesel fuel in several countries such as China, India, Thailand, Philippine, Sri Lanka, Egypt, and Germany (Bhumiratana *et al.*, 1984; Mahin, 1984; Stuckey, 1984). Sludge from biogas production is utilized as organic fertilizers replace chemical fertilizers such as urea. This sludge protects physical properties of soil. Pathogens and weeds can be destroyed during the fermentation (Office of Energy Efficiency and Renewable Energy, 2003).

In Thailand, biogas has been used as an energy source in rural areas (Bunchueydee, 1984). Most of biogas production is in small scale. There were about 5000 family-size digesters for generating biogas for cooking in 1984 (Chantovorapap, 1984). In China, there were 4 million biogas digesters installed in rural areas in 1984. The reactors could operate for 7 to 8 months in a year. About 1.5 billion m^3 of biogas are produced annually in China (Smil, 1984).

Table 1. Physical and chemical characteristics of methane.

Parameters	Characteristics
Chemical formula	CH_4
Molecular weight	16.042
Boiling point (760 mm)	-161.49°C
Freezing point (760 mm)	-182.48°C
Critical pressure	47.363 kg/cm^2
Critical temperature	-82.50°C
Specific gravity	
-liquid (-164°C)	0.415
-gas (25°C; 760 mm)	0.000658
-specific volume (15.5°C; 760mm)	1.47 L/g
Calorific value (15.5°C; 760mm)	38130.71 kJ/m^3
Air required for combustion	9.53
Flammability limits	5-15% by volume
Octane rating	130.00
Ignition temperature	650.00°C
Combustion equation	$CH_4+2O_2 \rightarrow CO_2+2H_2O$
O_2/CH_4 for complete combustion	3.98 by weigh
O_2/CH_4 for complete combustion	2.00 by volume
CO_2/CH_4 from complete combustion	2.74 by weigh
CO_2/CH_4 from complete combustion	1.00 by volume

Source: Kunawanakit (1986).

Industry and agriculture wastes are currently used in biogas production in large scale. For example, energy from methane gas is used to operate an internal combustion engine for mechanical and electric power. The large scale may be able to sell the gas to natural gas companies or power station (Office of Energy Efficiency and Renewable Energy, 2003). In Thailand, biogas production from pig manure has been operated in the large scale. The total volumes of biogas digester are 10,000 m^3 for 60,000 pigs in 1996-1997 and 40,000 m^3 for 240,000 pigs in 1998-2002. In 2006, the total volume of biogas digester could be constructed of 0.21 million m^3 for 1.3 million pigs; biogas could be produced as 39 million m^3/year that compared with 17.5

kg/year of LPG and produced electric power of 46.8 million kWh/year (Energy Policy and Planning Office, 2003).

2.1.7 Economic analysis

The economic analysis is conducted to determine the feasibility of various sizes of biogas plant. The return back period, the local climate, type of organic material, and production cost are considered for biogas plant construction (Bunchueydee, 1984; Office of Energy Efficiency and Renewable Energy, 2003). The general curves of capital cost, operation and maintenance cost of various biological treatments and anaerobic biogas treatment techniques are used for economic analysis in several biogas plant constructions (Figures 11A and 11B), respectively. The annual cost of various biological treatments and anaerobic biogas treatment techniques has been proposed (Figure 11C) (Bunchueydee, 1984). The benefit from biogas production and annual cost of anaerobic biogas treatment technique will be compared for economic analysis of minimum volume of hydraulic load of anaerobic biogas treatment technique.

An example of the economic analysis of biogas production is the gas production from tapioca wastewater using contact anaerobic process in Thailand (Bunchueydee, 1984). The minimum size of a contact anaerobic system was 2,500 m^3 for the return back period. However, the size of digester for 12-year return back period was 5,000 m^3 of digester. The comparison of production cost between fuel oil and biogas produced from 50 m^3 of biogas plant was calculated at various interest rates. The results showed that biogas production from tapioca wastewater was not competitive with fuel oil. The return back period of biogas production can not be achieved within 30 years.

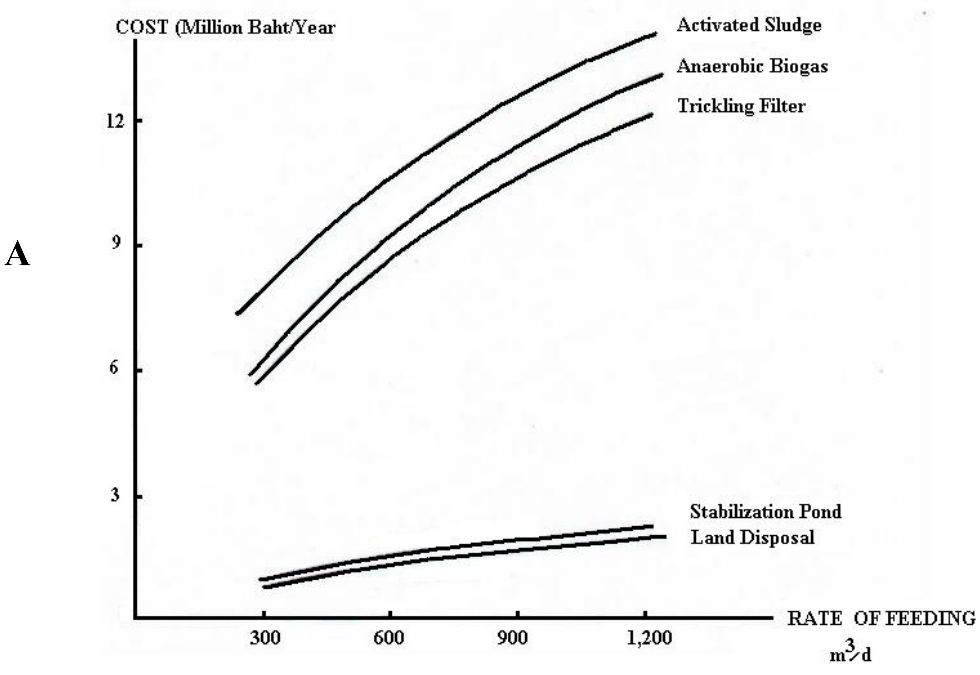


Figure 11. The cost of various biological treatments and anaerobic biogas treatment technique (A) the capital cost, (B) the operation and maintenance cost, and (C) the annual cost

Source: Bunchueydee (1984).

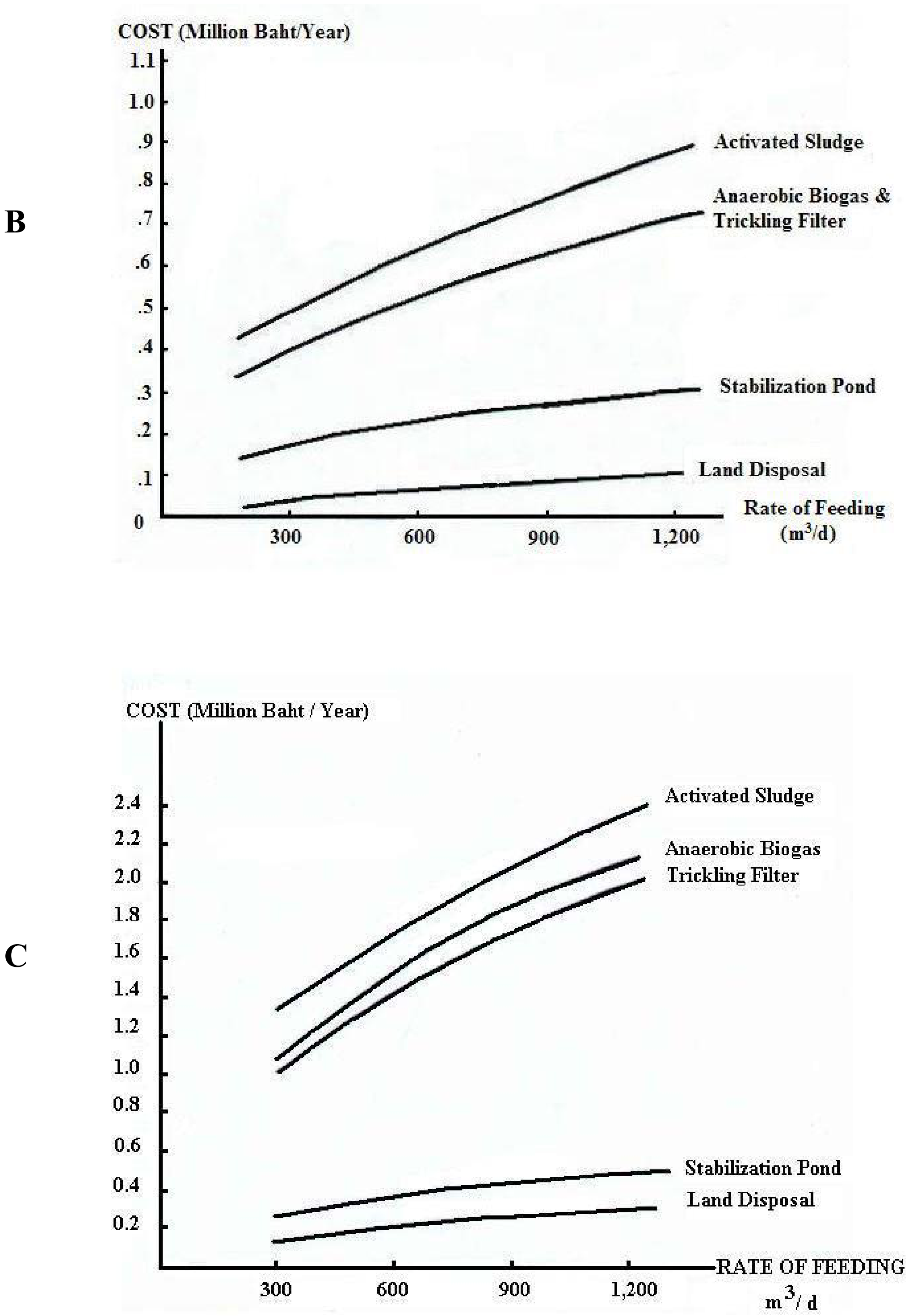


Figure 11. (Continued)

2.2 Cassava tubers

Cassava *(Manihot esculenta* Cranz*)* has been considered as an important source of food for a large population in tropical countries (Pandey *et al*., 2000). Cassava tubers are the underground part of the cassava plant responsible for the plant nutrition (Soccol, 1996; Pandey *et al*., 2000). The fresh weight of each tuber may vary between a few hundred grams and 5 kg (Pandey *et al*., 2000).

Physical and chemical compositions of cassava tubers are presented in Table 2. Fresh cassava tubers have the average of 20-30% of starch (Soccol, 1996).

Table 2. Physical and chemical compositions of cassava tubers.

Composition (100g)	Fresh weight	**Dry weight**
Calories (g)	135.00	335.00
Moisture (%)	65.50	15.70
Protein (g)	1.00	1.40
Lipid (g)	0.20	0.50
Starch (g)	32.40	80.60
Fiber (g)	1.10	1.20
Ash (g)	0.90	1.80
Calcium (mg)	26.00	96.00
Phosphorus (mg)	32.00	81.00
Iron (mg)	0.90	7.90
Sodium (mg)	2.00	ND
Potassium (mg)	394.00	ND
Vitamin B2 (mg)	0.04	0.06
Vitamin C (mg)	34.00	0.00
Niacine (mg)	0.60	0.80
Cyanide (%)	ND	1.60

ND = Not determined

Source: Pandey *et al.* (2000).

In Thailand, cassava can be grown well under rainfed and low soil fertility conditions when compared to other field crops. Cassava is frequently planted in the early rainy season between March and June, and the late rainy season between October and November. It is usually harvested within 8-12 months after planting (Limsila and Limsila, 2002). In 2001, there were 18.396 million tons of cassava tubers in Thailand (Table 3) (Office of Agricultural Economics, 2003). The Northeast region, particularly Nakhon Ratchasima Province, is the major planting area, which is about 57% of the total planting areas (Limsila and Limsila, 2002). Plantation areas and cassava productions in Nakhon Ratchasima Province in 2002 were 1,320,722 rai and 3.796432 million tons, respectively (Office of Agricultural Economics, 2003). The other planting areas are found in the East region about 22%, Central and the West region about 20% and the North region about 1% of the total plantation areas (Limsila and Limsila, 2002). Cassava tubers are an abundant and cheap agriculture product in Thailand (Table 4). The tubers are used for various applications (Figure 12).

Table 3. Cassava: harvested area, production and yield of major countries, 2000-2001.

Country	Harvested area (1000 rai)		Production(1000 tons)		Yield per rai (Kgs)	
	2000	2001	2000	2001	2000	2001
World total	106,291	105,791	174,807	139,827	1,645	1,322
Nigeria	19,594	19,594	33,854	33,854	1,728	1,728
Brazil	10,761	10,880	23,336	24,088	2,169	2,214
Thailand	7,068	6,558	19,064	18,396	2,697	2,805
Indonesia	8,500	8,500	15,351	15,800	1,806	1,859
Congo	12,188	11,890	15,959	15,436	1,309	1,298
Ghana	3,751	3,750	8,107	8,512	2,161	2,270
India	1,563	1,563	5,800	5,800	3,711	3,711
Tanzania	5,301	4,757	5,758	5,500	1,086	1,156
Mozambique	5,787	5,787	5,362	5,362	927	927
Uganda	2,388	2,388	4,966	4,966	2,080	2,080
Other	29,390	30,124	37,250	2,113	1,267	70

Source: Office of Agricultural Economics (2003).

Table 4. Cassava; area, production, farm price, and farm value, 1993-2002.

Year	Planted area (1000 rai)	Production (1000 tons)	Farm price (Bath per kg)	Farm value (million bath)
1993	9,100	20,203	0.66	13,334
1994	8,817	19,091	0.58	11,073
1995	8,093	16,217	1.15	18,650
1996	7,885	17,388	0.98	17,040
1997	7,907	18,084	0.71	12,840
1998	6,694	15,591	1.26	19,645
1999	7,200	16,507	0.91	15,021
2000	7,406	19,064	0.63	12,010
2001	6,918	18,396	0.69	12,693
2002	6,224	16,868	1.05	17,711

Source: Office of Agricultural Economics (2003).

Cassava tubers are rich in starch but poor in protein (Table 2) (Pandey *et al.*, 2000). About 60% of world cassava production is used for human food. It is consumed in natural form as flour or in fermented form. Another large consumer of cassava is the animal feed industry, using about 33% of the world production. The remaining (7%) is used by industrial such as textile, paper, food, and fermentation (Soccol, 1996).

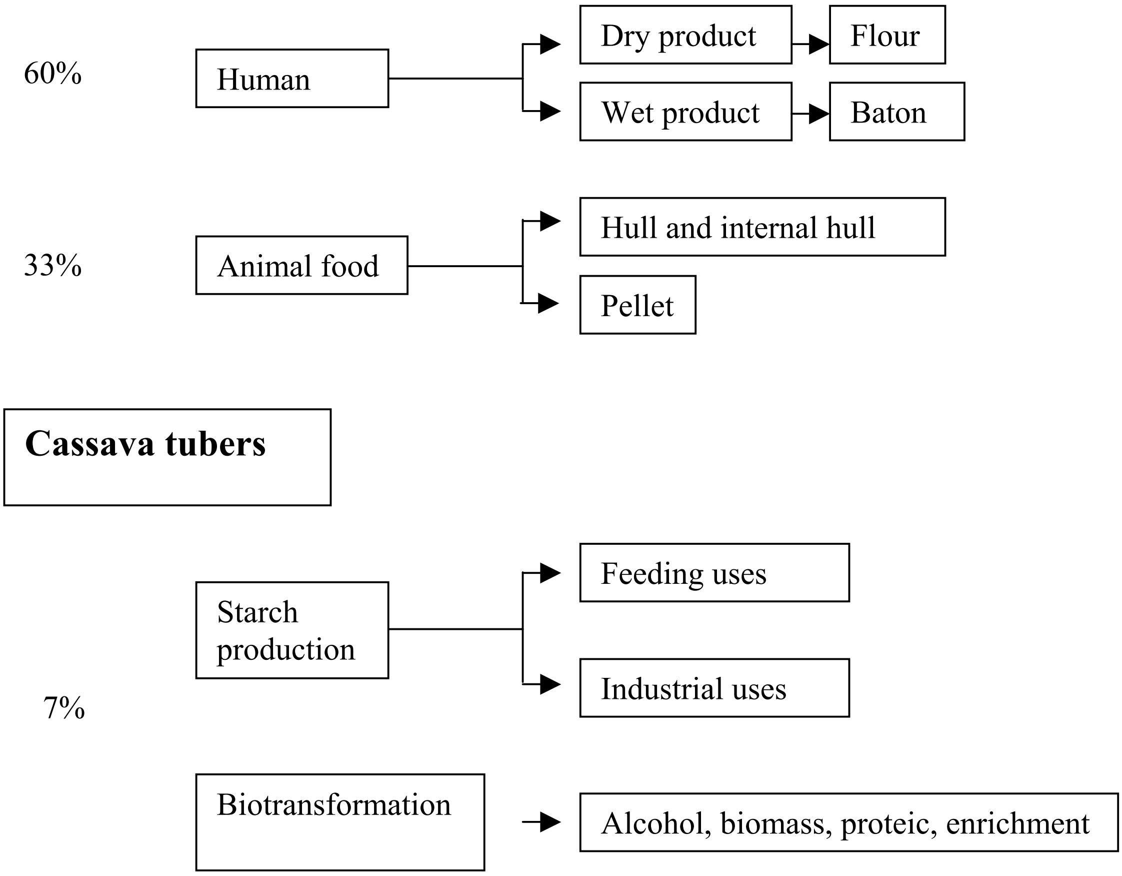


Figure 12. Utilization of cassava tubers.

Source: Soccol (1996).

CHAPTER III

MATERIALS AND METHODS

3.1 Materials

3.1.1 Chemicals

All chemicals used were laboratory grades and analytical grades, and purchased from Asia Pacific Chemicals Limited, (Ajax, Australia), Carlo Erba Reagenti, (Carlo Erba, Italy), and Sigma-Aldrich Co., (Sigma, U.S.A.).

3.2 Methods

3.2.1 Collection and preparation of a raw material for biogas production

Fresh cassava tubers, a raw material for biogas production, were collected from their plantation areas in Nakhon Ratchasima Province. The whole tubers (without peeling) were washed using clean water, air dry, and chopped into pieces (approximately 1 cm^3), then dried under sun light over the two-day period. Chopped cassava tuber was then crushed into small pieces (<0.2 cm^3) using blender (Waring Commercial, U.S.A.) and stored at 4°C until it was used for biogas production.

3.2.2 Determination of some physical and chemical compositions of fresh cassava tubers and dry cassava preparation

Some physical and chemical compositions of fresh cassava tubers and dry preparation were determined: moisture, TS, ash, VS, total carbon, total nitrogen, and phosphorus contents.

3.2.2.1 Moisture and total solids contents

Moisture and TS contents were determined using standard methods of Association of Official Analytical Chemists (1990). The empty porcelain dishes in triplicate were dried at 105°C for 1 hour or until weight becomes constant. The empty porcelain dishes were allowed to cool to room temperature in desiccator. Then, the empty porcelain dishes were weighed and recorded in the unit of g. About 10 g of cassava samples was placed in each porcelain dish. The porcelain dishes were weighed again and recorded. The dishes containing cassava samples were heated in the hot air oven at 105°C for 6 hours, then weighed until the constant weight was obtained. The different in weight of the material after drying was the moisture content of the cassava material and the weight of dry material was TS.

Calculation:

$$\text{Moisture content (\%)} = \frac{(\text{A} - \text{B}) \times 100}{\text{Sample weight, (g)}}$$

$$\text{Total solids (\%)} = 100 - \text{moisture content (\%)}$$

Where:

A = Sample weight before heating and porcelain dish weight (g),

B = Sample weight after heat and porcelain dish weight (g), and

C = Initial sample weight (g).

3.2.2.2 Ash and volatile solids

Ash and VS of cassava tubers were determined by standard methods of Association of Official Analytical Chemists (1990). Ten grams of dried cassava samples were placed in porcelain dishes in triplicate, weighted, and heated in muffle furnace at 550°C for 30 min. The organic solids burnt off on ignition were VS (or organic matter) which the residue contributed to the ash content.

Calculation:

$$\text{Ash (\%)} = \frac{\text{Sample weight after burnt} + \text{porcelain dish} \times 100}{\text{Sample weight}}$$

$$\text{Volatile solids (\%)} = 100 - \text{ash (\%)}$$

A = Sample weight after burning and porcelain dish weight (g) and

B = Sample weight before burning.

3.2.2.3 Total carbon and total nitrogen contents

Total carbon and total nitrogen contents were determined using the CNS-2000 Elemental Analyzer (Leco Corporation, U.S.A). Dry cassava sample (0.2 g) were put into ceramic boats, and loaded into the CNS-2000 Elemental Analyzer, where they were combusted with the pure oxygen of the furnace. Combustion gases were collected in 4.5-L ballast after being pulled through anhydrone to scrub out water. Individual Infrared (IR) cell detected carbon and a thermal conductivity cell detected nitrogen. Results of the analysis were reported as % of C and % of N using computer software (Leco Corporation, 2004).

3.2.2.4 Phosphorus content

The spectrophotometric Molydate-Vanadate method was used for the phosphorus analysis (Association of Official Analytical Chemists, 1990). Dry cassava sample (0.5 g) were transferred to 75 mL of a digestion tube, then six mL of mixed acid (Appendix A 1.1) were added. The mixture was digested in the fume hood at 200°C until solution was cleared. The digested slurry was cooled to room temperature, and adjusted volume to 50 mL with distilled water, then filtered through filter paper (Whatman No. 1, England). One mL of aliquot sample was transferred to 10-mL test tube. Two mL of HNO_3 2 N was dropped into 1 mL of aliquot sample. Then fine 5 mL of distilled water and 1 mL of molybdate-Vanadate reagent (Appendix A 1.2) were added. After that the volume of slurry was adjusted to 10 mL using distilled water. The mixture was well mixed and left for 20 min. Then the absorbence was spectrophotometrically determined at the wavelength of 420 nm. The standard curve of phosphorus was prepared by monobasic potassium phosphorus (KH_2PO_4) (Appendix A 1.4). The phosphorus content was obtained by calculation comparing to the standard curve of phosphorus (Figure 2B in Appendix B).

Calculation: From standard curve

$$\text{Phosphorus (\%)} = \frac{\text{A} \times \text{B} \times 50 \times 100}{10^6 \times \text{sample weight, (g)} \times \text{aliquot volume, (mL)}}$$

Where:

A = ppm from standard curve,

B = Final volume (mL),

C = Sample weight (g), and

D = Aliquot volume (mL).

3.2.2.5 Starch content

Starch concentration of cassava tubers was basically detected by spectrophotometry at 580 nm absorbance in the soluble form and presence of iodine (Gales, 1990; Plummer, 1971). One gram of dry cassava tuber was added into 100 mL of distilled water, and heated nearly to boiling point. Then cassava slurry was diluted with distilled water to the ratio of 1:50-1:1000 in 10 mL of the total sample. One mL of iodine solution (Appendix A 3.2) was added to the diluted cassava tuber slurry. After it was well mixed, the absorbance was spectrophotometrically determined at the wavelength of 580 nm. The standard curve of cassava starch (commercial flour) was prepared. Starch content was obtained by calculation comparing to the standard curve of starch (Figure 1B in Appendix B).

3.2.3 Microbial inoculum (seed culture) preparation for biogas production

Microbial inocula (seed cultures) were prepared by mixing animal manure (chicken dung), molasses, and liquid waste collected from the open-anaerobic pond of cassava starch production factory in Nakhon Ratchasima Province. Two and a half-kg chicken dung were mixed with 1 kg of molasses in a 50-L close container, then 25 L of water were added and incubated at room temperature for two weeks. After that the chicken dung slurry was filtered. Then, one kg/L of liquid waste from the open-anaerobic pond of cassava starch production factory was added to the chicken dung slurry to obtain the final volume of 50 L. Slurry was kept at room temperature for 3 months with manual stirring twice a day and adding 100 g of cassava starch every three days and 100 g of molasses every week. The pH of slurry was measured daily, and it was maintained greater than 6.8 using sodium hydrogen carbonate ($NaHCO_3$).

3.2.4 Biogas production from cassava tubers using single-state digesters

3.2.4.1 Substrate

Raw cassava tubers were used as a substrate for biogas production. The substrate was prepared as described in section 3.2.1, and determined some physical and chemical compositions as mentioned in section 3.2.2.

3.2.4.2 Laboratory scale digesters

Three sizes of laboratory scale digesters were used in this study in order to investigate the potential production of biogas from raw cassava tubers. Then, the digestion volumes increased to obtain the consistency and trend of increasing biogas production capacity.

A. The simple single-state digester with working volume of 5 L

Experiments were conducted in 7.50-L anaerobic digester (5-L working volume) (Figure 13). The digester was a white plastic rectangle tank (15 cm of width, 25 cm of height, and 20 cm of length). The biogas fermentation equipment composed of two parts: a digestion vessel and a gas collector. Gas produced during the digestion flowed through silicone rubber tube into 5 L-graduated gas collector (Figure 13). The volume of biogas produced in the digester was measured by the downward displacement of water. The pH of water in this gas collector was adjusted to 2 to avoid carbon dioxide dissolution (Bardiya *et al.*, 1996).

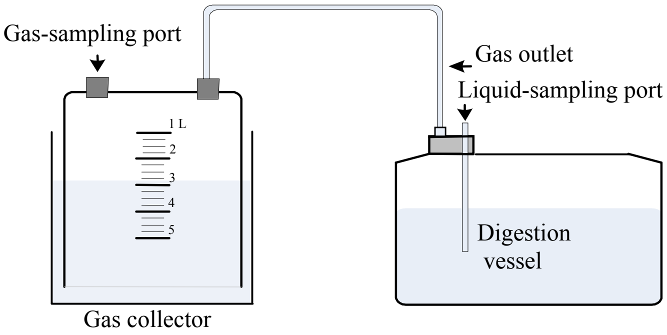


Figure 13. Single-state digester of 5-L working volume.

B. The simple single-state digester with working volume of 20 L

The digester was 26-L black plastic rectangle tank (18 cm of width, 41.5 cm of height, and 35 cm of length), with 20-L working volume (Figure 14). The biogas digestion equipment comprised of a digestion vessel and two gas collectors. The measurement of biogas produced in the digester was performed as mentioned in section 3.2.4.2A.

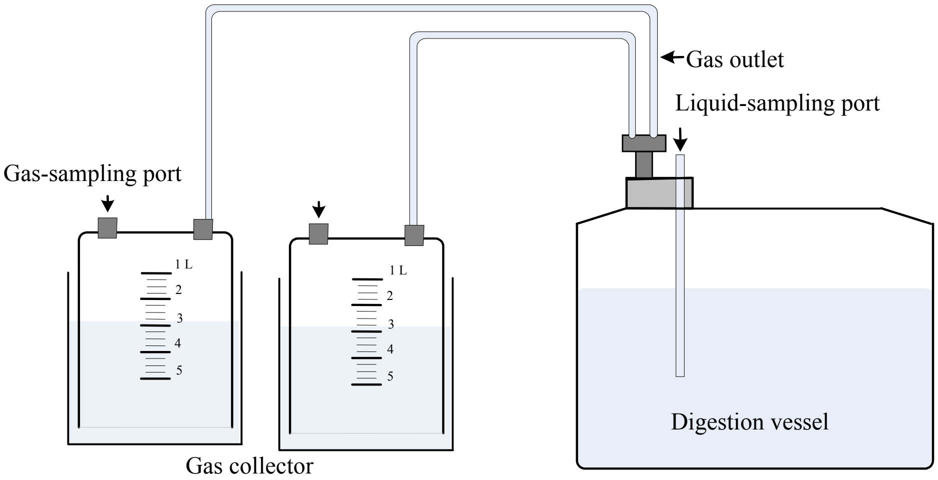


Figure 14. Single-state digester of 20-L working volume.

C. The simple single-state digester with working volume of 50 L

The anaerobic digester was a stainless steel tank having an inner diameter of 29 cm, a total height of 80 cm, and a working volume of 50 L. The digester also had the inlet for feeding substrate and outlet for gas produced and effluent (Figure 15). The volume of biogas produced was measured by the method as stated in section 3.2.3.2A.

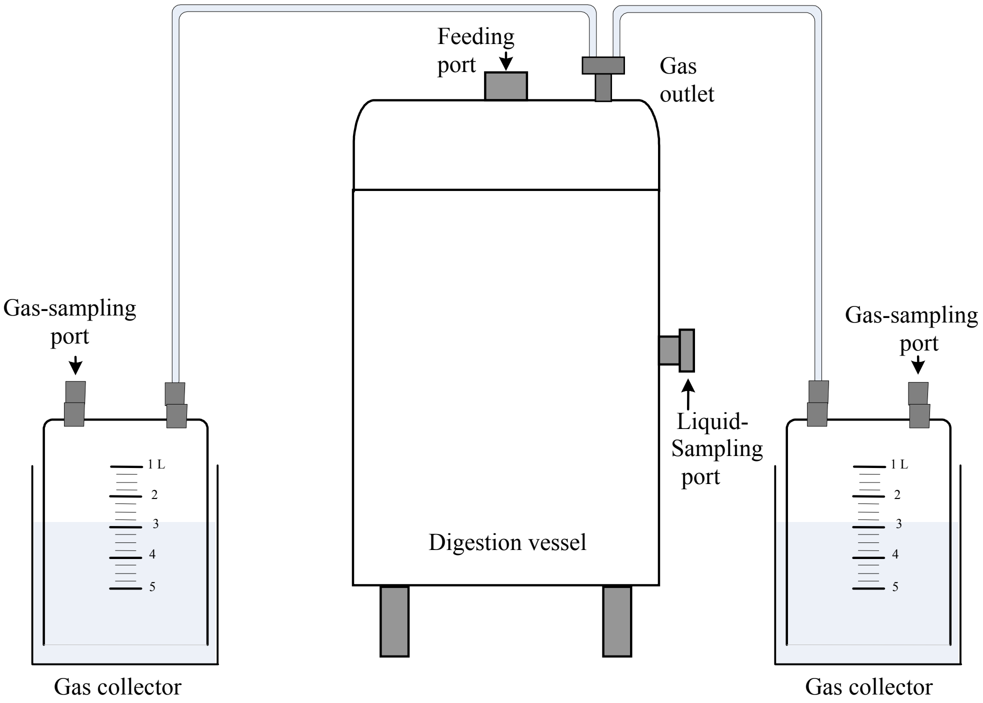


Figure 15. Single-state digester of 50-L working volume.

3.2.4.3 Biogas production using a simple single-state digester of 5-L digestion volume

Since the high carbon-to-nitrogen ratio (approximately 80:1) of cassava root (dry weight) has been reported (Soccol, 1996), the optimum ratios for the maximum biogas generation have been suggested to be 20-30:1 (Sanders and Bloodgood, 1965; Polprasert, 1989). Results of the chemical composition analysis of substrate (section 3.2.2) were applied for calculating the dry cassava tuber concentration in this experiment. The anaerobic digestion process was carried out in 7.5-L digestion tank (5-L working volume) in triplicate. The digesters were fed on a batch basis with the slurry of dry cassava tuber and 10% (v/v) of microbial inocula (seed cultures).

3.2.4.4 Optimization of some biogas production conditions

Since the amount of main nutrients (carbon and nitrogen sources) affected the growth of microorganisms and the production of biogas, the optimal concentrations of TS (carbon source) and nitrogen source added were determined. The biogas fermentation was then operated at ambient temperature for 30 days. No mechanical mixing was performed. For stabilizing pH of cassava slurry during the anaerobic digestion, the addition of $NaHCO_3$ (0.25%, w/v) was considered whenever the VFA-to-alkalinity ratio was greater than 0.8. The volume of gas produced was determined directly from the amount of water displaced by the gas in gas collector. Parameters during biogas production were determined as described in section 3.2.4.7.

A. The optimum concentration of total solids content (carbon source)

Preliminary studies were performed on dry cassava samples in term of TS in order to determine the most suitable TS content for biogas production from cassava tubers. In this study, various TS concentrations were applied to the 5-L working volume to obtain the optimum TS content. The initial dry weight of cassava tubers loaded into the digester was calculated from physical and chemical compositions of dry cassava tuber (section 3.2.2). Dry cassava tuber of 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, and 16.00% (w/v) TS were added into the digesters, respectively.

B. The optimum concentration of nitrogen source

The optimal concentration of TS from section 3.2.4.4A was applied using the single-state digester with working volume of 5 L. The optimum concentration of urea

(46% of nitrogen) as nitrogen source for the biogas production from cassava tubers at control (0.00% urea, w/v) and urea addition at carbon-to-nitrogen ratio of about 80:1, 30:1, 25:1, 20:1, 10:1, and 5:1, respectively.

3.2.4.5 Biogas production using the optimum conditions and a single-state digester of 5-L digestion volume

The digesters were loaded in triplicate on a batch basis of the optimal concentrations of both TS and nitrogen (section 3.2.4.4) with 10% (w/v) of microbial inocula (seed cultures) to produce biogas in 5-L working volume at ambient temperature for 30 days.

3.2.4.6 Biogas production using scaled-up digester of 20-L and 50-L digestion volumes

In order to obtain the amount of biogas production rates in the large scales, the digester size and digestion volume were increased. The optimal concentrations of both TS and urea addition (section 3.2.4.4) were applied to produce biogas in the scaled-up digesters, 20-L and 50-L working volumes. The digesters were fed on a batch basis with 10% of (w/v) of microbial inocula (seed cultures) at ambient temperature for 30 days. Total biogas and total methane yields were recorded and compared to different bioreactor sizes.

3.2.4.7 Analytical methods

Parameters during the production of biogas from raw cassava tubers in sections 3.2.4.4, 3.2.4.5, and 3.4.4.6 were monitored as follows:

A. Biogas yield and gas composition analysis

Biogas production was measured daily. The total biogas yields were calculated from the total biogas production of the digester divided by the total amount of TS initially fed into the digester. Gases were collected over water (Kalia *et al.,* 2000). Biogas composition was analyzed by using a gas analyzer (Shimadzu, class-GC14B, Japan) equipped with a thermal conductivity detector (TCD) and a 1-M porapak Q (80-100 mesh) column. Helium was used as a carrier gas at a flow rate of 25 mL/min. The oven, injector, and detector temperatures were 80, 120, and 120°C, respectively. Gas sample (0.1 mL) was taken from the headspace of the gas collector through the gas-sampling port with syringe. The syringe was redrawn, and the sample was injected directly into a gas analyzer where the mass of methane, carbon dioxide, and other traces gas was detected by comparing to the standard gas mixture of methane, nitrogen, and carbon dioxide.

B. Total solids and volatile solids contents

Total solids and VS contents of cassava tuber slurry before and after fermentation were determined using standard methods (American Public Health Association, 1990). Fifty mL of well-mixed slurry sample were pipetted to pre-weighed porcelain dishes. Then porcelain dishes were evaporated to dryness on drying oven at 105°C for 24 hours. The porcelain dishes were allowed to cool to room temperature in desiccator. Then, the porecelain dishes were weigh and recorded in the unit of mg. The cycle of drying, cooling, desiccating, and weighing were repeated to obtain a constant weight, or until weight change was less then 4% of previous weight or 0.5 mL, whichever was less.

Calculation:

$$\text{Total solids (mg/L)} = \frac{(\text{A} - \text{B}) \times 1000}{\text{Sample volume, (mL)}}$$

Where:

A = Weight of dried residue and porcelain dish weight (mg),

B = Weight of porcelain dish (mg), and

C = Sample volume (mL).

The residue produced from TS analysis was ignited to a constant weight in a muffle furnace at a temperature of 550°C for 2 hours. The porcelain dishes were allowed to cool to room temperature in the desiccator. Then it was removed from the descicator to weight.

Calculation:

$$\text{Volatile solids (mg/L)} = \frac{(\text{A} - \text{B}) \times 1000}{\text{Sample volume, (mL)}}$$

Where:

A = Weight of residue and porcelain dish before ignition (mg),

B = Weight of residue porcelain dish after ignition (mg), and

C = Sample volume (mL).

C. Volatile acids analysis

Volatile acids (acetate, propionic, and butyric acids) were detected using a gas analyzer (Shimadzu, class-GC14B, Japan) equipped with a flame ionization detector (FID) and DB-FFAP column. Helium was used as a carried gas at a flow rate of 40 cm/sec whereas nitrogen was used as a makeup gas at a flow rate of 30 mL/min. The oven, injector, and detector temperatures were 100, 250, and 300°C, respectively. Split ratio was 100:1. Before starting the analysis of the samples taken from the digester, calibration curves of standard acetic, propionic, and butyric acids were prepared by analyzing known concentrations of these volatile acids by the gas analyzer. For preparation of fermenting slurry, suspended solids were removed before injecting into the column to prevent any clogging in the gas chromatograph by centrifuging and filtering. Filled samples were subjected to centrifugation for 20 min at 4500 rpm, and then filtered through a membrane filter (0.45 µm), in order to obtain suspended solids-free content samples. Peaks areas were used to calculate concentrations by reference to calibration curves prepared from the standard volatile acids (Figures 3B-5B in Appendix B).

D. pH

The measurement of pH value was also performed daily using a Mettler delta 320 (Mettler-Toledo LTD, England).

E. Alkalinity and volatile fatty acids

Alkalinity and VFA were determined by the direct titration with 0.1 N of sulfuric acid (H_2SO_4) to the pH of 5.1, 4.3, and 3.5 (มั่นสิน ตัณฑุลเวศน์ และ มั่นรักษ์ ตัณฑุลเวศน์, 2545). Alkalinity was expressed in milligram per litre of equivalent calcium carbonate (mg as $CaCO_3$/L) whereas VFA was expressed in milligram per litre of equivalent acetic (mg as acetate/L) (Dugan, 2004; University of Florida, 2002). Alkalinity was measured by the equivalent concentration of 0.1 N H_2SO_4 added to slurry to lower the pH to 4.3 whereas volatile fatty acid was the amount of 0.1 N H_2SO_4 added to fermenting slurry to adjust pH of 5.1 from 3.5.

$$\text{Volatile fatty acids alkalinity (mg as CaCO}_3\text{/L)} = \frac{A \times B \times 50000}{C}$$

Calculation:

$$\text{Volatile fatty acids alkalinity (mg as acetate/L)} = \frac{A \times B \times 50000 \times 1.5}{C}$$

Where:

A = mL of H_2SO_4 titrant used to reduce pH to between 5.1 and 3.5

B = Normality of H_2SO_4, and

C = mL of sample.

Calculation: $$\text{Alkalinity (mg as CaCO}_3\text{/L)} = \frac{D \times B \times 50000}{C}$$

Where:

D = mL of H_2SO_4 titrant used to reduce pH between initial and 4.3

B = Normality of H_2SO_4, and

C = mL of sample.

Calculation: $$\text{Bicarbonate (mg as CaCO}_3\text{/L)} = \text{Alkalinity} - \text{volatile fatty acid alkalinity}$$

F. Starch concentration

Starch concentration of fermenting slurry was detected using the method which was mentioned in section 3.2.2.5. Fermenting slurry was heated in boiling water. Fermenting slurry (0.2 mL) was added to 0.8 mL of distilled water. One hundred μL of iodine solution (Appendix B 3.2) was filled to the mixture of fermenting slurry and distilled water. After it was well mixed, the absorbance was spectrophotometrically determined at the wavelength of 580 nm. Starch concentration was obtained by calculation comparing to standard curve of starch (Figure 1B in Appendix B).

G. Temperature

Temperature surrounding anaerobic digesters and temperature of slurry samples were measured daily by thermometer (Brannan, England).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Some physical and chemical compositions of cassava tubers

Four cassava tuber varieties (CMC 76, KU 50, Rayong 60, and Rayong 90) were collected from Nakhon Ratchasima Province (Table 5). Some physical and chemical compositions of cassava tubers were determined (Table 6).

Cassava tubers were rich in organic matters, and were starchy food low in protein, minerals, and vitamins, with the exception of vitamin C (Grace, 1977; Lancaster *et al.,* 1982). The starch contents of fresh and dry mass of cassava tubers were reported to be 32.40% and 80.60%, respectively, while the protein contents of fresh and dry mass of cassava tubers were 1.00% and 1.41%, respectively (Pandey *et al.,* 2000*).* Soccol (1996) stated that fresh cassava roots had 65.00% of moisture, 0.90% of ash, and 0.03% of phosphorus. Carbohydrates were known to be easily and rapidly converted via hydrolysis to simple sugars and subsequently fermented to VFA (Cohen, 1982). In Thailand, cassava plant variety KU 50 was one of the dominant varieties cultivated. The production yield was approximately 22.90% of total production yields of cassava tubers in Nakhon Ratchasima Province (Office of Agricultural Economics, 2003). KU 50 gave the high yield of 23 tons/hectare and high starch content of 23% (Limsila and Limsila, 2002).

Table 5. Cassava tubers collected from Nakhon Ratchasima Province.

Cassava variety	Plant morphology	Collection
CMC 76	1.6-2.0 metres of height Dark green mature leaf Root with white flesh, brown skin, and 20-25 centimetres of length	Fresh cassava tubers were collected from Suranaree village, Suranaree Sub-district, Muang District, Nakhon Ratchasima. About 20 kilograms of root was harvested for this experiment.
KU 50	2.0-3.0 metres of height Silver-green stem Dark violet-green mature leaf Root with white flesh, brown skin, and 20 centimetres of length	Fresh cassava tubers were collected from Bueng noi village, Phungtea Sub-district, Phatongkhum District, Nakhon Ratchasima. About 20 kilograms of root was harvested for this experiment.
Rayong 60	1.7-2.5 metres of height Light brown stem Dark green mature leaf Root with creamy flesh, light brown skin, and 20 centimetres of length	Fresh cassava tubers were collected from Suranaree village, Suranaree Sub-district, Muang District, Nakhon Ratchasima. About 20 kilograms of root was harvested for this experiment.
Rayong 90	1.6-2.0 metres of height Orange brown stem Dark green mature leaf Root with white flesh, brown skin, and 20-25 centimetres of length	Fresh cassava tubers were collected from Suranaree village, Suranaree Sub-district, Muang District, Nakhon Ratchasima. About 20 kilograms of root was harvested for this experiment.

