IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)
e-ISSN: 2319-2402, p- ISSN: 2319-2399.Volume 19, Issue 7 Ser. 2 (July 2025), PP 21-31
www.iosrjournals.org

Process Optimization For Enhanced Biogas Production From Starchy Agricultural Wastes

Gopinathan Chengalath [1], Aparna Menon Ambalakode [2]
[1](Department Of Biotechnology, University Of Calicut, India)
[2](Department Of Biotechnology, University Of Calicut, India)

Abstract:
First generation of biofuel like ethanol obtained from sources like sugarcane and corn were replaced by second generation of fuel from cellulose, hemicellulose and lignin which are chiefly based on non-food-based sources. Countries generating vast amount of agricultural and animal-based waste products started using these as sources for biofuel production. This study mainly focuses on utilizing tapioca-based material as source of generation of biofuel. Studies were conducted to devise methods for efficient, high yielding and cost-effective process for biogas generation by varying different process parameters. This study devises methods for effective management of waste generated in tapioca cultivation and processing industries as well as investigates ways for value addition of different by-products which when discarded unscientifically could attract environment pollution and associated hazards.

Background: *India being a chief producer of vegetables, the volume of waste generated from this field will also be higher. Waste materials, especially those containing starch, mainly from corn, potato and tapioca processing industries, when subjected to appropriate hydrolytic enzymes for the cleavage of a-1,4 linkage of the starch molecule coverts it into corresponding monomers, which can easily be subjected to fermentation employing methanogenic microbial consortia, which would ultimately result in the generation of biogas. The generated biogas can be used as a source of biofuel, to power electricity and the slurry formed as the end-product also finds application as manure in farming and agricultural sector benefiting a large proportion of the population. Effect of variations in process parameters as well incorporation of different additives to the fermentation mixture had been tested to check for method for high yield of the end product. This study also focusses on value-addition to materials that are otherwise discarded as waste material, which when unscientifically disposed could result in major environmental pollution and likely result in health issues.*

Materials and Methods*: In this study, starch from tapioca was used as the substrate for the production biogas. The process parameters were varied to check for maximum yield. The raw material was subjected to pretreatment like aeration, soaking, acid hydrolysis (using HCl), incubation and cooking to check for variations in gas production. Additives like ferric chloride and EDTA were also incorporated to investigate effect on biogas production.*

Results*: There was a 125 % increase in biogas production in fermentation mixture containing tapioca starch on comparison with the mixture devoid of it. Aerated samples produced lesser biogas than control samples. However, there was 25 % increment in biogas production in samples subjected to 24 h incubation. Pre-soaking resulted in 22 % increase in biogas production compared to controls. Pre-cooking resulted in considerable improvement in biogas production (50 % increase). There was 122 % and 50 % increment in biogas production respectively in samples upon addition of ferric chloride and ferric chloride and EDTA combined. Acid hydrolyzed samples showed 88 % increment in biogas production. Synergistic effect of acid hydrolysis and pre-cooking showed 122 % increase in biogas generation.*

Conclusion: *Aeration had limited beneficial impact on biogas production, while pre-incubation, pre-cooking and pre-soaking of the tapioca starch could result in an increase in biogas production. Addition of ferric chloride, EDTA and acid hydrolysis showed beneficial roles in biogas production.*

Key Word: *Anaerobic digestion, biofuel, tapioca starch, biogas, cost efficiency, renewable energy, vegetable leftovers.*

Date of Submission: 16-07-2025 Date of Acceptance: 26-07-2025

I. Introduction

Bio methanation or methanogenesis is a process coordinated by a group microorganisms known as methanogens, which results in the production of chiefly methane (biogas) along with other subsidiary products as a result of the anaerobic microbial metabolism resulting in the decomposition of biomass. Unlike in fermentation, which facilitates the anaerobic breakdown of larger organic compounds, methanogenesis involves

the removal of semi-final products like hydrogen, smaller organic fractions and carbon dioxide formed during the biomass decay. In methanogenesis which can also occur in ruminant gut, the anaerobic organisms digest cellulose to other easily usable forms which is absorbed in the intestine and the methane is released during eructation of the animal. Anaerobic respiration plays a major role in global nitrogen, sulfur and carbon cycles upon reduction of oxyanions of nitrogen, sulfur and carbon respectively.

Methanogenesis is a form of carbon-mediated respiration wherein methane gas with high calorific value is produced that could be used as a sustainable alternative to fossil fuels which could otherwise contribute to environmental pollution. However, a downside of uncontrolled methanogenesis which is likely to occur in huge land-fills is the massive release of methane gas which in turn is a green-house gas, the buildup of which can lead to problems like global warming.

The three-stage degradation of organic material follows-1) Liquefaction (hydrolysis), 2) Acidogenesis followed by 3) Methanogenesis. Hydrolysis involves the activity of hydrolytic bacteria (degraders) which results in the breakdown of polymers like polysaccharides, lipids and proteins into smaller units like monosaccharides, formate, acetate, ethanol, hydrogen, carbon dioxide using exoenzymes like proteases, amylases, cellulases, lipase or pectinase. This process is followed by acidogenesis involving acetogenic bacteria which ferments monomers like glucose, amino acids to form ethyl alcohol, butyrate, hydrogen and carbon dioxide. The formation of these end-products has a great potential to affect the pH and redox potential of the working media. In methanogenesis, methane is formed by the acetoclastic methanogenic process from carbon dioxide and hydrogen by hydrogenoclastic mechanisms. In the degradative process, the hydrolysis and methanogenesis are the rate limiting processes. The multiplication rate of methanogens is slow and are dependent on the pH of the media, where the build-up of an acidic environment can result in destruction of methanogens.