Table 6. Physical and chemical compositions of cassava tubers collected from Nakhon Ratchasima Province.

Composition (%)		Cassava variety			
		CMC 76	KU 50	Rayong 60	Rayong 90
Moisture	Fresh weight	54.20±1.50	61.66±0.69	69.42±0.32	74.07±0.09
	Dry weight	17.96±0.40	18.65±0.41	19.00±0.88	18.88±0.22
Total solids	Fresh weight	45.80±1.50	38.34±0.69	30.58±0.32	25.93±0.09
	Dry weight	82.04±0.40	81.35±0.41	81.00±0.88	81.12±0.22
Volatile solids	Fresh weight	99.08±0.04	99.12±0.005	98.86±0.03	98.80±0.14
	Dry weight	98.09±0.02	98.05±0.004	98.20±0.04	97.94±0.05
Total carbon	Fresh weight	22.89±0.20	18.64±0.20	13.84±0.74	13.02±0.12
	Dry weight	41.00±0.20	39.56±0.20	36.67±0.74	40.72±0.12
Total nitrogen	Fresh weight	0.11±0.016	0.22±0.01	0.18±0.003	0.33±0.004
	Dry weight	0.20±0.016	0.46±0.01	0.47±0.003	1.04±0.004
Starch	Fresh weight	ND	17.96±0.11	ND	ND
	Dry weight	ND	38.10±0.11	ND	ND
Ash	Fresh weight	0.92±0.04	0.88±0.01	1.14±0.03	1.20±0.14
	Dry weight	1.91±0.02	1.95±0.004	1.80±0.04	2.06±0.05
Phosphorus	Fresh weight	0.11±0.02	0.08±0.03	0.04±0.02	0.09±0.04
	Dry weight	0.20±0.02	0.18±0.03	0.11±0.20	0.27±0.04

ND = not determined

From this study, the fresh tuber variety KU 50 has approximately 17.96% of starch, 61.66% of moisture, 0.88% of ash, and 0.08% of phosphorus. The dry starchy material of variety KU 50 contained 18.65% of moisture, 81.35% of TS, 1.95% of ash, 98.05% of VS, 39.56% of total carbon, 38.10% of starch, 0.46% of total nitrogen, and 0.18% of phosphorus. It was used to prepare slurry to feed the simple single-state digesters for the production of biogas. The average carbon-to-nitrogen ratio of cassava tuber was 86:1, which was very high ratio compared to the optimum ratios of 20-30:1 for the maximum biogas generation suggested by Sanders and Bloodgood (1965) and Polprasert (1989). In this experiment, no cyanide content of cassava tubers was measured but it was reported by Pandey *et al.* (2000) that dry cassava tubers had 1.60% of cyanide.

4.2 Biogas production from cassava tubers using single-state digesters

Dry cassava tuber of variety KU 50 (Figure 16A) was used as the substrate for biogas production. The production was performed in the simple single-state digesters with the addition of seed cultures (Figure 16B) at 10% (v/v) inoculum size. The substrate contained 18.65% of moisture content.

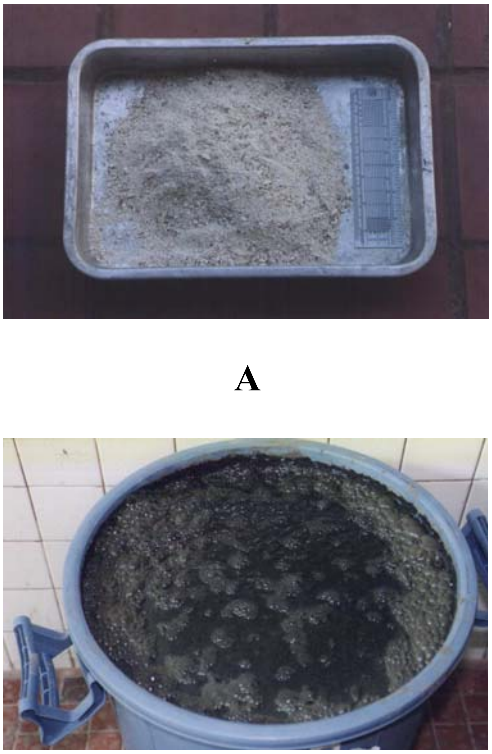


Figure 16. Dry cassava sample (A) and seed cultures (B) prepared for biogas production.

4.2.1 Biogas production using a simple single-state digester of 5-L digestion volume

Dry cassava material of variety KU 50 of 64 g was added into the digester of 5-L working volume (Figure 18) to obtain 1.00% (w/v) TS. The anaerobic digestion was performed at ambient temperature (22-26.5°C) for 30 days. It was found that the gas yield of 1.60 L/day containing the maximum methane content of 67.17% at 19-day retention time. The fermentation reactions were ceased after 25-day retention time (Figure 17). The total biogas yield and VS reduction were 355.57 L/kg TS fed and 41.88%, respectively.

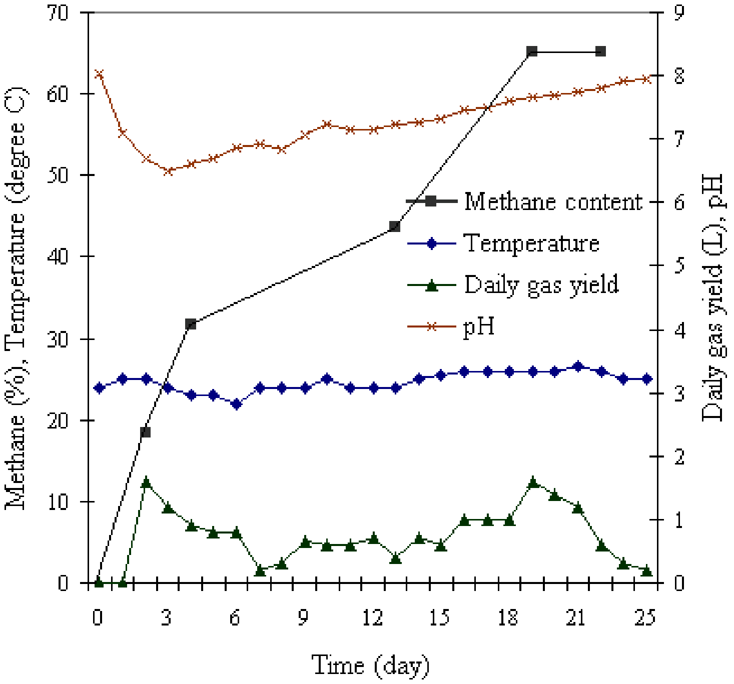
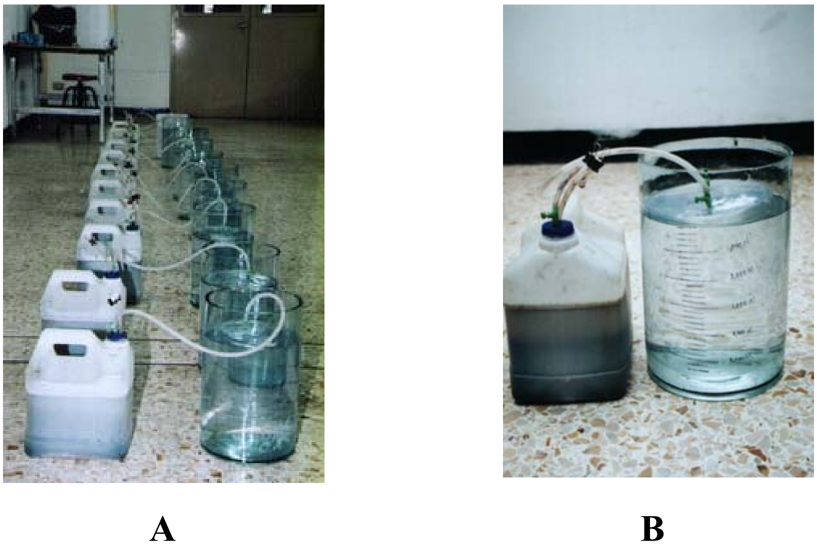


Figure 17. Biogas production from cassava tubers using 1.00% (w/v) total solids in the single-state digester of 5-L digestion volume.


A

B

Figure 18. Single-state digester of 5-L working volume.

4.2.2 Optimization of some biogas production conditions

Nutrients have been considered to be the main factor affecting microorganisms in biogas production. Like any other biological process, methanogenesis involves consortia of microorganisms that convert organic matters into methane, carbon dioxide, and traces of other gases. The overall rates of organic matter utilization and methane production depend on the extent to which the nutritional requirements of the methanogenic bacteria and non-methanogenic bacteria could be met by constituents of the organic matters and by primary or secondary metabolites produced by one species and utilized by another (Bardiya and Gaur, 1999). Main nutrients necessary for microorganisms in biogas production include carbon and nitrogen. The optimal ratio of carbon-to-nitrogen was suggested to be approximate 20-30:1 (Sanders and Bloodgood, 1965; Polprasert, 1989). Microorganisms in the anaerobic digestion commonly used carbon as an energy source for growth and nitrogen to built cell structure (University of Florida, 2002). The biogas components and biogas yields depend on a feed materials due to the difference of material characteristics in each raw material (Calzada *et al*., 1984; Cuzin *et al*., 1992; Kalia *et al*., 2000; National Research Council, 1977; Prema *et al*., 1992; Zhang and Zhang, 1999). Thus, the optimum of main nutrition factor (carbon and nitrogen) for the biogas production from cassava tubers was optimized using 5-L reaction volume.

4.2.2.1 Determination of the optimum concentration of total solids (carbon source)

The TS concentrations from 0.25 to 16.00% (w/v) TS were investigated to obtain the optimal concentration. The simple single-state digester with working volume of 5 L was performed at ambient temperature (22-30°C) for 30 days. The methane content decreased much more rapidly with each increase in TS concentration (Figure 19). A slight increase in methane production was observed when the TS concentrations decreased from about 16.00 to 1.00% (w/v). At the beginning of fermentation, the methane content obtained from 1.00 to 8.00% (w/v) TS were relatively low, and had the high carbon dioxide content. This could be the results of the occurrence of fermentative stage and acid-forming stage. Fermentative bacteria and acid-forming bacteria were predominant in these periods. The complex organic materials were hydrolyzed by fermentative bacteria to simple organic materials. Then, the acid-forming bacteria used these simple organic materials as substrates to produce volatile acids, hydrogen, and carbon dioxide. Finally, the methanogenic bacteria became established in the digester, and used end products from the acid-forming bacteria digestion to produce methane. In this stage, methanogenic bacteria were predominant. Thus, the methane content of the biogas was increased. The methane production increased with digestion time increased. The maximum of methane content obtained from 1.00, 2.00, 4.00, and 8.00% (w/v) TS, were 64.35% at 22-day, 61.24% at 31-day, 40.47% at 31-day, and 20.81% at 25-day retention times, respectively (Figures 19C-19F). While the maximum methane content obtained from 16.00% (w/v) TS was 4.22% at the first day retention time, and there was no methane in the digester after 10 days of the fermentation (Figure 19G). On the other hand, when the TS was lower than 0.50% (w/v), the methane content varied from 8.81 to 45.48% (Figure 19B). A slight increase in methane production was found when the TS concentrations increased from 0.50 to 1.00% (w/v). The maximum methane content from 0.50% (w/v) TS was 45.48% at 19-day retention time.

The biogas yield of 1.20 L/day containing 54.91% methane was obtained at 19-day retention time (Figure 19C). Whereas the maximum methane content of 64.35% and the biogas yield of 0.60 L/day were obtained at 22-day retention time (Figure 19C). The fermentation reactions were ceased after operating for 25 day. The total biogas yield from 0.25, 0.50, 2.00, 4.00, 8.00, and 16.00% (w/v) TS were 216.98, 251.29, 281.34, 177.95, 150.00, and 72.93 L/kg fed, respectively (Figure 20). The maximum of total biogas yield of 356.35 L/kg TS fed was achieved from 1.00% (w/v) TS (Figure 20). When TS concentrations were increased from 1.00% to 2.00, 4.00, 8.00, and 16.00% (w/v), the total biogas yields were declined by 21.05, 50.06, 57.91, and 79.53%, respectively. When TS concentrations were decreased from 1.00% to 0.50 and 0.25% (w/v) TS, the total biogas yields were declined by 39.11 and 29.48%, respectively.

The maximum VS reduction of fermenting slurry of 39.10% was achieved from 1.00% (w/v) TS. Volatile solids reductions of 10.75, 15.24, 36.15, 33.95, 33.77, and 34.47% were obtained from cassava slurry of 0.25, 0.50, 2.00, 4.00, 8.00, and 16.00% (w/v) TS, respectively. When compared with the maximum VS at 1.00% (w/v) TS, the utilization of VS was 72.51, 61.02, 7.54, 13.17, 13.63, and 11.84% at 0.25, 0.50, 2.00, 4.00, 8.00, and 16.00% (w/v) TS, respectively. At 1.00% (w/v) TS showed the maximum utilization of all constituents resulting in the highest biogas yield and methane production compared to other TS concentrations. The reduction of

VS might be attributed to both underfed and overfed TS concentrations. Bardiya *et al.* (1996) found that the utilization of VS reflected biogas production patterns.

During 30 days of operation, the pH ranges of 6.80-8.07, 6.72-8.09, 6.48-8.03, 6.68-7.61, 6.51-7.29, and 6.56-7.30 were found in the digester containing 0.25, 0.50, 1.00, 2.00, 4.00, and 8.00% (w/v) TS, respectively (Figures 19A-19F). When using 1.00, 2.00, 4.00, and 8.00% (w/v) TS respectively, the initial drops in pH from 8.03 to 6.48, 7.23 to 6.74, 7.19 to 6.51, and 7.09 to 6.69, were observed at 2-day retention time. Subsequently, pH rapidly increased near neutral pH (7) at 3-day retention time. The pH of digestion slurry prepared from 4.00 and 8.00% (w/v) TS was lower and greater variation than the digestion slurry prepared from 1.00 and 2.00% (w/v) TS. When using 16.00% (w/v) TS, a drop in pH from 7.13 to 4.72 was observed at 13-day retention time. And no biogas was produced in the digester when pH reached 4.79 (Figure 19G). A low pH value inactivated microorganisms involved in the biogas production especially methanogenic bacteria (Vicenta *et al.*, 1984). Cuzin *et al.* (1992) provided data showing that bacterial counts were less than 10 and 100 times of the fermentative bacteria and the methanogenic bacteria, respectively, at the same loading rate before acidification. The biogas production was also reduced to 20% of the normal production.

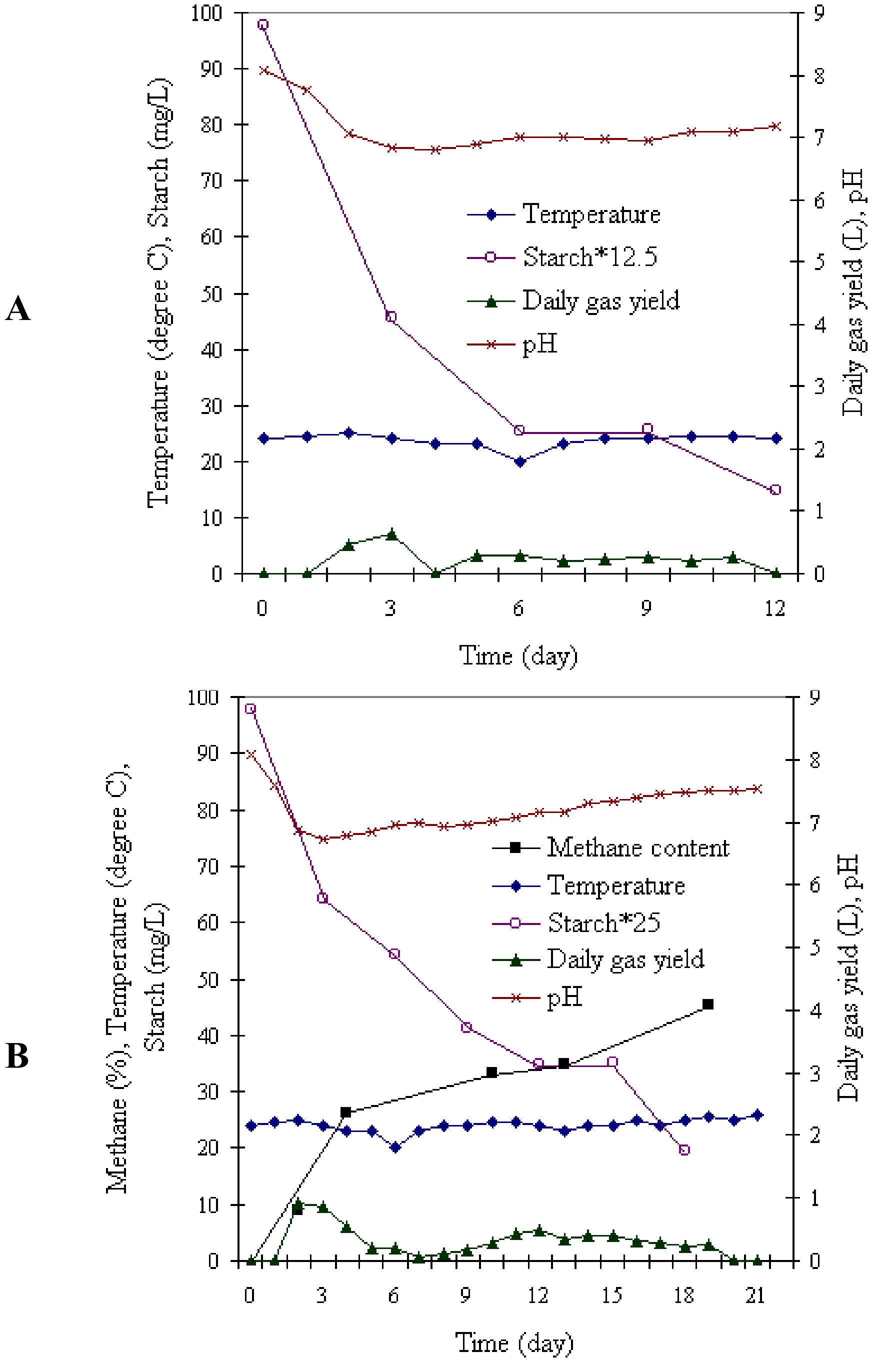


Figure 19. Biogas production from cassava tubers using (A) 0.25, (B) 0.50, (C) 1.00, (D) 2.00, (E) 4.00, (F) 8.00, and (G) 16.00% (w/v) total solids in the single-state digester of 5-L digestion volume.

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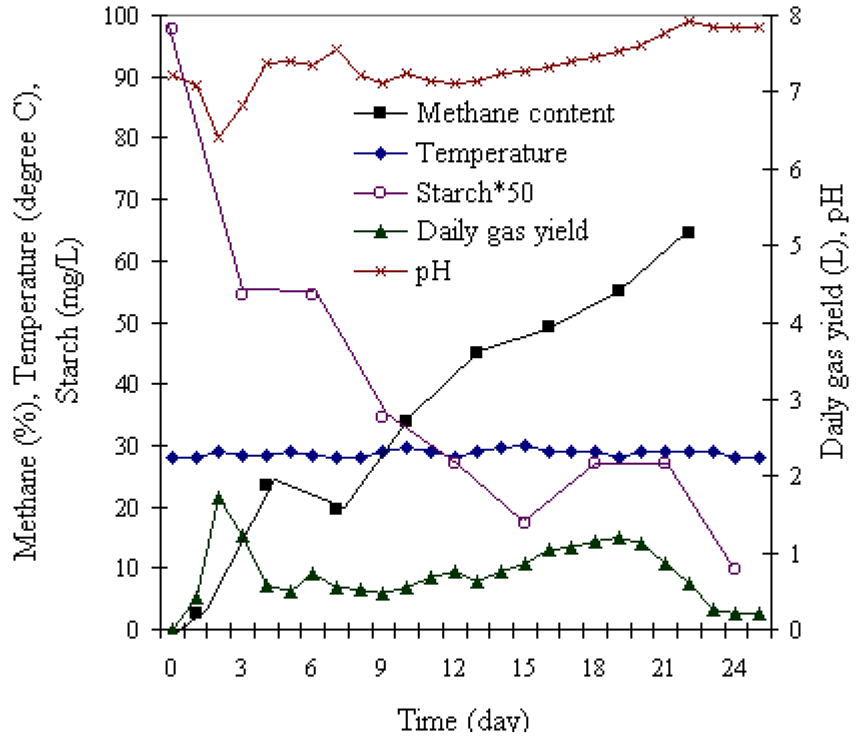

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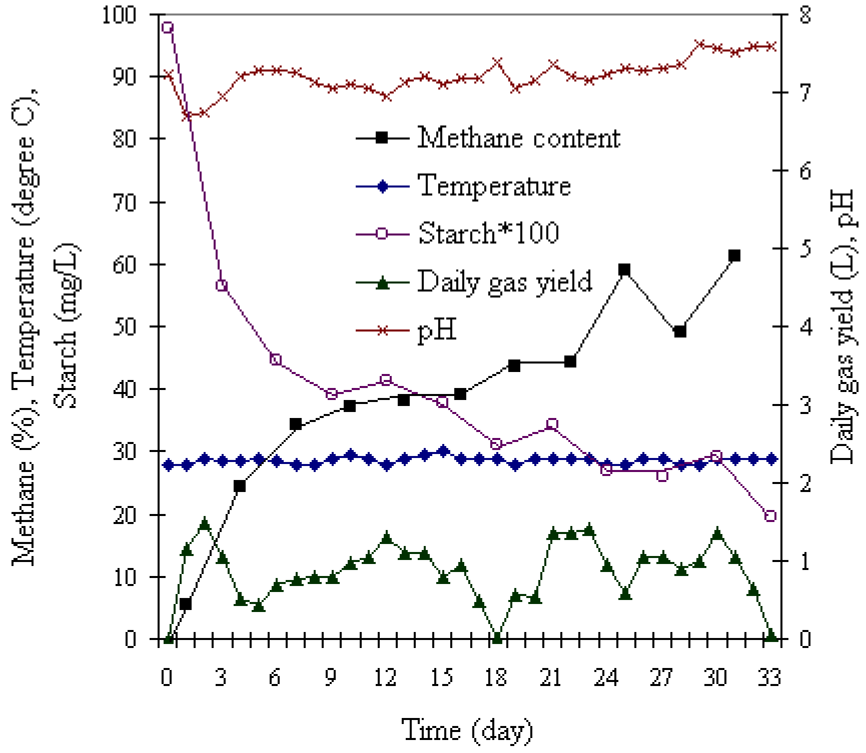

Figure 19. (Continued)

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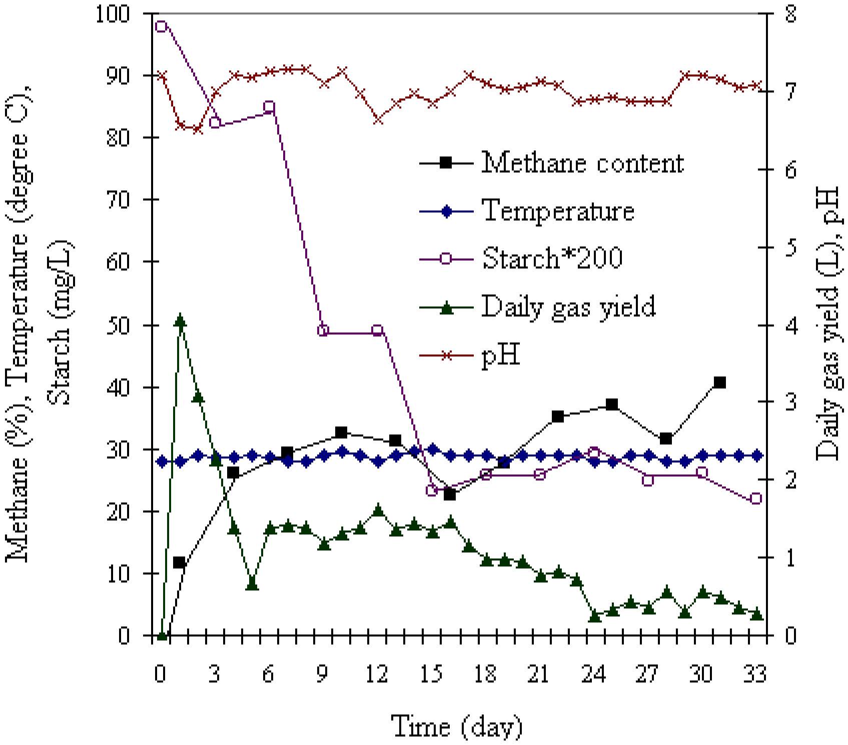

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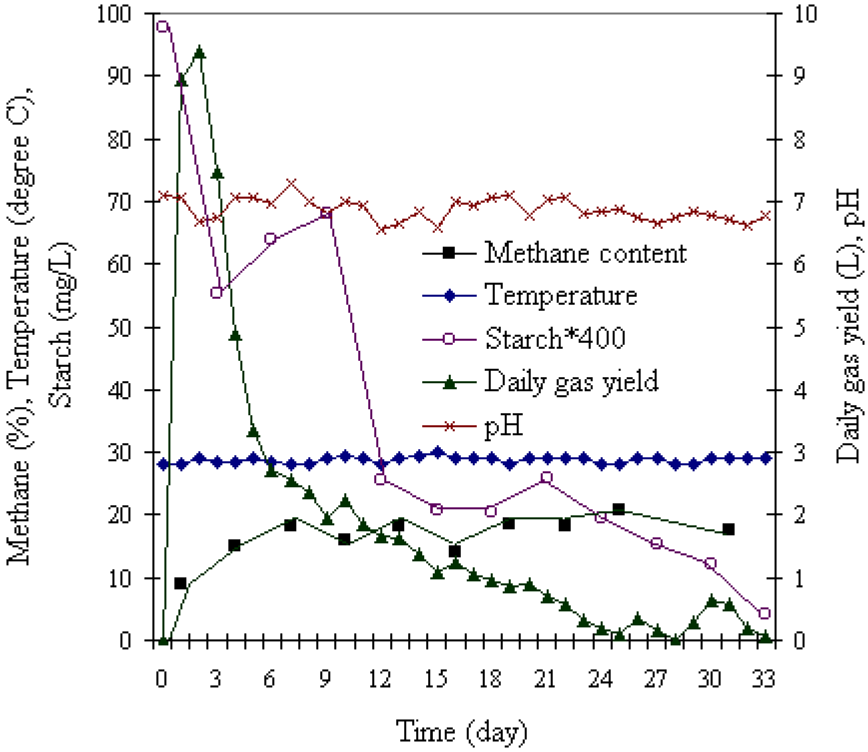

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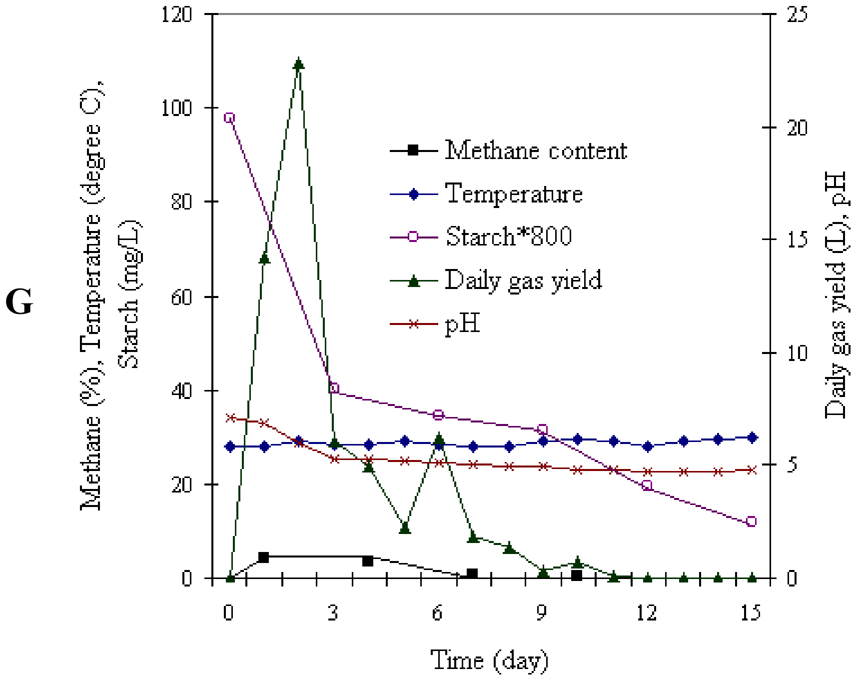

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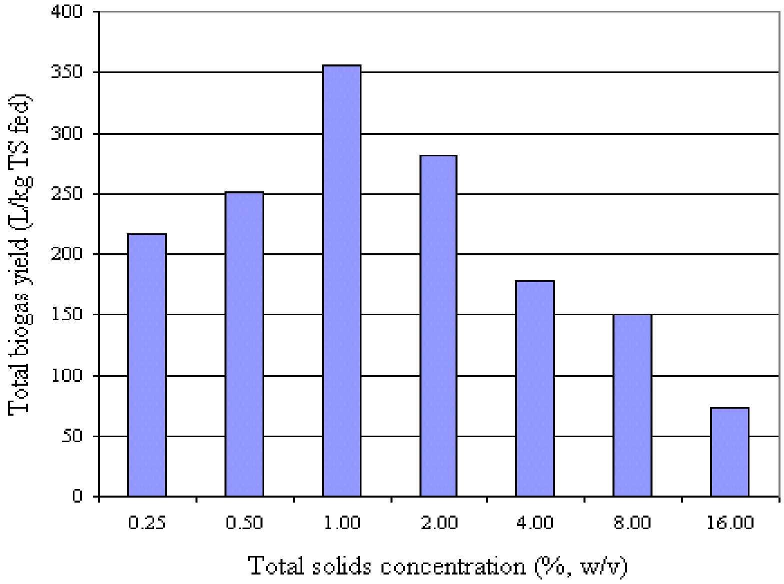


Figure 20. Total biogas yields from various concentrations of total solids performed in the single-state digester of 5-L digestion volume.

Volatile fatty acids were intermediate compounds in the metabolic pathway of methane fermentation, and could cause microbial stress if there were in high concentrations, which resulted in a decrease in pH, ultimately leading to failure of the digester. Therefore, the monitor of VFA concentration was very important for the operation performance of the anaerobic digester (Buyukkamaci and Filibeli, 2004). In this study, the VFA concentrations obtained from 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, and 16.00% (w/v) TS, varied from 510.0-1350.0, 555.0-2325.0, 1222.5-4650.0, 1312.5-6675.0, 2250.0-11850.0, 4200.0-25650.0, and 6487.5-26700.0 mg as acetate/L, respectively (Figure 21). These experimental studies showed that the higher influent substrate concentration led to the higher formation of VFA. This agreed with Buyukkamaci and Filibeli (2004) who reported that VFA concentrations increased at influent substrate concentrations increased. After the first day of fermentation, VFA concentrations increased for all TS concentrations applied. This was due to fermentative and acid-forming bacteria broken down complex organic matters into VFA (carbohydrates were broken down into simple sugars such as glucose and then into VFA) (University of Florida, 2002). During the commencement of methane fermentation, there were low numbers of methanogenic bacteria because they were slowest growing organisms in the anaerobic digestion. Hence, there were high concentrations of VFA accumulated in the digester. The VFA concentration appeared to be maximum (26700 mg as acetate/L) at 16.00% (w/v) TS (Figure 21G). The maximum concentration of VFA was obtained after operating for 3 days. The digestion failed when 16.00% (w/v) TS was applied because the excess TS concentration was added to the digester. Then, the acid-forming bacteria could convert the organic matters to VFA before the methanogenic bacteria could use these VFA. Thus, VFA were accumulated in the digester. This was resulted in the decrease in pH. If the pH dropped below 6.5, the methanogenic bacteria began to die, and bacterial population became unbalanced (Tanticharoen *et al*., 1984). The digester slurry became acidified, and no biogas was produced.

The alkalinity in the digester was used for monitoring anaerobic processes, but was considered as an intensive indicator of process instability. The measurements of alkalinity would reflect both levels of VFA and bicarbonate (Sáchez *et al*., 2001). Upon instability, the increase in VFA concentrations would cause a decrease in bicarbonate concentration resulting in a constant alkalinity concentration. In this investigation, the alkalinity concentration was found in the range of 1750 and 22850 mg as $CaCO_3$/L for overall experiment (Figure 21). The highest alkalinity (7950-22850 mg as $CaCO_3$/L) was observed from 8.00% (w/v) TS. The lowest alkalinity (2200-2560 mg as $CaCO_3$/L) was achieved from 0.25% (w/v) TS. The alkalinity ranges were 2200-4000, 1750-7325, 2550-8000, 4150-11850, and 9525-11425 mg as $CaCO_3$/L, when the digesters were operated at the TS concentrations of 0.50, 1.00, 2.00, 4.00, and 16.00% (w/v), respectively. These results reveal that the alkalinity in digester increased while the TS concentrations increased. The findings were coincident with those obtained by Sáchez *et al*. (2001).

A

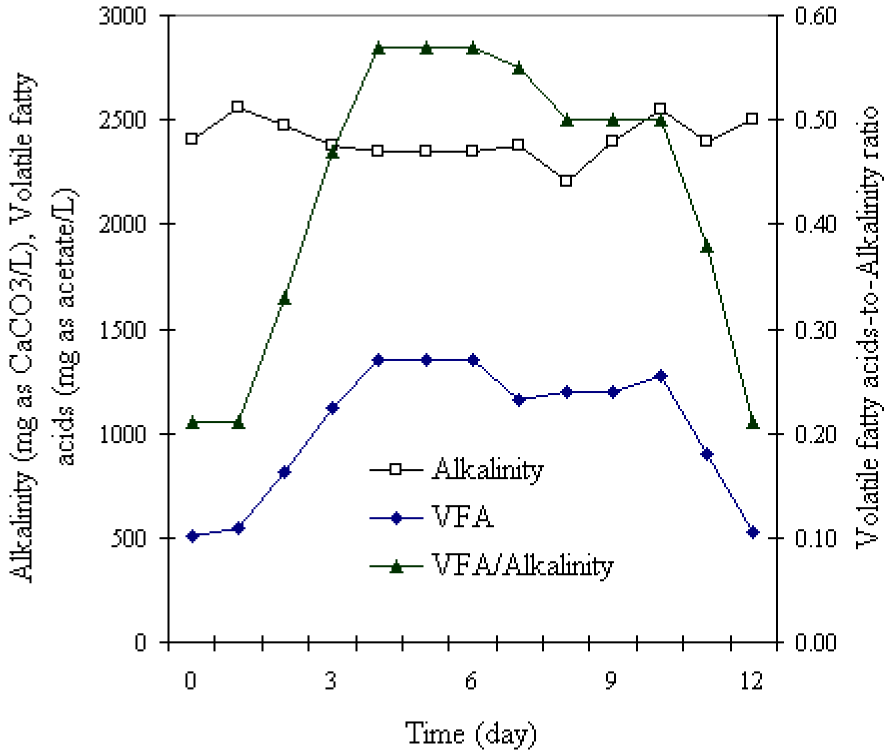
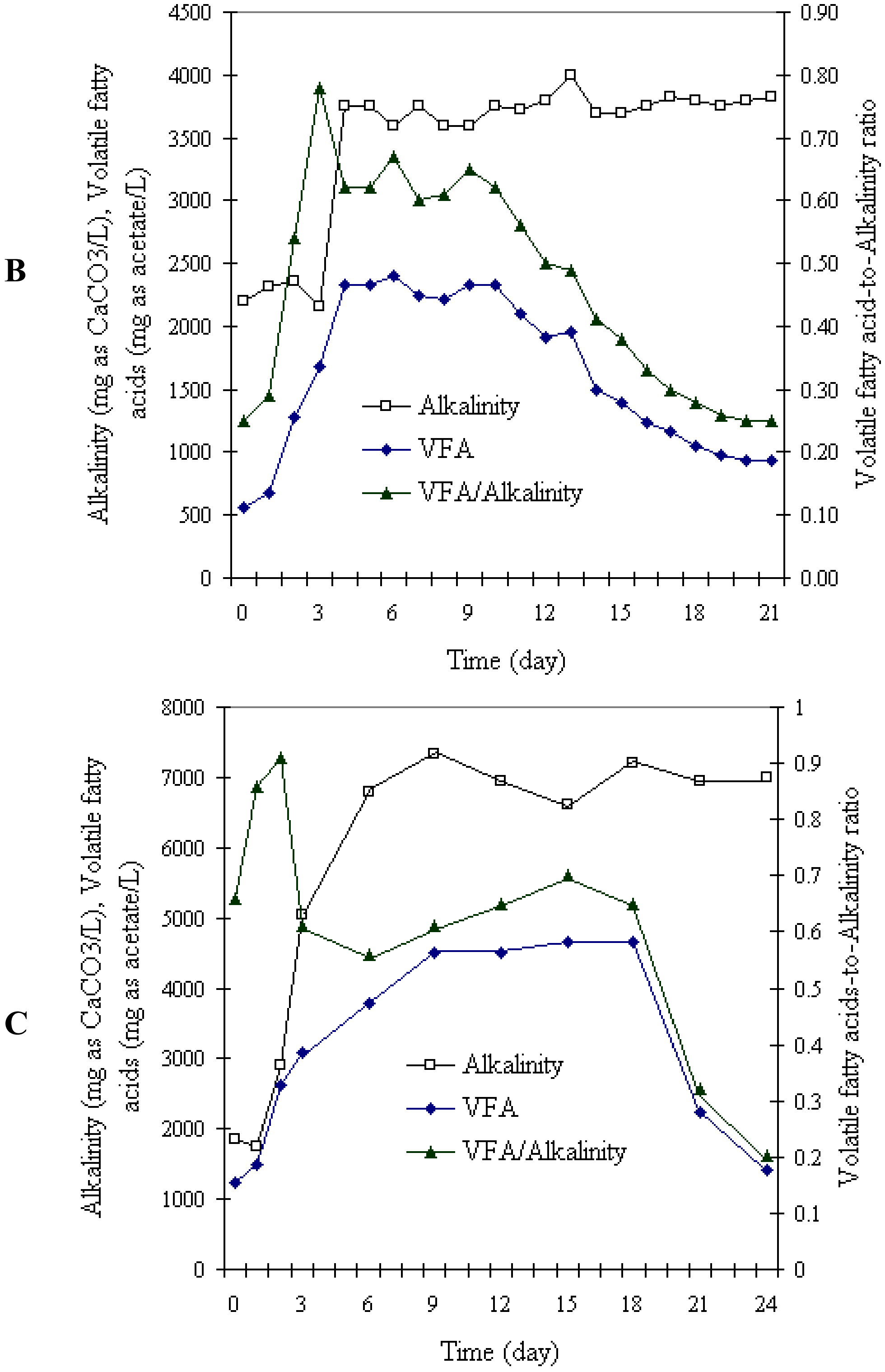


Figure 21. Alkalinity, volatile fatty acids and volatile fatty acids-to-alkalinity ratio measured during biogas production from cassava tubers from (A) 0.25, (B) 0.50, (C) 1.00, (D) 2.00, (E) 4.00, (F) 8.00, and (G) 16.00% (w/v) total solids in the single-state digester of 5-L working volume.



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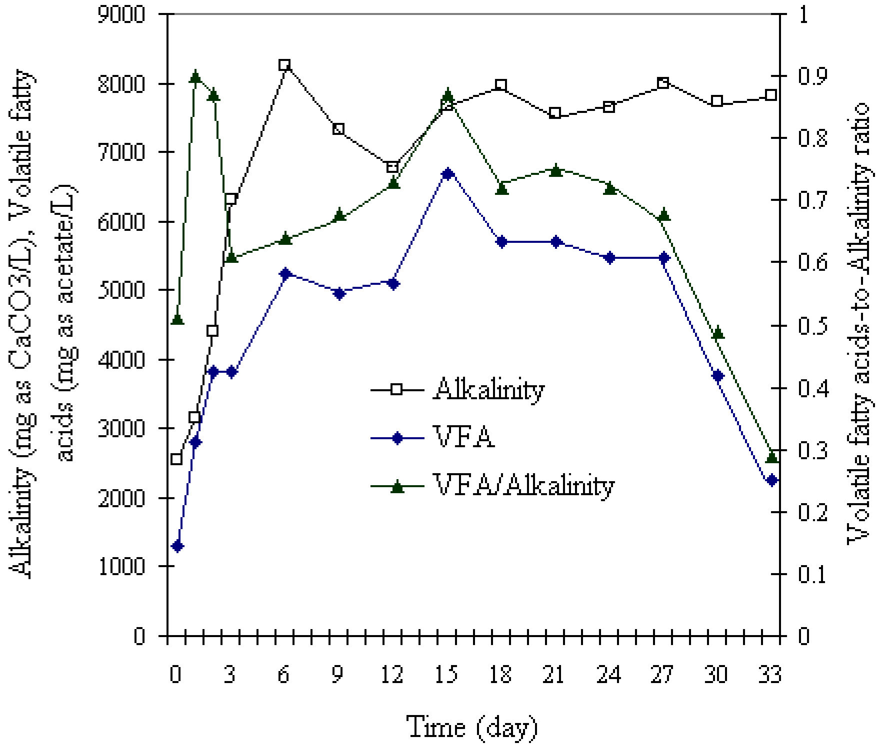

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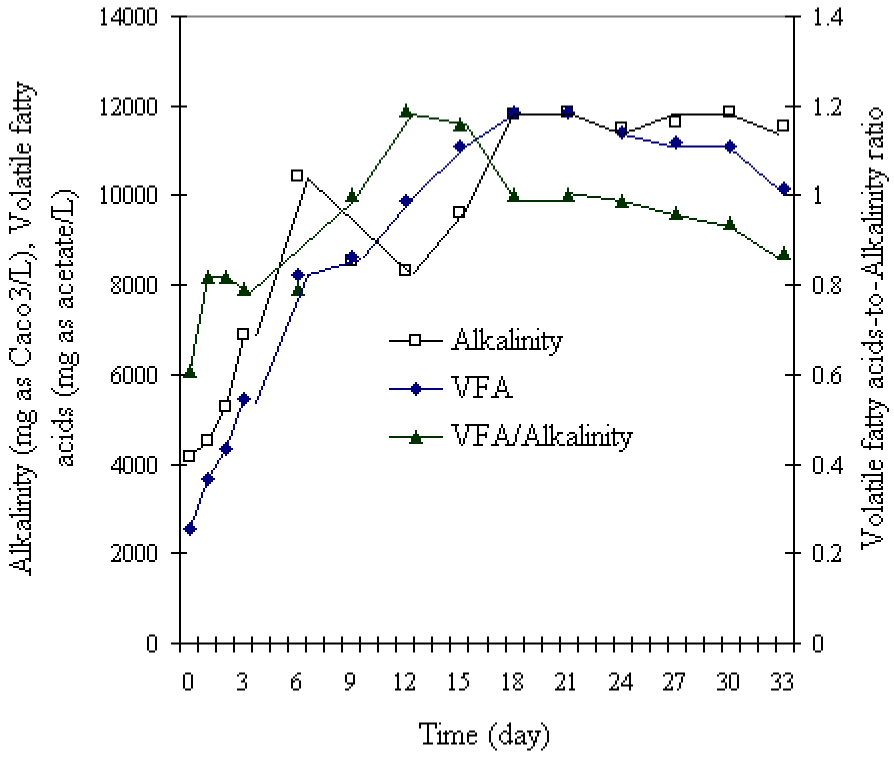

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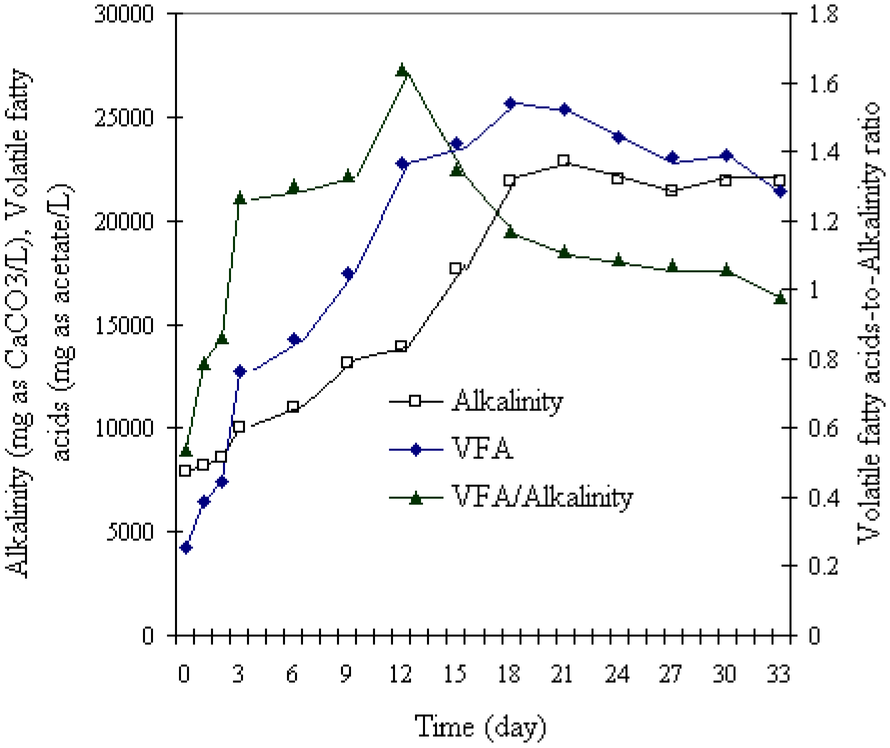

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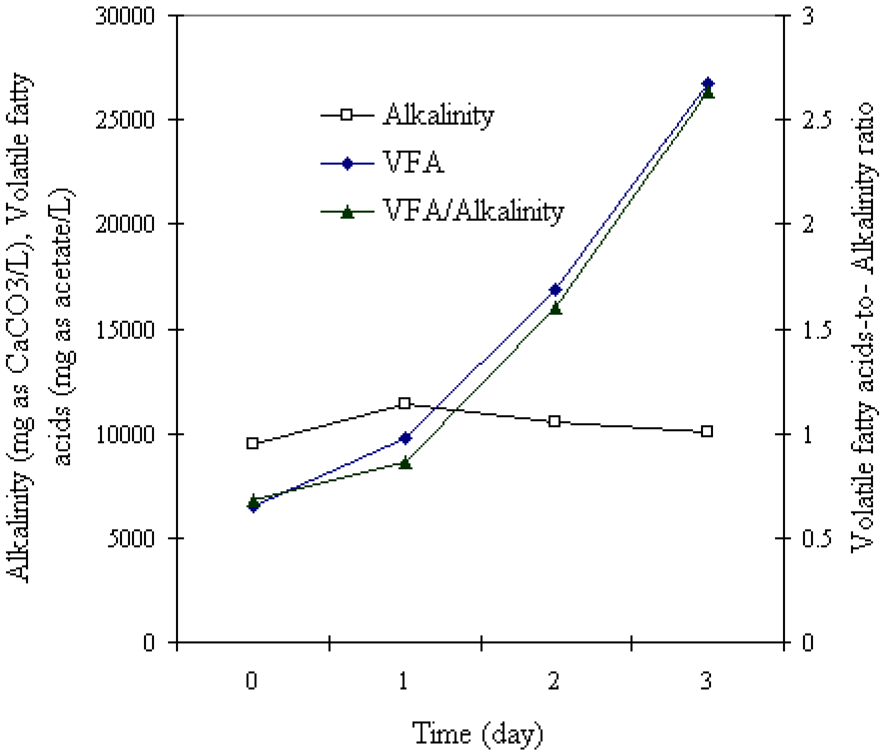

Figure 21. (Continued)

In order to allow the methanogenic bacteria to grow, digesters should be properly fed, and buffered by raising alkalinity to 2500-5000 mg as acetate/L. Sodium hydrogen carbonate ($NaHCO_3$) was used to increase alkalinity or buffering capacity of fermenting slurry in this study. It was added to the digester whenever the VFA-to-alkalinity ratio was greater than 0.8. If the ratio of the VFA-to-alkalinity exceeded 0.8, pH depression and inhibition of methane production occurred. The proper ratio for the VFA-to-alkalinity was between 0.1 and 0.2 (Water Pollution Control Federation, 1987). The ratios of the VFA-to-alkalinity obtained from 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, and 16.00% (w/v) TS ranged from 0.21 to 0.57, 0.25 to 0.67, 0.20 to 0.91, 0.29 to 0.90, 0.61 to 1.19, 0.54 to1.27, and 0.68 to 2.64, respectively, which were higher than the normal range indicated in the literature (Water Pollution Control Federation, 1987) especially at high TS concentrations (4.00-16.00%, w/v TS). It was probably caused by the high TS concentrations loaded to the digester. Although the alkalinity was high, the accumulation of VFA at high concentrations (8625-26700 mg as acetate/L) in the digester was also found. The pH value was low whereas the VFA-to-alkalinity ratio was high. These results reveal that methane could be produced from raw cassava tuber in high the VFA-to-alkalinity ratio. The optimal range of the VFA-to-alkalinity ratio range for biogas production from cassava tubers was 0.2-0.4. As the acid-forming bacteria produced VFA, the methanogenic bacteria utilized the acids and maintained a neutral pH. Since the reaction rate involving acid-forming bacteria proceeded much faster than the reaction involving methanogenic bacteria, a larger population of methanogenic bacteria must be fed and maintained (University of Florida, 2002). When the digester was initially heavily fed, acid forming-bacteria quickly produced acids. The methanogenic bacteria population might not be adequate to consume the acids produced and maintain a neutral pH resulting in declining pH below the neutral pH and diminishing growth of methanogenic bacteria and methanogenesis. The pH could be maintained by adding $NaHCO_3$ to increase alkalinity. At high TS concentrations (4.00-16.00%, w/v) required more $NaHCO_3$ concentration to stabilize the pH during anaerobic digestion than at low TS concentrations (Figure 21).

Acetic acid was the best precursor for more than 70% of methane formation in most anaerobic processes (Horiuchi *et al*., 2002; Yu *et a*l., 2002). Other short-chain organic acids such as propionic and butyric acids were also the methane formation. High concentrations of these specific acids could cause failure of anaerobic digestion systems, due to their relatively high toxicity to methanogenic bacteria compared to that of acetic acid (Stronach *et al*., 1986). Acetic, propionic, and butyric acids were the major volatile acids present during anaerobic digestion and their concentrations provided a useful measurement of digester performance. At 1.00 to 8.00% (w/v) TS feeding, the concentrations of acetic, propionic, and butyric acids accumulated, were lower than at 16.00% (w/v) TS, and the concentration of propionic and butyric acids were found to be higher than acetic acid (Figure 22). The maximum concentrations of acetic, propionic, and butyric acids (3227.54, 8309.70, and 2992.15 ppm, respectively) were achieved from 16.00% (w/v) TS (Figure 22G). The high acetic acid accumulation usually implies the failure of methanogenic bacteria to convert acetic acid to methane and carbon dioxide (Buswell and Mueller, 1952). The accumulation of volatile acid, especially propionic acid, at the higher TS concentration was typical in stressed or sour digesters (Mackie and Bryant, 1995). The acid-forming bacteria produced the basic feed for the methanogenic bacteria. Then, the methanogenic bacteria removed the metabolic end products of acid-forming bacteria, and converted them into gases, which escaped from the system. If this conversion did not occur, conditions in the digester would become to acids that even the acid-forming bacteria would not survive, and the methane-forming population would also decrease (Aiman *et al.*, 1981).

The alkalinity, VFA concentration, and pH were very important in digester operation. The pH was the key indicator of operational stability (Tanticharoen *et al.*, 1984). Vicenta (1984) reported that the optimum pH for the biogas production from pineapple peelings was in the range of 6.8 to 7.2 with the limitation of the range for operation without significant inhibition being 6.5 to 7.6. Methanogenic bacteria could occasionally grow at the pH range of 6.5-8.2 (Buyukkamaci and Filibeli, 2004). Viswanath *et al.* (1992) mentioned that there was a perfect link of the acidogenic and methanogenic phases when the pH was remained at 7 and there was no drastic increase in acidity or alkalinity. Increased concentration of VFA might lead to a decrease in the buffering bicarbonate concentration, as the bicarbonate became protonated, and was released as carbon dioxide (Jantsch and Mattiasson, 2004). The increase in the initial substrate concentration caused an increase in the VFA concentration, alkalinity, and the VFA-to-alkalinity ratio with a consequent reduction of the pH (Sânchez *et al.*, 2001).

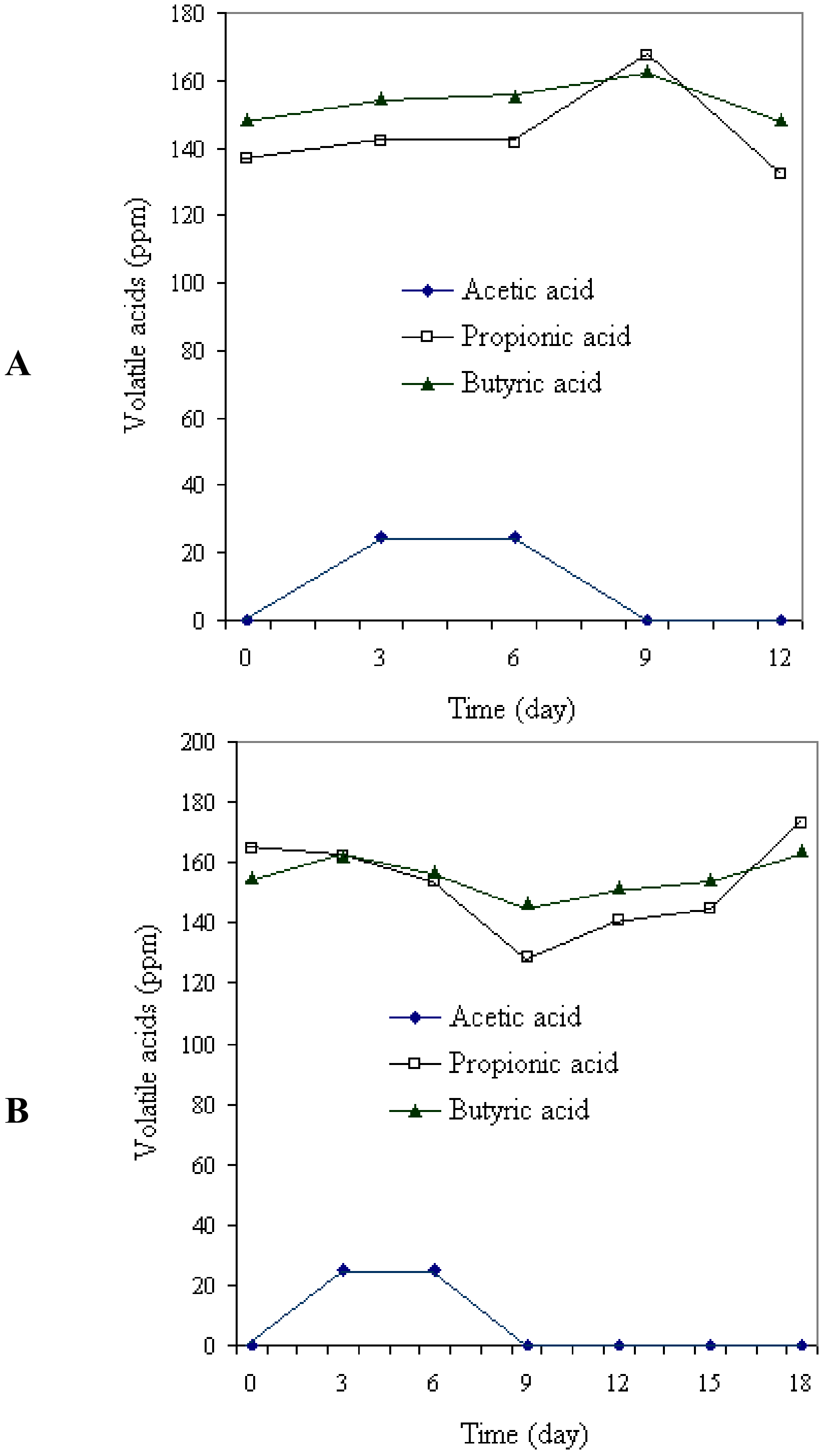

Figure 22. Volatile acids accumulation during cassava tuber fermentation from (A) 0.25, (B) 0.50, (C) 1.00, (D) 2.00, (E) 4.00, (F) 8.00, and (G) 16.00% (w/v) total solids in the single-state digester of 5-L working volume.

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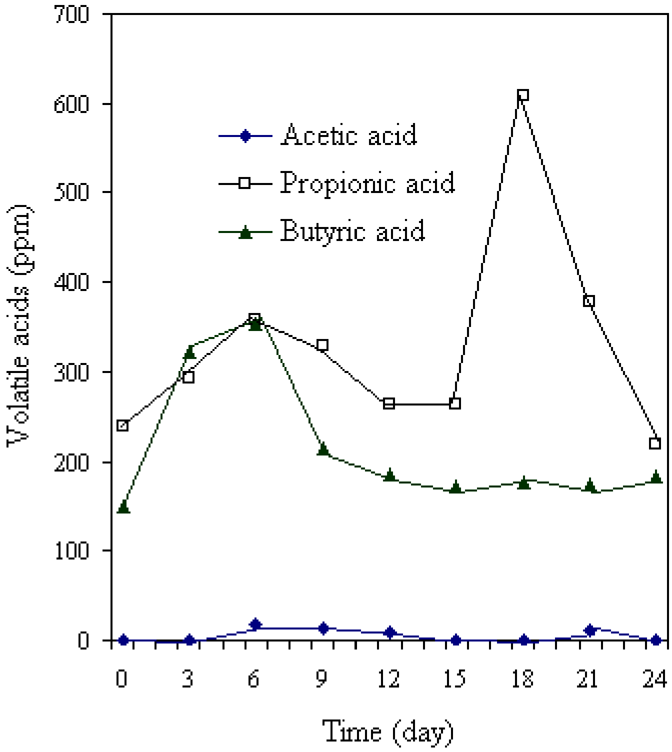

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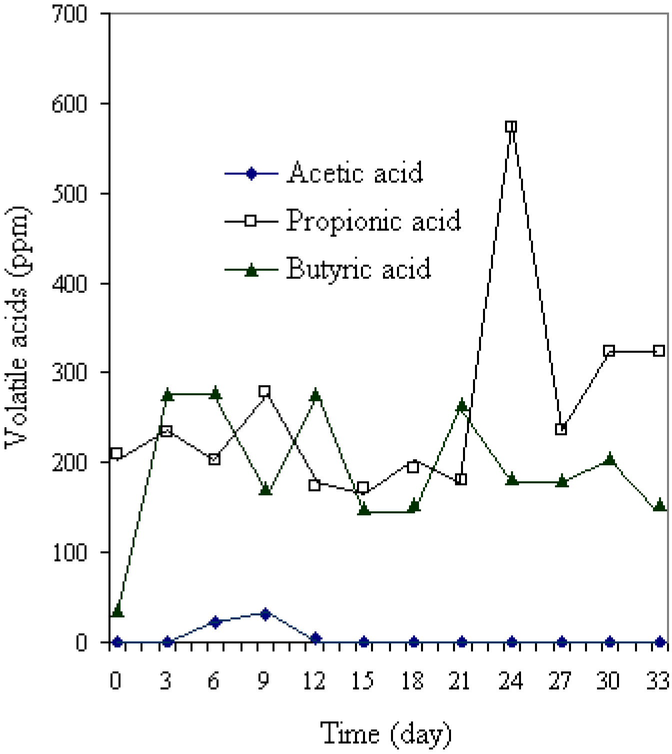

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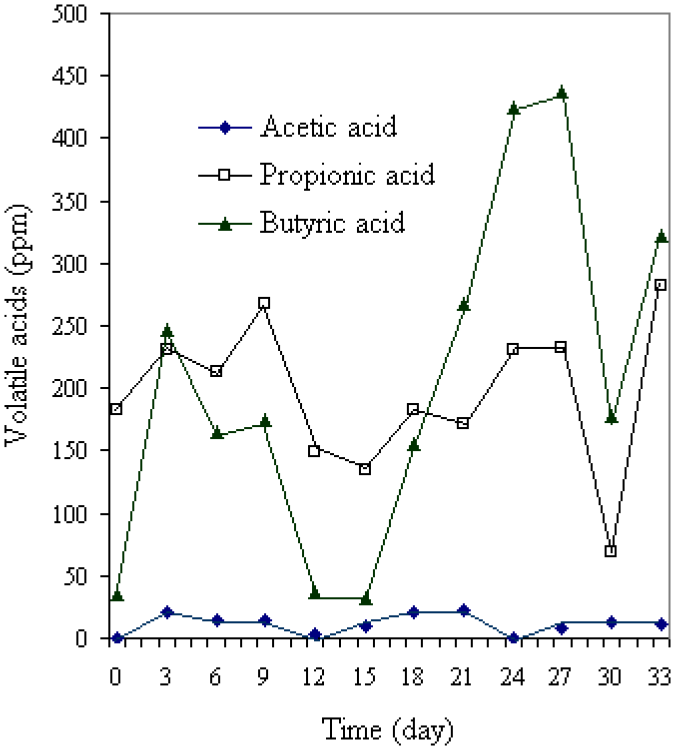

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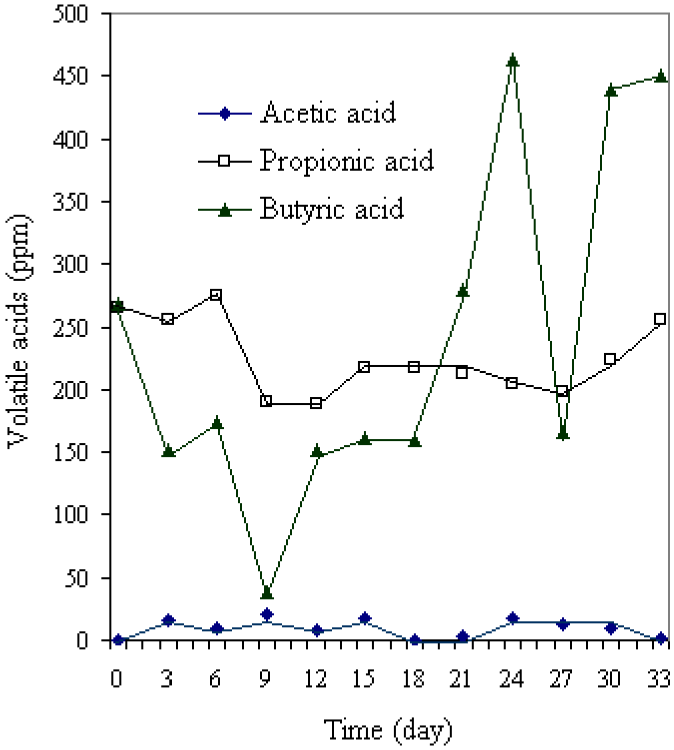

Figure 22. (Continued)

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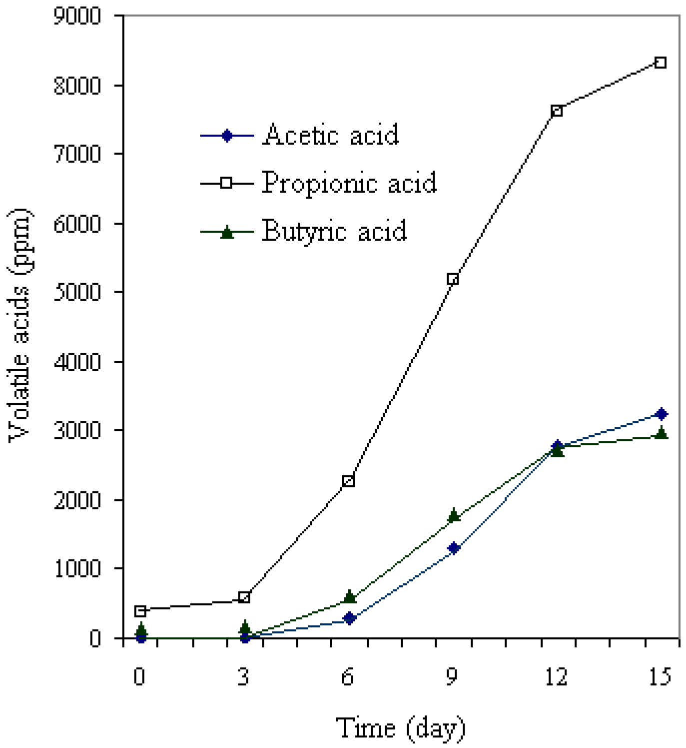


Figure 22. (Continued)

Pandey *et al.* (2000) reported that dry cassava tubers (15.70% of moisture) composed of 1.60% of cyanide. In Thailand, the cyanide contents of fresh cassava tuber, native chip, and native pellet were 122.09, 30.48, and 13.76 ppm, respectively (Department of Animal Science, 1992). The pure culture of methanogenic bacteria was highly sensitive to cyanide (<1 mg/L) but tolerated up to 5-6 mg cyanide/L during the methanogenic fermentation in the digester (Cuzin *et al.*, 1992). The cynanide content of cassava in Thailand was lower than the concentration that effected methanogenesis. In this study, if the cyanide concentration was calculated according to Department of Animal Science (1992), TS of 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, and 16.00% (w/v) might contain 0.10, 0.20, 0.39, 0.78, 1.56, 3.12, and 6.24 mg cyanide/L, respectively. The ability of methanogenic bacteria to adapt to cyanide was previously reported by Feddorak *et al.* (1986). However, results from the biogas production from raw cassava tubers reveal that methanogenic bacteria were able to produce methane from cassava tubers. The TS of 1.00% (w/v) was found to be suitable TS for biogas production from cassava tubers using 10% (v/v) inocula, and the digestion failed when the TS was increase to 16.00% (w/v).

4.2.2.2 The optimum concentration of nitrogen source

Dry cassava substrate prepared for biogas production contained 0.46% of nitrogen and 39.58% of carbon, which had a carbon-to-nitrogen ratio of around 86:1. The high carbon-to-nitrogen ratio (86) of dry cassava tuber might induce excess acid production and nitrogen deficiency. The inhibitory effects of high acid production and nitrogen deficiency on cassava waste methanogenesis in fermenters were reported by Wurster (1985). The lack of specific elements required for bacterial growth would also limit the biogas production. The proper carbon-to-nitrogen ratio for biogas production was 20 to 30:1 (Sanders and Bloodgood, 1965; Polprasert, 1989). Thus nitrogen supplement should be considered to enhance the biogas production from raw cassava tubers. Nitrogen could be added in inorganic form (e.g. ammonia) or in organic form (e.g. urea, animal manure or food waste). Once nitrogen was released from the organic matters, it became ammonium (NH_4^+) which was water-soluble (Zhang and Zhang, 1999). In this study, urea was selected as the nitrogen source due to its was easily digested to ammonia by a variety of microorganisms (Sterling *et al.,* 2001). The addition of urea to experimental digester caused the increase in nitrogen and ammonia concentrations (Sterling *et al.*, 2001), then increased the alkalinity concentration due to the increased ammonium ion as well as pH (Kroeker *et al.*, 1979; Georgacakis *et al.,* 1982; Sterling, 2001). This also benefited to the biogas production process (Pound *et al.*, 1981; Bardiya *et al.*, 1996). However, Vicenta *et al.* (1984) reported that the addition of urea to the biogas production digester using pineapple peelings could not increase the gas production. The overload of urea could be inhibited the methane production (Sterling *et al.*, 2001). For the biogas production from raw cassava tubers, various concentration of urea was performed from 0.00 (control), 0.02, 0.03, 0.04, 0.10, and 0.20% (w/v), and it was found that urea stimulated the biogas production (Figures 23 and 24).

A

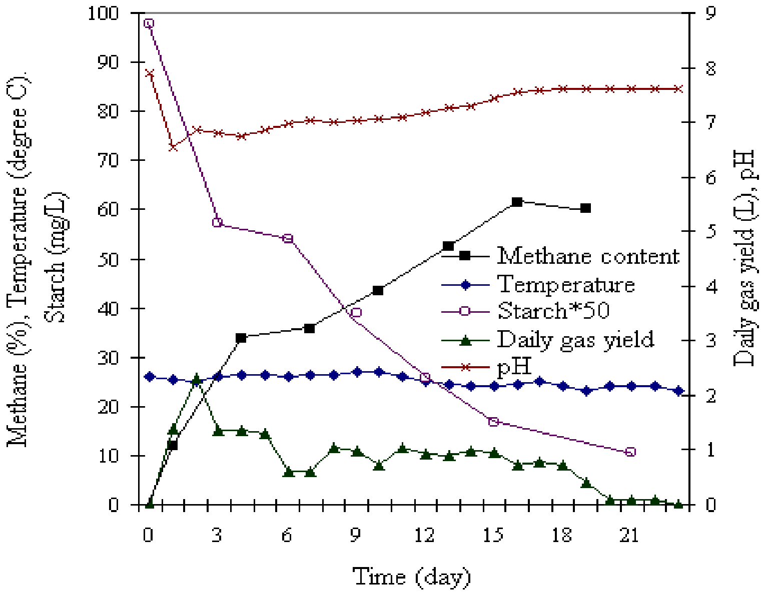
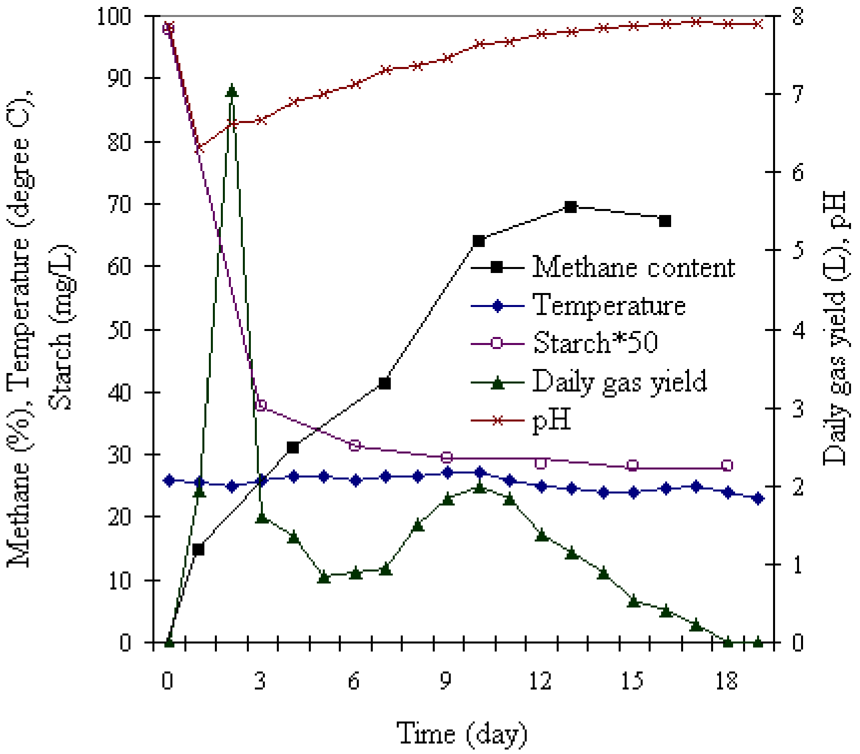


Figure 23. Biogas production from cassava tubers using 1.00% (w/v) total solids and urea supplements at various concentrations: (A) 0.00, (B) 0.02%, (C) 0.03, (D) 0.04, (E) 0.10, and (F) 0.20% (w/v), in the single-state digester of 5-L digestion volume.

B



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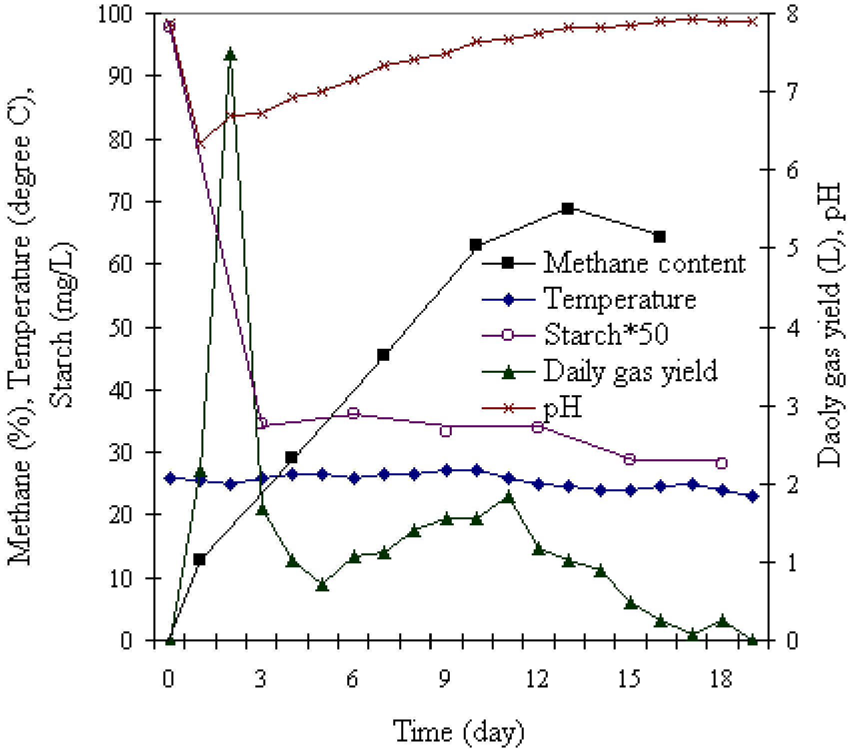

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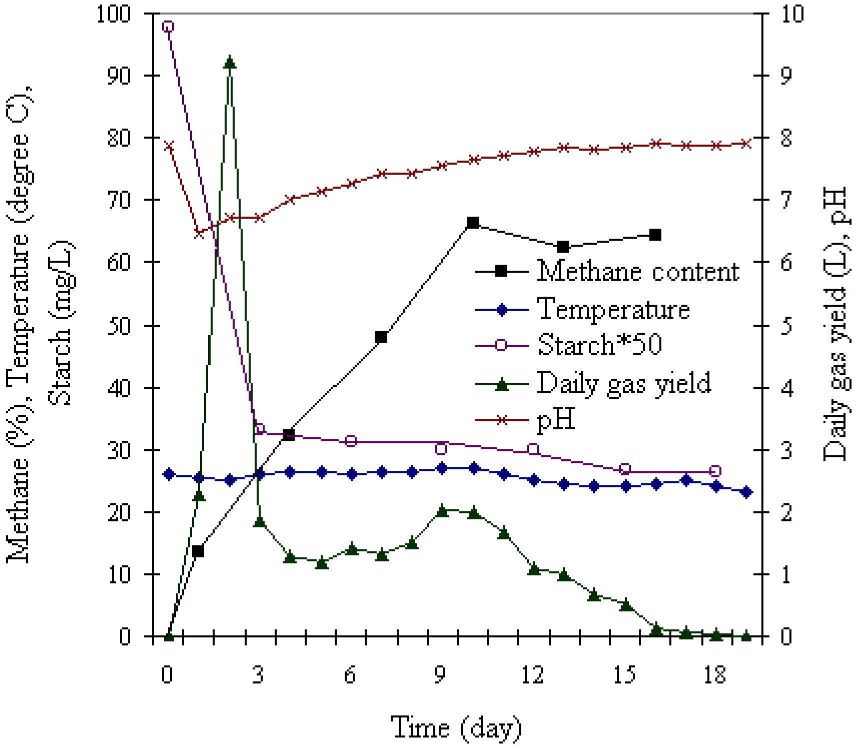

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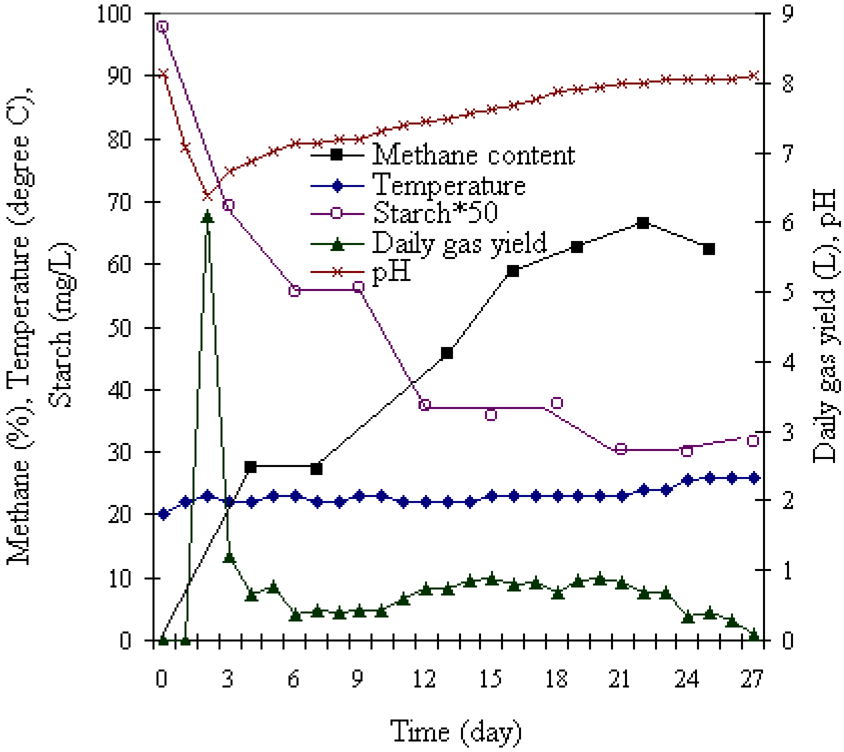

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F

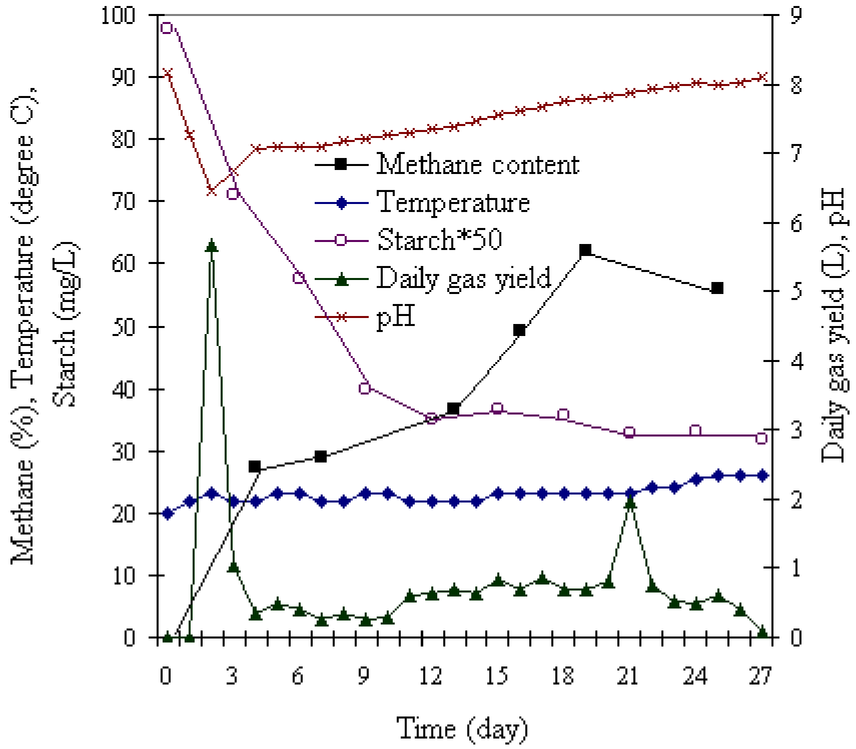


Figure 23. (Continued)

Temperatures of the cassava slurry during fermentation were found to be between 20 and 27.5°C (Figure 23). The optimum concentration of urea for biogas production from raw cassava tubers was 0.04% (w/v) (Figures 23D and 24). Whereas the addition of urea either more than 0.04% (w/v) (0.10 and 0.20%) or lower than 0.04% (w/v) (0.02 and 0.03%) resulted in low gas yields.

The maximum of total biogas yield of this experiment was obtained from 0.04% (w/v) urea as 561.38 L/kg TS fed (Figure 23). The maximum biogas yield of 1.98 L/day and maximum methane composition of 66.13% were obtained at 10-day retention time (Figure 23D). But the fermentation reactions were ceased after 19-day operation. When urea additions were decreased from 0.04% to 0.03, 0.02, and 0.00% (w/v), total biogas yields were declined by 11.77, 9.92, and 33.97%, respectively.

When urea additions were increased from 0.04% to 0.10 and 0.20%, total biogas yields were declined by 24.46 and 27.60%, respectively.

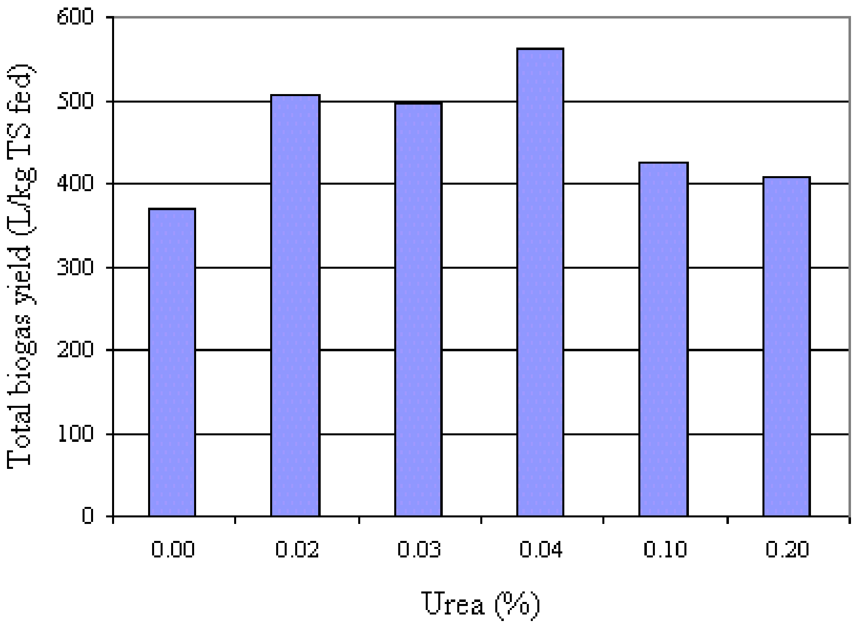


Figure 24. Total biogas yields using 1.00% (w/v) total solids and urea supplements at various concentrations: 0.00, 0.02, 0.03, 0.04, 0.10, and 0.20% (w/v), in the single-state digester of 5-L digestion volume.

The supplementation of urea also effected the quality as well as quantity of the biogas. The addition of urea of 0.02, 0.03, 0.04, 0.10, and 0.20% (w/v) could increase methane content compared to the control (0.00%, w/v, urea). The increase in methane content was observed after 7 days of fermentation. The maximum methane content achieved from experiments of 0.00, 0.02, 0.03, 0.04, 0.10, and 0.20% (w/v) urea addition were 61.56% on 16 days, 69.46% on 13 days, 68.58% on 13 days, 66.13% on 10 days, 66.41% on 22 days, and 62.08% on 19 days, respectively (Figure 22).