The major players of bio methanation are a consortium of bacteria which helps in the conversion of biomaterial into methane and the terminal step is carried out by a class of ancient, unusual group of bacteria called archaea, which are similar to prokaryotes in most aspects of cell structure and metabolism but similar to eukaryotes in terms of genetic transcription and translation. They are also extremophiles which survive in temperatures above 100°C like rocks near hydrothermal vents, geysers and black smokers. They are also mesophiles and are common inhabitants of marshlands, sewage, highly saline, acidic or alkaline waters and in the digestive tracts of ruminants, termites and in humans.

Methanogenic archaea have unusual type of metabolism where they use hydrogen, carbon dioxide formate, methylated chloride compounds or acetate as energy source and uses carbon sources for growth resulting in the production of methane as the chief end-product of their metabolism in a unique energy-generating process under sulfate-limiting conditions. They are also important for both recycling of carbon compounds and for the maintenance of global carbon flux on earth. This study focusses on optimization of process for enhanced production of biogas from agricultural waste products which are high in starch content. This could add to the economic value of such products as well as generation of additional revenue with the generation of biogas which could be used for various applications as cooking fuel or for electrification owing to its high calorific value.

II. Material And Methods

Materials: Tapioca starch, cow dung, tap water, 1L conical flask, rubber stoppers with 1 and 2 holes, 5mm diameter rubber tube, wooden stand for gas collection, ferric chloride, ethylene diamine tetra acetate (EDTA), hydrochloric acid (HCl).

Methods:

Determination of biogas production with raw tapioca starch as substrate

350g fresh cow dung was mixed with water so as to make 700mL final volume. To this slurry 5g tapioca starch was added and mixed well. The slurry was then poured into 1L conical flask and closed with single holed rubber stopper connected with tubing to an inverted conical flask filled with water and closed with double holed rubber stopper. The outlet tubing of the inverted conical flask was placed in a conical flask containing 100mL of water. Fermentation gases produced were collected in the bottle by water displacement method and daily displaced volume was measured using a measuring cylinder which was equal to the daily biogas produced from the particular set up. This set up was used throughout the study to assess the cumulative biogas production over a period of 10 days.

Determination of biogas production from pre-aerated tapioca starch containing slurry

350g cow dung was mixed with water and made up to 700mL. 5g tapioca starch was added to the slurry and mixed well. This slurry was aerated using aerator (air blower) for 24 hours. After aeration, the slurry was poured into 1-liter conical flask and the gas production was measured using water displacement method as explained above. The slurry without aeration was used as control in this experiment.

Determination of biogas production with limited pre-aerated starch containing slurry

350g cow dung was mixed with water so that final volume was 700mL. To this slurry 5g tapioca starch was added and mixed well. This slurry was aerated using aerator (air blower) for 6 hours. After aeration, the slurry was poured into 1-liter glass bottle, wherein the gas production was measured using water displacement method. The slurry without aeration was used as control in this experiment.

Determination of biogas production from pre-incubated tapioca starch containing slurry

350g cow dung along with this 5g tapioca starch were mixed with water so that final volume was 700 mL. This mixture was pre-incubated for 24h before setting up the experiment. After pre-incubation, the slurry was poured into 1-liter glass bottle and gas production measured using water displacement method. The slurry not subjected to pre-incubation was used as control in this experiment.

Determination of biogas production from pre-soaked tapioca starch containing slurry

350g cow dung was mixed with water so that final volume was 700mL. This mixture was pre-incubated for 24 hours. To this 5g pre-soaked (for 24 hours) tapioca starch was added and mixed well just before setting up the experiment, after which the slurry was poured into 1L conical flask and gas production measured using water displacement method. Control for this experiment was the same slurry with unsoaked tapioca starch.

Determination of biogas production from pre-cooked starch containing slurry

350g cow dung was mixed with water so that final volume was 700ml and this mixture was kept for 24 hours, to which 5g pre-cooked (for 24 hours) tapioca starch was added and mixed well just before setting up the experiment. After that, the slurry was poured into 1-liter conical flask and gas production measured as in above section. Control employed for this experiment was the same slurry containing 5g tapioca starch but without pre-cooking.

Effect of ferric chloride on bio methanation of tapioca starch containing slurry

350g cow dung was mixed with water so that final volume was 700mL. To this mixture 5g tapioca starch was added and pre-incubated for 24 hours. To this slurry, 17.5mg ferric chloride was added and mixed well just before setting up the experiment, after which it was poured into 1-liter conical flask and gas production assessed as above. Slurry containing 5g tapioca starch without ferric chloride was used as control for this experiment.

Synergistic effect of ferric chloride and EDTA on bio methanation of tapioca starch containing slurry

350g cow dung was mixed with water so that final volume was 700mL. To this, 5g tapioca starch was added and pre-incubated for 24h. 17.5mg ferric chloride and 17.5mg EDTA was added to this mixture and mixed well just before setting up the experiment. The slurry was then poured into 1-liter conical flask and the gas production was measured using water displacement method. Slurry containing 5g tapioca starch and ferric chloride was used as control for this experiment.