For overall experiment, the VS reductions varied between 40.13 and 59.23%. The maximum reduction of VS (59.23%) occurred at 0.04 % (w/v) urea. The VS reduction of 40.13, 58.71, 58.28, 48.69, and 44.44% were found at 0.00, 0.02, 0.03, 0.10, and 0.20% (w/v) urea, respectively, which were 67.75, 99.12, 98.40, 82.20, and 75.03% compared to 0.04% (w/v) urea. The VS reduction reflected the methane production, which agreed with Sterling *et al.* (2001) who mentioned that the methane production increased when the VS reduction increased, and the methane production decreased when the VS reduction decreased.

The pH value, alkalinity, VFA concentration, and volatile acids (acetic, propionic, and butyric acids) accumulation during the fermentation of cassava tubers with the addition of various concentration of urea were monitored (Figures 23, 25, and 26). At the first day of operation, pH of fermenting slurry was high (7.87-8.16), then pH rapidly declined. The VFA formation in acetogenesis stage was rapid (Figure 25). During the first week of fermentation, urea supplementation pH decreased more highly than without urea due to VFA concentrations increased. This could explain that the urea addition (nitrogen source) could increase the rate of microorganisms growth. When adding 0.02, 0.03, 0.04, 0.10, and 0.20% (w/v) urea to the digester, pH was initially dropped from 7.87 to 6.63, 7.87 to 6.69, 7.88 to 6.72, 8.15 to 6.37, and 8.16 to 6.45 within 2 days of operation. Whereas without the addition of urea, the initial drop in pH from 7.89 to 6.74 was observed at 4 days retention time. Then, the rise in pH value was found to be higher in the case of urea addition than without urea addition. The final pH values were 7.6, 7.91, 7.91, 7.90, 8.12, and 8.09 for the addition of urea at 0.00, 0.02, 0.03, 0.04, 0.10, and 0.02% (w/v), respectively. pH values did not increase proportionally to the amount of urea added.

For VFA concentrations, they were varied from 625.5-2850.0, 664.5-3465.0, 630.0-3325.5, 619.5-3550.5, 712.5-4912.5, and 675.0-4950.0 mg as acetate/L at 0.00, 0.02, 0.03, 0.04, 0.10, and 0.20% (w/v) urea, respectively (Figure 25). The volatile fatty acids concentration appeared to be maximum at 0.20% (w/v) urea (Figure 25F).

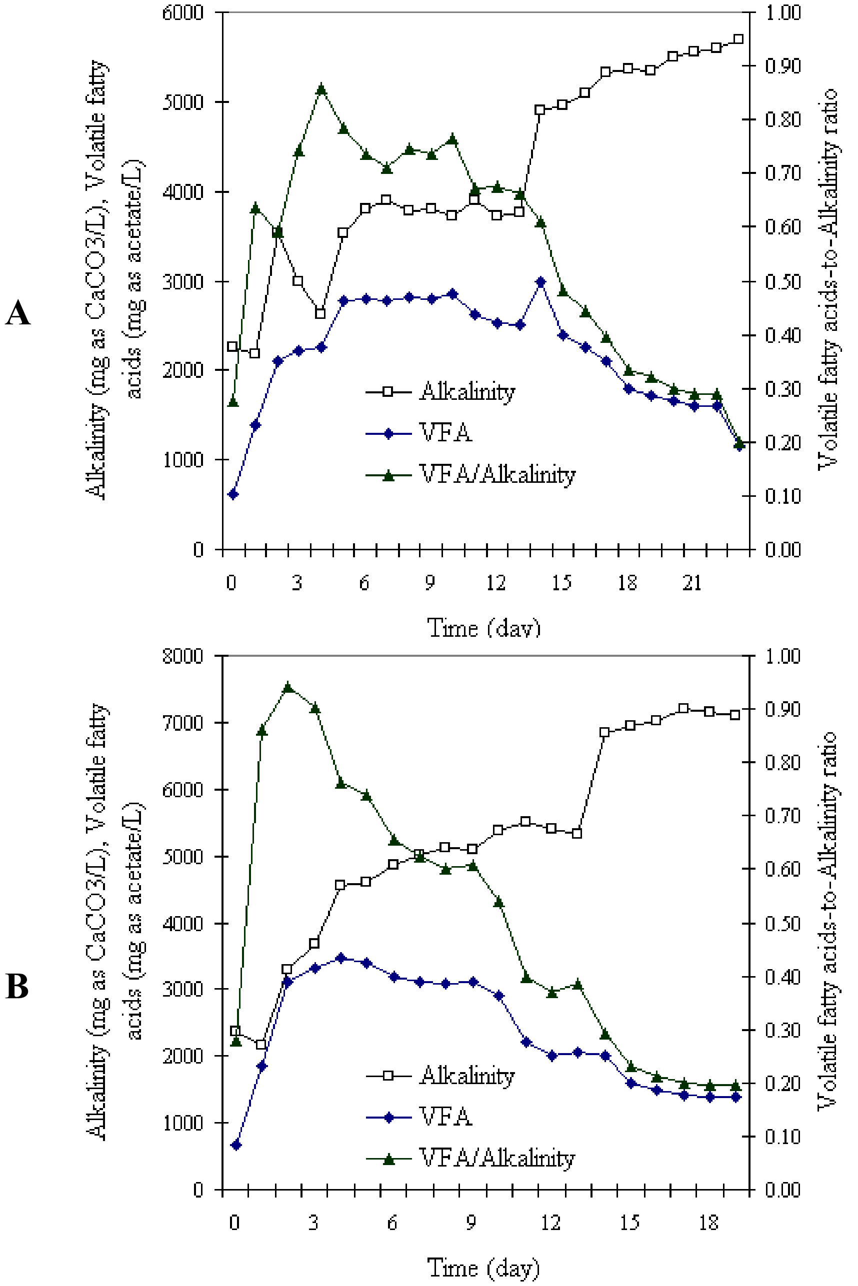
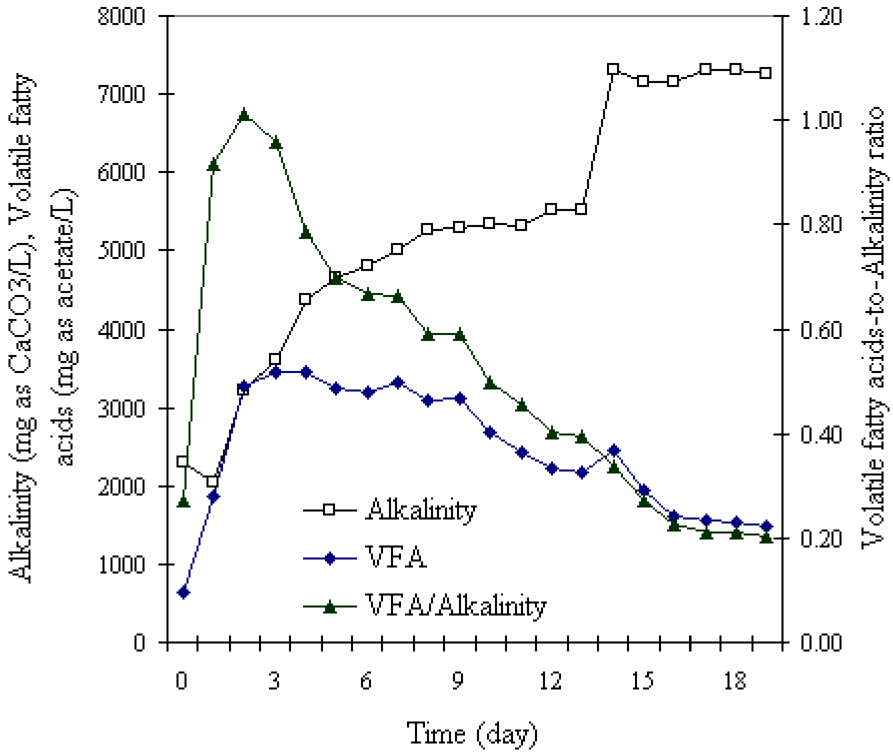


Figure 25. Alkalinity, volatile fatty acids, and volatile fatty acids-to-alkalinity ratio measured during biogas production from cassava tubers using 1.00% (w/v) total solids and urea supplement at various concentrations: (A) 0.00, (B) 0.02, (C) 0.03, (D) 0.04, (E) 0.10, and (F) 0.20% (w/v), in the single-state digester of 5-L working volume.

C



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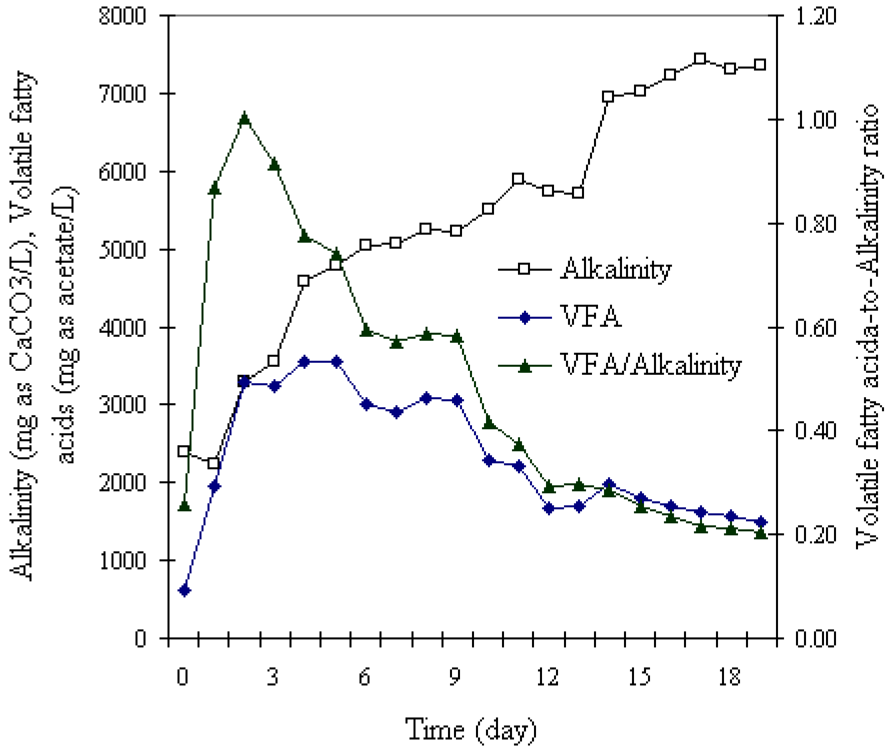

Figure 25. (Continued)

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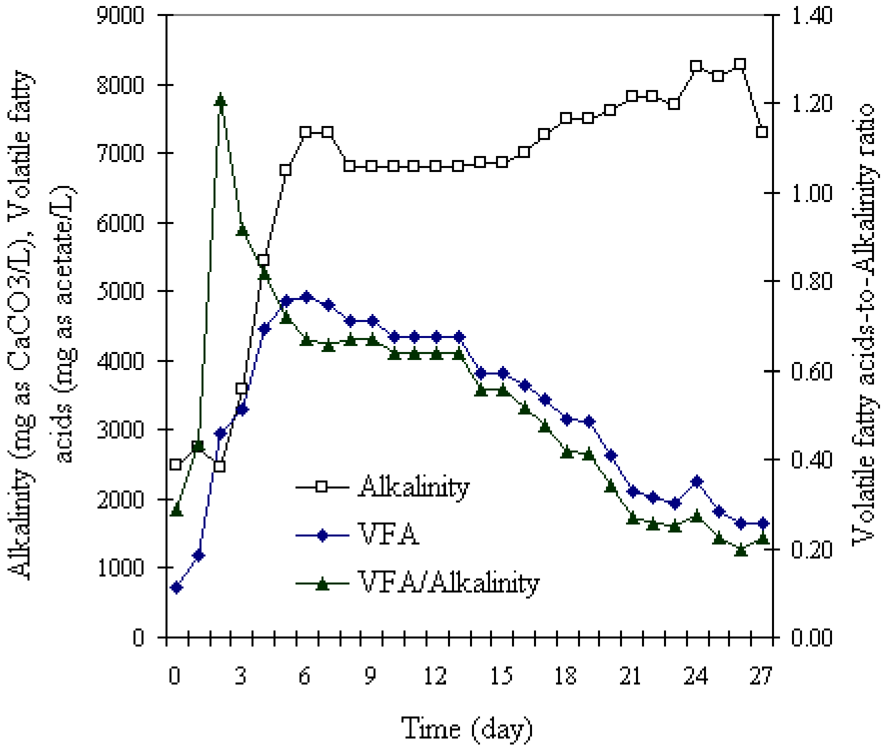

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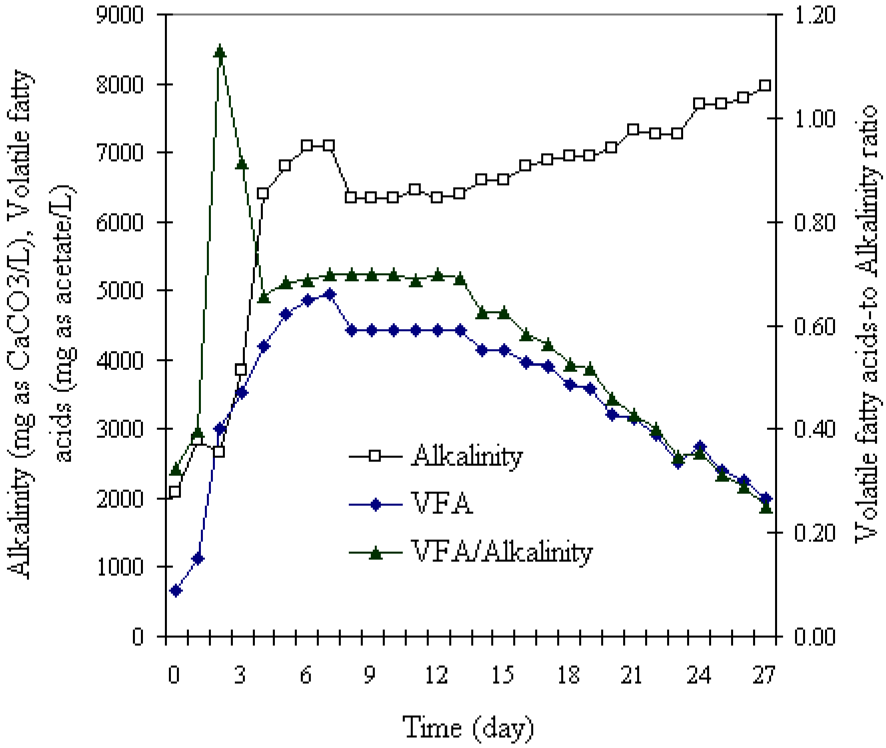


Figure 25. (Continued)

All digesters had similar initial alkalinity concentration (2000-2500 mg as $CaCO_3$/L). The alkalinity ranges of 2150-7200, 2050-7317, 2250-7433, and 1850-7950 mg as $CaCO_3$/L were detected when urea concentrations of 0.02, 0.03, 0.04, and 0.20% (w/v) were added to the digesters (Figure 25A). Sodium hydrogen carbonate ($NaHCO_3$) was used for increasing digester alkalinity whenever VFA-to alkalinity ratio was greater than 0.8 as same as previous section. Two and a half gram of $NaHCO_3$ was added once, thrice, thrice, thrice, twice, and once during the first week of fermentation for 0.00, 0.02, 0.03, 0.04, 0.10, 0.20% (w/v) urea supplement, respectively. The low concentration of $NaHCO_3$ was added when 0.00 and 0.20% (w/v) urea was operated compared to other urea additions. Although 0.2% (w/v) urea needed as much $NaHCO_3$ as control but the alkalinity of 0.2% (w/v) urea was higher than control due to excess ammonia contributed to the increased alkalinity of the experiment digester.

For volatile acids accumulated in the digester, concentrations of propionic and butyric acids were higher than acetic acid for all experiments. The accumulative acetic, propionic, and butyric acids were in the ranges of 0-24.73, 106.82-219.14, and 0-402.30 ppm, respectively (Figure 26). The low concentration of acetic acid accumulated probably resulted from the bacterial utilization to form methane.

A

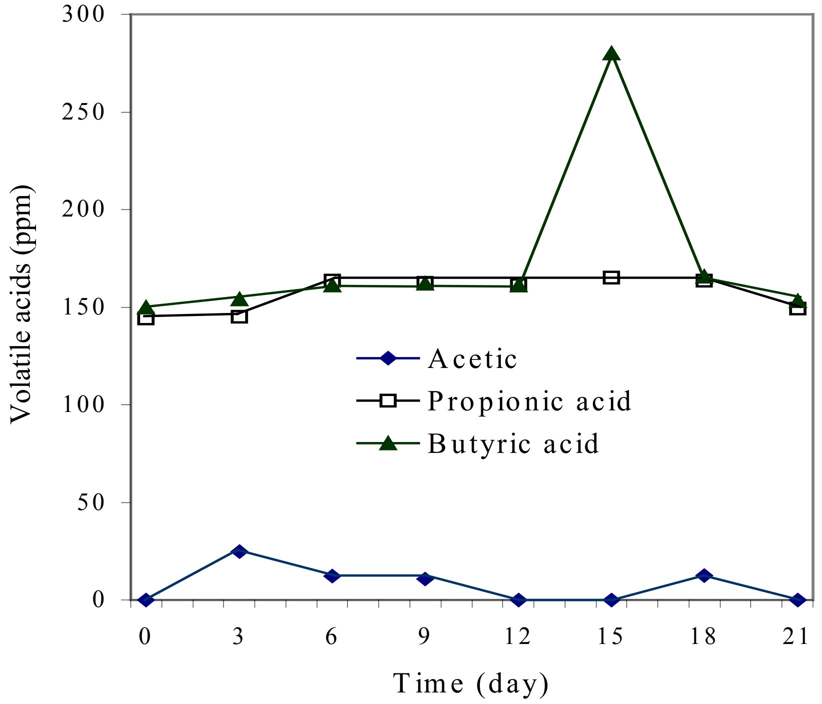
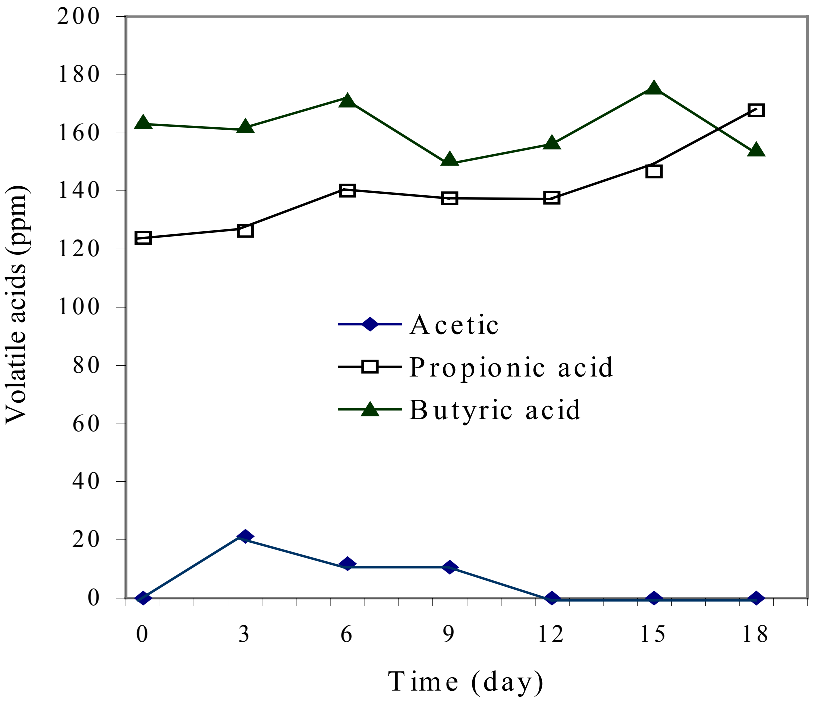


Figure 26. Volatile acids accumulation during cassava tuber fermentation using 1.00% (w/v) total solids and urea supplement at various concentrations:(A) 0.00, (B) 0.02, (C) 0.03, (D) 0.04, (E) 0.10, and (F) 0.20% (w/v), in the single-state digester of 5-L working volume.

B



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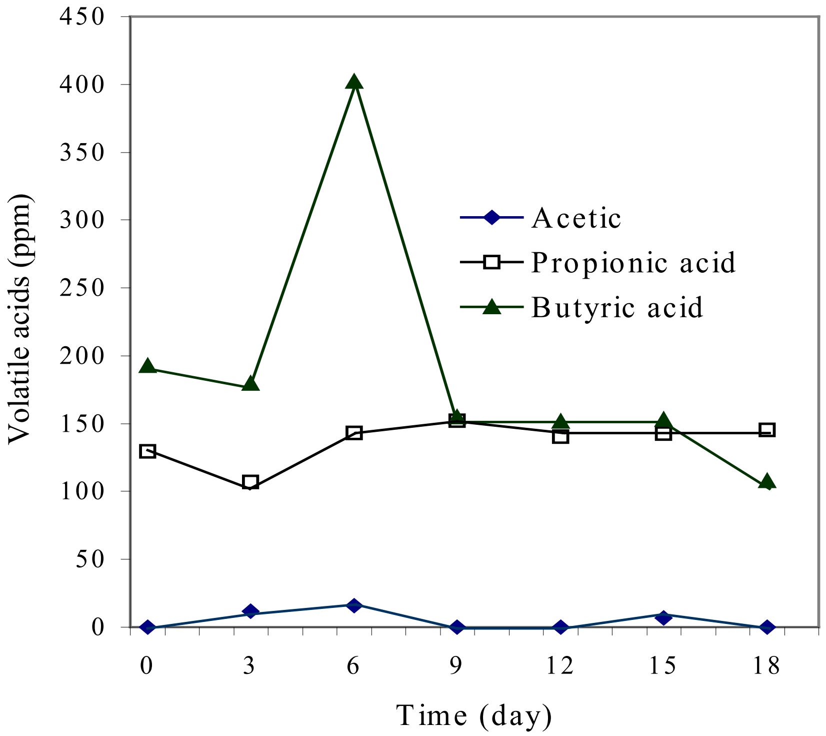

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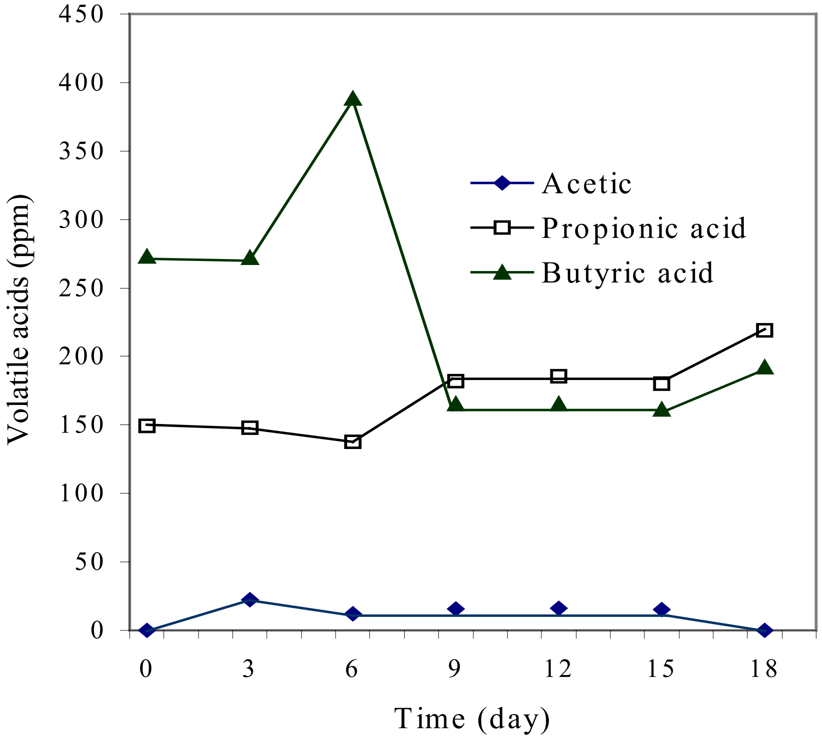

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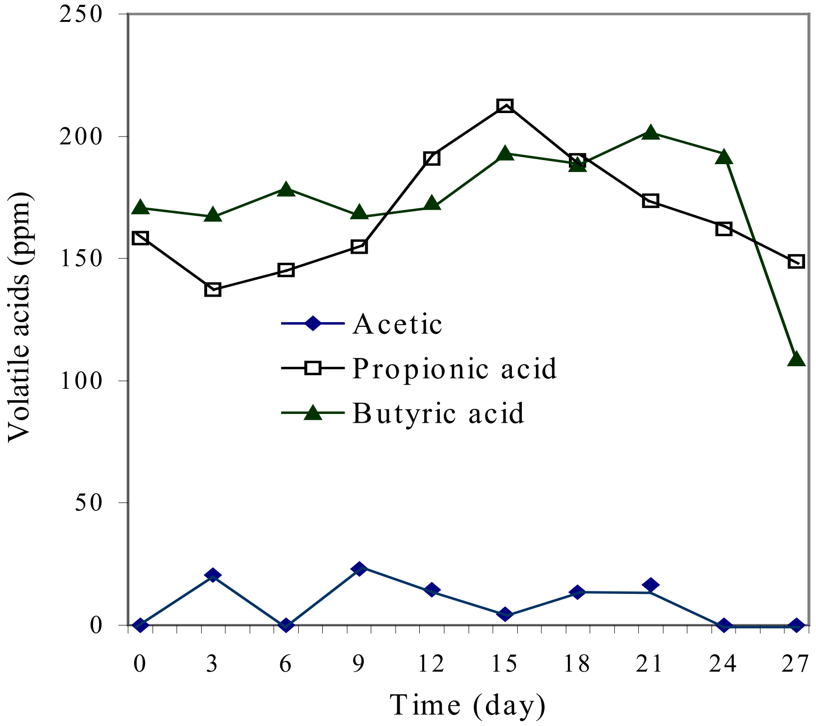

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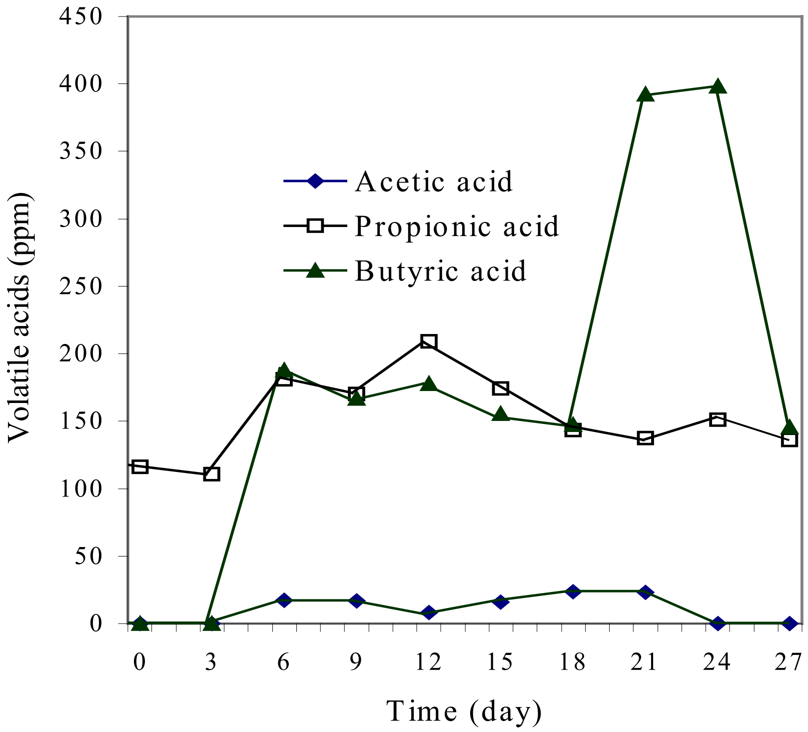


Figure 26. (Continued)

Urea supplement at 0.04% (w/v) resulted in an increase rate of the biogas production. The higher biogas yields, the higher methane production, and the greater reduction of VS (Figures 23 and 24). The addition of carbon-to-nitrogen ratio was expressed as 20:1. These results were supported by Polprasert (1989) and Sanders and Bloodgood (1965) who reported that the optimum carbon to nitrogen ratio was between 20 and 30:1. In addition, Pohland and Bloodgood (1963) stated that if the carbon-to-nitrogen ratio exceeded 16:1, the capacity of microorganisms for organic digestion would not increase. The gas generation failed whenever the carbon-to-nitrogen ratio was higher than 52:1 (Sander and Bloodgood, 1965). For the production of biogas from cassava tubers, the addition of urea higher and lower than

0.04% (w/v) had adverse effect on various biogas production parameters. Increasing amount of urea to about 0.10 and 0.20% (w/v) caused the decrease in biogas production. This might be attributed to ammonia inhibition at higher urea addition (lower carbon to nitrogen ratio) (Sterling *et al*., 2001).

4.2.3 Biogas production using the optimum conditions and a single-state digester of 5-L digestion volume

The biogas productions were performed in the simple single-state digester of 5-L digestion volume at ambient temperature (29-31°C). Optimum concentrations of both TS (1.00%, w/v) and urea (0.04%, w/v) (20:1 of carbon-to-nitrogen ratio) were applied the simple single-state digester with working volume of 5 L. The yield of total biogas of 530.96 L/kg TS fed was obtained (Table 7). The gas yield of 1.95 L/day containing the maximum methane content of 67.92% was achieved at 10-day retention time (Figures 27, 30, and 31). The utilization of VS was 56.83% (Table 7). But the fermentation reactions were ceased after 16-day operation. Time required for the anaerobic digestion depended on the temperature of digestion (Bunchueydee, 1984). The biogas production rate could be stimulated at high temperature more than low temperature. Thus, the digestion time of this experiment (29-31°C and 16-day retention time) was 3 days shorter than previous experiment (24-27°C and 19-day retention time). The average methane content for overall reactions of 5 L was 49%. These single-state digesters converted at least 70% of the starch to biogas (Figure 27).

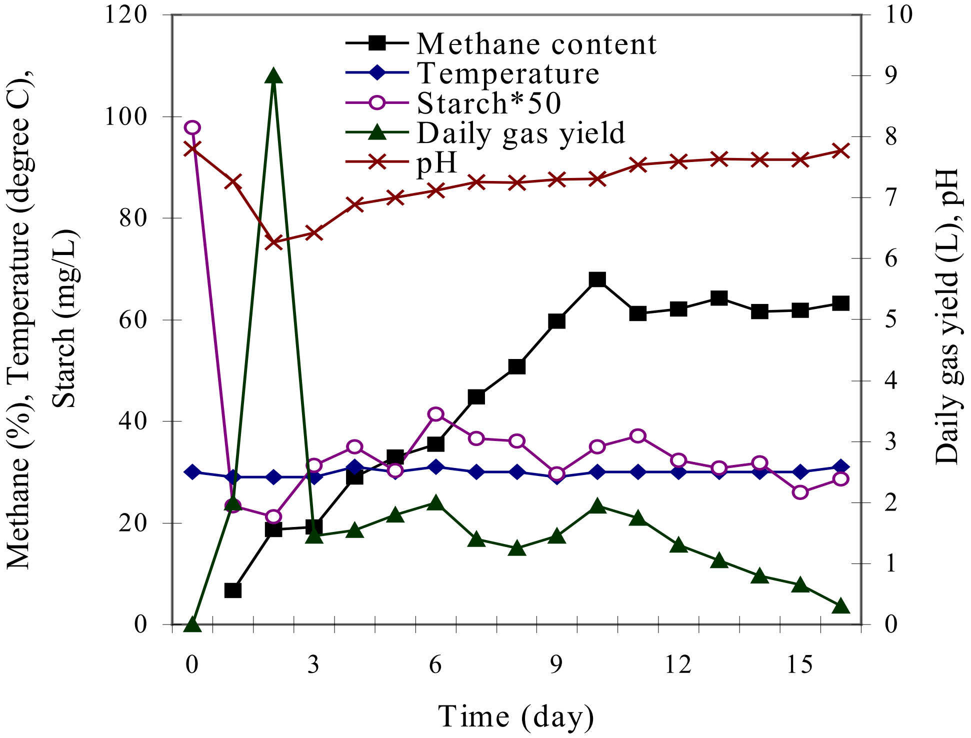


Figure 27. Biogas production from cassava tubers using 1.00% (w/v) total solids and 0.04% (w/v) urea supplement in the single-state digester of 5-L digestion volume.

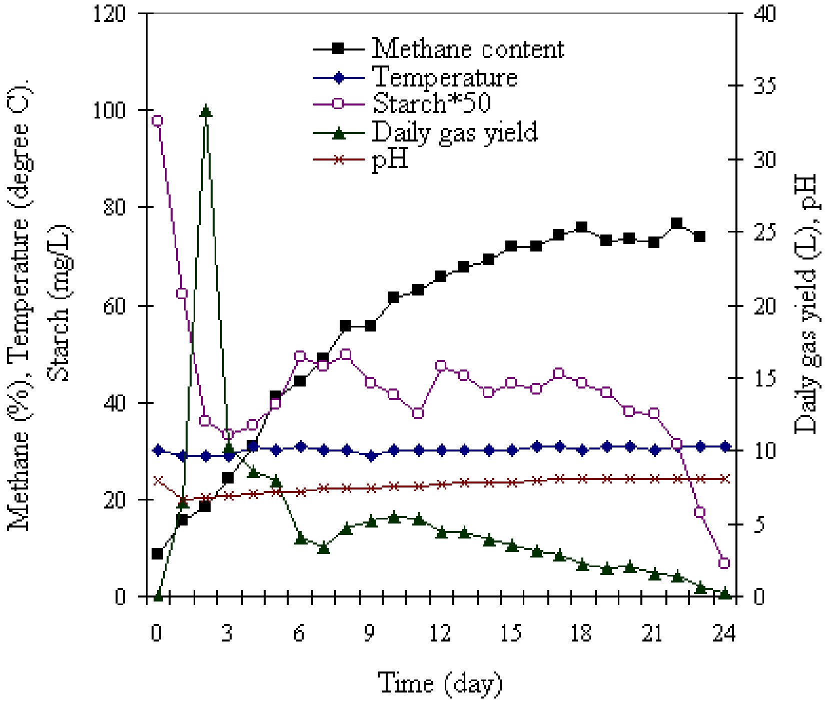
4.2.4 Biogas production using scaled-up digesters of 20-L and 50-L digestion volumes

To investigate the amount of the biogas production rates from raw cassava tubers when the digesters were scaled up to the large scales, the anaerobic digesters of 20-L and 50-L working volumes (Figure 28) were used. When the optimal concentrations of TS (1.00%, w/v) and urea addition of 0.04% (w/v) were applied to the scaled-up experiments of 20-L reaction volume, the gas yield of 5.50 L/day containing 55.70% methane was obtained at 10-day retention time form working volume of 20 L (Figures 29A, 30, and 31). Whereas the methane content of 67.57 % and the gas yield of 3.88 L/day were obtained at 14-day retention time (Figures 29A, 30, and 31). The VS reduction was 61.51% (Table 7). The fermentation reactions were ceased after 24-day operation. When the digester was scaled up to 50 L, the maximum gas yield of 24.40 L/day and the methane composition of 68.65% were obtained at 10-day retention time (Figures 29B, 30, and 31). Whereas the methane content of 69.79% and the gas yield of 9.95 L/day were obtained at 14-day retention time (Figures 29B, 30, and 31). The utilization of VS was 61.98% (Table 7). The fermentation reactions were ceased after operating for 21 days. The average methane contents for overall reactions of 20-L and 50-L digestion mixtures were 57.57 and 57.21%, respectively. These single-state digesters converted at least 80% of the starch to biogas (Figure 29).

A **B**

Figure 28. Single-state digesters of (A) 20-L and (B) 50-L working volumes.

A



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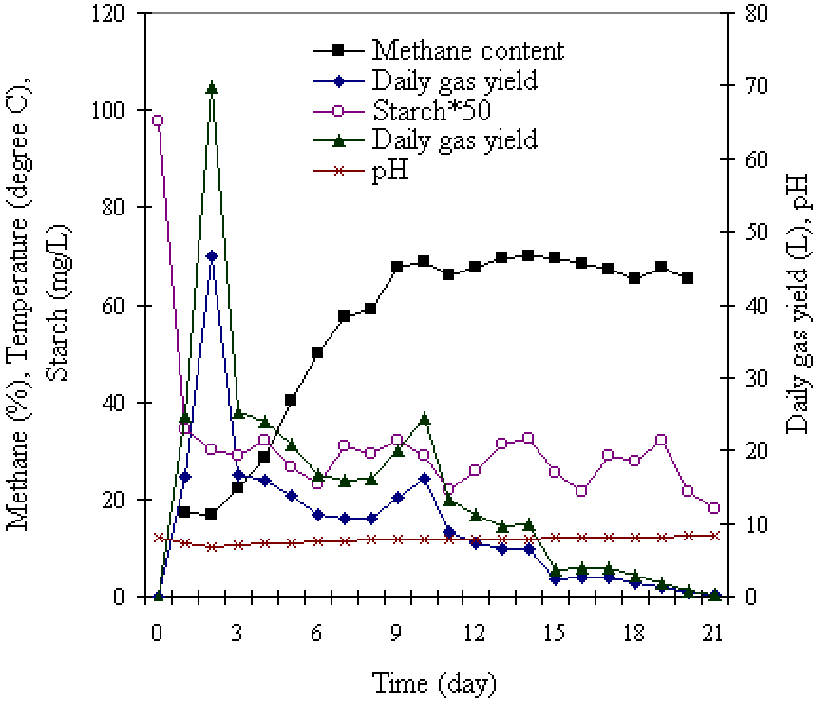

Figure 29. Biogas production from cassava tubers using 1.00% (w/v) total solids and 0.04% (w/v) urea supplement in the single-state digesters of (A) 20-L and (B) 50-L digestion volumes.

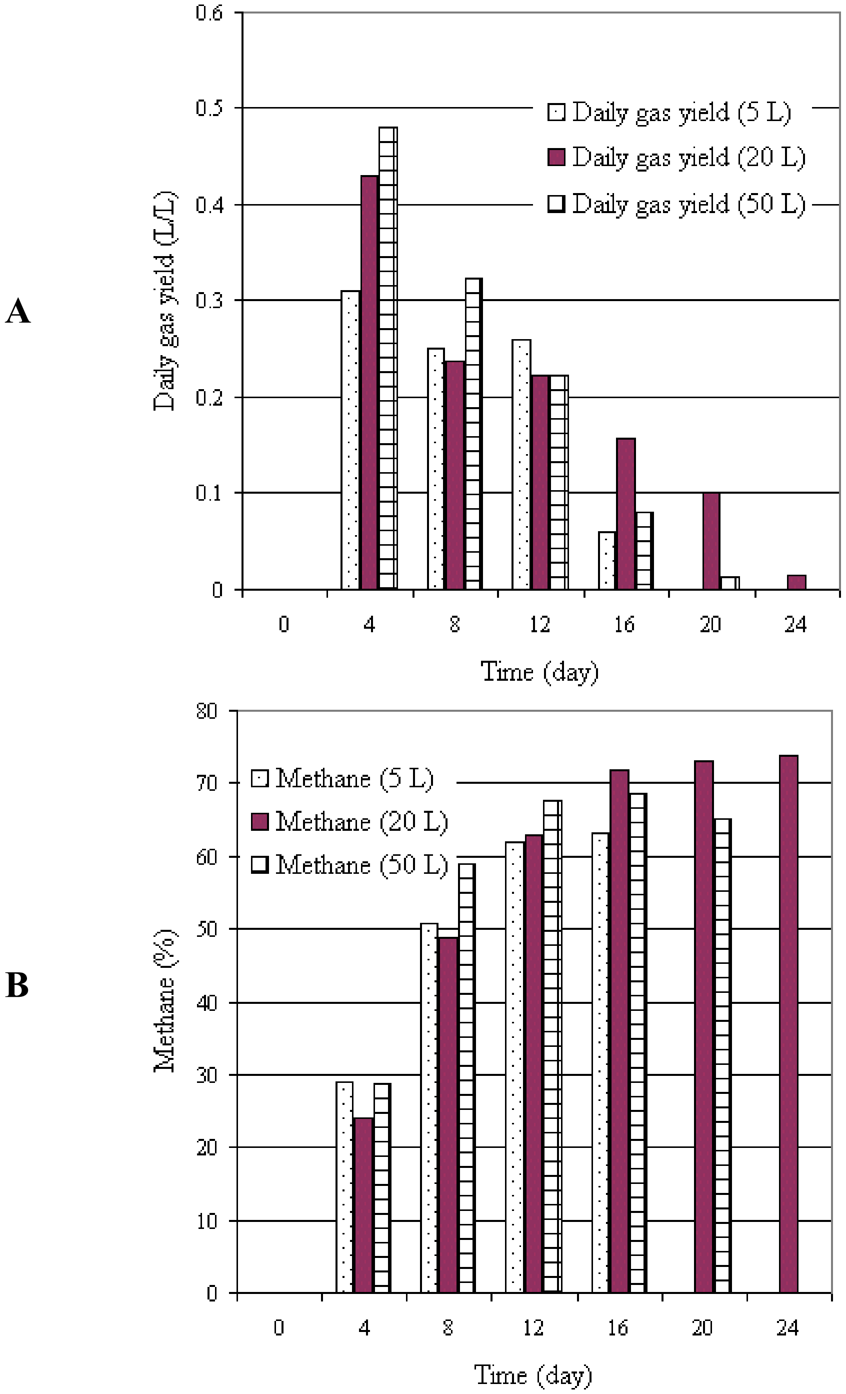

Figure 30. Biogas production from cassava tubers using 1.00% (w/v) total solids and 0.04% (w/v) urea supplement in the single-state digesters of 5-L, 20-L, and 50-L digestion volumes. (A) Daily gas yield and (B) methane content.

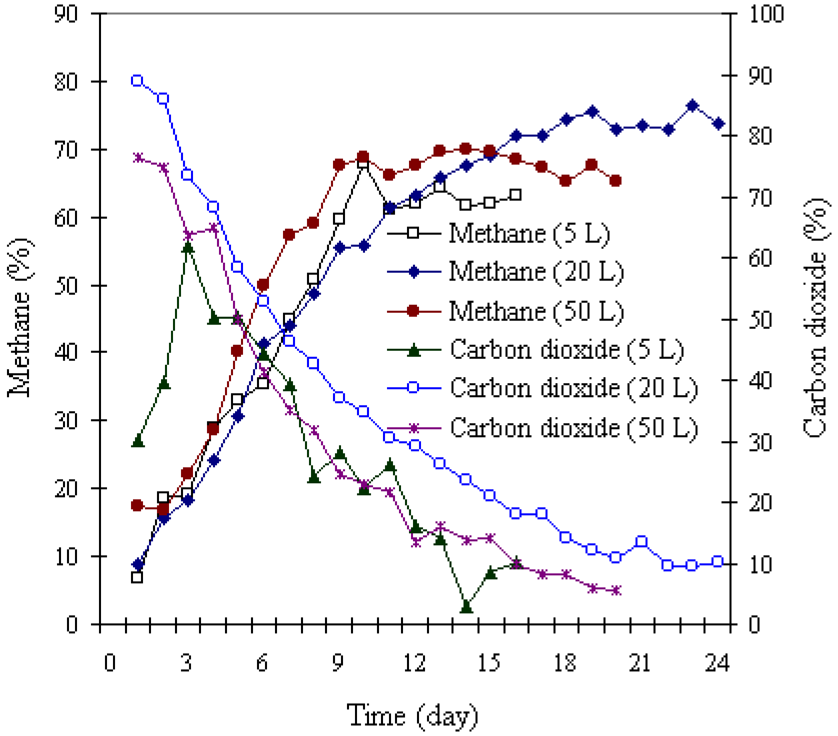

Figure 31. Methane and carbon dioxide composition of gas measured during cassava tuber fermentation in the single-state digesters of 5-L, 20-L, and 50-L working volumes.

The comparison of total biogas and total methane yields, and VS reduction from three bioreactor sizes were performed (Table 7). The biogas yields of 5 L, 20 L, and 50 L were 530.96, 580.41, and 564.29 L/kg TS fed, respectively. The maximum yield of total biogas was obtained from 20-L working volume. When working volume of 20 L was applied, the total biogas yield was 8.52 and 2.78% higher than 5 L and 50 L, respectively. The highest VS reduction was achieved from 50 L (61.98%) followed by 20 L (61.51%), and 5 L (56.83%). Whereas the maximum of total methane yield of 334.14 L/kg TS fed was achieved from 20 L followed by 50 L

(322.83 L/kg TS fed) and 5 L (259.32 L/kg TS fed) (Table 7). When the fermentation was scaled up from 5-L to 20-L and 50-L working volumes the efficiency of digesters is slightly increased. Whereas the comparison of total biogas yield from the first day operation to 16-day retention time from three bioreactor sizes were presented in Figure 32. The biogas yield of 5 L, 20 L, and 50 L were 530.96, 517.40, and 546.00 L/kg TS fed, respectively. The maximum yield of total biogas was obtained from 50-L working volume. When working volume of 50 L was applied, the total biogas yield was 2.75 and 5.24% higher than 5 L and 20 L, respectively. The maximum of total methane yield of 299.15 L/kg TS fed was achieved from 50 L followed by 5 L (259.32 L/kg TS fed) and 20 L (252.70 L/kg TS fed) (Figure 32)

.

Table 7. Biogas production from cassava tubers in laboratory scale experiments.

Parameters	Reaction volume (L)		
	5	20	50
Total biogas yields (L/kg TS fed)	530.96±3.13	580.41±4.02	564.29
Total biogas yields (L/kg VS fed)	440.52±3.13	481.55±4.02	468.18
Total methane yields (L/kg TS fed)	259.32±4.94	334.14±3.29	322.83
Volatile solid reduction (%)	56.83±0.36	61.51±0.52	61.98

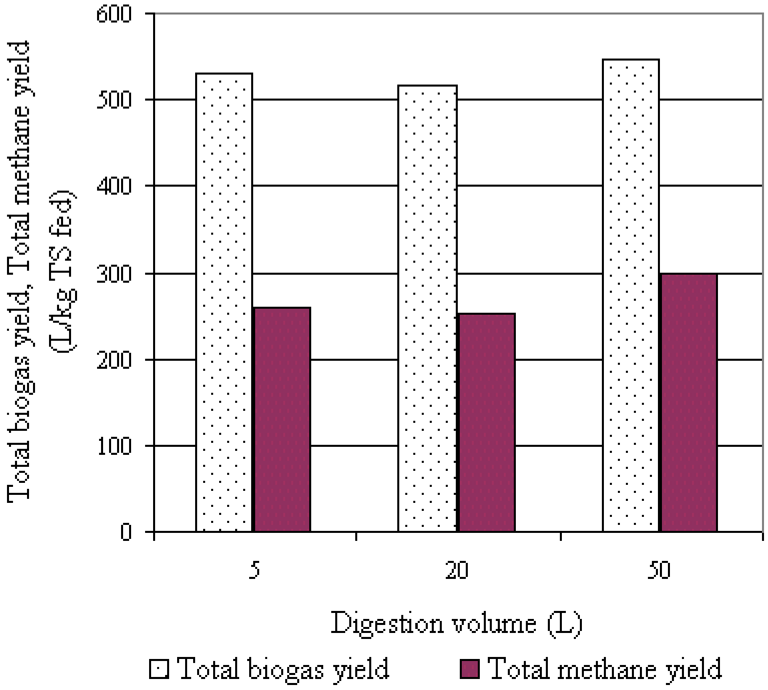

Figure 32. Total gas yields and total methane yields of 5-L, 20-L, and 50-L working volumes monitored during 0 to 16-day retention times.

When the digester was initially fed, acid-forming bacteria quickly produced VFA resulting in declining pH below the neutral pH and diminishing growth of methanogenic bacteria and methanogenesis. The pH could be maintained by adding $NaHCO_3$ to increase alkalinity concentration. In this study, 0.25% (w/v) $NaHCO_3$ was added four, four, and three times during the first week of fermentation for bioreactor sizes of 5 L, 20 L, and 50 L, respectively. Afterwards the digester could maintain themselves (Figures 33 and 34). At daily methane content of more than 50%, digesters 5 L, 20 L, and 50 L cassava tuber slurry, respectively, operated at the pH ranges of 7.2 to 7.8, 7.4 to 8.1, and 7.6 to 8.2 with the alkalinity concentrations of 7000 to 7550, 6800 to 9400, and 6700 to 8000 mg as $CaCO_3$/L, VFA concentrations of 1585 to 4218, 2250 to 4350, and 1550 to 2400 mg as acetate/L, and VFA-to-alkalinity ratios of 0.6 to 0.2, 0.6 to 0.2, and 0.5 to 0.2 (Figures 27, 29, 31, 33, and 34). The anaerobic reactors were operated with VFA-to alkalinity ratio suitable for methanogenic bacterial activity, showing that there was no accumulation of volatile acids (Figures 33 and 35). The maximum methane contents of biogas for working volumes of 5 L, 20 L, and 50 L, were obtained from the VFA-to-alkalinity ratios of 0.5, 0.5, and 0.4, respectively (Figures 27, 29, 31, and 33).

Volatile acids (acetic, propionic, and butyric acids) accumulation during cassava tuber fermentation was detected (Figure 35). Acetic acid was known to be the immediate precursor of approximately 70% of all methane formed during the digestion and the rest (30%) was formed from carbon dioxide and hydrogen (Aiman *et al.*, 1981). Similarly, propionic acid was the immediate precursor to approximately 70% of acetic acid (Hill *et al.*, 1987). The concentrations of propionic and butyric acids were higher than that of acetic acid in three digester sizes (Figure 33). The maximum accumulation was found for butyric acid followed by propionic and acetic acids. Hill *et al.* (1987) proposed that acetic acid level in excess of 800 mg/L indicated digester failure. The acetic acid concentration ranges of 0 to 56.72, 0 to 11.44, and 0 to 46.93 ppm were obtained from 5-L, 20-L, and 50-L digestion volumes, respectively. The low accumulation of acetic acid could imply the successful methane production.

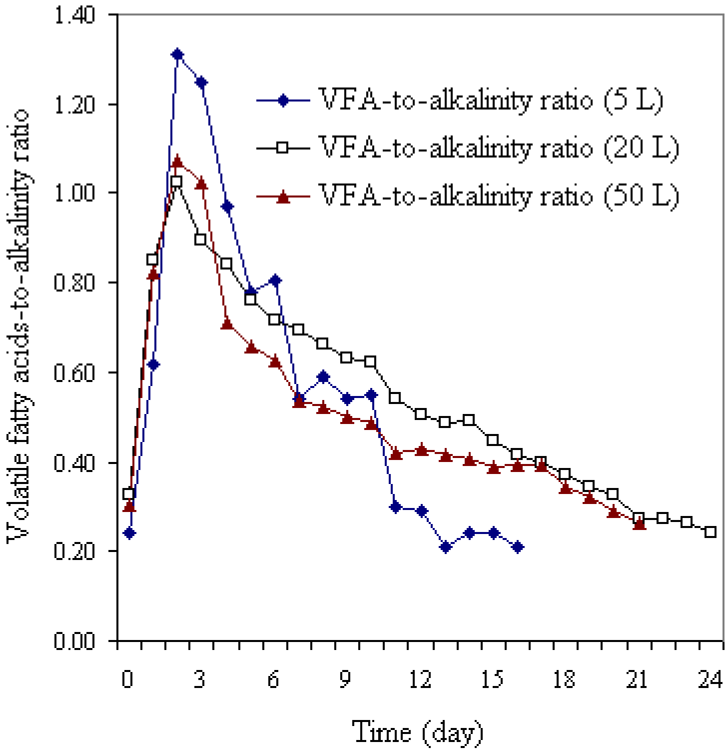

Figure 33. Volatile fatty acids-to-alkalinity ratio measured during cassava tuber fermentation in the single-state digesters of 5-L, 20-L, and 50-L working volumes.

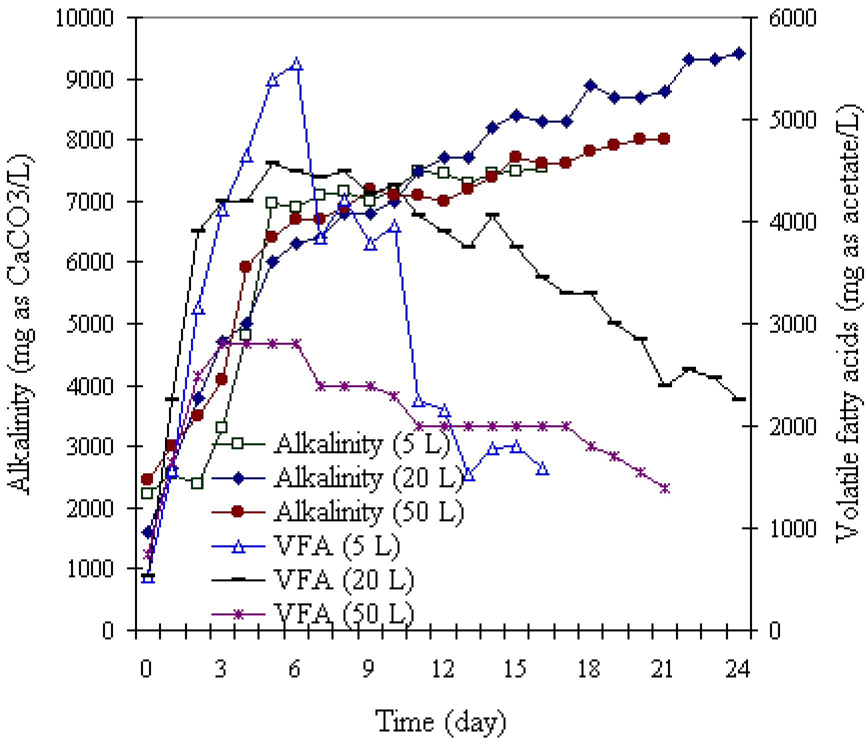

Figure 34. Alkalinity and volatile fatty acids measured during biogas production from cassava tubers in the single-state digesters of 5-L, 20-L, and 50-L working volumes.

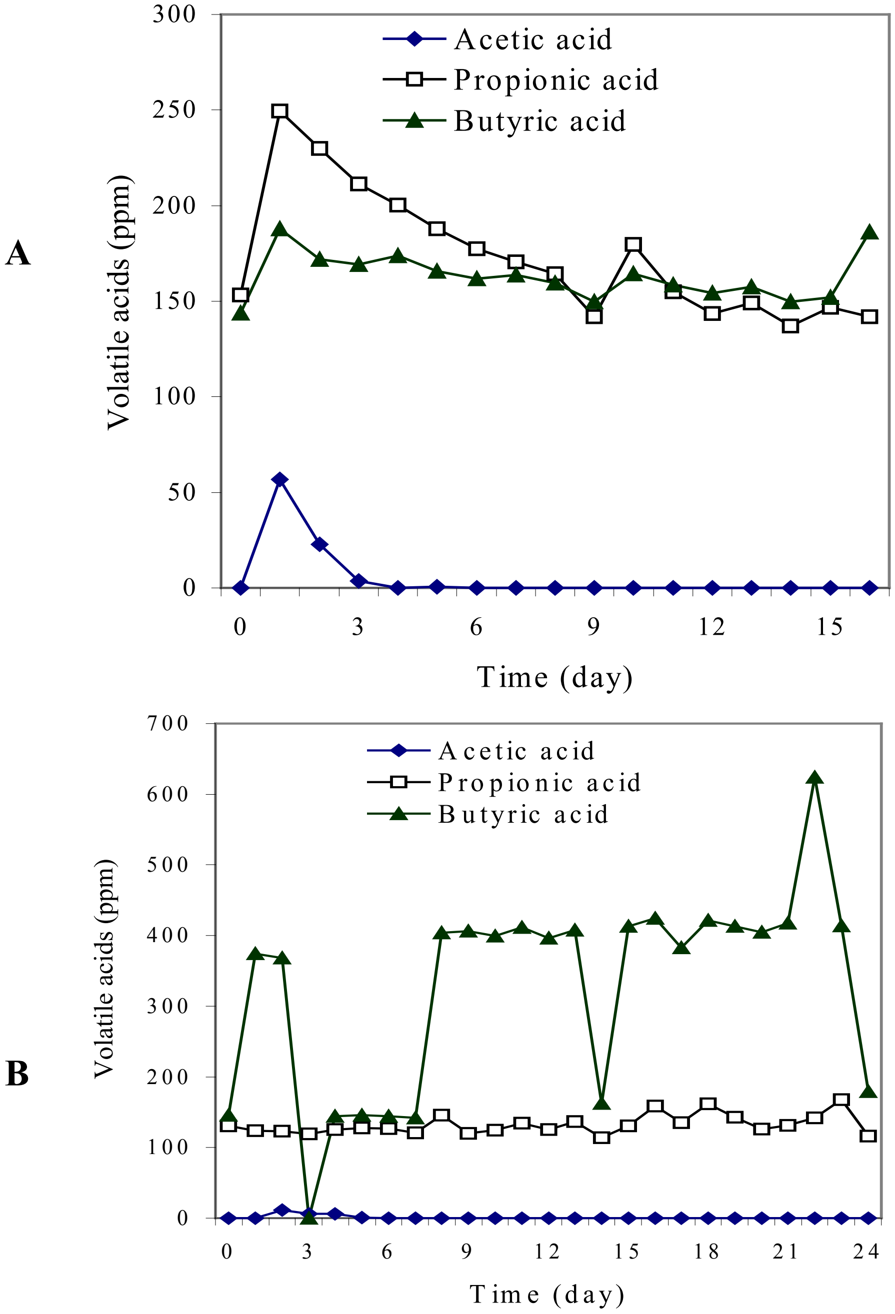

Figure 35. Volatile acids accumulation during cassava tuber fermentation in the single-state digesters of (A) 5-L, (B) 20-L, and (C) 50-L working volumes.

C

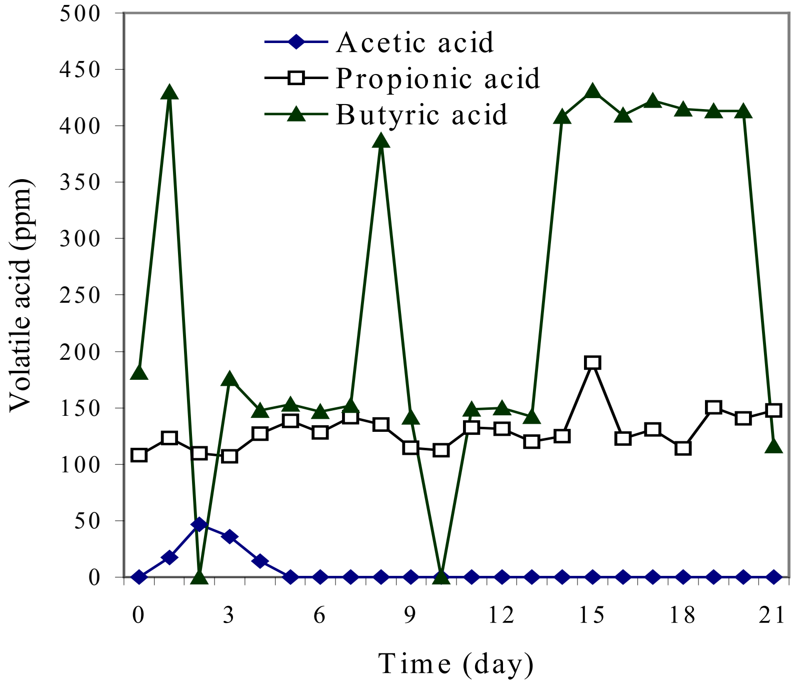

Figure 35. (Continued)

The maximum yield of total biogas was achieved from 50-L digestion volume at 16-day retention time (546.00 L/kg TS fed with 54.79% of average methane content). Thus, the results from 50 L would be chosen to basically calculate the capital cost of production. One kg TS of dry cassava tuber was obtained from 1.23 kg of the total dry mass prepared from the whole tuber (containing 18.65% of moisture). And one kg of the dry cassava mass achieved from 2.11 kg of fresh cassava tuber (containing 61.66% of moisture). Thus, one kg TS of dry cassava tuber was obtained from 2.60 kg of fresh cassava tubers. From these calculation results, one kg of dry cassava tuber could be biologically converted to 443.90 L of biogas, and one kg of fresh cassava tuber produced 210.00 L biogas. In comparison to the biogas yield reported by Cuzin *et a*l. (1992), the biogas production from cassava peels using a pilot plug flow digester gave the gas yield of 65 L/kg cassava roots, with a peeling yield of 30%. The average methane content of the biogas was 57%. Total methane yield of 299.15 L/kg TS fed was obtained from 50-L working volume. Thus, one gram of TS could convert to 0.299 L of methane. Specific volume of methane was 1.47 L/g (Kunawanakit, 1986). Thus, methane of 0.20 g obtained from 1 g TS. From this calculation result, one g of dry cassava tuber could be biologically converted to 0.17 g of methane.

The theoretical biogas yield from carbohydrates has been reported to be 886 L/kg VS fed (Burford and Varani, 1976). From our experiments using cassava tuber, the total biogas yields per kg VS fed throughout the anaerobic digestion were 440.52 L, 481.55 L, and 468.18 L from 5-L, 20-L, and 50-L digestion volumes, respectively (Table 7). The total biogas yields of biogas production from cassava tubers were low when compared with the theoretical biogas yields because some components in cassava tubers could not be converted into biogas. Carbohydrates were only the predominant components form, in comparison to the biogas yields that have been reported by National Research Council (1977), the biogas yields of 333, 390, 480, 470, 455, and 310 L/kg VS fed were obtained from flax straw, grass clippings, potatoes, papyrus, rice straw, and wheat straw. From our experiment, the higher gas yield of 440.52-481.55 L/kg VS fed has been recorded. The relatively higher biogas yield might be due to dry cassava tuber had a high carbon source in term of TS for microorganism growth involved in the biogas production. And urea supplement improved the rate of microbial growth. The study showed an effective bioconversion of cassava tubers to biogas.

Biogas provided energy for cooking, lighting, and fuel. It could be burnt when methane content was at least 45% but it would not be burnt when methane content was less than 45% (University of Florida, 2002). When 5-L digestion volume was performed for biogas production for raw cassava tubers, the average methane content accumulated in the digester was higher than 45% after operating for 15 days. While 20-L and 50-L digestion volumes were applied, the average methane content were higher than 45% after operating for 16 and 12 days, respectively. Whereas daily methane contents of 5-L, 20-L, and 50-L digestion volumes were higher than 45% after operating for 8, 11, and 6 days, respectively. From the results, biogas produced from cassava tubers at 1.00% (w/v) TS with 0.04% (w/v) urea supplement in batch digester could be properly used for heating, cooking, and lighting.

Thus, the results obtained on biomethanation of cassava tubers suggest their potential and suitability for economically viable biogas technology through anaerobic digestion. The energy generated in the form of methane when utilized efficiently not only provides alternative energy source but also improves the utilization and production of cassava tubers.

CHAPTER V

CONCLUSIONS

Cassava tubers from 4 varieties; CMC 76, KU 50, Rayong 60, and Rayong 90, were collected from their plantation areas in Nakhon Ratchasima Province. The fresh tubers had the average contents of 65% of moisture, 18% of starch, 17% of total carbon, 0.20% of total nitrogen, and 35% of total solids. For biogas production using the simple single-state digester, the fresh tubers of variety KU 50 were chosen to be used as a raw material. The tuber had approximately 18% of starch, 62% of moisture, 0.9% of ash, and 0.08% of phosphorus. For feeding into the biogas bioreactor, the dry cassava tuber sample containing 18.65% of moisture content, was prepared. The dry material had 81% of TS, 98% of VS, 1.95% of ash, 38% of starch, 40% of total carbon, 0.5% of total nitrogen, and 0.2% of phosphorus. The average carbon-to-nitrogen ratio of the dry cassava tuber sample was 86:1. Sixty-four grams of the raw material were added into the digester of 5-L working volume to obtain 1.00% (w/v) TS. Ten percents (v/v) of seed cultures were inoculated into the digester. The anaerobic digestion was performed at ambient temperature for 30 days. It was found that the total biogas yield of 355.57 L/kg TS fed was achieved. The gas yield of 1.60 L/day containing the maximum methane content of 67.17% was obtained at 19-day retention time. The fermentation reactions were ceased after operating for 25 days. To obtain the optimal concentration of TS, which referred to the amount of raw cassava tubers, for biogas production, various TS concentrations: 0.25, 0.50, 1.00, 2.00, 4.00, 8.00, and 16.00% (w/v), were applied to the simple single-state digester with working volume of 5 L. The biogas production was also performed at ambient temperature (22-30°C) for 30 days. The maximum yield of total biogas of 356.35 L/kg TS fed was achieved from 1.00% (w/v) TS. The biogas yield of 1.20 L/day containing 54.91% methane was obtained at 19-day retention time. Whereas the maximum methane content of 64.35% and the biogas yield of 0.60 L/day were obtained at 22-day retention time. The fermentation reactions were ceased after operating for 25 days. Then, various urea concentrations: 0.00 (control), 0.02, 0.03, 0.04, 0.10, and 0.20% (w/v), were added to the digestion slurry to maximize the biogas production. The biogas production was performed in the simple single-state digester with working volume of 5 L at ambient temperature. It was found that urea stimulated the biogas production. At this experiment period, temperatures of fermenting slurry fluctuated between 23 and 27°C. The maximum yield of total biogas of 561.38 L/kg TS fed was obtained when urea (0.04%, w/v) was applied. For this urea supplement, the biogas yield of 1.98 L/day with the maximum methane content of 66.13% was obtained at 10-day retention time. But the fermentation reactions were ceased after operating for 19 days. When optimum concentrations of both TS (1.00%, w/v) and urea (0.04%, w/v), which could represent as carbon-to-nitrogen ratio of 20:1, were applied to produce biogas in the simple single-state digester with working volume of 5 L at ambient temperature, the total biogas yield of 530.96 L/kg TS fed containing the total methane yield of 259.32 L/kg TS fed was obtained. Temperatures of fermenting slurry varied from 29 to 31°C. The fermentation reactions were ceased after operating for 16 days, which was shorter than the previous experiments. This could be because of temperature effects. The fermentation rate could be stimulated at high temperature (29-31°C) more than lower temperature (23-27°C). When the digesters were scaled up to 20-L and 50-L reaction volumes, the total biogas yields of 517.40 and 546.00 L/kg TS fed containing the total methane yields of 252.70 and 299.15 L/kg TS fed respectively were obtained at 16-day retention time. The fermentation reactions of the two digester sizes were ceased after operating for 24 and 21 days, with the total biogas yields of 580.41 and 564.29 L/kg TS fed containing the total methane yields of 334.14 and 322.83 L/kg TS fed respectively. When the potential production of biogas using three sizes of bioreactors was compared at 16-day retention time, the total biogas yield obtained from 50-L working volume was 2.75 and 5.24% higher than the yields obtained from 5 L and 20 L respectively. The total biogas yield of 546.00 L/kg TS fed containing the total methane yield of 299.15 L/kg TS fed, which was achieved from 5-L working volume, was chosen to basically calculate the capital cost of only the raw material (raw cassava tuber) for the production of biogas. One kilogram TS of dry cassava tuber was obtained from 1.23 kg of the total dry mass prepared from the whole tuber (containing 18.65% of moisture). And one kg of the dry cassava mass was achieved from 2.11 kg of fresh cassava tuber (containing 61.66 % of moisture). Thus, one kg TS of dry cassava tuber was obtained from 2.60 kg of fresh cassava tubers. One kg of dry cassava tuber could be biologically converted to 443.90 L of biogas, and one kg of fresh cassava tuber produced 210.00 L of biogas. If the energy value of biogas (50-70% of methane) was 22000-26000 kJ/m^3 (National Research Council, 1977), one kg of fresh and dry cassava tubers used as raw materials for the biogas production, could produce energy of 4620 kJ and 9765.8 kJ respectively. The market price of fresh cassava tuber (during 1-15 September 2004) was 1.30 Baht/kg (The Thai Tapioca

Trade Association, 2004). Therefore, the capital cost of only the raw material (fresh cassava tuber) for the production of biogas of 1 m^3 could be 6.19 Baht. One $m^3$of biogas has been reported to equal to 0.46 kg of liquefied petroleum gas (LPG), or 0.67 L of benzene, or 0.60 L of diesel (Energy Policy and Planning Office, 2002). The market prices of LPG, benzene 95, benzene 91, and diesel were approximately 16.80 Baht/kg, 21.79 Baht/L, 20.99 Baht/L, and 14.59 Baht/L, respectively (25 September 2004) (Energy Policy and Planning Office, 2004). In conclusion, one kg of fresh cassava tubers (1.30 Baht) could produce 0.210 m^3 of biogas containing approximately 55% methane, which equals to 4620 kJ or 0.1 kg of LPG, and costs 1.68 Baht; or 0.14 L of benzene 95, and costs 3.05 Baht; or 0.14 L of benzene 91, and costs 2.93 Baht; or 0.13 L of diesel, and costs 1.90 Baht.

This research was focused on the production of biogas as an energy source from raw cassava tubers, a cheap and abundant agricultural product in Thailand. The potential biogas production from raw cassava tubers could be evaluated as mentioned above. Data concerning biogas quantity and quality, biogas production process, and some factors affecting the gas production from cassava tubers were also obtained. Consequently, the utilization and economic value of raw cassava tubers could be increased.

For future investigation of biogas production from raw cassava tubers, based on the obtainable results of this study, TS concentration of 1.00% (w/v) (containing 18.65% of moisture content) and 0.04% (w/v) urea, or the carbon-to-nitrogen ratio of 20:1, could support the maximum biogas yield. Also, during digestion in the simple single-stage digester, approximately 70-80% of total biogas yield was produced in the first 10 days of operation, and methane content was higher than 55%. The results would be useful for increasing productivity of biogas and methane yields using the semi-continuous digester, in which the operation could be run with the organic loading rate at 1.00% (w/v) TS at 10 days of HRT. Moreover, the high concentration of TS (>1%, w/v) could be performed when the two-state digester is introduced. The two-state digester, which consists of the acidogenic and the methanogenic reactors, could be used to increase the efficiency of biogas production. Only organic acids produced from raw cassava tuber would take place in the acidogenic reactor, and the methanogenic reactor is responsible for methane production.

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APPENDICES

APPENDIX A

REAGENTS PREPARATION

1 Reagents for Spectrophotometric Molydate-Vanadate method for the phosphorus analysis

1.1 Solution of mixed acid

Nitric acid (HNO_3)	500.00	mL
Sulfuric acid (H_2SO_4)	100.00	mL
Perchloric acid ($HClO_4$)	200.00	mL

1.2 Molypdate-Vanadate reagents

Ammonium molybdate ($(NH_4)_6 Mo_7 O_{24} .4H_2O$)	25.00	g
Ammonium vanadate (NH_4VO_3)	1.25	g
HNO_3	500.00	mL
Distilled water	500.00	mL

Prepared molybdate solution by dissolving 25 g of $(NH_4)_6 Mo_7 O_{24}.4H_2O$ in 250 mL of distilled water with warming, and adjusted volumes to 500 mL using distilled water. Dissolved 1.25 g of NH_4VO_3 in 500 mL of 1 N HNO_3, and gradually added molybdate solution to vanadate solution with stirring.

1.3 HNO_3 (2 N)

HNO_3	130.00	mL
Distilled water	870.00	mL

1.4 Standard phosphorus (25 ppm, stock solution)

KH_2PO_4	0.11	g
HNO_3	2.00	mL
Distilled water	1.00	L

Dissolved 0.11 g of KH_2PO_4 in 1.00 L of distilled water. Dispensed 0, 1, 2, 3, 4, 5, and 6 mL of stock solution to each 25-mL test tube then added 2.00 mL of 2 N HNO_3 to obtain 0, 2.5, 5.0, 7.5, 10.0, 12.5, and 15.0 ppm of phosphorus, respectively.

2 Solution for the determination of alkalinity and volatile fatty acids

2.1 H_2SO_4 (0.1 N)

H_2SO_4	2.80	mL
Distilled water	997.20	mL

3 Solution for the determination of starch concentration

3.1 Standard starch solution

Soluble starch	1.00	g
Distilled water	100.00	mL

Dissolved 1.00 g of soluble starch in 100 mL of distilled water to obtain to 1.00% (w/v) of starch, heat nearly to boiling point to make starch slurry. After cooling, 0.100, 0.067, 0.050, 0.040, 0.033, 0.025, and 0.020 mL of starch slurry were diluted with 9.900, 9.933, 9.950, 9.960, 9.967, 9.975, and 9.980 mL of distilled water to obtain concentrations of 0.100, 0.067, 0.050, 0.040, 0.033, 0.028, and 0.020 mg/L, respectively.

3.2 Iodine solution

Potassium iodide (KI)	6.60	g
Iodine (I_2)	0.66	g
Distilled water	165.00	mL

Dissolved 6.60 g of KI and 0.66 g of I_2 in 165.00 mL of distilled water.

4 Standard solution for gas chromatography

4.1 Propionic acid (98.0%)

4.2 Butyric acid (99.0%)

4.3 Acetic acid (99.8%)

APPENDIX B

STANDARD CURVES

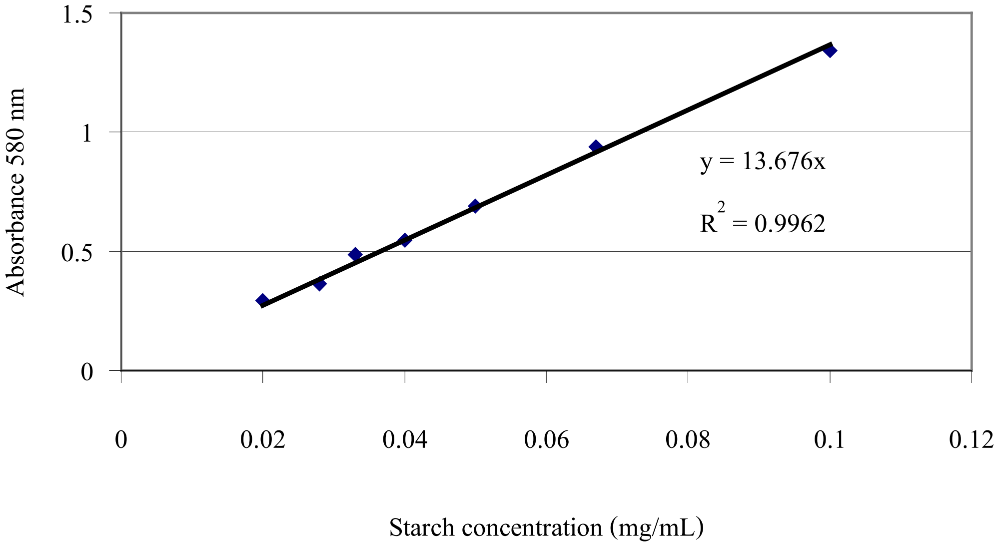


Figure 1B. Standard curve of starch.

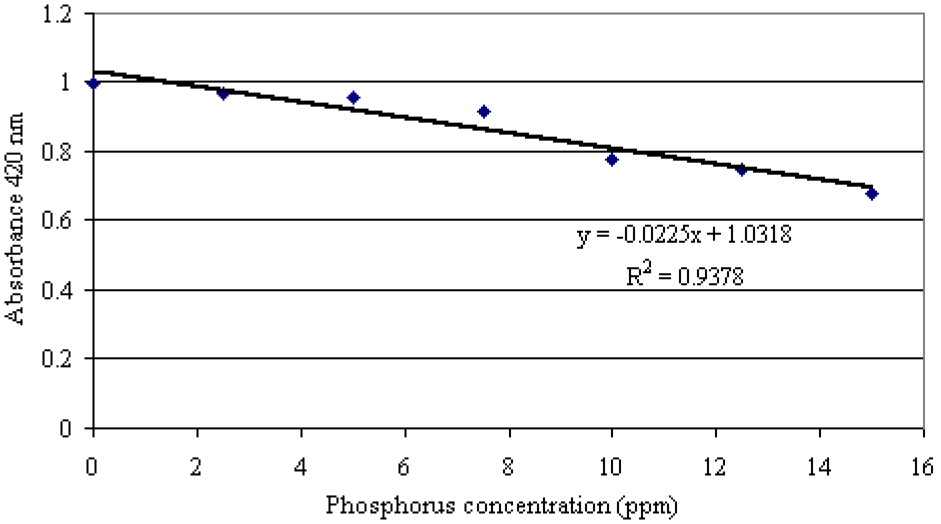


Figure 2B. Standard curve of phosphorus.

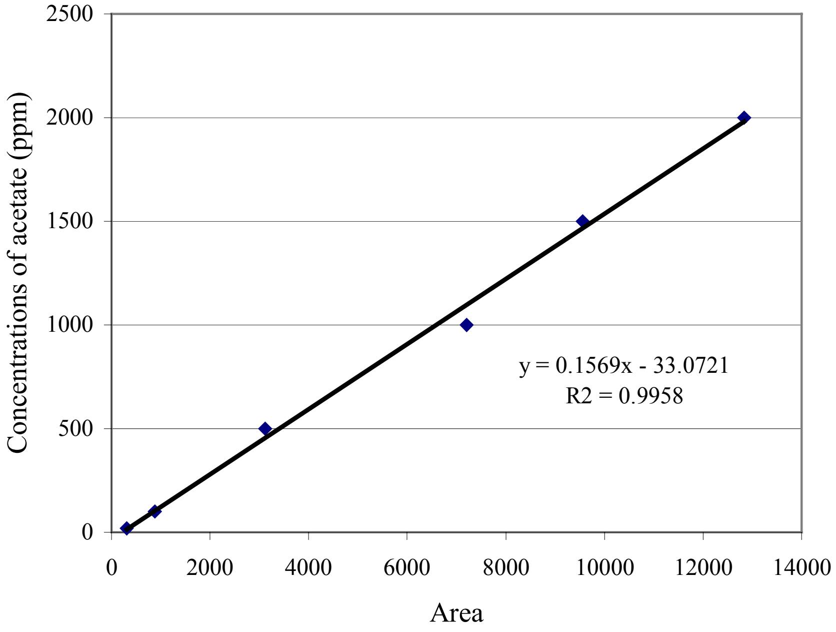


Figure 3B. Standard curve of acetate from gas chromatography analysis.

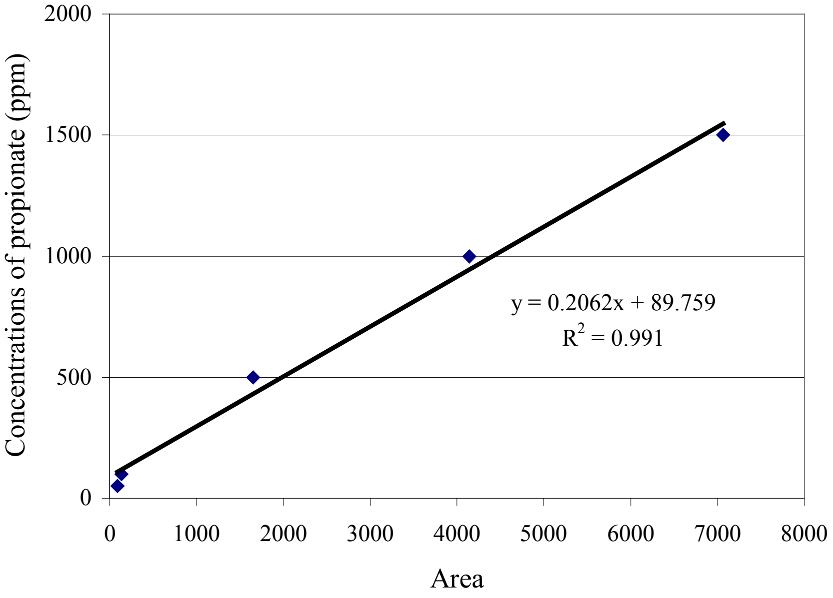


Figure 4B. Standard curve of propionate from gas chromatography analysis.

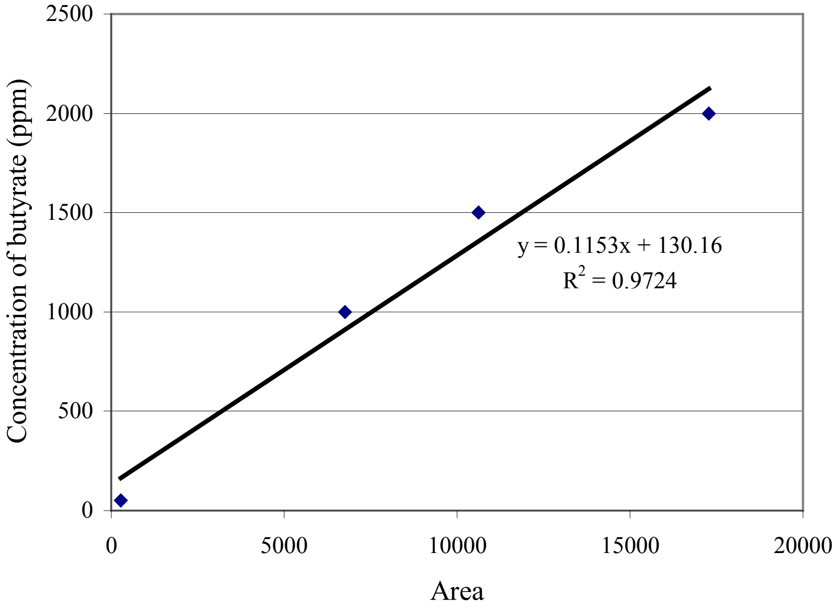
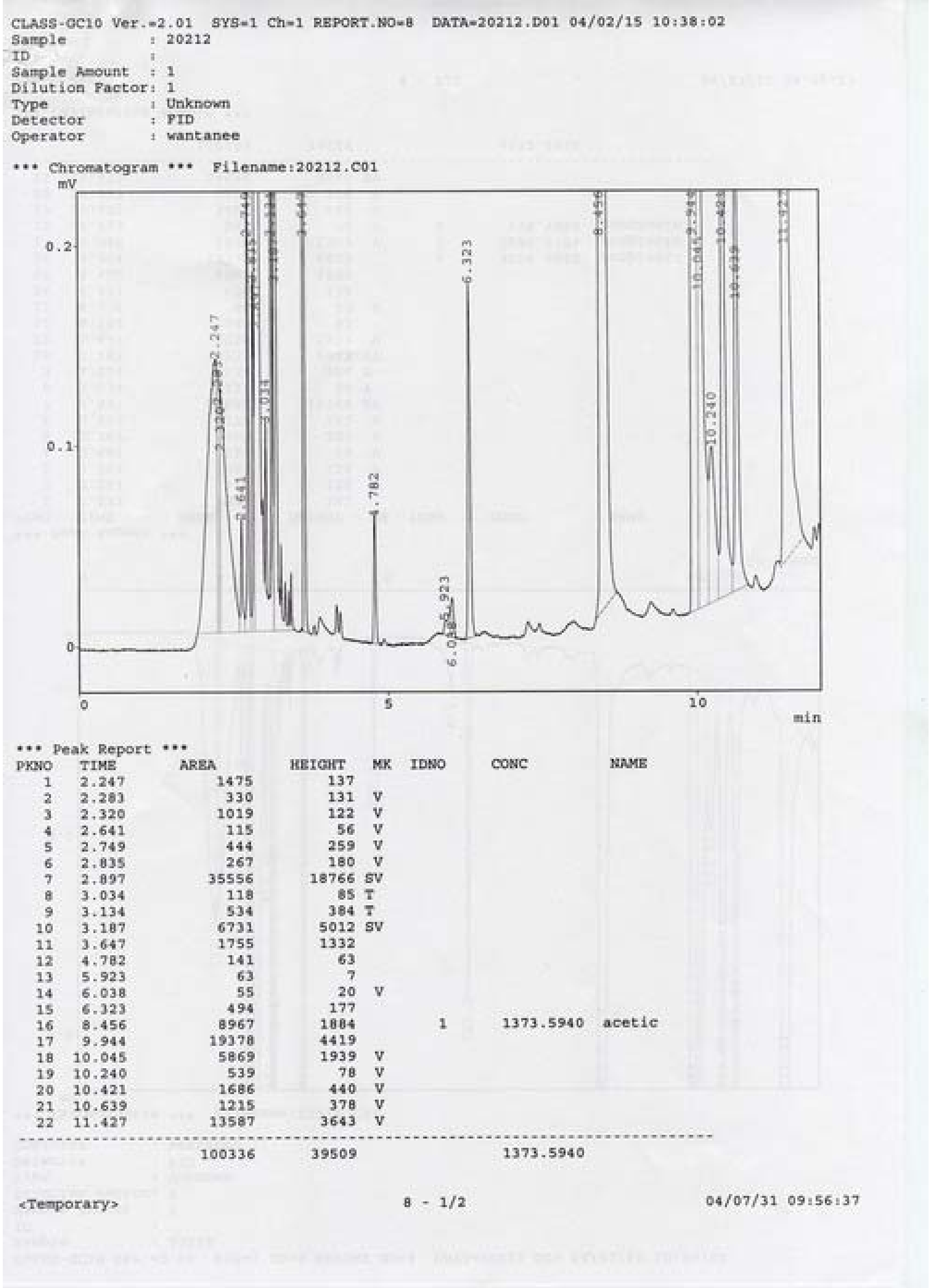


Figure 5B. Standard curve of butyrate from gas chromatography analysis.