Effect of hydrolysis (HCl) on bio methanation of tapioca starch containing slurry

350g cow dung was mixed with water so that final volume was 700mL. This mixture was kept for 24 hours and to this 5g hydrolyzed (for 30 min using 1N HCl) starch was added and mixed well just before setting up the experiment. The slurry was then poured into 1-liter conical flask and gas production was measured. Slurry containing unhydrolyzed tapioca starch was used as control in the experiment.

Synergistic effect of acid hydrolysis (HCl) and boiling on bio methanation of tapioca starch containing slurry

350g cow dung was mixed with water so that final volume was 700mL. This mixture was kept for 24 hours and to this 5g hydrolyzed (for 30 min using 0.2N HCl) starch which was boiled for 15 min was added and mixed well just before setting up the experiment, after which the slurry was poured into 1-liter conical flask and gas production measured. Slurry containing unhydrolyzed tapioca starch was used as control for this experiment.

III. Results

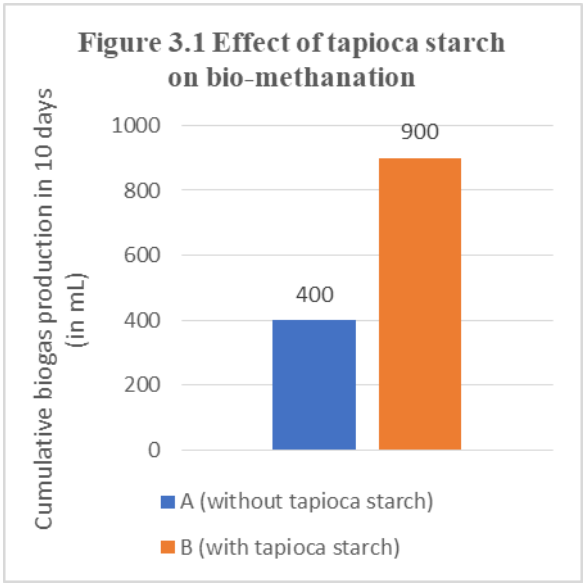

Table 3.1 Effect of tapioca starch on bio-methanation

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without tapioca starch)	350mL water+ 350g cow dung	400mL
B (with tapioca starch)	350mL water+ 350g cow dung+ 5g tapioca starch	900mL

*Percentage increase in biogas production is 125%

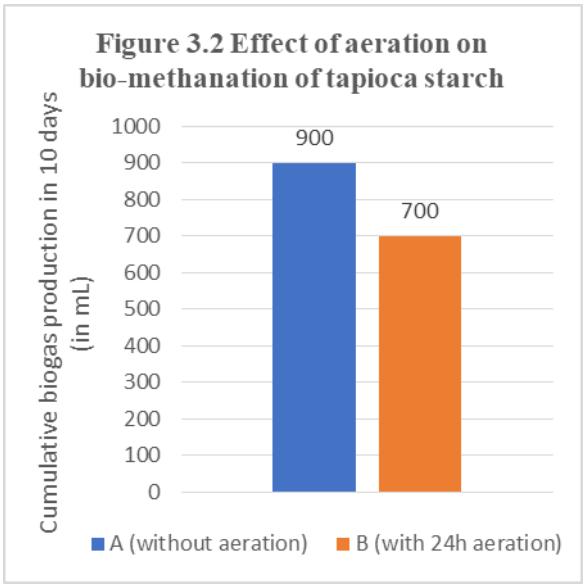

Table 3.2 Effect of aeration on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without aeration)	350mL water+ 350g cow dung+ 5g tapioca starch	900mL
B (with 24h aeration)	350mL water+ 350g cow dung+ 5g tapioca starch	700mL

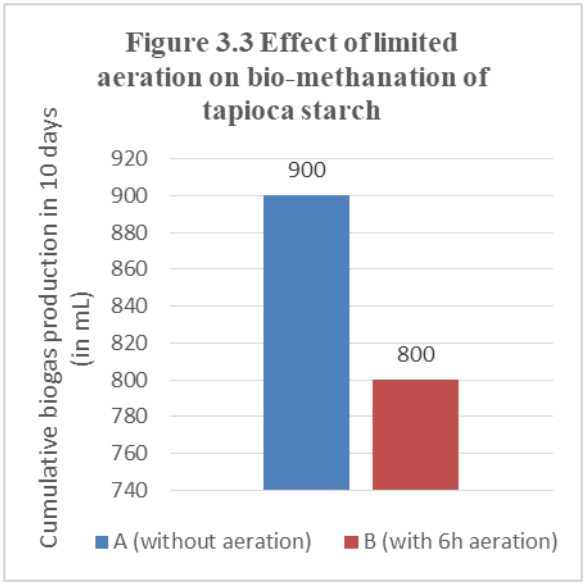

Table 3.3 Effect of limited aeration on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without aeration)	350mL water+ 350g cow dung+ 5g tapioca starch	900mL
B (with 6h aeration)	350mL water+ 350g cow dung+ 5g tapioca starch	800mL