APPENDIX C

EXAMPLES OF VOLATILE ACIDS AND GAS COMPOSITION REPORTS, AND VOLATILE ACIDS AND GAS CHROMATOGRAMS



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Detector        : FID
Operator        : wantanee

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   1   2.247        1475         137
   2   2.283         330         131   V
   3   2.320        1019         122   V
   4   2.641         115          56   V
   5   2.749         444         259   V
   6   2.835         267         180   V
   7   2.897       35556       18766  SV
   8   3.034         118          85  T
   9   3.134         534         384  T
  10   3.187        6731        5012  SV
  11   3.647        1755        1332
  12   4.782         141          63
  13   5.923          63           7
  14   6.038          55          20   V
  15   6.323         494         177
  16   8.456        8967        1884        1     1373.5940  acetic
  17   9.944       19378        4419
  18  10.045        5869        1939   V
  19  10.240         539          78   V
  20  10.421        1686         440   V
  21  10.639        1215         378   V
  22  11.427       13587        3643   V
-----------------------------------------------------------------------------
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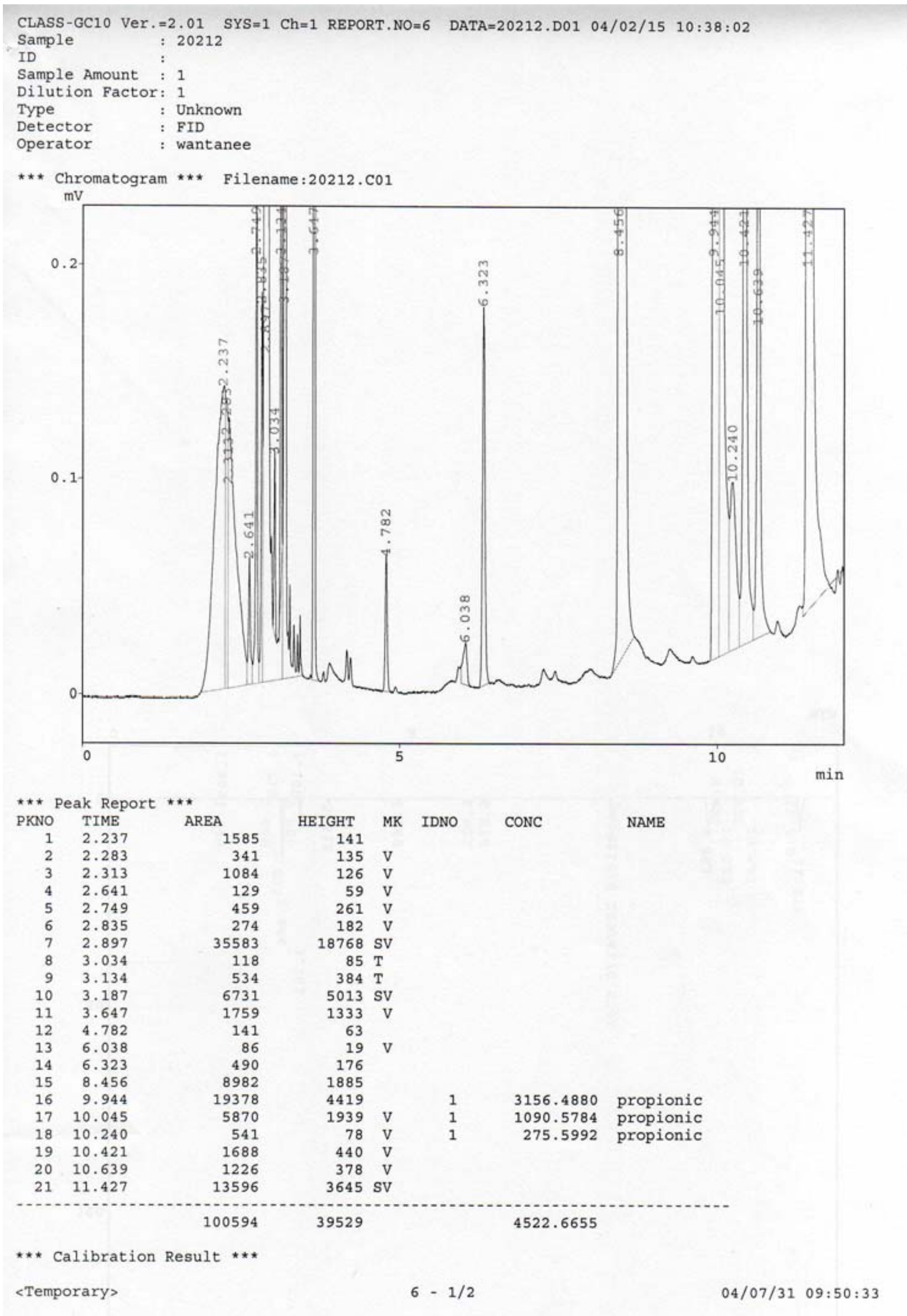
Figure 1C. Chromatogram and report of standard acetic acid analyzed by the Gas Analyser (Shimadzu, Japan) equipped with a flame ionization detector (FID) and DB-FFAP column.



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   3    2.313       1084          126  V
   4    2.641        129           59  V
   5    2.749        459          261  V
   6    2.835        274          182  V
   7    2.897      35583        18768 SV
   8    3.034        118           85 T
   9    3.134        534          384 T
  10    3.187       6731         5013 SV
  11    3.647       1759         1333  V
  12    4.782        141           63
  13    6.038         86           19  V
  14    6.323        490          176
  15    8.456       8982         1885
  16    9.944      19378         4419         1      3156.4880  propionic
  17   10.045       5870         1939  V      1      1090.5784  propionic
  18   10.240        541           78  V      1       275.5992  propionic
  19   10.421       1688          440  V
  20   10.639       1226          378  V
  21   11.427      13596         3645 SV
----------------------------------------------------------------------------
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*** Calibration Result ***

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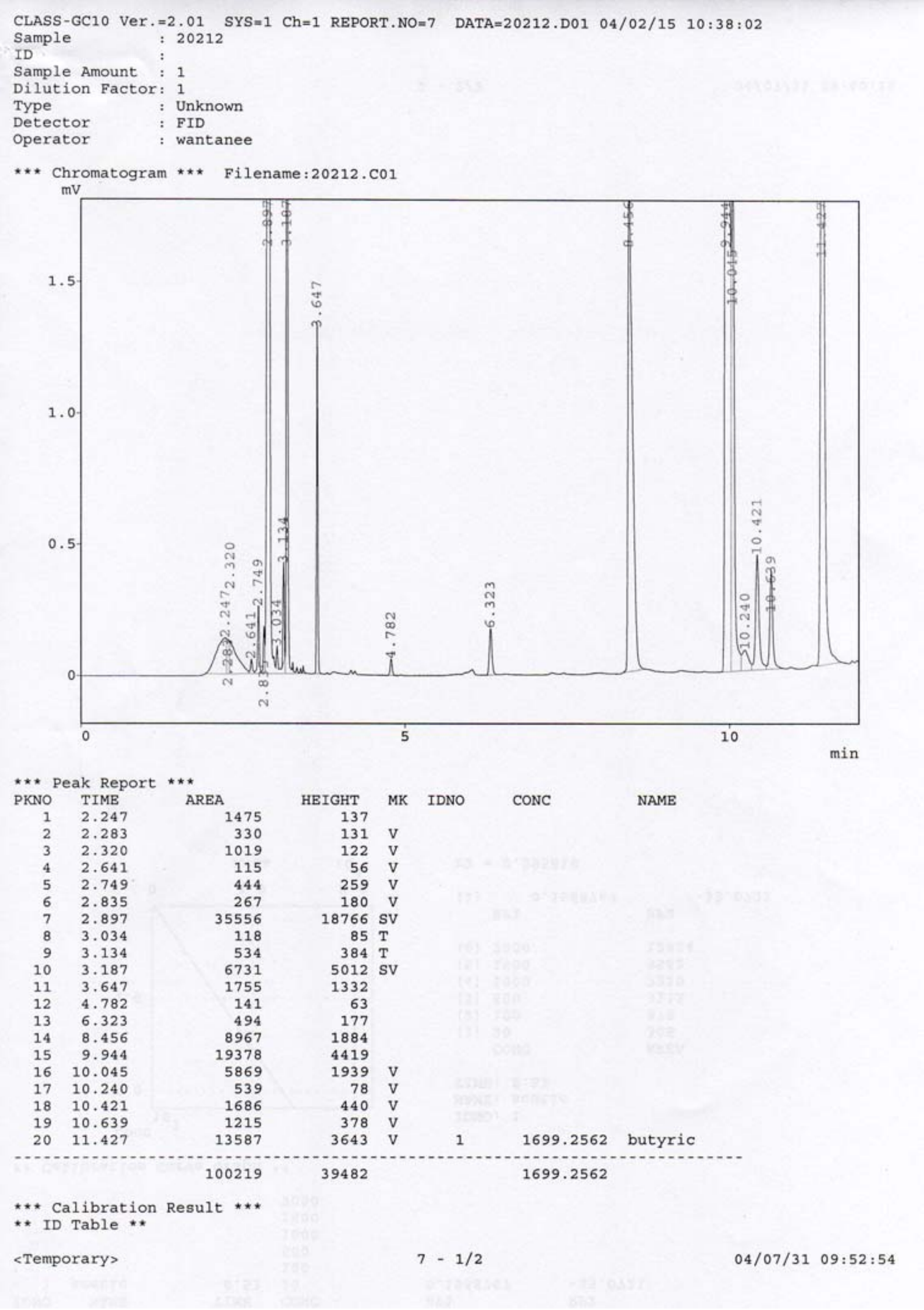
Figure 2C. Chromatogram and report of standard propionic acid analyzed by the Gas Analyser (Shimadzu, Japan) equipped with a flame ionization detector (FID) and DB-FFAP column.



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   2   2.283         330          131   V
   3   2.320        1019          122   V
   4   2.641         115           56   V
   5   2.749         444          259   V
   6   2.835         267          180   V
   7   2.897       35556        18766  SV
   8   3.034         118           85  T
   9   3.134         534          384  T
  10   3.187        6731         5012  SV
  11   3.647        1755         1332
  12   4.782         141           63
  13   6.323         494          177
  14   8.456        8967         1884
  15   9.944       19378         4419
  16  10.045        5869         1939   V
  17  10.240         539           78   V
  18  10.421        1686          440   V
  19  10.639        1215          378   V
  20  11.427       13587         3643   V     1      1699.2562  butyric
------------------------------------------------------------------------------
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*** Calibration Result ***
** ID Table **

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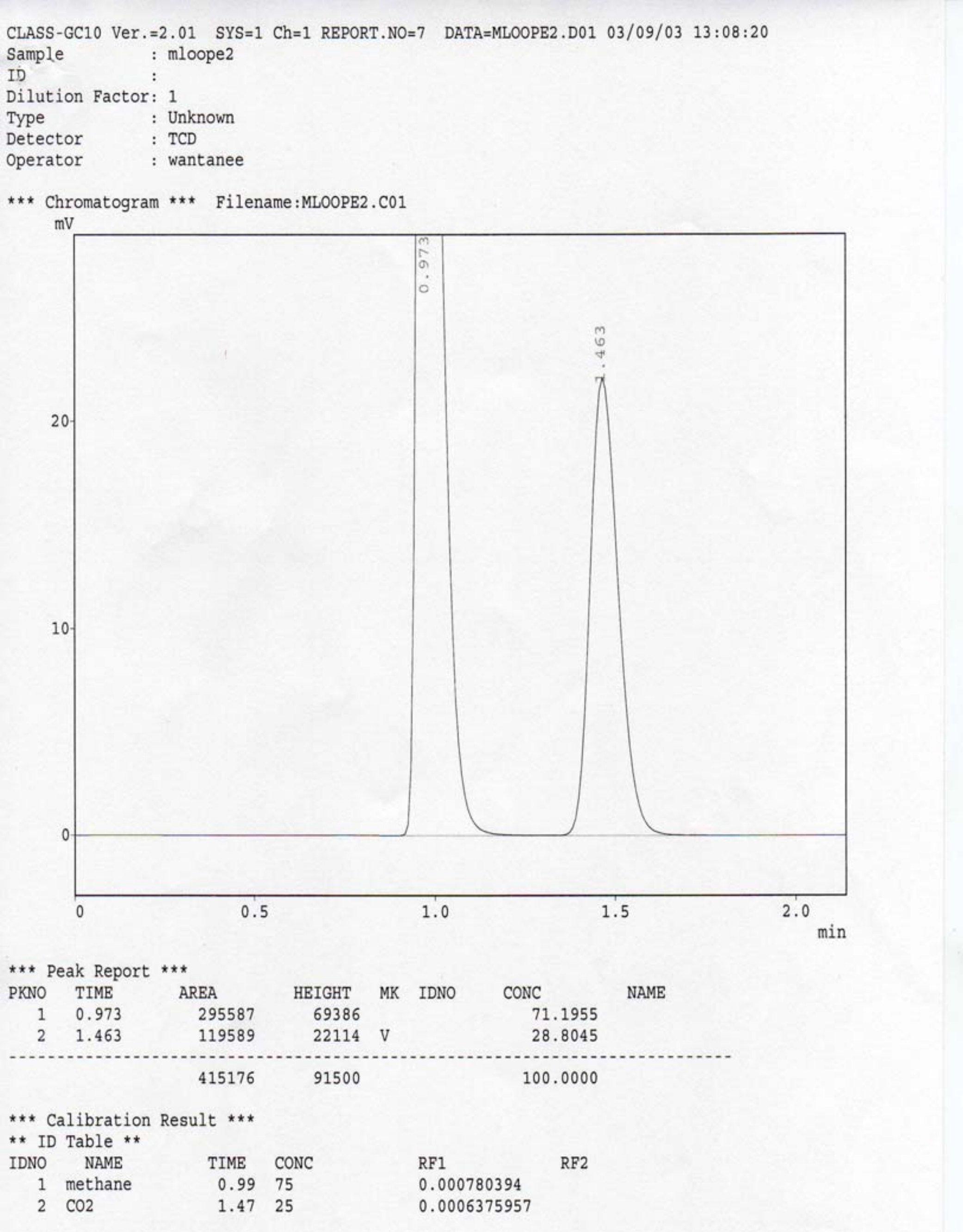
Figure 3C. Chromatogram and report of standard butyric acid analyzed by the Gas Analyser (Shimadzu, Japan) equipped with a flame ionization detector (FID) and DB-FFAP column.



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*** Calibration Result ***
** ID Table **
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   2  CO2            1.47  25             0.0006375957
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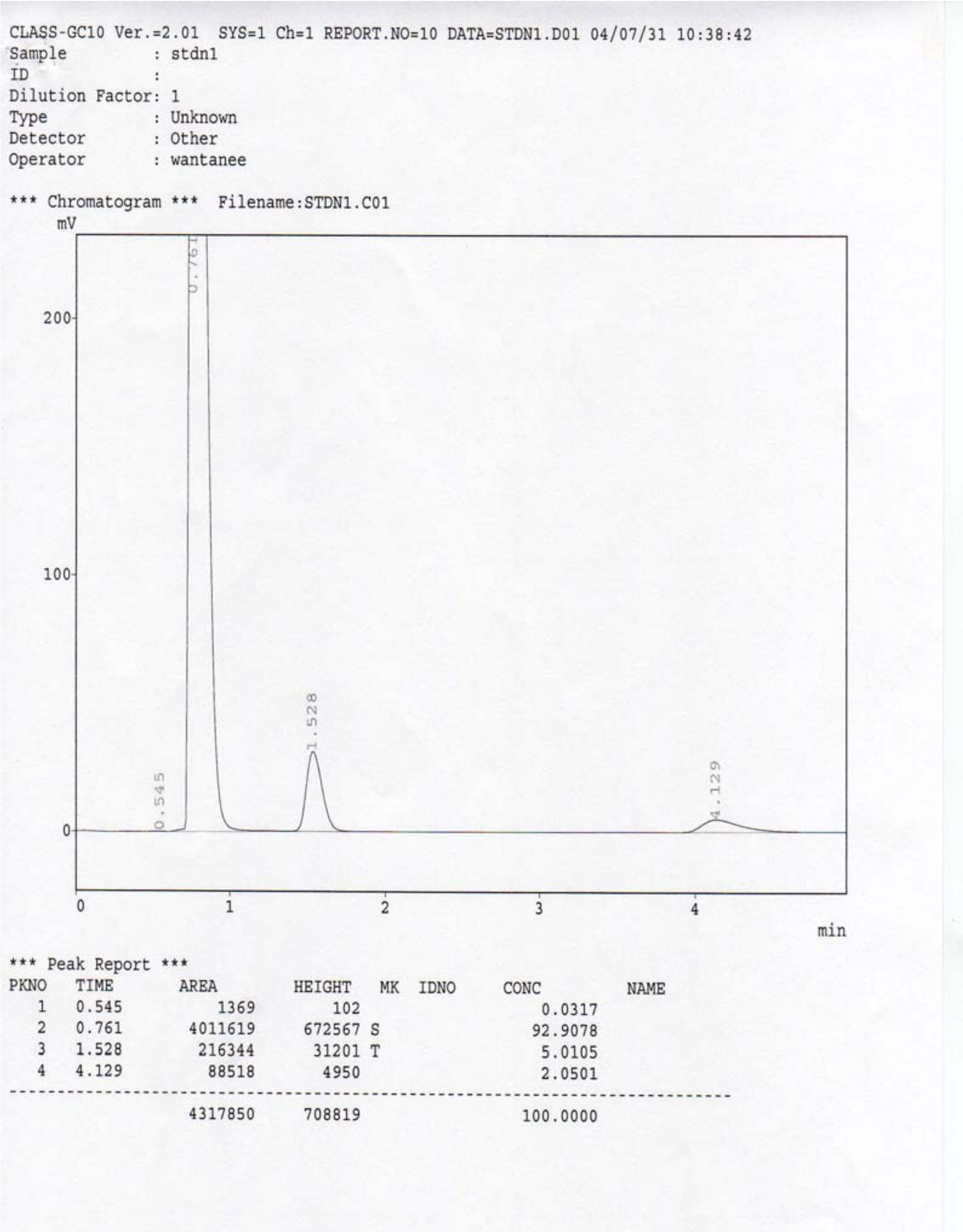
Figure 4C. Chromatogram and report of standard methane and standard carbon dioxide analyzed by the Gas Analyser (Shimadzu, Japan) equipped with a thermal conductor detector (TCD) and DB-FFAP column.



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Operator        : wantanee

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   2  0.761      4011619    672567 S                92.9078
   3  1.528       216344     31201 T                 5.0105
   4  4.129        88518      4950                   2.0501
----------------------------------------------------------------------------
                 4317850    708819                 100.0000
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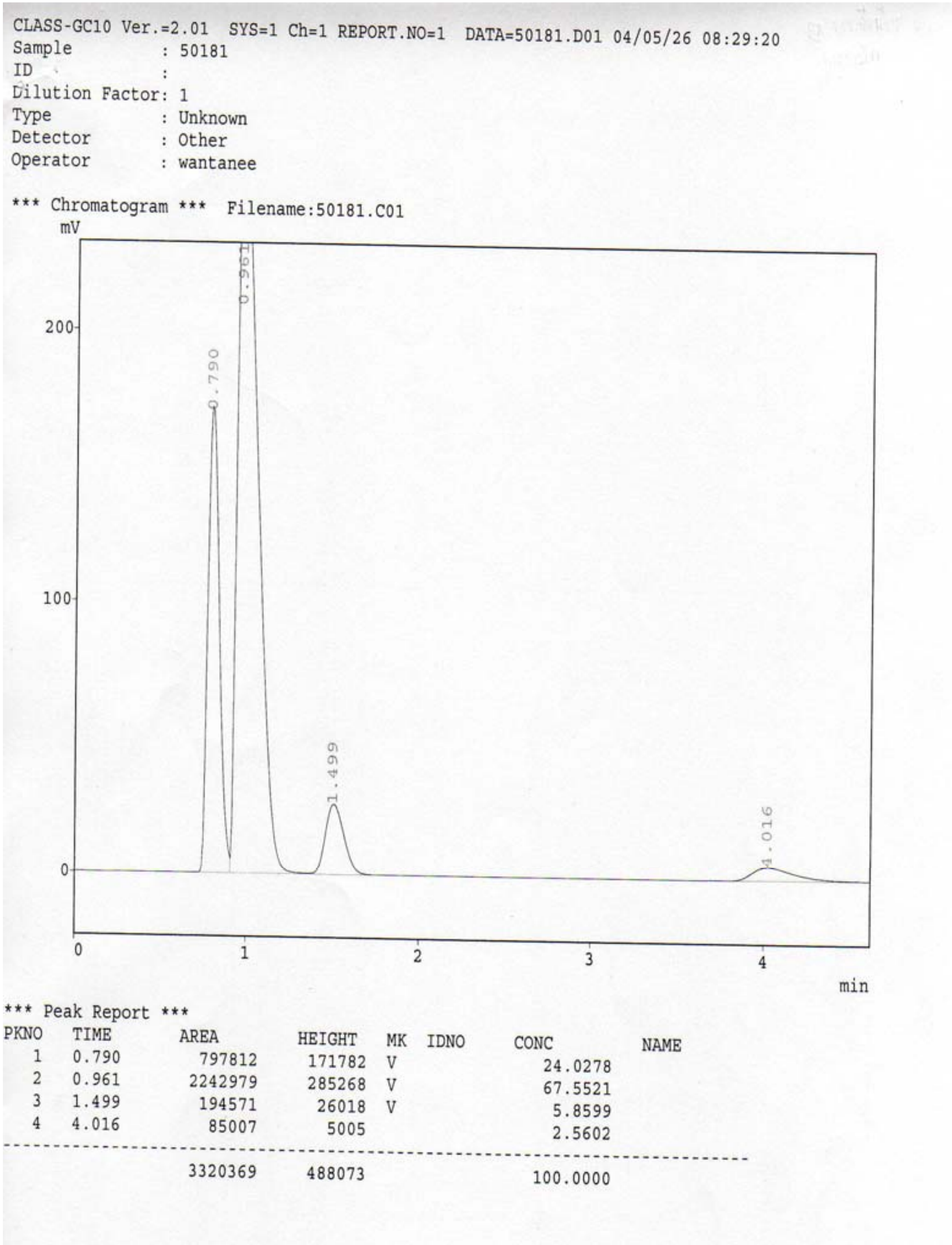
Figure 5C. Chromatogram and report of standard nitrogen, carbon dioxide, and hydrogen analyzed by the Gas Analyser (Shimadzu, Japan) equipped with a thermal conductor detector (TCD) and DB-FFAP column.



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Operator        : wantanee

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   2  0.961    2242979      285268  V               67.5521
   3  1.499     194571       26018  V                5.8599
   4  4.016      85007        5005                   2.5602
------------------------------------------------------------------------
               3320369      488073                 100.0000
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Figure 6C. Chromatogram and report of biogas produced from cassava tubers when analysis by the Gas Analyser (Shimadzu, Japan) equipped with a thermal conductor detector (TCD) and DB-FFAP column.

APPENDIX D

PARAMETERS AND RESULTS OF BIOGAS PRODUCTION FROM CASSAVA TUBERS USING THE SIMPLE SINGLE-STAGE DIGESTER

Table 1D. Biogas production from cassava tubers using 1.00% (w/v) total solids in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	24.0	24.0	8.03	0.00	ND	ND	ND	ND	2350	975	0.41	1700	0	196.41	407.13	4886.71
1	25.0	24.5	7.09	0.00	ND	ND	ND	ND	2500	1050	0.42	1800	ND	ND	ND	ND
2	25.0	25.0	6.69	1.65	18.38	19.79	60.47	1.36	2300	1500	0.65	1300	ND	ND	ND	ND
3	24.0	24.0	6.48	1.25	ND	ND	ND	ND	2100	2400	1.14	500	0	135.74	394.45	2717.01
4	23.0	23.0	6.60	1.10	31.77	33.13	33.74	1.67	3400	3150	0.93	1300	ND	ND	ND	ND
5	23.0	23.0	6.70	0.85	ND	ND	ND	ND	4900	3600	0.73	2500	ND	ND	ND	ND
6	22.0	20.0	6.85	0.85	ND	ND	ND	ND	4900	3900	0.80	2300	0	199.79	423.33	2714.35
7	24.0	23.0	6.92	0.20	ND	ND	ND	ND	4800	4050	0.84	2100	ND	ND	ND	ND
8	24.0	24.0	6.84	0.30	ND	ND	ND	ND	6500	4350	0.67	3600	ND	ND	ND	ND
9	24.0	24.0	7.07	0.65	ND	ND	ND	ND	6400	3900	0.61	3800	0	208.51	176.97	1727.41
10	25.0	24.5	7.23	0.80	ND	ND	ND	ND	6300	4050	0.64	3600	ND	ND	ND	ND
11	24.0	24.5	7.16	0.90	ND	ND	ND	ND	6400	4050	0.63	3700	ND	ND	ND	ND
12	24.0	24.0	7.14	0.75	ND	ND	ND	ND	6400	4200	0.66	3600	0	260.99	194.83	1357.25
13	24.0	23.0	7.24	0.40	43.71	5.77	49.23	1.29	5900	3750	0.64	3400	ND	ND	ND	ND
14	25.0	24.0	7.26	0.70	ND	ND	ND	ND	6000	3600	0.60	3600	ND	ND	ND	ND
15	25.5	24.0	7.32	0.90	ND	ND	ND	ND	6000	3600	0.60	3600	0	222.67	181.22	863.71
16	26.0	25.0	7.47	1.20	ND	ND	ND	ND	6200	3300	0.53	4000	ND	ND	ND	ND
17	26.0	24.0	7.50	1.20	ND	ND	ND	ND	6200	3000	0.48	4200	ND	ND	ND	ND
18	26.0	25.0	7.61	1.30	ND	ND	ND	ND	6200	2700	0.44	4400	0	208.56	174.35	1357.25
19	26.0	25.5	7.66	1.60	65.17	11.78	22.1	0.95	6200	2550	0.41	4500	ND	ND	ND	ND
20	26.0	25.0	7.70	1.40	ND	ND	ND	ND	6400	2250	0.35	4900	ND	ND	ND	ND
21	26.5	26.0	7.76	1.20	ND	ND	ND	ND	6600	2250	0.34	5100	0	224.50	186.87	1357.25
22	26.0	26.0	7.80	0.60	65.12	7.68	12.83	14.36	6500	2100	0.32	5100	ND	ND	ND	ND
23	25.0	26.0	7.92	0.20	ND	ND	ND	ND	6970	1455	0.21	6000	ND	ND	ND	ND
24	25.0	25.0	7.95	0.20	ND	ND	ND	ND	7000	1425	0.20	6050	0	218.30	183.80	495.27
25	27.0	28.0	7.85	0.20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not detectable

Table 2D. Biogas production from cassava tubers using 0.25% (w/v) total solids in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature (°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA (mg as acetate/L)	VFA /A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	24.0	24.00	8.07	0.00	ND	ND	ND	ND	2410.0	510.0	0.32	1900.0	0.00	137.00	148.14	1221.68
1	25.0	24.5	7.76	0.00	ND	ND	ND	ND	2560.0	547.5	0.32	2012.5	ND	ND	ND	ND
2	25.0	25.0	7.05	0.45	ND	ND	ND	ND	2475.0	810.0	0.49	1665.0	ND	ND	ND	ND
3	24.0	24.0	6.82	0.65	ND	ND	ND	ND	2375.0	1125.0	0.71	1250.0	24.54	142.26	154.48	572.60
4	23.0	23.0	6.80	0.00	ND	ND	ND	ND	2350.0	1350.0	0.86	1000.0	ND	ND	ND	ND
5	23.0	23.0	6.90	0.30	ND	ND	ND	ND	2350.0	1350.0	0.86	1000.0	ND	ND	ND	ND
6	22.0	20.0	7.00	0.30	ND	ND	ND	ND	2350.0	1350.0	0.86	1000.0	24.68	141.56	155.48	316.29
7	24.0	23.0	7.00	0.20	ND	ND	ND	ND	2375.0	1162.5	0.73	1212.5	ND	ND	ND	ND
8	24.0	24.0	6.98	0.23	ND	ND	ND	ND	2200.0	1200.0	0.82	1000.0	ND	ND	ND	ND
9	24.0	24.0	6.94	0.25	ND	ND	ND	ND	2400.0	1200.0	0.75	1200.0	0.00	167.20	162.53	320.20
10	25.0	24.5	7.08	0.20	ND	ND	ND	ND	2550.0	1275.0	0.75	1275.0	ND	ND	ND	ND
11	24.0	24.5	7.09	0.25	ND	ND	ND	ND	2400.0	900.0	0.56	1500.0	ND	ND	ND	ND
12	24.0	24.0	7.17	0.00	ND	ND	ND	ND	2500.0	525.0	0.32	1975.0	0.00	132.18	148.50	183.50

ND = Not detectable

Table 3D. Biogas production from cassava tubers using 0.50% (w/v) total solids in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature (°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA (mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate (ppm)	Butyrate (ppm)	Starch (mg/L)
0	24.0	24.0	8.09	0.000	ND	ND	ND	ND	2200.0	555.0	0.25	1645.0	0.00	164.62	154.66	2443.35
1	25.0	24.5	7.59	0.000	ND	ND	ND	ND	2315.0	675.0	0.29	1640.0	ND	ND	ND	ND
2	25.0	25.0	6.87	0.925	8.81	10.35	79.49	1.35	2360.0	1275.0	0.54	1085.0	ND	ND	ND	ND
3	24.0	24.0	6.72	0.860	ND	ND	ND	ND	2150.0	1687.5	0.78	462.5	24.96	162.22	162.13	1606.26
4	23.0	23.0	6.78	0.550	26.11	13.25	58.86	1.79	3750.0	2325.0	0.62	1425.0	ND	ND	ND	ND
5	23.0	23.0	6.84	0.200	ND	ND	ND	ND	3750.0	2325.0	0.62	1425.0	ND	ND	ND	ND
6	22.0	20.0	6.95	0.200	ND	ND	ND	ND	3600.0	2400.0	0.67	1200.0	25.07	153.96	156.73	1360.46
7	24.0	23.0	6.98	0.050	ND	ND	ND	ND	3750.0	2250.0	0.60	1500.0	ND	ND	ND	ND
8	24.0	24.0	6.94	0.110	ND	ND	ND	ND	3600.0	2212.5	0.61	1387.5	ND	ND	ND	ND
9	24.0	24.0	6.95	0.185	ND	ND	ND	ND	3600.0	2325.0	0.65	1275.0	0.00	128.81	146.64	1030.12
10	25.0	24.5	7.01	0.300	33.11	4.55	60.17	2.17	3750.0	2325.0	0.62	1425.0	ND	ND	ND	ND
11	24.0	24.5	7.06	0.425	ND	ND	ND	ND	3725.0	2100.0	0.56	1625.0	ND	ND	ND	ND
12	24.0	24.0	7.16	0.500	ND	ND	ND	ND	3800.0	1912.5	0.50	1887.5	0.00	140.78	151.60	873.99
13	24.0	23.0	7.17	0.350	34.78	14.6	50.17	0.46	4000.0	1950.0	0.49	2050.0	ND	ND	ND	ND
14	25.0	24.0	7.31	0.400	ND	ND	ND	ND	3700.0	1500.0	0.41	2200.0	ND	ND	ND	ND
15	25.5	24.0	7.33	0.410	ND	ND	ND	ND	3700.0	1387.5	0.38	2312.5	0.00	144.61	154.53	882.54
16	26.0	25.0	7.40	0.330	ND	ND	ND	ND	3750.0	1237.5	0.33	2512.5	ND	ND	ND	ND
17	26.0	24.0	7.44	0.285	ND	ND	ND	ND	3825.0	1162.5	0.30	2662.5	ND	ND	ND	ND
18	26.0	25.0	7.48	0.225	ND	ND	ND	ND	3800.0	1050.0	0.28	2750.0	0.00	172.81	164.12	488.91
19	26.0	25.5	7.50	0.250	45.48	1.63	51.84	1.06	3750.0	975.0	0.26	2775.0	ND	ND	ND	ND
20	26.0	25.0	7.51	0.000	ND	ND	ND	ND	3800.0	937.5	0.25	2862.5	ND	ND	ND	ND
21	26.5	26.0	7.54	0.000	ND	ND	ND	ND	3825.0	937.5	0.25	2887.5	ND	ND	ND	ND

ND = Not detectable

Table 4D. Biogas production from cassava tubers using 1.00% (w/v) total solids in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	29.0	28.0	7.22	0.00	ND	ND	ND	ND	1850	1222.5	0.66	1035.0	0.00	238.28	150.49	4886.71
1	28.5	28.0	7.09	0.42	2.65	3.35	91.99	2.01	1750	1500.0	0.86	750.0	ND	ND	ND	ND
2	30.0	29.0	6.4	1.73	ND	ND	ND	ND	2900	2625.0	0.91	1150.0	ND	ND	ND	ND
3	29.0	28.5	6.84	1.22	ND	ND	ND	ND	5050	3075.0	0.61	3000.0	0.00	293.04	321.31	2717.01
4	28.0	28.5	7.37	0.57	23.55	15.05	59.28	2.12	ND	ND	ND	ND	ND	ND	ND	ND
5	29.0	29.0	7.41	0.49	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	29.0	28.5	7.35	0.73	ND	ND	ND	ND	6800	3787.5	0.56	4275.0	17.79	358.55	353.61	2714.35
7	29.0	28.0	7.57	0.55	19.57	15.26	61.25	3.91	ND	ND	ND	ND	ND	ND	ND	ND
8	29.0	28.0	7.22	0.51	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	29.0	29.0	7.12	0.47	ND	ND	ND	ND	7325	4500.0	0.61	4325.0	12.81	327.66	215.39	1727.41
10	29.0	29.5	7.25	0.54	33.77	14.25	49.25	2.74	ND	ND	ND	ND	ND	ND	ND	ND
11	29.0	29.0	7.14	0.67	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	29.0	28.0	7.12	0.75	ND	ND	ND	ND	6950	4500.0	0.65	3950.0	8.79	263.32	185.87	1357.25
13	29.0	29.0	7.13	0.63	45.11	17.74	35.34	1.82	ND	ND	ND	ND	ND	ND	ND	ND
14	29.0	29.5	7.25	0.76	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	29.0	30.0	7.26	0.86	ND	ND	ND	ND	6600	4650.0	0.7	3500.0	0.00	263.63	171.41	863.71
16	29.0	29.0	7.31	1.04	49.13	19.76	29.73	1.39	ND	ND	ND	ND	ND	ND	ND	ND
17	28.0	29.0	7.39	1.08	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	28.0	29.0	7.46	1.14	ND	ND	ND	ND	7200	4650.0	0.65	4100.0	0.00	607.98	177.63	1357.25
19	28.5	28.0	7.52	1.20	54.91	16.91	25.87	2.31	ND	ND	ND	ND	ND	ND	ND	ND
20	29.0	29.0	7.62	1.12	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	29.0	29.0	7.77	0.87	ND	ND	ND	ND	6950	2250.0	0.32	5450.0	12.12	377.38	173.66	1357.25
22	28.0	29.0	7.93	0.60	64.35	10.3	23.10	2.25	ND	ND	ND	ND	ND	ND	ND	ND
23	29.0	29.0	7.84	0.26	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	27.0	28.0	7.85	0.20	ND	ND	ND	ND	7000	1425.0	0.20	6050.0	0	218.30	183.80	495.27
25	27.0	28.0	7.85	0.20	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND = Not detectable

Table 5D. Biogas production from cassava tubers using 2.00% (w/v) total solids in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH4	%CO2	%N2	%H2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate (ppm)	Starch (mg/L)
0	29.0	28.0	7.23	0.000	ND	ND	ND	ND	2550.0	1312.5	0.51	1675.0	0.00	209.61	36.61	9773.41
1	28.5	28.0	6.68	1.150	5.30	12.08	80.51	2.11	3150.0	2812.5	0.90	1275.0	ND	ND	ND	ND
2	30.0	29.0	6.74	1.500	ND	ND	ND	ND	4400.0	3825.0	0.87	1850.0	ND	ND	ND	ND
3	29.0	28.5	6.95	1.050	ND	ND	ND	ND	6300.0	3825.0	0.61	3750.0	0.00	233.86	276.23	5635.35
4	28.0	28.5	7.21	0.510	24.44	21.59	50.98	2.10	ND	ND	ND	ND	ND	ND	ND	ND
5	29.0	29.0	7.29	0.435	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	29.0	28.5	7.28	0.700	ND	ND	ND	ND	8250.0	5250.0	0.64	4750.0	23.39	203.70	279.77	4462.54
7	29.0	28.0	7.26	0.775	34.23	26.26	37.20	2.50	ND	ND	ND	ND	ND	ND	ND	ND
8	29.0	28.0	7.13	0.800	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	29.0	29.0	7.05	0.800	ND	ND	ND	ND	7325.0	4950.0	0.68	4025.0	31.44	278.35	171.00	3910.34
10	29.0	29.5	7.11	0.980	37.30	29.14	31.54	2.02	ND	ND	ND	ND	ND	ND	ND	ND
11	29.0	29.0	7.05	1.060	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	29.0	28.0	6.94	1.300	ND	ND	ND	ND	6775.0	5100.0	0.73	3375.0	5.34	173.07	277.52	4119.49
13	29.0	29.0	7.14	1.100	38.27	32.6	26.85	2.27	ND	ND	ND	ND	ND	ND	ND	ND
14	29.0	29.5	7.20	1.100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	29.0	30.0	7.10	0.800	ND	ND	ND	ND	7675.0	6675.0	0.87	3225.0	0.00	172.15	149.73	3781.33
16	29.0	29.0	7.17	0.950	39.02	27.16	34.80	2.07	ND	ND	ND	ND	ND	ND	ND	ND
17	28.0	29.0	7.18	0.500	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	28.0	29.0	7.38	0.000	ND	ND	ND	ND	7950.0	5700.0	0.72	4150.0	0.00	194.91	153.38	3099.15
19	28.5	28.0	7.04	0.575	43.66	24.94	30.54	1.86	ND	ND	ND	ND	ND	ND	ND	ND
20	29.0	29.0	7.15	0.550	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	29.0	29.0	7.36	1.350	ND	ND	ND	ND	7550.0	5700.0	0.75	3750.0	0.00	179.49	265.69	3429.49
22	28.0	29.0	7.21	1.350	44.37	24.21	29.17	2.25	ND	ND	ND	ND	ND	ND	ND	ND
23	29.0	29.0	7.15	1.400	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	27.0	28.0	7.24	0.950	ND	ND	ND	ND	7650.0	5475.0	0.72	4000.0	0	573.68	182.11	2685.73
25	27.0	28.0	7.30	0.600	58.97	18.94	19.92	2.17	ND	ND	ND	ND	ND	ND	ND	ND
26	28.0	29.0	7.29	1.050	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
27	28.0	29.0	7.31	1.050	ND	ND	ND	ND	8000.0	5475.0	0.68	4350.0	23.39	235.55	181.07	2606.57
28	28.0	28.0	7.37	0.900	49.01	11.23	38.85	0.90	ND	ND	ND	ND	ND	ND	ND	ND
29	29.0	28.0	7.61	1.000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
30	29.0	29.0	7.57	1.350	ND	ND	ND	ND	7725.0	3750.0	0.49	5225.0	5.34	322.85	204.87	2906.61
31	29.0	29.0	7.52	1.050	61.24	10.41	25.19	3.15	ND	ND	ND	ND	ND	ND	ND	ND
32	29.0	29.0	7.60	0.650	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
33	29.0	29.0	7.58	0.060	ND	ND	ND	ND	7825.0	2250.0	0.29	6325.0	31.44	322.39	153.52	1968.37