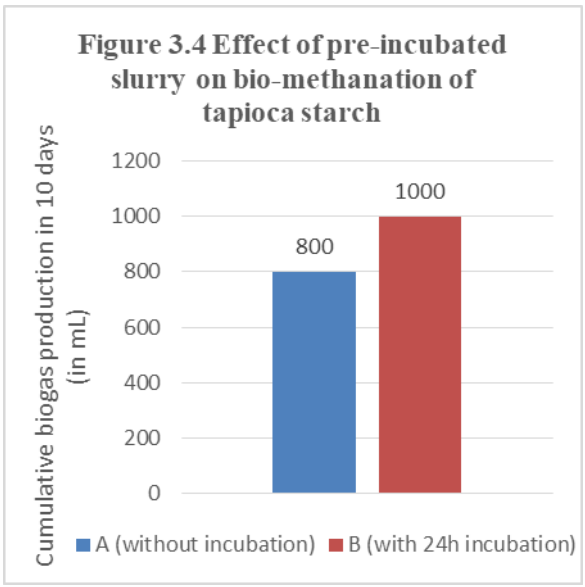

Table 3.4 Effect of pre-incubated slurry on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without incubation)	350mL water+ 350g cow dung+ 5g tapioca starch	800mL
B (with 24h incubation)	350mL water+ 350g cow dung+ 5g tapioca starch	1000mL

*Percentage increase in biogas production is 25%

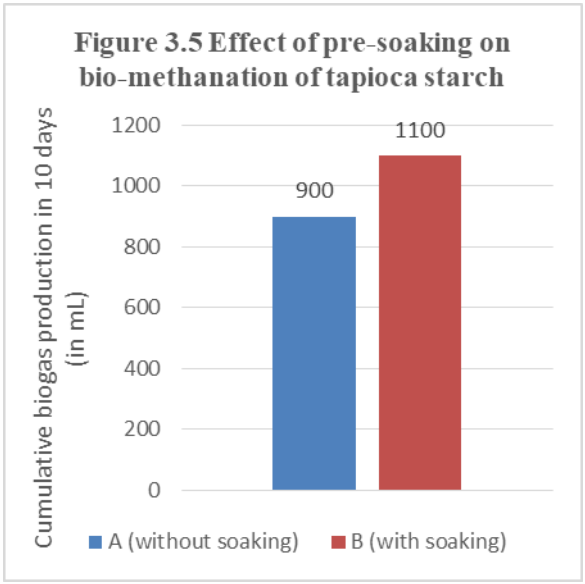

Table 3.5 Effect of pre-soaking on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without soaking)	350mL water+ 350g cow dung+ 5g tapioca starch	900mL
B (with soaking)	350mL water+ 350g cow dung+ 5g tapioca starch	1100mL

*Percentage increase in biogas production is 22%

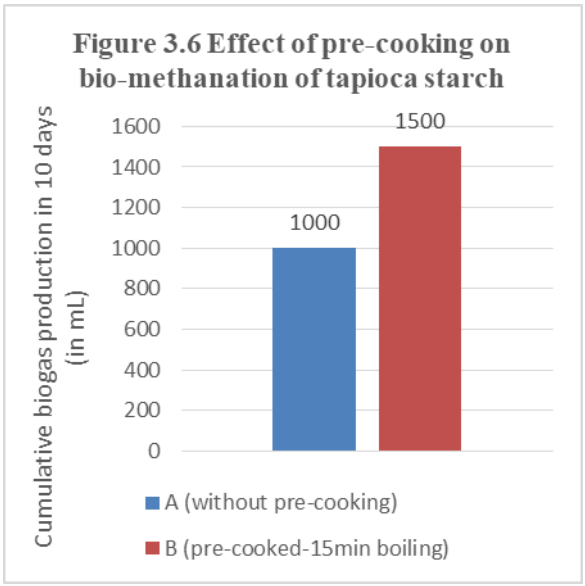

Table 3.6 Effect of pre-cooking on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without pre-cooking)	350mL water+ 350g cow dung+ 5g tapioca starch	1000mL
B (pre-cooked-15min boiling)	350mL water+ 350g cow dung+ 5g tapioca starch	1500mL

*Percentage increase in biogas production is 50%

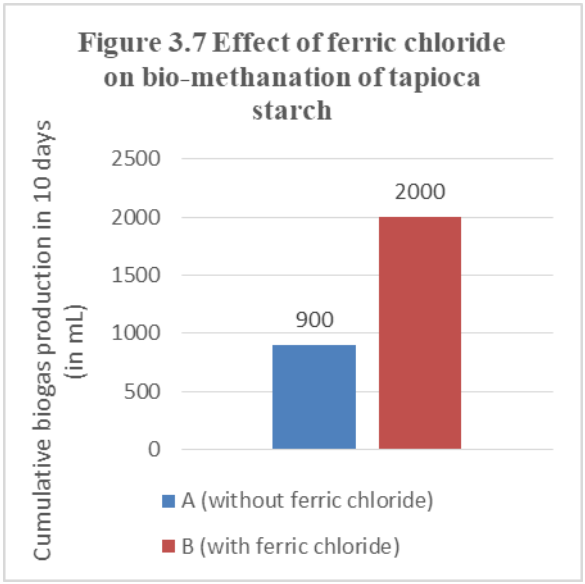

Table 3.7 Effect of ferric chloride on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without ferric chloride)	350mL water+ 350g cow dung+ 5g tapioca starch	900mL
B (with ferric chloride)	350mL water+ 350g cow dung+ 5g tapioca starch+ 17.5mg ferric chloride	2000mL

*Percentage increase in biogas production is 122%

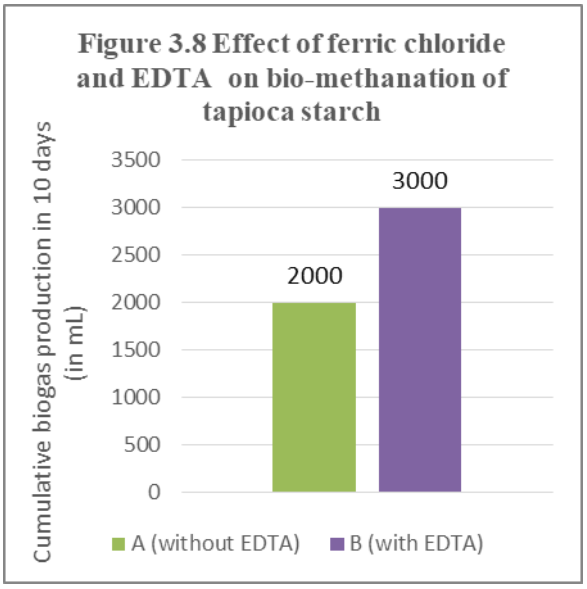

Table 3.8 Effect of ferric chloride and EDTA on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without EDTA)	350mL water+ 350g cow dung+ 5g tapioca starch+ 17.5mg ferric chloride	2000mL
B (with EDTA)	350mL water+ 350g cow dung+ 5g tapioca starch+ 17.5mg ferric chloride+ EDTA	3000mL

*Percentage increase in biogas production is 50%

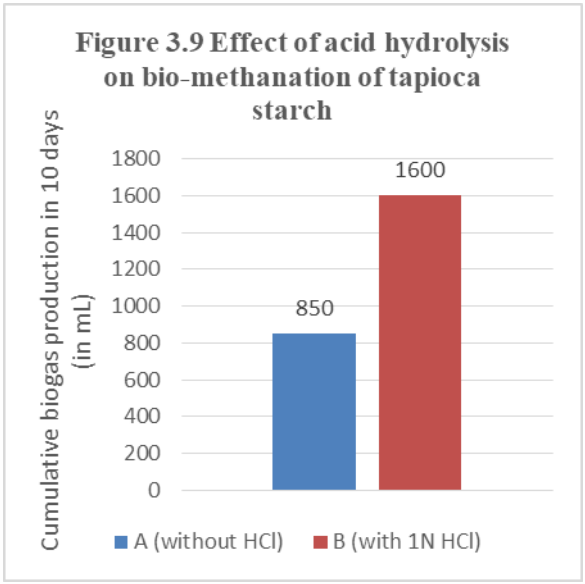

Table 3.9 Effect of acid hydrolysis on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without HCl)	350mL water+ 350g cow dung+ 5g tapioca starch	850mL
B (with 1N HCl)	350mL water+ 350g cow dung+ 5g tapioca starch+1N HCl	1600mL

*Percentage increase in biogas production is 88%

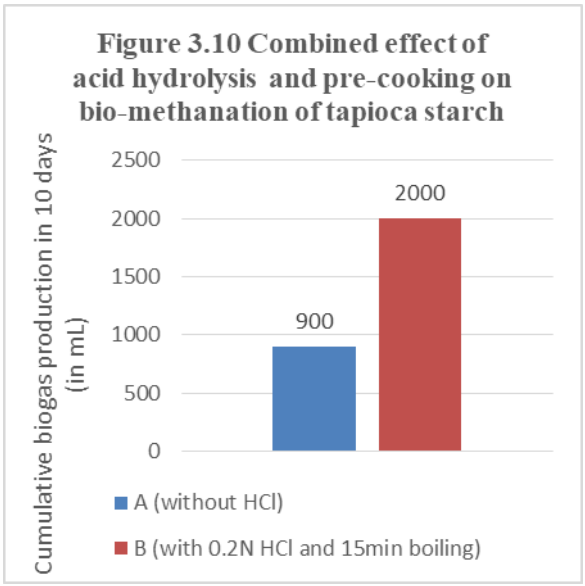

Table 3.10 Combined effect of acid hydrolysis and pre-cooking on bio-methanation of tapioca starch

Sample	Fermentation mixture	Cumulative biogas production in 10 days (in mL)
A (without HCl)	350mL water+ 350g cow dung+ 5g tapioca starch	900mL
B (with 0.2N HCl and 15min boiling)	350mL water+ 350g cow dung+ 5g tapioca starch+0.2N HCl	2000mL

*Percentage increase in biogas production is 122%

IV. Discussion

It is evident from the data that dry tapioca starch waste has the potential to generate more biogas than the conventional source available in India that is cow dung. Starch is mainly available from corn processing industry, potato processing industry, tapioca processing industry and other similar agriculture-based industries.