ND = Not detectable

Table 6D. Biogas production from cassava tubers using 4.00% (w/v) total solids in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH4	%CO2	%N2	%H2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA /A	Bicarbonate(mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	29.0	28.0	7.19	0.000	ND	ND	ND	ND	4150.0	2550.0	0.61	2450.0	0.00	183.02	34.55	19546.82
1	28.5	28.0	6.56	4.075	11.48	56.86	29.54	2.13	4500.0	3675.0	0.82	2050.0	ND	ND	ND	ND
2	30.0	29.0	6.51	3.075	ND	ND	ND	ND	5300.0	4350.0	0.82	2400.0	ND	ND	ND	ND
3	29.0	28.5	6.99	2.275	ND	ND	ND	ND	6875.0	5437.5	0.79	3250.0	20.66	231.17	246.01	16460.38
4	28.0	28.5	7.19	1.400	26.04	47.16	24.73	2.07	ND	ND	ND	ND	ND	ND	ND	ND
5	29.0	29.0	7.18	0.665	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	29.0	28.5	7.26	1.400	ND	ND	ND	ND	10400.0	8250.0	0.79	4900.0	14.59	213.80	164.32	16974.46
7	29.0	28.0	7.29	1.425	29.18	43.9	24.98	1.95	ND	ND	ND	ND	ND	ND	ND	ND
8	29.0	28.0	7.27	1.400	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	29.0	29.0	7.09	1.175	ND	ND	ND	ND	8550.0	8625.0	1.00	2800.0	14.03	267.74	175.10	9773.41
10	29.0	29.5	7.26	1.305	32.48	44.8	20.74	2.00	ND	ND	ND	ND	ND	ND	ND	ND
11	29.0	29.0	6.98	1.395	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	29.0	28.0	6.63	1.625	ND	ND	ND	ND	8300.0	9900.0	1.19	1700.0	3.26	148.29	37.65	9773.41
13	29.0	29.0	6.85	1.360	31.14	46.26	20.50	2.08	ND	ND	ND	ND	ND	ND	ND	ND
14	29.0	29.5	6.97	1.440	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	29.0	30.0	6.83	1.325	ND	ND	ND	ND	9600.0	11100.0	1.16	2200.0	9.72	134.55	32.56	4628.69
16	29.0	29.0	6.99	1.475	22.47	43.7	31.85	1.98	ND	ND	ND	ND	ND	ND	ND	ND
17	28.0	29.0	7.20	1.170	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	28.0	29.0	7.10	0.985	ND	ND	ND	ND	11800.0	11850.0	1.00	3900.0	20.69	182.16	155.91	5144.72
19	28.5	28.0	7.02	0.975	27.61	36.01	34.38	2.00	ND	ND	ND	ND	ND	ND	ND	ND
20	29.0	29.0	7.06	0.940	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	29.0	29.0	7.13	0.775	ND	ND	ND	ND	11850.0	11850.0	1.00	3950.0	22.26	171.02	267.11	5144.72
22	28.0	29.0	7.07	0.815	35.061	33.85	28.53	2.57	ND	ND	ND	ND	ND	ND	ND	ND
23	29.0	29.0	6.87	0.715	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	27.0	28.0	6.89	0.250	ND	ND	ND	ND	11500.0	11400.0	0.99	3900.0	0.73	231.48	423.90	5873.82
25	27.0	28.0	6.92	0.325	36.85	21.6	38.62	2.93	ND	ND	ND	ND	ND	ND	ND	ND
26	28.0	29.0	6.87	0.450	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
27	28.0	29.0	6.86	0.360	ND	ND	ND	ND	11650.0	11175.0	0.96	4200.0	8.22	232.70	437.82	4980.53
28	28.0	28.0	6.86	0.560	31.63	18.05	44.89	5.42	ND	ND	ND	ND	ND	ND	ND	ND
29	29.0	28.0	7.21	0.300	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
30	29.0	29.0	7.20	0.575	ND	ND	ND	ND	11850.0	11100.0	0.94	4450.0	0.00	69.13	178.52	5220.96
31	29.0	29.0	7.15	0.500	40.47	17.53	39.82	2.17	ND	ND	ND	ND	ND	ND	ND	ND
32	29.0	29.0	7.06	0.350	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
33	29.0	29.0	7.07	0.275	ND	ND	ND	ND	11525.0	10162.5	0.87	4750.0	0.00	282.10	322.86	4366.76

ND = Not detectable

Table 7D. Biogas production from cassava tubers using 8.00% (w/v) total solids in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH4	%CO2	%N2	%H2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	29.0	28.0	7.09	0.000	ND	ND	ND	ND	7950.0	4200.0	0.54	5100.0	0.00	265.90	267.85	39093.65
1	28.5	28.0	7.07	8.945	8.82	82.01	7.67	1.51	8200.0	6450.0	0.79	3900.0	ND	ND	ND	ND
2	30.0	29.0	6.69	9.400	ND	ND	ND	ND	8600.0	7425.0	0.86	3650.0	ND	ND	ND	ND
3	29.0	28.5	6.75	7.475	ND	ND	ND	ND	10050.0	12750.0	1.27	1550.0	16.73	256.20	151.08	22095.73
4	28.0	28.5	7.05	4.900	15.04	75.99	7.13	1.96	ND	ND	ND	ND	ND	ND	ND	ND
5	29.0	29.0	7.06	3.350	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	29.0	28.5	6.97	2.700	ND	ND	ND	ND	11000.0	14250.0	1.3	1500.0	8.89	274.31	173.43	25496.88
7	29.0	28.0	7.30	2.550	18.08	70.5	9.48	1.94	ND	ND	ND	ND	ND	ND	ND	ND
8	29.0	28.0	7.01	2.350	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	29.0	29.0	6.81	1.960	ND	ND	ND	ND	13125.0	17437.5	1.33	1500.0	21.31	190.16	38.56	27197.45
10	29.0	29.5	7.01	2.240	16.12	68.79	12.22	2.87	ND	ND	ND	ND	ND	ND	ND	ND
11	29.0	29.0	6.93	1.840	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	29.0	28.0	6.56	1.675	ND	ND	ND	ND	13900.0	22800.0	1.64	-1300.0	7.48	189.28	152.01	10199.53
13	29.0	29.0	6.66	1.625	18.13	65.8	14.15	1.92	ND	ND	ND	ND	ND	ND	ND	ND
14	29.0	29.5	6.85	1.365	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	29.0	30.0	6.58	1.100	ND	ND	ND	ND	17650.0	23775.0	1.35	1800.0	16.82	217.36	161.19	8264.40
16	29.0	29.0	6.99	1.240	14.19	59.14	24.70	1.97	ND	ND	ND	ND	ND	ND	ND	ND
17	28.0	29.0	6.94	1.060	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
18	28.0	29.0	7.05	0.950	ND	ND	ND	ND	21900.0	25650.0	1.17	4800.0	0.00	216.73	159.97	8162.75
19	28.5	28.0	7.10	0.850	18.53	55.47	24.27	1.73	ND	ND	ND	ND	ND	ND	ND	ND
20	29.0	29.0	6.78	0.900	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
21	29.0	29.0	7.02	0.700	ND	ND	ND	ND	22850.0	25350.0	1.11	5950.0	2.80	212.41	279.82	10367.64
22	28.0	29.0	7.07	0.575	18.36	46.6	32.99	2.05	ND	ND	ND	ND	ND	ND	ND	ND
23	29.0	29.0	6.82	0.335	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
24	27.0	28.0	6.83	0.185	ND	ND	ND	ND	22000.0	24000.0	1.09	6000.0	17.89	203.82	463.48	7807.00
25	27.0	28.0	6.88	0.085	20.81	34.61	42.64	1.94	ND	ND	ND	ND	ND	ND	ND	ND
26	28.0	29.0	6.75	0.350	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
27	28.0	29.0	6.64	0.150	ND	ND	ND	ND	21450.0	23025.0	1.07	6100.0	12.17	198.12	165.37	6153.34
28	28.0	28.0	6.74	0.000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
29	29.0	28.0	6.85	0.300	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
30	29.0	29.0	6.76	0.625	ND	ND	ND	ND	21875.0	23175.0	1.06	6425.0	9.68	223.31	438.96	4812.43
31	29.0	29.0	6.72	0.575	17.45	36.90	40.16	3.28	ND	ND	ND	ND	ND	ND	ND	ND
32	29.0	29.0	6.60	0.200	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
33	29.0	29.0	6.76	0.050	ND	ND	ND	ND	21875.0	21450.0	0.98	7575.0	1.60	256.27	450.99	1700.57

ND = Not detectable

Table 8D. Biogas production from cassava tubers using 16.00 (w/v) total solids in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA (mg as acetate/L)	VFA/ A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	29.0	28.0	7.13	0.000	ND	ND	ND	ND	9525.0	6487.5	0.68	5200.0	0.00	379.11	152.20	78187.30
1	28.5	28.0	6.87	14.250	4.22	88.18	5.50	2.10	11425.0	9787.5	0.86	4900.0	ND	ND	ND	ND
2	30.0	29.0	5.96	22.875	ND	ND	ND	ND	10550.0	16875.0	1.60	-700.0	ND	ND	ND	ND
3	29.0	28.5	5.31	6.050	ND	ND	ND	ND	10100.0	26700.0	2.64	-7700.0	0.00	571.40	166.87	32213.17
4	28.0	28.5	5.25	4.950	3.64	87.54	6.94	1.88	ND	ND	ND	ND	ND	ND	ND	ND
5	29.0	29.0	5.19	2.220	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
6	29.0	28.5	5.10	6.240	ND	ND	ND	ND	ND	ND	ND	ND	283.29	2248.34	601.69	27756.49
7	29.0	28.0	5.00	1.825	0.92	76.35	20.87	1.87	ND	ND	ND	ND	ND	ND	ND	ND
8	29.0	28.0	4.98	1.375	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
9	29.0	29.0	4.94	0.300	ND	ND	ND	ND	ND	ND	ND	ND	1297.74	5170.44	1788.94	25176.31
10	29.0	29.5	4.81	0.700	0.22	68.54	29.35	1.90	ND	ND	ND	ND	ND	ND	ND	ND
11	29.0	29.0	4.79	0.100	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
12	29.0	28.0	4.74	0.000	ND	ND	ND	ND	ND	ND	ND	ND	2773.31	7618.96	2714.76	15559.27
13	29.0	29.0	4.72	0.000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
14	29.0	29.5	4.73	0.000	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
15	29.0	30.0	4.76	0.000	ND	ND	ND	ND	ND	ND	ND	ND	3227.54	8309.70	2992.15	9617.04

ND = Not detectable

Table 9D. Biogas production from cassava tubers using 1.00% (w/v) total solids and no urea addition in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	26.0	26.0	7.89	0.000	ND	ND	ND	ND	2250.0	625.5	0.28	1833.0	0.00	144.10	149.73	4886.71
1	26.0	25.5	6.53	1.400	12.05	24.4	62.34	1.21	2183.0	1390.5	0.64	1256.0	ND	ND	ND	ND
2	26.0	25.0	6.86	2.310	ND	ND	ND	ND	3533.0	2095.5	0.59	2136.0	ND	ND	ND	ND
3	26.0	26.0	6.79	1.350	ND	ND	ND	ND	2983.0	2220.0	0.74	1503.0	24.73	145.17	154.12	2857.01
4	26.0	26.5	6.74	1.347	33.85	48.1	15.40	2.66	2617.0	2250.0	0.86	1117.0	ND	ND	ND	ND
5	26.0	26.5	6.86	1.293	ND	ND	ND	ND	3533.0	2775.0	0.79	1683.0	ND	ND	ND	ND
6	26.0	26.0	6.97	0.600	ND	ND	ND	ND	3800.0	2800.5	0.74	1933.0	12.21	163.67	161.34	2706.35
7	26.0	26.5	7.04	0.600	35.61	44.55	17.62	2.23	3900.0	2775.0	0.71	2050.0	ND	ND	ND	ND
8	27.0	26.5	6.99	1.033	ND	ND	ND	ND	3783.0	2824.5	0.75	1900.0	ND	ND	ND	ND
9	27.0	27.0	7.02	0.983	ND	ND	ND	ND	3800.0	2800.5	0.74	1933.0	10.72	162.44	161.97	1937.41
10	27.0	27.0	7.06	0.733	43.47	34.29	20.37	1.87	3717.0	2850.0	0.77	1817.0	ND	ND	ND	ND
11	27.0	26.0	7.10	1.033	ND	ND	ND	ND	3900.0	2625.0	0.67	2150.0	ND	ND	ND	ND
12	26.0	25.0	7.19	0.933	ND	ND	ND	ND	3733.0	2524.5	0.68	2050.0	0.00	161.29	161.09	1287.25
13	25.0	24.5	7.26	0.883	52.3	23.42	22.02	2.26	3767.0	2500.5	0.66	2100.0	ND	ND	ND	ND
14	25.0	24.0	7.30	0.973	ND	ND	ND	ND	4900.0	3000.0	0.61	2900.0	ND	ND	ND	ND
15	24.0	24.0	7.43	0.950	ND	ND	ND	ND	4967.0	2400.0	0.48	3367.0	0.00	165.20	279.98	836.71
16	25.0	24.5	7.56	0.717	61.56	15.48	20.56	2.14	5100.0	2260.5	0.44	3593.0	ND	ND	ND	ND
17	25.0	25.0	7.59	0.777	ND	ND	ND	ND	5317.0	2100.0	0.39	3917.0	ND	ND	ND	ND
18	24.0	24.0	7.60	0.723	ND	ND	ND	ND	5367.0	1800.0	0.34	4167.0	12.50	163.64	165.65	9977.25
19	23.0	23.0	7.60	0.400	60.06	9.99	23.03	6.92	5350.0	1725.0	0.32	4200.0	ND	ND	ND	ND
20	24.0	24.0	7.60	0.100	ND	ND	ND	ND	5500.	1650.0	0.3	3850.0	ND	ND	ND	ND
21	25.0	24.0	7.62	0.100	ND	ND	ND	ND	5550.0	1600.0	0.29	3950.0	0.00	149.25	153.02	532.25
22	26.5	26.0	7.54	0.000	ND	ND	ND	ND	3825.0	937.5	0.25	2887.5	ND	ND	ND	ND

ND = Not detectable

Table 10D. Biogas production from cassava tubers using 1.00% (w/v) total solids and urea addition at 0.02% (w/v), in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature (°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	26.0	26.0	7.87	0.00	ND	ND	ND	ND	2367.0	664.5	0.28	1924.0	0.00	123.91	149.73	4886.71
1	26.0	25.5	6.31	1.950	14.62	32.47	49.54	2.21	2150.0	1849.5	0.86	917.0	ND	ND	ND	ND
2	26.0	25.0	6.63	7.050	ND	ND	ND	ND	3293.0	3100.5	0.94	1226.0	ND	ND	ND	ND
3	26.0	26.0	6.68	1.600	ND	ND	ND	ND	3667.0	3310.5	0.90	1460.0	21.23	126.18	154.12	2857.01
4	26.0	26.5	6.91	1.367	30.85	51.31	16.32	1.52	4550.0	3465.0	0.76	2240.0	ND	ND	ND	ND
5	26.0	26.5	7.00	0.833	ND	ND	ND	ND	4600.0	3400.5	0.74	2333.0	ND	ND	ND	ND
6	26.0	26.0	7.14	0.900	ND	ND	ND	ND	4867.0	3199.5	0.66	2734.0	11.74	140.12	161.34	2706.35
7	26.0	26.5	7.31	0.933	41.28	42.03	12.45	4.25	5017.0	3124.5	0.62	2934.0	ND	ND	ND	ND
8	27.0	26.5	7.36	1.517	ND	ND	ND	ND	5117.0	3075.0	0.60	3067.0	ND	ND	ND	ND
9	27.0	27.0	7.46	1.833	ND	ND	ND	ND	5100.0	3100.5	0.61	3033.0	10.63	137.42	161.97	1937.41
10	27.0	27.0	7.64	1.983	63.77	22.19	10.63	3.41	5383.0	2899.5	0.54	3450.0	ND	ND	ND	ND
11	27.0	26.0	7.67	1.833	ND	ND	ND	ND	5500.0	2200.5	0.40	4033.0	ND	ND	ND	ND
12	26.0	25.0	7.76	1.383	ND	ND	ND	ND	5400.0	1999.5	0.37	4067.0	0.00	137.74	161.09	1287.25
13	25.0	24.5	7.80	1.150	69.46	13.42	15.09	2.04	5333.0	2050.5	0.38	3966.0	ND	ND	ND	ND
14	25.0	24.0	7.84	0.883	ND	ND	ND	ND	6833.0	1999.5	0.29	5500.0	ND	ND	ND	ND
15	24.0	24.0	7.88	0.533	ND	ND	ND	ND	6933.0	1600.5	0.23	5866.0	0.00	146.63	279.98	836.71
16	25.0	24.5	7.90	0.400	67.2	7.56	21.90	3.35	7033.0	1489.5	0.21	6040.0	ND	ND	ND	ND
17	25.0	25.0	7.92	0.233	ND	ND	ND	ND	7200.0	1425.0	0.20	6250.0	ND	ND	ND	ND
18	24.0	24.0	7.91	0.000	ND	ND	ND	ND	7150.0	1395.0	0.20	6220.0	0.00	167.68	165.65	9977.25
19	23.0	23.0	7.91	0.000	ND	ND	ND	ND	7100.0	1387.5	0.20	6175.0	ND	ND	ND	ND

ND = Not detectable

Table 11D. Biogas production from cassava tubers using 1.00% (w/v) total solids and urea addition at 0.03% (w/v), in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature (°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	26.0	26.0	7.87	0.000	ND	ND	ND	ND	2310.0	630.0	0.27	1890.0	0.00	129.39	392.72	4886.71
1	26.0	25.5	6.35	2.183	12.71	31.00	53.98	1.97	2050.0	1875.0	0.91	800.0	ND	ND	ND	ND
2	26.0	25.0	6.69	7.483	ND	ND	ND	ND	3233.0	3270.0	1.01	1053.0	ND	ND	ND	ND
3	26.0	26.0	6.72	1.683	ND	ND	ND	ND	3593.0	3439.5	0.96	1300.0	11.87	106.82	179.51	1729.52
4	26.0	26.5	6.92	1.023	29.11	46.24	21.63	3.02	4383.0	3450.0	0.79	2083.0	ND	ND	ND	ND
5	26.0	26.5	7.00	0.717	ND	ND	ND	ND	4650.0	3250.5	0.70	2483.0	ND	ND	ND	ND
6	26.0	26.0	7.16	1.075	ND	ND	ND	ND	4800.0	3199.5	0.67	2667.0	15.84	143.12	402.30	1803.14
7	26.0	26.5	7.33	1.125	45.25	34.43	17.09	3.22	5017.0	3325.5	0.66	2800.0	ND	ND	ND	ND
8	27.0	26.5	7.40	1.400	ND	ND	ND	ND	5267.0	3100.5	0.59	3200.0	ND	ND	ND	ND
9	27.0	27.0	7.50	1.567	ND	ND	ND	ND	5283.0	3124.5	0.59	3200.0	0.00	152.08	154.70	1654.03
10	27.0	27.0	7.64	1.567	62.99	23.25	11.19	2.64	5350.0	2674.5	0.50	3567.0	ND	ND	ND	ND
11	27.0	26.0	7.67	1.833	ND	ND	ND	ND	5317.0	2425.5	0.46	3700.0	ND	ND	ND	ND
12	26.0	25.0	7.74	1.183	ND	ND	ND	ND	5533.0	2224.5	0.40	4050.0	0.00	140.36	151.72	1699.87
13	25.0	24.5	7.82	1.017	68.58	13.24	15.65	2.53	5517.0	2175.0	0.39	4067.0	ND	ND	ND	ND
14	25.0	24.0	7.81	0.900	ND	ND	ND	ND	7300.0	2449.5	0.34	5667.0	ND	ND	ND	ND
15	24.0	24.0	7.85	0.483	ND	ND	ND	ND	7167.0	1950.0	0.27	5867.0	6.61	142.77	153.22	1444.59
16	25.0	24.5	7.91	0.267	64.34	7.05	26.87	1.74	7160.0	1620.0	0.23	6080.0	ND	ND	ND	ND
17	25.0	25.0	7.92	0.067	ND	ND	ND	ND	7317.0	1549.5	0.21	6284.0	ND	ND	ND	ND
18	24.0	24.0	7.90	0.267	ND	ND	ND	ND	7300.0	1530.0	0.21	6280.0	0.00	145.42	108.08	1409.09
19	23.0	23.0	7.91	0.000	ND	ND	ND	ND	7250.0	1470.0	0.20	6270.0	ND	ND	ND	ND

ND = Not detectable

Table 12D. Biogas production from cassava tubers using 1.00% (w/v) total solids and urea addition at 0.04% (w/v), in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature (°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	26.0	26.0	6.47	2.267	13.41	31.88	50.51	1.70	2250.0	1950.0	0.87	950.0	ND	ND	ND	ND
1	26.0	25.5	6.72	9.237	ND	ND	ND	ND	3283.0	3289.5	1.00	1090.0	ND	ND	ND	ND
2	26.0	25.0	6.73	1.867	ND	ND	ND	ND	3550.0	3250.5	0.92	1383.0	22.46	147.75	272.18	1648.02
3	26.0	26.0	7.02	1.300	32.03	44.68	21.37	1.92	4567.0	3550.5	0.78	2200.0	ND	ND	ND	ND
4	26.0	26.5	7.14	1.183	ND	ND	ND	ND	4783.0	3550.5	0.74	2416.0	ND	ND	ND	ND
5	26.0	26.5	7.26	1.400	ND	ND	ND	ND	5033.0	3000.0	0.60	3033.0	11.87	137.49	388.32	1552.49
6	26.0	26.0	7.44	1.317	48.07	31.61	18.44	1.88	5067.0	2899.5	0.57	3134.0	ND	ND	ND	ND
7	26.0	26.5	7.44	1.500	ND	ND	ND	ND	5250.0	3075.0	0.59	3200.0	ND	ND	ND	ND
8	27.0	26.5	7.55	2.017	ND	ND	ND	ND	5233.0	3049.5	0.58	3200.0	15.75	181.97	165.33	1497.23
9	27.0	27.0	7.64	1.983	66.13	20.51	11.37	2.00	5500.0	2284.5	0.42	3977.0	ND	ND	ND	ND
10	27.0	27.0	7.71	1.683	ND	ND	ND	ND	5883.0	2200.5	0.37	4416.0	ND	ND	ND	ND
11	27.0	26.0	7.79	1.100	ND	ND	ND	ND	5733.0	1675.5	0.29	4616.0	15.87	185.28	165.39	1500.06
12	26.0	25.0	7.84	0.983	62.51	10.06	21.72	5.71	5700.0	1699.5	0.30	4567.0	ND	ND	ND	ND
13	25.0	24.5	7.82	0.683	ND	ND	ND	ND	6933.0	1975.5	0.28	5616.0	ND	ND	ND	ND
14	25.0	24.0	7.83	0.517	ND	ND	ND	ND	7033.0	1800.0	0.26	5833.0	15.11	180.17	161.14	1332.16
15	24.0	24.0	7.91	0.133	64.33	8.76	25.04	1.87	7240.0	1699.5	0.23	6107.0	ND	ND	ND	ND
16	25.0	24.5	7.88	0.077	ND	ND	ND	ND	7433.0	1615.5	0.22	6356.0	ND	ND	ND	ND
17	25.0	25.0	7.89	0.040	ND	ND	ND	ND	7300.0	1560.0	0.21	6260.0	0.00	219.14	192.24	1311.48
18	24.0	24.0	7.90	0.000	ND	ND	ND	ND	7350.0	1500.0	0.20	6350.0	ND	ND	ND	ND
19	23.0	23.0	7.91	0.000	ND	ND	ND	ND	7250.0	1470.0	0.20	6270.0	ND	ND	ND	ND

ND = Not detectable

Table 13D. Biogas production from cassava tubers using 1.00% (w/v) total solids and urea addition at 0.10% (w/v), in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	22.0	20.0	8.15	0.000	ND	ND	ND	ND	2475.0	712.5	0.29	2000.0	0.00	158.28	171.01	4886.71
1	22.0	22.0	7.08	0.000	ND	ND	ND	ND	2750.0	1200.0	0.44	1950.0	ND	ND	ND	ND
2	22.0	23.0	6.37	6.100	ND	ND	ND	ND	2450.0	2962.5	1.21	475.0	ND	ND	ND	ND
3	22.0	22.0	6.74	1.200	ND	ND	ND	ND	3600.0	3300.0	0.92	1400.0	20.44	137.15	168.20	3462.28.
4	22.0	22.0	6.87	0.675	27.48	48.5	22.31	1.72	5450.0	4462.5	0.82	2475.0	ND	ND	ND	ND
5	22.0	23.0	7.03	0.790	ND	ND	ND	ND	6750.0	4875.0	0.72	3500.0	ND	ND	ND	ND
6	22.0	23.0	7.13	0.360	ND	ND	ND	ND	7300.0	4912.5	0.67	4025.0	0.00	145.31	178.42	2779.77
7	22.0	22.0	7.13	0.425	27.28	46.08	25.53	1.38	7300.0	4800.0	0.66	4100.0	ND	ND	ND	ND
8	22.0	22.0	7.19	0.400	ND	ND	ND	ND	6800.0	4575.0	0.67	3750.0	ND	ND	ND	ND
9	22.0	23.0	7.2	0.440	ND	ND	ND	ND	6800.0	4575.0	0.67	3750.0	22.97	154.76	169.04	2819.30
10	22.0	23.0	7.3	0.435	ND	ND	ND	ND	6800.0	4350.0	0.64	3900.0	ND	ND	ND	ND
11	22.0	22.0	7.39	0.615	ND	ND	ND	ND	6800.0	4350.0	0.64	3900.0	ND	ND	ND	ND
12	22.0	22.0	7.44	0.760	ND	ND	ND	ND	6800.0	4350.0	0.64	3900.0	14.47	190.76	172.73	1874.15
13	22.0	22.0	7.48	0.750	45.55	23.73	29.24	1.48	6800.0	4350.0	0.64	3900.0	ND	ND	ND	ND
14	22.0	22.0	7.55	0.850	ND	ND	ND	ND	6850.0	3825.0	0.56	4300.0	ND	ND	ND	ND
15	23.0	23.0	7.62	0.900	ND	ND	ND	ND	6850.0	3825.0	0.56	4300.0	4.40	212.45	193.13	1796.15
16	23.0	23.0	7.67	0.800	58.79	17.57	21.72	1.92	7000.0	3637.5	0.52	4575.0	ND	ND	ND	ND
17	23.0	23.0	7.76	0.825	ND	ND	ND	ND	7250.0	3450.0	0.48	4950.0	ND	ND	ND	ND
18	23.0	23.0	7.88	0.700	ND	ND	ND	ND	7500.0	3150.0	0.42	5400.0	13.52	190.16	188.36	1881.28
19	23.0	23.0	7.9	0.850	62.51	10.93	22.52	4.03	7500.0	3112.5	0.42	5425.0	ND	ND	ND	ND
20	24.0	23.0	7.94	0.900	ND	ND	ND	ND	7625.0	2625.0	0.34	5875.0	ND	ND	ND	ND
21	24.0	23.0	7.98	0.822	ND	ND	ND	ND	7800.0	2100.0	0.27	6400.0	16.42	173.50	201.59	1512.36
22	25.0	24.0	8.00	0.700	66.41	7.74	23.9	1.97	7825.0	2025.0	0.26	6475.0	ND	ND	ND	ND
23	25.0	24.0	8.04	0.700	ND	ND	ND	ND	7700.0	1950.0	0.25	6400.0	ND	ND	ND	ND
24	26.0	25.5	8.06	0.350	ND	ND	ND	ND	8250.0	2250.0	0.27	6750.0	0.00	162.16	191.63	1501.26
25	26.5	26.0	8.05	0.400	62.31	5.2	30.85	1.64	8100.0	1837.5	0.23	6875.0	ND	ND	ND	ND
26	27.0	26.0	8.05	0.300	ND	ND	ND	ND	8275.0	1650.0	0.20	7175.0	ND	ND	ND	ND
27	27.5	26.0	8.12	0.100	ND	ND	ND	ND	7300.0	1650.0	0.23	6200.0	0.00	148.84	108.87	1587.88

ND = Not detectable

Table 14D. Biogas production from cassava tubers using 1.00% (w/v) total solids and urea addition at 0.20% (w/v), in the single-state digester of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	22.0	20.0	8.16	0.000	ND	ND	ND	ND	2075.0	675.0	0.33	1625.0	0.00	116.25	0	4886.71
1	22.0	22.0	7.27	0.000	ND	ND	ND	ND	2825.0	1125.0	0.40	2075.0	ND	ND	ND	ND
2	22.0	23.0	6.45	5.675	ND	ND	ND	ND	2650.0	3000.0	1.13	650.0	ND	ND	ND	ND
3	22.0	22.0	6.73	1.050	ND	ND	ND	ND	3850.0	3525.0	0.92	1500.0	0.00	110.69	0	3550.17
4	22.0	22.0	7.05	0.350	27.46	35.58	35.46	1.51	6400.0	4200.0	0.66	3600.0	ND	ND	ND	ND
5	22.0	23.0	7.08	0.500	ND	ND	ND	ND	6800.0	4650.0	0.68	3700.0	ND	ND	ND	ND
6	22.0	23.0	7.09	0.400	ND	ND	ND	ND	7100.0	4875.0	0.69	3850.0	17.43	181.13	187.93	2879.10
7	22.0	22.0	7.09	0.250	28.84	29.96	39.81	1.39	7100.0	4950.0	0.70	3800.0	ND	ND	ND	ND
8	22.0	22.0	7.17	0.350	ND	ND	ND	ND	6350.0	4425.0	0.70	3400.0	ND	ND	ND	ND
9	22.0	23.0	7.21	0.250	ND	ND	ND	ND	6350.0	4425.0	0.70	3400.0	16.73	170.26	166.86	2000.97
10	22.0	23.0	7.26	0.300	ND	ND	ND	ND	6350.0	4425.0	0.70	3400.0	ND	ND	ND	ND
11	22.0	22.0	7.30	0.600	ND	ND	ND	ND	6450.0	4425.0	0.69	3500.0	ND	ND	ND	ND
12	22.0	22.0	7.36	0.650	ND	ND	ND	ND	6350.0	4425.0	0.70	3400.0	8.36	209.18	177.77	1755.53
13	22.0	22.0	7.38	0.700	36.58	12.16	49.36	1.91	6400.0	4425.0	0.69	3450.0	ND	ND	ND	ND
14	22.0	22.0	7.46	0.650	ND	ND	ND	ND	6600.0	4125.0	0.63	3850.0	ND	ND	ND	ND
15	23.0	23.0	7.56	0.850	ND	ND	ND	ND	6600.0	4125.0	0.63	3850.0	16.07	173.97	155.72	1826.17
16	23.0	23.0	7.62	0.700	49.14	8.99	33.83	8.05	6800.0	3975.0	0.58	4150.0	ND	ND	ND	ND
17	23.0	23.0	7.68	0.870	ND	ND	ND	ND	6900.0	3900.0	0.57	4300.0	ND	ND	ND	ND
18	23.0	23.0	7.76	0.700	ND	ND	ND	ND	6950.0	3652.5	0.53	4515.0	23.47	143.12	147.23	1781.21
19	23.0	23.0	7.79	0.700	62.08	6.97	28.94	2.00	6950.0	3600.0	0.52	4550.0	ND	ND	ND	ND
20	24.0	23.0	7.82	0.820	ND	ND	ND	ND	7050.0	3225.0	0.46	4900.0	ND	ND	ND	ND
21	24.0	23.0	7.88	1.970	ND	ND	ND	ND	7325.0	3150.0	0.43	5225.0	23.05	137.33	392.47	1632.14
22	25.0	24.0	7.93	0.750	ND	ND	ND	ND	7275.0	2925.0	0.40	5325.0	ND	ND	ND	ND
23	25.0	24.0	7.96	0.520	ND	ND	ND	ND	7250.0	2512.5	0.35	5575.0	ND	ND	ND	ND
24	26.0	25.5	8.02	0.500	ND	ND	ND	ND	7700.0	2737.5	0.36	5875.0	0.00	151.03	398.47	1650.12
25	26.5	26.0	7.98	0.600	56.07	4.31	37.34	2.27	7700.0	2400.0	0.31	6100.0	ND	ND	ND	ND
26	27.0	26.0	8.03	0.400	ND	ND	ND	ND	7775.0	2250.0	0.29	6275.0	ND	ND	ND	ND
27	27.5	26.0	8.09	0.100	ND	ND	ND	ND	7950.0	1987.5	0.25	6625.0	0.00	135.99	145.48	1598.78

ND = Not detectable

Table 15D. Biogas production from cassava tubers in laboratory scale experiment with working volume of 5-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	31.0	30.0	7.8.0	0.00	ND	ND	ND	ND	2225.0	534.00	0.24	1869.0	0.00	153.36	143.67	4886.71
1	29.0	29.0	7.27	2.00	6.66	30.11	58.45	3.92	2550.0	1575.0	0.62	1500.0	56.72	249.52	187.91	1166.96
2	29.0	29.0	6.27	9.00	18.67	39.53	39.95	1.86	2400.0	3150.0	1.31	300.0	22.89	229.91	171.99	1060.87
3	30.0	29.0	6.43	1.45	19.21	62.11	16.14	2.55	3300.0	4125.0	1.25	550.0	3.75	211.38	169.1	1564.78
4	31.0	31.0	6.89	1.55	29.02	50.29	15.87	4.82	4800.0	4650.0	0.97	1700.0	0.00	200.18	173.79	1750.43
5	31.0	30.0	7.00	1.80	32.99	50.15	12.47	4.41	6950.0	5400.0	0.78	3350.0	0.75	187.68	165.54	1511.74
6	31.0	31.0	7.12	2.00	35.5	44.26	18.19	2.04	6900.0	5550.0	0.80	3200.0	0.00	177.41	161.83	2068.70
7	30.0	30.0	7.26	1.40	44.75	39.39	13.95	1.92	7100.0	3834.0	0.54	4544.0	0.00	170.57	163.69	1830.00
8	30.0	30.0	7.24	1.25	50.73	24.24	20.25	4.60	7150.0	4218.0	0.59	4338.0	0.00	164.32	159.38	1803.48
9	29.0	29.0	7.30	1.45	59.71	28.24	8.7	3.34	7000.0	3780.0	0.54	4480.0	0.00	141.99	149.6	1485.22
10	30.0	30.0	7.31	1.95	67.92	22.18	5.9	4.00	7200.0	3960.0	0.55	4560.0	0.00	179.7	164.19	1750.43
11	30.0	30.0	7.54	1.75	61.14	26.10	10.27	2.49	7500.0	2250.0	0.30	6000.0	0.00	154.82	158.39	1856.52
12	30.0	30.0	7.59	1.30	62.04	16.04	19.34	1.80	7450.0	2160.0	0.29	6010.0	0.00	143.45	154.37	1617.83
13	30.0	30.0	7.63	1.05	64.25	14.11	14.51	2.16	7300.0	1533.0	0.21	6278.0	0.00	149.17	157.42	1538.26
14	30.0	30.0	7.62	0.80	61.59	2.86	33.84	1.71	7450.0	1788.0	0.24	6258.0	0.00	137.02	149.59	1591.30
15	30.0	30.0	7.62	0.65	61.86	8.49	28.29	1.36	7500.0	1800.0	0.24	6300.0	0.00	146.84	151.94	1299.57
16	31.0	31.0	7.77	0.30	63.17	10.15	24.38	2.30	7550.0	1585.5	0.21	6493.0	0.00	141.89	186.31	1432.17

ND = Not detectable

Table 16D. Biogas production from cassava tubers in laboratory scale experiment with working volume of 20-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH4	%CO2	%N2	%H2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	31.0	30.0	7.9 7	0.000	ND	ND	ND	ND	1600.0	525.0	0.33	1250.0	0.00	130.96	145.97	4886.71
1	29.0	29.0	6.64	6.450	8.72	88.98	2.28	0.00	2650.0	2250.0	0.85	1150.0	0.00	123.91	374.38	3111.41
2	29.0	29.0	6.72	33.40	15.65	86.02	0.00	0.00	3800.0	3900.0	1.03	1200.0	11.44	123.02	368.44	1801.34
3	30.0	29.0	6.92	10.30	18.29	73.5	6.05	2.16	4700.0	4200.0	0.89	1900.0	6.41	119.33	0.00	1670.34
4	31.0	31.0	7.00	8.580	24.06	68.32	5.13	2.48	5000.0	4200.0	0.84	2200.0	5.75	124.92	144.3	1768.59
5	31.0	30.0	7.12	7.940	30.72	58.47	7.57	3.24	6000.0	4575.0	0.76	2950.0	0.70	127.58	145.52	1965.10
6	31.0	31.0	7.22	4.050	41.22	52.7	5.7	0.39	6300.0	4500.0	0.71	3300.0	0.00	126.94	144.56	2456.38
7	30.0	30.0	7.49	3.375	44.07	46.21	9.28	0.44	6400.0	4425.0	0.69	3450.0	0.00	120.67	142.17	2358.12
8	30.0	30.0	7.40	4.750	48.77	42.63	2.32	2.34	6800.0	4500.0	0.66	3800.0	0.00	145.68	403.92	2489.13
9	29.0	29.0	7.42	5.275	55.50	36.96	5.32	2.22	6800.0	4275.0	0.63	3950.0	0.00	119.58	405.9	2194.36
10	30.0	30.0	7.56	5.500	55.70	34.84	7.06	2.4	7000.0	4350.0	0.62	4100.0	0.00	124.17	399.37	2063.36
11	30.0	30.0	7.58	5.300	61.29	30.56	5.47	2.68	7500.0	4050.0	0.54	4800.0	0.00	134.30	411.81	1866.85
12	30.0	30.0	7.65	4.450	63.01	29.26	5.19	2.54	7700.0	3900.0	0.51	5100.0	0.00	125.43	395.94	2358.12
13	30.0	30.0	7.80	4.475	65.73	26.09	5.66	2.52	7700.0	3750.0	0.49	5200.0	0.00	136.32	407.36	2259.87
14	30.0	30.0	7.81	3.875	67.57	23.75	6.28	2.41	8200.0	4050.0	0.49	5500.0	0.00	114.05	162.8	2096.11
15	30.0	30.0	7.88	3.550	69.09	20.92	8.19	1.81	8400.0	3750.0	0.45	5900.0	0.00	130.34	413.28	2194.36
16	31.0	31.0	8.00	3.150	71.94	18.09	7.14	2.83	8300.0	3450.0	0.42	6000.0	0.00	158.90	424.25	2128.86
17	31.0	31.0	8.03	2.850	71.95	18.04	7.41	2.60	8300.0	3300.0	0.40	6100.0	0.00	135.49	382.77	2292.62
18	30.0	30.0	8.08	2.200	74.34	14.19	9.50	1.98	8900.0	3300.0	0.37	6700.0	0.00	161.43	421.48	2194.36
19	31.0	31.0	8.09	2.000	75.68	12.03	9.99	2.13	8700.0	3000.0	0.34	6700.0	0.00	143.02	412.88	2096.11
20	31.0	31.0	8.09	2.025	72.98	10.74	12.83	3.50	8700.0	2850.0	0.33	6800.0	0.00	125.95	404.43	1899.60
21	31.0	30.0	8.07	1.600	73.57	13.41	10.51	2.43	8800.0	2400.0	0.27	7200.0	0.00	131.43	417.43	1866.85
22	31.0	31.0	8.14	1.475	72.77	9.43	15.09	2.71	9300.0	2550.0	0.27	7600.0	0.00	142.01	624.26	1572.08
23	31.0	31.0	8.12	0.700	76.51	9.36	11.9	2.22	9300.0	2475.0	0.27	7650.0	0.00	167.64	414.03	851.54
24	31.0	31.0	8.10	0.300	73.81	10.21	13.65	2.33	9400.0	2250.0	0.24	7900.0	0.00	116.09	178.87	327.52

ND = Not detectable

Table 17D. Biogas production from cassava tubers in laboratory scale experiment with working volume of 50-L digestion volume.