Starch is a polymer of glucose connected in α-1,4 linkage through glycosidic bond. With the help of appropriate hydrolytic enzyme this polymer can be cleaved into its oligomeric form or ultimately to its dimer or monomer. monomers can be fermented through the methanogenic pathway with the help of microbial consortia producing biogas which consist of mainly methane as its inflammable component.

Considering the huge availability of starch containing agricultural wastes in this country there is a great potential to convert reduced carbon sources to methane through anaerobic pathway and is further used for power generation. This work assumes a lot of importance considering the huge population of India which is second largest in the world and its corresponding energy demand. At present India is importing 80% of its crude oil mainly from gulf countries in order to feed its huge economy. Crude oil and other conventional energy sources like coal and wood being non-renewable, is contributing not only environmental pollution, but also continuous price hike of various products manufactured using this material. Depletion of natural resources being a reality there is very much less we can do to solve this problem.

In this context alternate renewable source of energy has to be explored which are not seen as a supplement to existing energy sources but has the potential to replace them in the future. Out of the various options available, solar energy, wind energy, tidal energy and bio energy show much promise for the future. All these energy sources are ultimately driven by the universal source of energy which is sun and from that point of view solar radiation falling on the earth's surface a day is equivalent to the 1/5th of all fossil reserve energy available. The plants have the ability to trap this solar energy through photosynthesis and to lock this energy in various biomolecules.

Unleashing energy present in biomolecules in plant can all together eliminate present world energy crisis. Considering amount of biomass synthesized on day-to-day basis appropriate technology should be developed to extract this cosmic energy which is hidden in the biomass and which can be made available to serve various needs of individuals, industries and countries. Bio-methanation being an anaerobic process efficiently converts carbon Containing substrates like starch, protein and fats to methane which is most reduced form of carbon. When methane is burnt no smoke will be there which contribute to environmental pollution. This is also significant in the context of global warming, the major problem of present world. Microbes taking pan in bio-methanogenesis pathway (hydrolytic, acidogenic, acetogenic, and methanogenic), out of these methanogens are very sensitive to temperature and pH and they are strict anaerobes. The other 3 groups of bacteria can tolerate slightly acidic pH and fluctuations in temperature. This calls for phase separation of biochemical processes involved leading to better efficiency in minimal time.

Fig: 3.1 — Table 3.1 show bio-methanation potential of tapioca starch. it is evident from the data that dry tapioca starch generated 500 mL more gas compares to control.

Fig: 3.2 — Table 3.2 show bio-methanation potential of tapioca starch in aerated and non-aerated condition. In aerated gas production was lesser than control, proving that oxygen is toxic to methanogens and even if rate of hydrolytic enzymes secretion increased resulting in increased monomer production, ultimate gas production will be low since methanogens are affected by oxygen.

Fig: 3.3 — Table 3.3 show bio-methanation potential of tapioca starch under limited aerated condition (6 hours) data show that 6h aerated culture produced 400mL, lesser gas than non-aerated one which produced 900mL gas. This proves that methanogens were still affected but that the damage is less.

Fig: 3.4 — Table 3.4 show bio-methanation potential of tapioca starch which was pre-incubated for 24h without aeration. 24h pre-incubated fermentation mixture produces 1000mL gas compared to 900mL gas production in control. This increase in gas could be attributed to the fact that glucose present as part of starch polysaccharide is converted to monomeric acids and to acetate. The accumulation of acetate in high concentration in fermentation broth helps to boost biogas production which is transferred to anaerobic digester and acetate being the immediate precursor.

Fig: 3.5 — Table 3.5 show bio-methanation potential of tapioca starch which was soaked for 24h prior to bio-methanation. Soaking helps to swell the polymer making easy for hydrolytic enzymes to cleave the glycosidic bond and to produce corresponding monomers. This will naturally help to increase acetate concentration in the pre digestor which will produce excess biogas when digested anaerobically.

Fig: 3.6 — Table 3.6 show bio-methanation potential of pre-cooked tapioca starch. Exposing organic polymer to steam under high pressure will help to soften the material which enhances the rate of catalysis by hydrolytic enzymes.

Pre-cooking not only soften the material but also reduces the pre-treatment time compared to soaking in water, precooked starch produced 1500mL gas compared to control which produced only 1000mL biogas.

Fig: 3.7 — Table 3.7 show bio-methanation potential of tapioca starch in the presence of ferric chloride. Various metals have important roles in bio-methanogenesis and they act as cofactors of key enzymes. Iron will help to enhance the rate conversion of acetate to methane. 25ppm ferric chloride containing fermentation broth produced 1500mL gas compared to control (1000mL).

Fig: 3.8 — Table 3.8 show bio-methanation potential of tapioca starch in the presence of ferric chloride and EDTA. Biogas mixture contains methane, carbon dioxide, hydrogen sulphide etc. In the presence of hydrogen sulphide, ferric ions form ferric sulphate. Once the metal ion is precipitated it will not be available to the methanogenic bacteria. In order to address this problem chelating agent EDTA was used, which will complex with ferric ion thus avoiding precipitation. From the data it is very evident that the presence of EDTA increased the bioavailability of metal ion leading to more biogas production.

Fig: 3.9 — Table 3.9 show bio-methanation potential of tapioca starch when hydrolyzed with 1N hydrochloric acid. From the data it is evident that there was significant increase in biogas production due to acid hydrolysis.

Fig: 3.10 Table 3.10 show bio-methanation potential of tapioca starch when hydrolyzed with 0.2N hydrochloric acid and boiled for 15 minutes. Boiling and acid hydrolysis caused cleavage of the glycosidic bond of starch which give rise to monomers and hence enhances biogas production. From the data it is evident that there was significant increase in biogas production due to acid hydrolysis and boiling.

V. Conclusion

Fermentation mixture when pre-incubated produced more biogas because of higher acetate accumulation.

Since methanogens are sensitive to oxygen, the biogas production was lesser when the fermentation mixture was aerated.

Pre-soaking increased biogas production because it makes starch prone to hydrolytic enzymes, similar effect was also seen in the case of pre-cooking.

When tapioca starch containing fermentation mixture was supplemented with ferric chloride, biogas production increased because these metal ions act as cofactor and as prosthetic group for functional enzymatic action.

Acid hydrolysis leads to cleavage of starch polymer leading to high monomer production and hence enhanced biogas production.

Acid hydrolysis along with precooking further increased biogas production. This technique minimizes environmental pollution caused by acids.

References

[1] A. Molino , F. Nanna , Y. Ding, B. Bikson, Biomethane Production By Anaerobic Digestion Of Organic Waste. Fuel (2012) 173-190.

[2] A.Asankulova And A.D. Obozov (2007). Biogas In Kyrgyzstan, National Academy Of Sciences Of The Kyrrgyz Republic, Vol.43, No.4, 262-265.

[3] Babu Z-Fathepure, (1987) Factors Affecting The Methanogenic Activity Of Methanothrix Soehngenii Vnbf, Applied And Enviormental Microbiology, Vol.53, No. 12, P.2978-2982.

[4] Burak, Demiral And Orhan Yenigun, Two Phase Anaerobic Digestion Process-A Review. J Chem Technol Bioctehnol (2002) .

[5] Baumer S. , Ide T., Jacobi C., Johann A., Gottischalk G. And Deppenmeier U.(2000). J.Bi01.Chem. 275, 17968-17973.

[6] Deppenmeier U.2002. The Unique Biochemistry Of Methanogenesis.

[7] Prog Nucleic Acid Res Mol Biol. 71.•223-83.

[8] Ding Y.H.R., Zhang S.P., Tomb J.F. And Ferry J.G. (2002) Fems Microbiol. Lett.215, 127-132.

[9] Dr.Nirmala Bardiya And A.C. Gaur, Iron Supplementation Enhances Biogas Generation, Tata Energy Research Institute, United Nations Development Programmes.

[10] E.P.Ryzhkova (2003). Multiple Functions Of Corrinoids In Prokaryote Biology, Applied Biochemistry And Microbiology, Vol.39, No.2, 115139.

[11] Ellen Oelgeschlager, Michael Rother, (2008). Carbon Monoxidedependent Energy Metabolism In Anaerobic Bacteria And Archaea, Arch Microbial. 190, 257-269.

[12] Ernst Gunter Graf And Rudolf K. Thauver, (1981). Hydrogenase From Methanobacterium Thermoautotrophicum. A Nickel-Containing Enzyme, Vol. 136, No. L, 165-169.

[13] Ferry J.G. (1999). Enzymology Of One-Carbon Metabolism In Methanogenic Pathways. Fems Microbiol Rev. 23: 13-38.

[14] Forterre P., Brochier C., Philippe H. 2002. Evolution Of The Archaea.

[15] Theor Popul Biol. 61: 409-422.

[16] G.Zayed, J. Winter (2000) Inhibition Of Methane Production From Whey By Heavy Metals-Protective Effect Of Sulphide. Appl. Microbial Biotechnol (2000), 53:726-731.

[17] Graciela, Gonzalez-Gil, Robbert Kleerebezem And Getze Lettinga (Apr. 1999) Effect Of Nickel And Cobalt On Kinetics Of Methanol Conversion By Methanogenic Sludge As Assessed By On Line Ch4 Monitoring, Applied And Environmental Microbiology, P. 1789-1793.

[18] Grahame D.A. (2003). Trends Biochem. Sci-5. 221-224.

[19] H.L. Ehrilich (1997). Microbes And Metals Appl. Microbiol.Biotechnol. 48, 687-692.

[20] Hedderich R., Klimmek 0•, Kroger A., Dirmeier R., Keller M. And Stetter K.O. (1998). Fems Microbial Rev.22, 353-381.

[21] Hifjur Raheman, Subhrajit Mondol (2012). Biogas Production Potential Ofjatropha Seed Cake. Biomass And Bioenergy 3, 179-189.

[22] Leach G. (1987). Hoisehold Energy In South Asia Biomass, 12, 155184.

[23] Lehtomaki Biogas Production From Energy Crops And Crop Residues. Department Of Biological And Environmental Sciences, University Of Dyvaskyla P.O. Box 35, F-40014 Finland, Copyright C 2006.

[24] M. (Lule) Albek M.L., Yetis U., Gokcay C.F. (1997). Effect Of Ni (11) On Respirometric Oxygen Uptake. Appl. Microbiol Biotechnol. 48:636641.