Day	Room temperature (°C)	Slurry temperature(°C)	pH	Daily gas yield (L/day)	%CH_4	%CO_2	%N_2	%H_2 and other gas	Alkalinity (mg as $CaCO_3$/L)	VFA(mg as acetate/L)	VFA/A	Bicarbonate (mg as $CaCO_3$/L)	Acetate (ppm)	Propionate(ppm)	Butyrate(ppm)	Starch (mg/L)
0	31.0	30.0	8.05	0.00	ND	ND	ND	ND	2450.0	750.0	0.31	1950.0	0.00	108.30	181.40	4886.71
1	29.0	29.0	7.22	24.65	17.33	76.55	3.14	2.99	3000.0	1650.0	0.83	1350.0	17.58	123.10	429.91	1720.32
2	29.0	29.0	6.65	69.84	16.77	74.77	6.28	2.19	3500.0	2500.0	1.07	1000.0	46.93	109.64	0.00	1495.93
3	30.0	29.0	6.94	25.20	22.09	63.5	7.97	6.44	4100.0	2800.0	1.02	1300.0	35.67	107.10	176.10	1446.07
4	31.0	31.0	7.25	24.00	28.70	64.84	4.39	2.08	5900.0	2800.0	0.71	3100.0	14.08	126.96	147.73	1595.66
5	31.0	30.0	7.42	20.80	40.21	49.84	7.02	2.94	6400.0	2800.0	0.66	3600.0	0.00	138.61	152.99	1321.41
6	31.0	31.0	7.55	16.80	49.87	41.32	4.82	3.98	6700.0	2800.0	0.63	3900.0	0.00	128.30	146.73	1146.88
7	30.0	30.0	7.67	16.00	57.33	34.98	5.06	2.63	6700.0	2400.0	0.54	4300.0	0.00	141.80	151.91	1545.79
8	30.0	30.0	7.79	16.20	59.04	31.77	6.52	2.68	6900.0	2400.0	0.52	4500.0	0.00	135.22	387.26	1471.00
9	29.0	29.0	7.80	20.15	67.70	24.53	5.12	2.65	7200.0	2400.0	0.50	4800.0	0.00	114.47	141.75	1595.66
10	30.0	30.0	7.79	24.40	68.65	22.81	5.88	2.66	7100.0	2300.0	0.49	4800.0	0.00	112.34	0.00	1446.07
11	30.0	30.0	7.81	13.25	66.08	21.69	8.35	3.88	7100.0	2000.0	0.42	5100.0	0.00	132.63	148.60	1097.02
12	30.0	30.0	7.90	11.10	67.59	13.58	16.89	1.94	7000.0	2000.0	0.43	5000.0	0.00	131.41	149.96	1296.47
13	30.0	30.0	7.88	9.60	69.75	16.17	11.39	2.70	7200.0	2000.0	0.42	5200.0	0.00	119.76	141.98	1570.73
14	30.0	30.0	7.92	9.95	69.79	13.71	13.98	2.52	7400.0	2000.0	0.41	5400.0	0.00	125.06	408.09	1620.59
15	30.0	30.0	7.98	3.60	69.62	13.99	14.07	2.33	7700.0	2000.0	0.39	5700.0	0.00	189.95	430.91	1271.54
16	31.0	31.0	8.00	4.00	68.59	9.80	19.00	2.61	7600.0	2000.0	0.39	5600.0	0.00	122.79	409.56	1072.08
17	31.0	31.0	8.00	3.80	67.33	8.27	20.63	3.77	7600.0	2000.0	0.39	5600.0	0.00	130.68	422.13	1446.07
18	30.0	30.0	8.02	2.80	65.10	8.05	24.5	2.36	7800.0	1800.0	0.35	6000.0	0.00	114.11	414.58	1396.20
19	31.0	31.0	8.15	1.95	67.55	5.86	24.03	2.56	7900.0	1700.0	0.32	6200.0	0.00	150.53	413.03	1595.66
20	31.0	31.0	8.21	0.70	65.24	5.58	26.26	2.92	8000.0	1550.0	0.29	6450.0	0.00	140.69	413.34	1072.08
21	31.0	30.0	8.23	0.25	ND	ND	ND	ND	8000.0	1400.0	0.26	6600.0	0.00	147.66	116.19	897.56

ND = Not detectable

APPENDIX E

LIST OF PRESENTATIONS

Anunputtikul, W. and Rodtong, S. 2004. Investigation of the potential production of biogas from cassava tuber**. The 15th Annual Meeting of the Thai Society for Biotechnology and JSPS-NRCT Symposium,** 3-6 February 2004, Lotus Hotel Pang Suan Kaew, Chiang Mai, Thailand (Poster Presentation).

วันทนีย์ อนันต์พุฒิกุล และ สุรีลักษณ์ รอดทอง. 2547. ปัจจัยด้านสารอาหารหลักในการผลิตก๊าซชีวภาพจากหัวมันสำปะหลัง. **การประชุมเสนอผลงานวิจัยระดับบัณฑิตศึกษาแห่งชาติ ครั้งที่ 4**, 10-11 สิงหาคม 2547, ณ โรงแรมโลตัสปางสวนแก้ว จ. เชียงใหม่ (Poster Presentation).

Anunputtikul, W. and Rodtong, S. 2004. Laboratory scale experiments for biogas production from cassava tubers. **The Joint International Conference on "Sustainable Energy and Environment (SEE)",** 1-3 December 2004, Hilton Hua Hin Resort and Spa, Hua Hin, Thailand (Oral Presentation).

Investigation of the Potential Production of Biogas from Cassava Tuber

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Biogas is an alternative source of energy for substitution of natural energy, that is being reduced by human activities and becoming expensive. Cassava tuber, a cheap and abundant agriculture product produced in the Northeast Thailand, particularly in Nakhon Ratchasima province, is considered to be a suitable raw material for the production of biogas. The objective of this study is to investigate the potential production of biogas from cassava tuber using the single-state digester. It was found that cassava tuber collected from Nakhon Ratchasima province composed of the average content of 88.63% (dry weight) of total solid, 97.57% of volatile solid, 2.43% of ash, 39.48% of carbon, 0.54% of nitrogen, and 0.23% of phosphorus. The carbon-to-nitrogen ratio was 73:1. Dry cassava tuber (after chopping into <1 cm^3 pieces and containing 11.37% of moisture content) and organic loading rates at 1.25- 20% (w/v) total solid were applied. Biogas production was performed in anaerobic digesters with working volume of 5 liters at ambient temperature for 30 days. When using 1.25% (w/v) total solid, the maximum gas yield of 1.14 liters/day and the methane content of 54.91% were obtained on day 18 whereas the maximum methane content of 64.35% and the gas yield of 0.61 liters/day were obtained on day 21. The calorific value was basically estimated. At the maximum methane yield of 1.25% (w/v) total solid organic loading rates, 25.48 kJ per liter of biogas which compared to 1.38 liters of coal gas, 0.31 liters of propane, and 0.2 liters of butane were achieved.

ปัจจัยด้านสารอาหารหลักในการผลิตก๊าซชีวภาพจากหัวมันสำปะหลัง

Main nutrition factor in biogas production from cassava tubers

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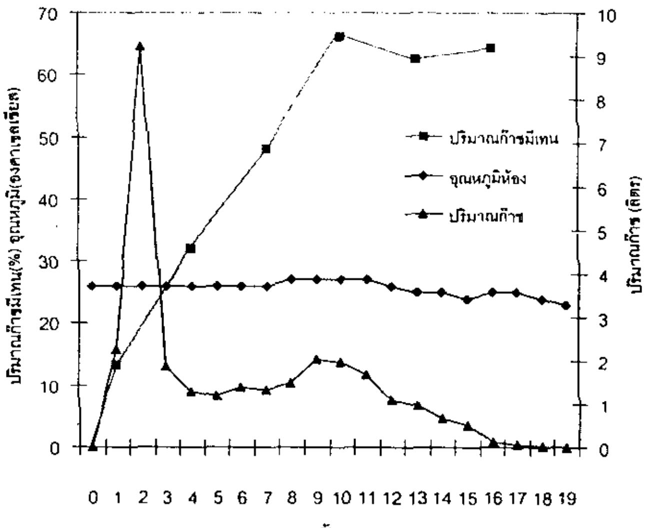
บทคัดย่อ: ก๊าซชีวภาพเป็นแหล่งพลังงานแหล่งหนึ่งที่สามารถใช้ทดแทนพลังงานจากธรรมชาติซึ่งมีการใช้กันมากและมีมูลค่าสูงขึ้นในปัจจุบัน เมื่อพิจารณาถึงวัตถุดิบในการผลิตก๊าซชีวภาพแล้วมันสำปะหลังเป็นวัตถุดิบที่หาได้ง่ายในประเทศไทย มีมูลค่าต่ำ และมีองค์ประกอบที่จุลินทรีย์ใช้เป็นแหล่งอาหารได้ดี การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาปัจจัยด้านสารอาหารหลักคือปริมาณหัวมันสำปะหลังแห้ง และชนิดและปริมาณของแหล่งไนโตรเจนในการผลิตก๊าซชีวภาพจากหัวมันสำปะหลังดิบ โดยใช้กระบวนการหมักแบบขั้นตอนเดียวในปริมาตรของการหมัก 5 ลิตร ที่อุณหภูมิห้อง (22-30°C) เป็นเวลา 30 วัน ซึ่งเมื่อใช้หัวมันแห้ง (ความชื้น 18.65%) พบว่าที่ 1.2% (w/v) ของของแข็งทั้งหมด และการเติมยูเรียที่ 0.4 กรัม/ลิตร (w/v) ให้ผลผลิตของก๊าซมากที่สุดคือ 1.98 ลิตร/วัน และปริมาณมีเทนมากที่สุดคือ 66.13% ที่ระยะเวลาการหมัก 10 วัน โดยกระบวนการหมักจะสิ้นสุดลงเมื่อหมักได้ 19 วัน

ระเบียบวิธีวิจัย: เตรียมวัตถุดิบเพื่อการหมักก๊าซชีวภาพโดยได้นำหัวมันสำปะหลังสดจากแหล่งเพาะปลูกในเขตจังหวัดนครราชสีมามาล้างทำความสะอาดแล้วหั่นเป็นชิ้นเล็กๆ (ขนาด 1 ซม.^{3}) ตากแดดเป็นเวลา 2 วันให้แห้ง แล้วนำไปปั่นโดยใช้โถปั่นให้ได้ชิ้นมันขนาดประมาณ 0.2 ซม.^{3} พร้อมกับการวิเคราะห์หาองค์ประกอบทางเคมีและทางกายภาพของหัวมันสำปะหลังสดและวัสดุแห้ง (1) และหมักที่ปริมาตรการหมัก 5 ลิตร โดยใช้หัวเชื้อจุลินทรีย์ซึ่งเตรียมจากของเสียจากบ่อบำบัดน้ำเสียของโรงงานแป้งมันสำปะหลังผสมกับมูลสัตว์ โดยใช้หัวเชื้อจุลินทรีย์ในปริมาณ 10% (v/v) และทดลองใช้ปริมาณหัวมันสำปะหลังแห้งที่ 0.3-18.6% (w/v) ของของแข็งทั้งหมด และเลือกยูเรียเป็นแหล่งไนโตรเจนเติมยูเรียที่ 0.2-2 กรัม/ลิตร (w/v) ที่อุณหภูมิห้อง เป็นเวลาทั้งหมด 30 วัน โดยรักษาระดับความเป็นกรดด่างที่ค่าสัดส่วนของกรดไขมันระเหยและค่าความเป็นด่างที่ ≤0.8 วัดปริมาณก๊าซที่เกิดขึ้นและวิเคราะห์องค์ประกอบของก๊าซตามวิธีที่ระบุใน Cuzin *et al.*(2)

ผลการวิจัย อภิปราย และสรุปผลการวิจัย จากการศึกษาองค์ประกอบของหัวมันสำปะหลังแห้ง (สายพันธุ์เกษตรศาสตร์ 50) พบว่าประกอบด้วยความชื้น 18.65 % ของแข็งทั้งหมด 81.35% ของแข็งระเหย 97.57 % เถ้า 2.43% ธาตุคาร์บอน 39.60% ธาตุไนโตรเจน 0.46% และธาตุฟอสฟอรัส 0.18% จากการหมักก๊าซชีวภาพในปริมาตร 5 ลิตร ที่อุณหภูมิห้อง (22-30°C) พบว่าเมื่อใช้หัวมันสำปะหลังแห้ง 1.2 % (w/v) ของของแข็งทั้งหมด และเติมยูเรียที่ 0.4 กรัม/ ลิตร (w/v) ให้ผลผลิตของก๊าซสูงคือ 1.98 ลิตร/วัน และมีปริมาณมีเทนเป็นองค์ประกอบมากที่สุดคือ 66.13 % ที่ระยะเวลาการหมัก 10 วัน (รูปที่ 1) โดยกระบวนการหมักจะสิ้นสุดลงเมื่อหมักได้ 19 วัน จากการทดลองนี้แสดงให้เห็นถึงศักยภาพของหัวมันสำปะหลังในการนำมาใช้เป็นวัตถุดิบในการผลิตก๊าซชีวภาพโดยการหมักแบบขั้นตอนเดียวเพราะมีปริมาณมีเทนเป็นองค์ประกอบถึง 66%.



รูปที่ 1 ปริมาณก๊าซทั้งหมดและก๊าซมีเทน และอุณหภูมิห้องที่เปลี่ยนแปลงในช่วงเวลาของการหมัก เมื่อใช้มันสำปะหลังแห้งที่ 1.2% (w/v) ของของแข็งทั้งหมดและเติมยูเรียที่ 0.4 กรัม/ลิตร

เอกสารอ้างอิง: (1) *Association of Official Analytical Chemists* (1990). Arington: The Association of Official Analytical Chemists, Inc.
(2) Cuzin, N., Farinet, J.L., Segretain, C., and Labat, M. (1992). *Bioresource Technology*. 41:259-264.

คำสำคัญ: ก๊าซชีวภาพ หัวมันสำปะหลัง

Laboratory Scale Experiments for Biogas Production from Cassava Tubers

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Abstract: The production of biogas, an alternative source of energy, from starch-rich tubers of cassava plant, was investigated in the laboratory scale using the simple single-state digesters of 5- and 20-liter working volumes. The digesters were fed on a batch basis with the slurry of dry cassava tuber containing the average moisture content of 18%, and operated at ambient temperature (29-31°C) for 30 days. When operating the single-state digester of 5-liter working volume fed with the optimal concentrations of carbon and nitrogen sources, 1.00% (w/v) total solids and 0.04% (w/v) urea, the gas yield of 1.95 liters/day containing the maximum methane content of 67.92% was achieved at 10-day retention time. The fermentation reactions were ceased after 16-day operation. The fermentation volume was then scaled up to 20 liters. The gas yield of 5.50 liters/day containing 55.70% methane was obtained at 10-day retention time. Whereas the methane content of 67.57% and the gas yield of 3.88 liters/day were obtained at 14-day retention time. The fermentation reactions were ceased after 24-day operation. Biogas containing 67% methane content could be achieved from the digestion of cassava tubers using simple single-state digesters.

Keywords: Biogas, Cassava, Cassava Tuber, Methane, Single-state Digester.

1. INTRODUCTION

Biogas, the gas generated from organic digestion under anaerobic conditions by mixed population of microorganisms, is an alternative energy source which has been commenced to be utilized both in rural and industrial areas at least since 1958 [17]. The gas generally composes of methane (55-65%), carbon dioxide (35-45%), nitrogen (0-3%), hydrogen (0-1%), and hydrogen sulfide (0-1%) [11]. The composition of biogas depends on feed materials. Organic waste has been mainly used for the biogas production, and several kinds of waste materials have been reported to be exploited (4, 6, 7, 9, 10, 20). Raw cassava tubers, the cheap and abundant agriculture product in Thailand [12, 13, 14], are initially investigated to be applied as a raw material for the bio-energy production in our previous [2] and this studies. In this study, the maximum production of biogas and methane from the starch-rich tuber is determined in laboratory scale using the simple single-state digesters.

2. MATERIALS AND METHODS

2.1 Preparation of the raw material for biogas production

Fresh cassava tubers were collected from their plantation area in Nakhon Ratchasima Province, Thailand. To obtain the consistency of the raw material for biogas production experiments, dry cassava tubers containing the average moisture content of 18% were prepared by chopping the whole tuber into pieces, dried under sun light over a two-day period, then crushed into small pieces (<0.2 cm^3) using blender (Waring Commercial, U.S.A.). Total solids (TS), volatile solids (VS), ash, and phosphorus contents of the raw material were determined using standard methods [1, 3]. Total carbon and nitrogen contents were also determined using CNS-2000 Elemental Analyzer (Leco Corporation, U.S.A). Starch concentration was basically detected by spectrophotometry at 580 nm absorbance in the soluble form and presence of iodine [8, 14].

2.2 Preparation of seed cultures

Seed cultures were prepared by mixing animal manure and liquid waste collected from the anaerobic pond of the cassava starch production factory in Nakhon Ratchasima Province, then kept in a closed container at room temperature with regular adding a small amount of cassava starch for 3 months before inoculating the biogas production digester.

2.3 Biogas production from cassava tubers

The production of biogas from raw cassava tuber was performed using the simple single-state digesters with working volumes of 5 and 20 liters (L) (Table 1, Fig. 1). The digesters were fed on a batch basis with the slurry of dry cassava tuber containing the average moisture content of 18% and 10% (v/v) of seed cultures. The biogas fermentation was then operated in triplicate at ambient temperature for 30 days.

Table 1 Physical characteristics of 5-L and 20-L working volume digesters

Parameter	5 L	20 L
Digester height (cm)	25.00	35.00
Liquid height (cm)	13.50	41.30
Empty volume (L)	7.50	26.00
Filled volume (L)	5.00	20.00

Since the amount of main nutrients (carbon and nitrogen sources) affects the growth of microorganisms and the production of biogas, the optimal concentrations of TS (carbon source) and nitrogen source added were determined. The high carbon-to-nitrogen ratio (approximately 80:1) of cassava root (dry weight) has been reported [16]. The optimum ratios for the maximum biogas generation have been suggested to be 20-30:1 [15, 19]. In this study, various TS concentrations: 0.25, 0.50, 1.00, 2.00, 4.00, and 8.00% (w/v), were applied to the 5-L reaction volume to obtain the optimum TS content. Then the addition of urea (46% of nitrogen) as a nitrogen source at 0.00, 0.02, 0.03, 0.04, 0.10, and 0.20% (w/v) was investigated.

For stabilizing pH of cassava slurry during the anaerobic digestion, the addition of sodium bicarbonate (0.25%, w/v) was considered whenever the volatile fatty acids-to-alkalinity ratio was greater than 0.8.

The volume of biogas produced in the digester was measured by the displacement of water in the gas holder compartment. The pH of water in this holder was adjusted to 2 to avoid carbon dioxide dissolution [1]. Gas production was measured daily. The composition of biogas collected over water, was analyzed using the Gas Analyser (Shimadzu,

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Class-GC14B, Japan) equipped with a thermal conductivity detector (TCD) and 1-M Porapak Q (80-100 mesh) column. Helium was used as a carrier gas at a flow rate of 25 mL/min. The oven, injector, and detector temperatures were 80, 120, and 120°C respectively.

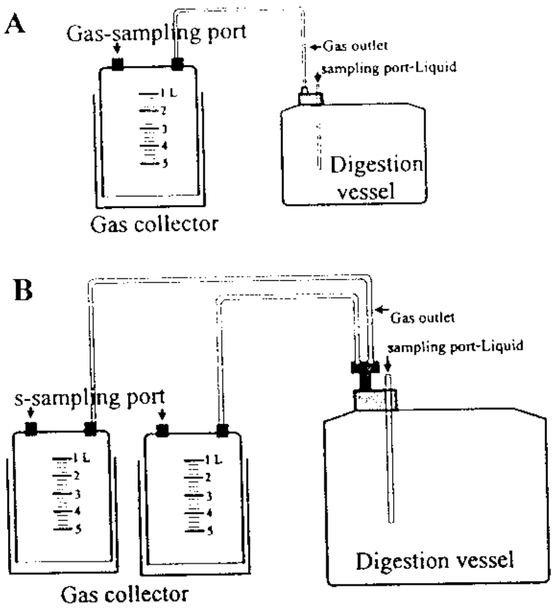


Fig. 1 Single-state digesters of (A) 5-L and (B) 20-L working volumes.

Volatile acids (acetic, propionic, and butyric acids) were analyzed using the Gas Analyser (Shimadzu, Class-GC14B, Japan) equipped with a flame ionization detector (FID) and DB-FFAP column. Helium was used as a carrier gas at a flow rate of 40 cm/sec whereas nitrogen was used as a makeup gas at a flow rate of 30 mL/min. The oven, injector, and detector temperatures were 100, 250, and 300°C, respectively. Peak areas were used to calculate concentrations by comparing to calibration curves prepared from standard solutions of acetic, propionic, and butyric acids.

Starch content, alkalinity, and volatile fatty acids (VFA) of cassava slurry during digestion were determined daily. Alkalinity and VFA were determined by the direct titration with sulfuric acid [1]. The VS content and the reduction of VS in the slurry were detected and calculated [20], respectively. The measurement of pH value and temperature was also performed.

The optimal concentrations of both total solids and nitrogen were applied to produce biogas in the scaled-up digester, 20-L working volume.

3. RESULTS AND DISCUSSION

3.1 Raw material for biogas production

Cassava plant variety KU 50 was one of dominant varieties cultivated in Nakhon Ratchasima Province. Fresh starch-rich tubers of the plant were collected. Some physical and chemical compositions of the tuber were analyzed (Table 2). The fresh tuber has approximately 18% of starch, 62% of moisture, 0.9% of ash, and 0.08% of phosphorus. Soccol (1996) stated that fresh cassava roots had 20-30% of starch, 65% of moisture, 0.9% of ash, and 0.03% of phosphorus [16]. The dry starchy material of variety KU 50 containing 18.65% of moisture, 81.35% of TS, 1.95% of ash, 98.05% of VS, 39.56% of total carbon, 38.10% of starch, 0.46% of total nitrogen, and 0.18% of phosphorus, was used to prepare slurry to feed the simple single-state digesters. The average carbon-to-nitrogen ratio of the dry cassava material is 86:1 which is very high ratio compared to the optimum ratios of 20-30:1 for the maximum biogas generation [15, 19].

Table 2 Compositions of cassava tuber, plant variety KU50, collected from the plantation area in Makhon Ratchasima Province

Composition (%)	Fresh weight	Dry weight
Moisture	61.66	18.65
Total solids (TS)	38.34	81.35
Volatile solids (VS)	99.12	98.05
Total carbon	18.64	39.56
Total nitrogen	0.22	0.46
Starch	17.96	38.10
Ash	0.88	1.95
Phosphorus	0.08	0.18

3.2 Biogas production from cassava tubers

When the single-state digester with working volume of 5 L was used for optimization of some biogas production conditions, the maximum yield of 356.35 L/kg TS fed of biogas was achieved from 1.00% (w/v) TS (Fig. 2). The gas yield of 1.20 liters/day composing the maximum methane content of 64.35% was obtained at 22-day retention time. The fermentation reactions were ceased after operating for 25 days. The volatile solids reduction of fermenting slurry was 39.10%.

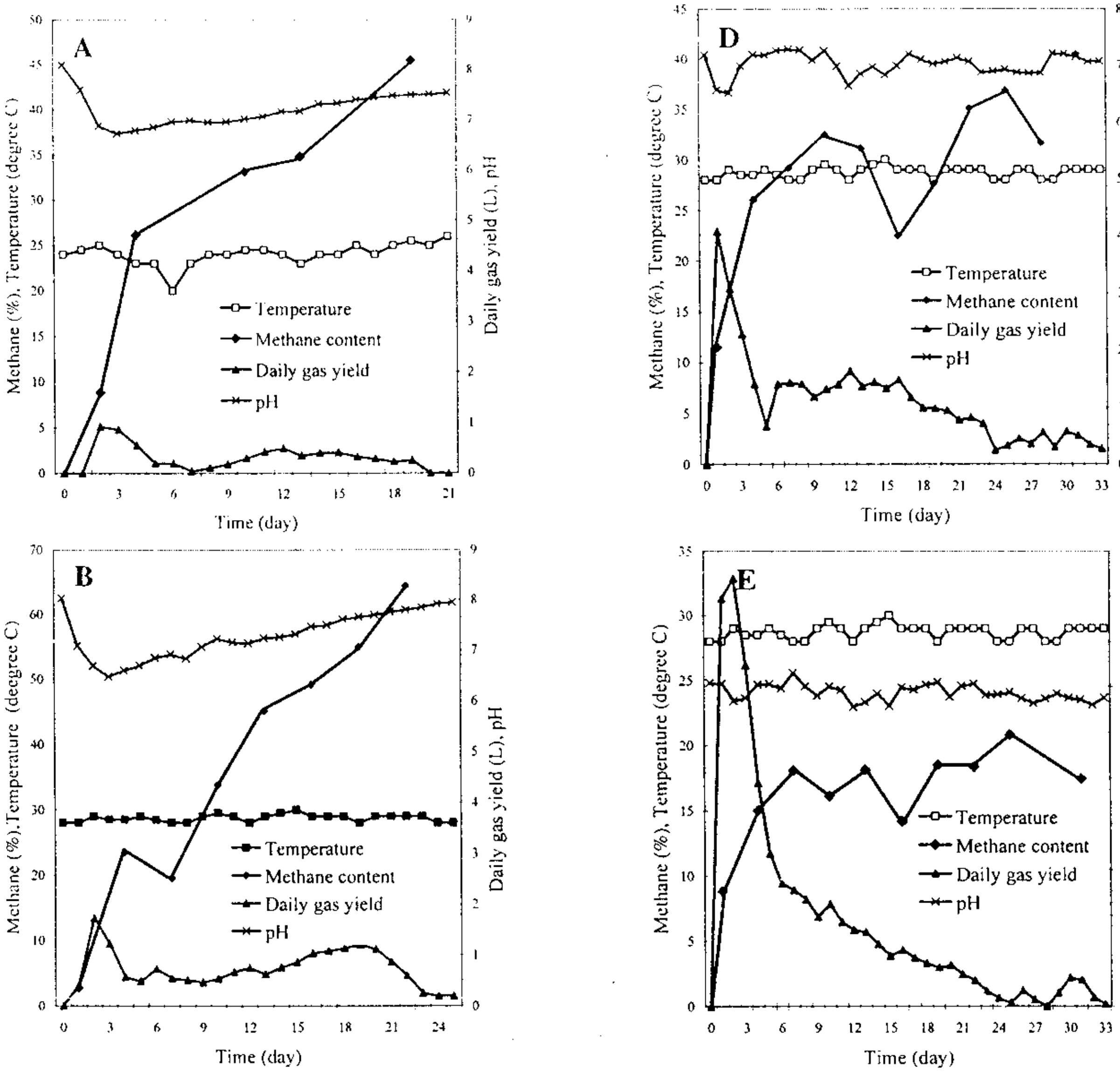
The supplement of urea (0.04%, w/v) to the cassava slurry (1.00%, w/v, TS) could stimulate the maximum biogas production. The maximum yield of total biogas was 569.29 L/kg TS fed. The gas yield of 1.95 liters/day containing the maximum methane content of 67.92% was achieved at 10-day retention time (Figs. 3C, 4A and 5). The utilization of volatile solids was 56.83%. But the fermentation reactions were ceased after 16-day operation (Fig. 4A).

When the optimal concentrations of total solids (1.00%, w/v) and urea 0.04% (w/v) were applied to the scaled-up experiment, 20-L reaction volume, the gas yield of 5.50 liters/day containing 55.70% methane was obtained at 10-day retention time. Whereas the methane content of 67.57% and the gas yield of 3.88 liters/day were obtained at 14-day retention time. The fermentation reactions were ceased after 24-day operation (Figs. 4B and 5).

When the digesters was initially fed, acid forming-bacteria quickly produced acid resulting in declining pH below the neutral pH and diminishing growth of methanogenic bacteria and methanogenesis. The pH could be maintained by adding sodium bicarbonate to increase digester alkalinity. In this study, sodium bicarbonate was added four times during the first week of fermentation for both bioreactor sizes. Afterwards the digesters could maintain themselves (Fig. 4). At the daily methane yield of more than 50% of biogas composition, the digesters operated at a pH range of 7.2 to 7.8 and 7.4 to 8.1 with the alkalinity of 7000-7550 and 6800-9400 mg/L, and VFA of 1585-4218 and 2250-4350 mg/L, for 5-L and 20-L cassava tuber slurry, respectively (Figs. 5 and 6).

Volatile acids (acetic, propionic, and butyric acids) accumulation during cassava tuber fermentation were detected (Fig. 7). The concentration of propionic and butyric acids were higher than that of acetic acid in both digester sizes.

Temperatures of the cassava slurry during fermentation were found to be between 29 and 31°C for all experiments



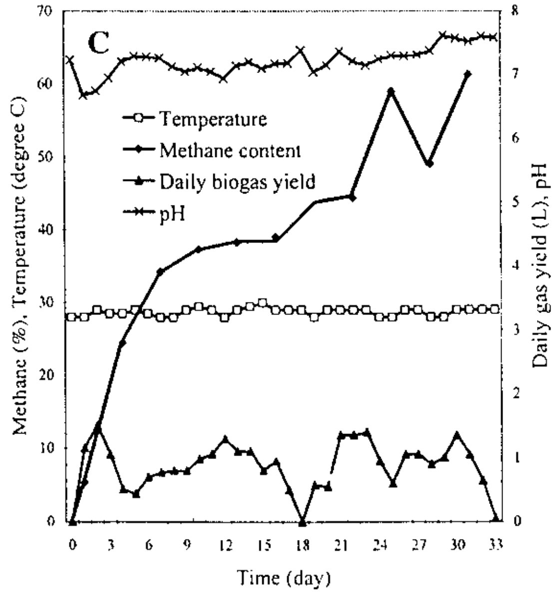
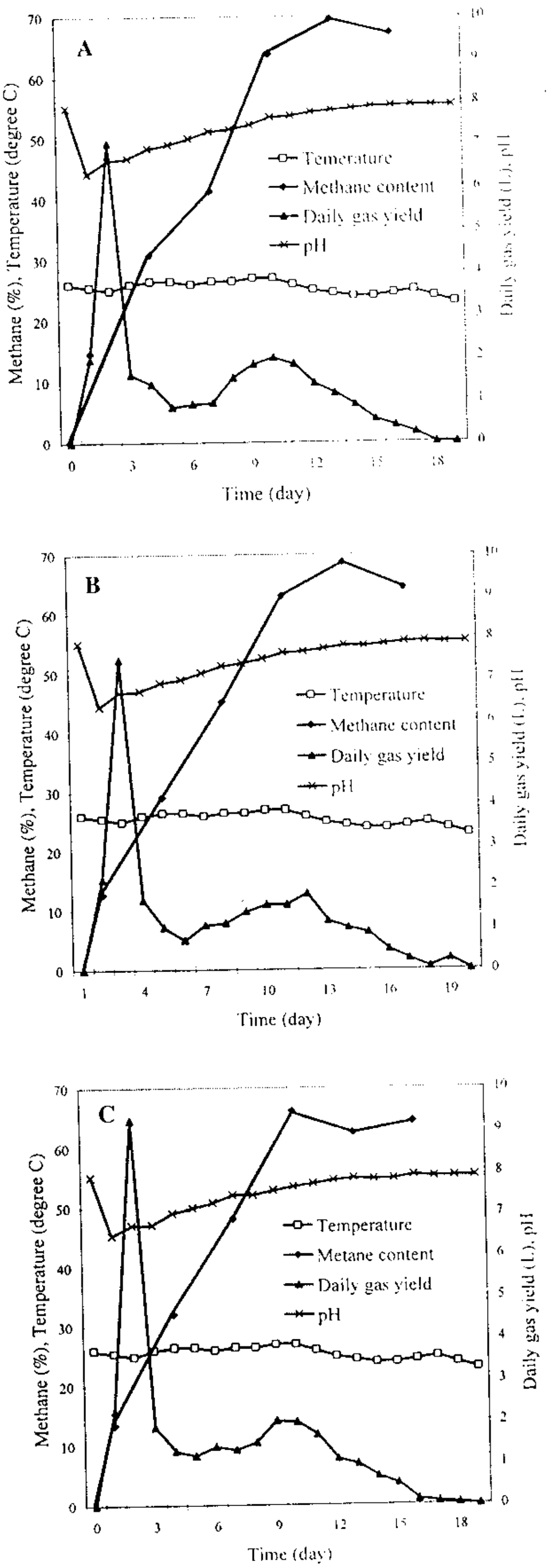


Fig. 2 Biogas production from cassava tubers using (A) 0.50, (B) 1.00, (C) 2.00, (D) 4.00, and (E) 8.00% (w/v) total solids without supplementing a nitrogen source in the single-state digester of 5-L digestion volume.

(Fig. 4).

Total biogas yield, total methane yield, and VS reduction obtained from the two bioreactor sizes were compared (Table 3). The total biogas yields of 5-L and 20-L cassava slurry were 569.29 and 611.32 L/kg TS fed respectively. The biogas yield from 20-L working volume was 6.88% higher than the yield from 5 L. The total methane yield was also higher (339.53 L/kg TS fed from 20 L and 263.90 L/kg TS fed from 5 L). But the average methane contents for overall reactions of 5-L and 20-L digestion mixtures were 46.22% and 55.54%, respectively.

The theoretical biogas yield from carbohydrate has been reported to be 886 L/kg VS fed [5]. From our experiments, the total biogas yields per kg VS fed were 474.67 L and 509.71 L from 5-L and 20-L digestion volumes, respectively (Table 3). The obtainable products were lower than theoretical yields.



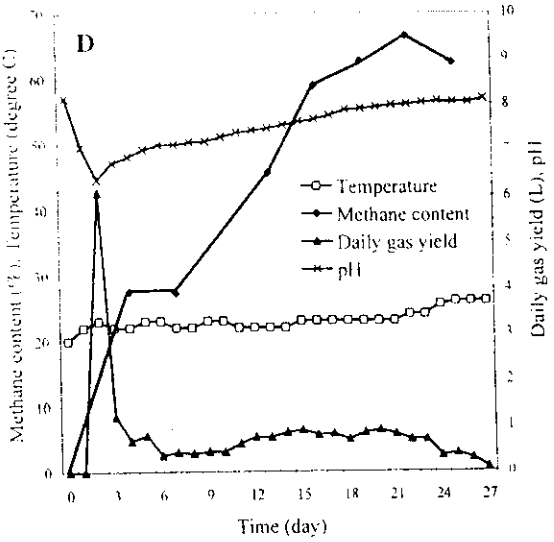


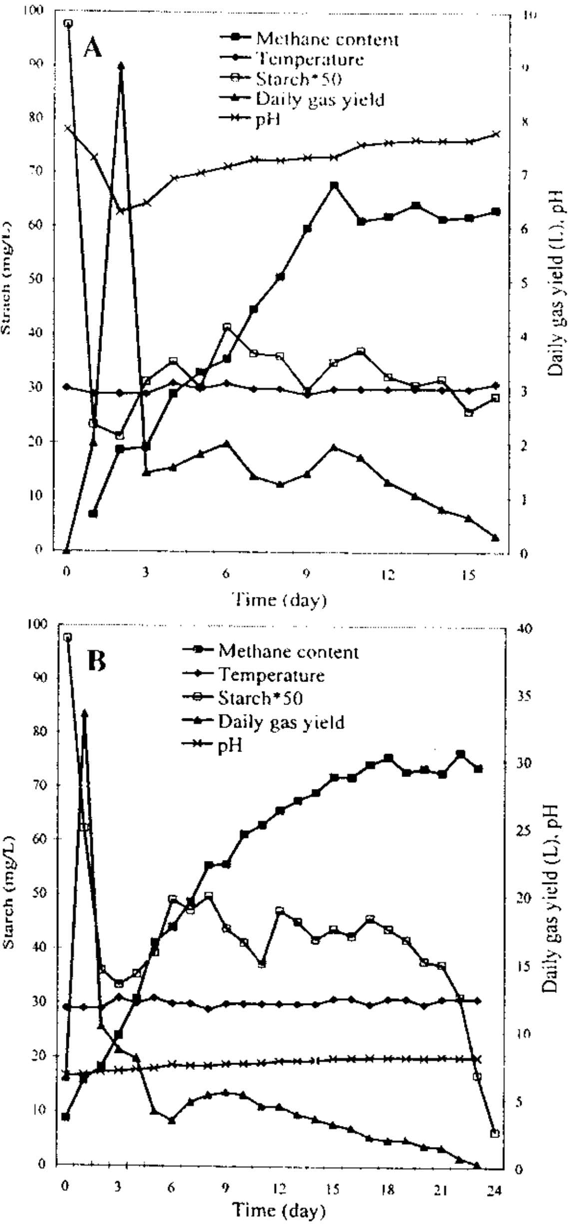
Fig. 3 Biogas production from cassava tubers using 1.00% (w/v) total solids and urea supplements at various concentrations: (A) 0.02, (B) 0.03, (C) 0.04, and (D) 0.10% (w/v), in the single-state digester of 5-L reaction volume.

Table 3 Biogas production from cassava tubers in laboratory scale experiments

Parameter	Reaction volume (L)	
	5	20
Total biogas yield (L/kg TS fed)	569.29	611.32
Total biogas yield (L/kg VS fed)	474.67	509.71
Total methane yield (L/kg TS fed)	263.90	339.53
Volatile solids (VS) reduction (%)	56.83	61.51

4. CONCLUSIONS

Biogas containing the methane content of 67% could be efficiently produced from cassava tuber slurry (1%, w/v, TS) and the supplement of urea (0.04%, w/v) in the simple single-state digester with both 5-L and 20-L reaction volumes. Cassava tubers used to prepared the slurry contain the average contents of 81% of TS, 40% of total carbon, 38% of starch, and 0.5% of total nitrogen. One kilogram (kg) TS of the dry tuber was obtained from 1.23 kg of the total dry mass prepared from the whole tuber. And one kg of the dry cassava mass was achieved from 2.11 kg of fresh cassava tuber. From these practical calculation results, one kg of dry cassava tuber could be biologically converted to 497.01 L of biogas, and one kg of fresh cassava tuber could produce 235.12 L of biogas. If the energy value of biogas (50-70% of methane content) was 22000-26000 kJ/m^3, one kg of fresh and dry cassava tubers used as raw materials for biogas production, could produce 5172.64 kJ and 10934.22 kJ energy, respectively.



, 4 Biogas production from cassava tubers using 1.00%) total solids and 0.04% (w/v) urea supplement in the le-state digesters of (A) 5-L and (B) 20-L working volumes.

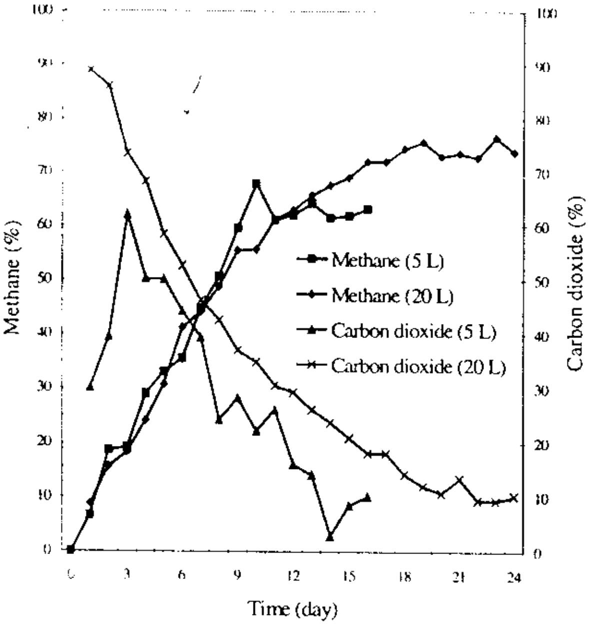


Fig. 5 Methane and carbon dioxide composition of gas measured during cassava tuber fermentation in the single-state digesters of 5-L and 20-L working volumes.

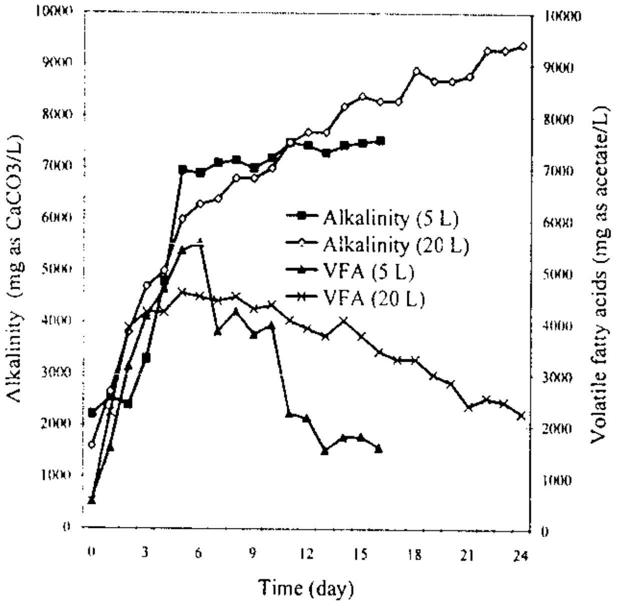


Fig. 6 Alkalinity and volatile fatty acids measured during biogas production from cassava tubers in the single-state digesters of 5-L and 20-L working volumes.

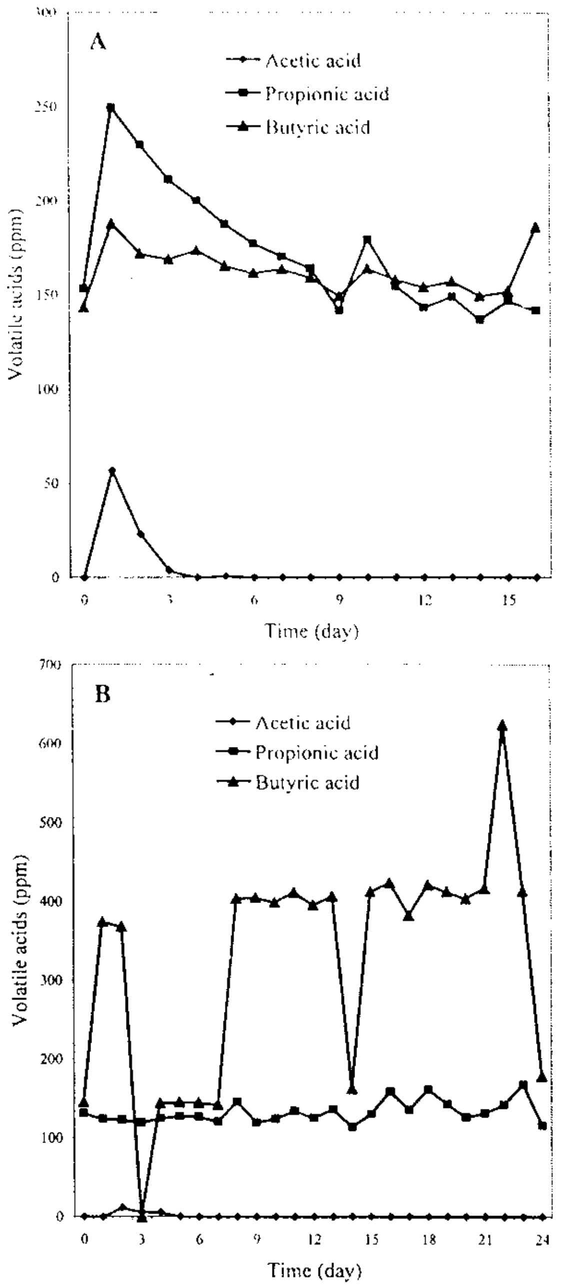


Fig. 7 Volatile acids accumulation during cassava tuber fermentation in the single-state digesters of (A) 5-L and (B) 20-L working volumes.

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