[25] M.Sai Ram, L.Singh, M.V.S. Suryanarayana And S.I.Alam (2000), Effect Of Iron, Nickel And Cobalt On Bacterial Activity And Dynamics During Anaerobic Oxidation Of Organic Matter, Water, Air And Soil Pollution, 1 17, 305-312.
[26] Marianne Lange, Peter Westermann, Birgitte Kiaer Ahring, 2005. Archaea In Protozoa And Metazoa, Appl. Microbiol. Biotechnol, 66:465-474.
[27] P.Preeti Rao And G.Seenayya 1994, Improvement Of Mcthanogenesis From Cow Dung And Poultry Litter Waste Digesters By Addition Of Ironworld Journal Of Microbiology And Biotechnology- 1 0, 211-214.
[28] Parimal Samir, 2005. Novel Techniques To Improve Biogas Production From Agrowastes, M.Sc. Biotechnology, Calicut University.
[29] Parkin Gf, Lynch Na, Kuo W, Van. Keuren El, Bhattarcharya Sk (1990), Interaction Between Sulphate Reducers And Methanogens Fed Acetate And Propionate, Res. J. Water Pollut. Control Fed-62, 780-788.
[30] Pradeep Kumar Nagar, 2009. Enhancement Of Biogas Production From Agrowaste Using Metal Ions As Additives, M.Sc. Biotechnology, Calicut University.
[31] Peter Weiland (2000). Anaerobic Waste Digestion In Germany-Status And Recent Developments, Kluwer Acadelnic Publishers, Netherlands, Vol. I L , No.415-421
[32] Raymond Ilovey, Sabine I,Ents, Arjnili Ehrenjeich, K'iwty Saltuon, Karla Saba, Gerhard Gottschalk, Robefl P (Gunsalus, I)Eppentueir, Dna Jnicroarray Analysis Of' Jnethanosarcina Inazei Gol Reseals
[33] Adaptation To Different Methanogenic Substrates. Mol Gen Genomics 2005, 273: 225-239.
[34] Scherer P. And H. Sahm 1981. Effect Of Trace Elements And Vitamins On The Growth Of Methanosarcina Barkeri. Acta Biotechnol. 1:57-65.
[35] Scherer P. And H. Sahm 1981. Influence Of Sulfer-Containing Compounds On The Growth Of Methanosarcina Barkeri In A Defined Medium Eur.J. Appl. Microbial. Biotechnol. 12: 28-35.
[36] Setzke E., R.Hedderich, S. Heiden And R.K. Thauer 1994. H2: Heterodisulfide Oxidoreductase Complex From Methanobacterium Thermoautotrophicum- Composition And Properties. Eur.J.Biochem. 220:139-148.
[37] Sorgenfrei O. , Muller S. , Pfeiffer M., Seiezko I. And Klein A. (1997). Arch Microbiol -167, 189-195.
[38] Subhash Chand Verma, 2009. Biogas Production From Pretreated Rice Husk, M.Sc. Biotechnology, Calicut University.
[39] Sunil Kumar, 2009. Biomethanation Of Sugar Cane Juice, M.Sc. Biotechnology, Calicut University.
[40] Venkateswara Rp, Baral Ss, Dey R, Mutnuri S. Biogas Generation Potential By Anaerobic Digestion For Sustainable Energy Development In India. Renewsustain Energy Rev 2010;14:2086—94
[41] Valle Bl., Ulner Dd. (1972) Biochemical Effects Of Mercury, Cadmium And Lead, Annu Rev Biochem. -41, 91-128.
[42] Walsh C., 1986. Acc. Chem Res. 19, 216-221.
[43] William D.Murray And L.Van Den Berg (1981). Effect Of Nickel Cobalt And Molybolenum On The Performance Of Methanogenic Fixed Film Reactors. Applied And Environmental Microbiology Sep. 1981, 502505.
[44] Wang K. (1994) Integrated Anaerobic And Aerobic Treatment Of Sewage. Ph.D. Thesis,Wageningen Agricultural University, Wageningen, Netherlands.
[45] Wilson F., Yu H., Tay J. And Gu G. (1998) An Empirical Model For Predicting The Organic Concentration Of Anaerobic Filter Effluents. Water Environ. Res. 70(3), 299-305.
[46] Witt E. R., Humphrey W. J. And Roberts T. E. (1979) Full-Scale Anaerobic Filter Treatshigh Strength Wastes. Proc. 34th Annual Ind.
[47] Waste Conf Purdue Univ. 229-234.
[48] Wu M., Wilson F. And Tay J. H. (2000) Influence Of Media-Packing Ratio On Performance Of Anaerobic Hybrid Reactors. Bioresource Tech.
[49] 71, 151-157. Yilmazer G. And Yenigun O. (1999) Two-Phase Anaerobic Treatment Of Cheese Whey. Wat. Sci. Tech. 40(1), 289-295.
[50] Young J. C. And Mccarty P. L. (1969) The Anaerobic Filter For Waste Treatment. Stanford University Technical Report No. 87.
[51] Young J. C. And Stewart M. C. (1979) Pbr-A New Addition To The Awt Family. Water & Wastes Engineering. 8, 20-25.
[52] Yu H., Tay Joo-Hwa And Wilson F. (1997) A Sustainable Municipal Wastewater Treatment Process For Tropical And Subtropical Regions In Developing Countries. Wat. Sci. Tech. 35(9), 191-198.