South Asian Journal of Life Sciences. 1 (1): 12 – 18
http://www.nexusacademicpublishers.com/journal/25

Research Article

Production of Biogas by Mesophilic Bacteria Isolated from Manure

Wardah Sharmeen Syed[1]*, Muhammad Nadeem[2], Ikram–ul–Haq[1], Farid Ahmed Khan[3]

[1]Government College University, Lahore, Pakistan; [2]PCSIR Laboratories Complex, Lahore, Pakistan; [3]University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding author: wardah_syed2002@yahoo.com

ARTICLE HISTORY

Received: 2013–09–29
Revised: 2013–11–14
Accepted: 2013–11–15

Key Words: *Methanosarcinae*, biogas production, GCMS, Applied microbiology, *Mesophilic* bacteria, Energy alternatives

ABSTRACT

Biogas, an unpolluted and environmentally benign form of energy is very productive for developing countries like Pakistan (specially its rural sector) where the recycling of the waste can be ensured. Moreover, it's the best supernumerary for the conventional forms of energy like oil and fossil fuels. The present study determined the biogas production potential of animal manure with bacterial strain and compared them with manure alone. Bacterial isolation was carried out using Hungate technique of anaerobic growth, GCMS and the production was made possible by following the rules of anaerobic digester. The isolate was gram positive, non–spore forming and non–motile with the spheroid shape and creamy yellowish colony also was catalase and indole positive. The production of biogas was enhanced by adding this strain in diluted form along with the manure. The results revealed that co–digestion could bring better and stable performances and may prove one of many options for efficient gas production.

ARTICLE CITATION: Syed WS, Nadeem M, Ikram–ul–Haq and Khan FA (2013). Production of biogas by mesophilic bacteria isolated from manure. *South Asian J. Life Sci.* 1 (1): 12 – 18.

INTRODUCTION

Our life style is basically based on most of the energy demanding processes. The total energy demand of the world is estimated to be 400EJ/Year (McKendry, 2002). Most of the recent reports have indicated that this would increase by the factor of two or three during this century (International Energy Agency, 2006). Globally the demand for energy has been encompassed to 88% by the use of fossil fuels. However, all the existing resources are scarce to fulfill the total energy claims.

Biofuel is the most promising alternative resource. The demand for bio–fuels had been enhanced by the past few decades to 30 billion ($3x10^9$) in 2003 (Stevens, 2004). This group includes bio–hydrogen, biodiesel, bio–ethanol and biogas (Kaparaju *et al*., 2009). Among these, biogas has to be used as a feedstock for producing a variety of materials and chemical, generation of electricity and heat (Weiland, 2010).

Biogas or bio–methane is a combination or a mixture of methane (60%), carbon dioxide (40%) so is "energy rich gas". When CO_2 is removed from the mixture, pure methane can be used in natural gas grid station and in the vehicles as a source of fuel. The remaining residue (after the removal of methane and carbon dioxide) is known as "digestate." All of the nutrients like potassium, phosphorous and nitrogen which are essential plant nutrients are stored in this digestate and hence they can be used in agricultural fields as fertilizers (Barglund, 2006).

Biomass occurs widely in nature and it can be exploited for the production of biogas and reduction in CO_2 emissions to intend decrease global warming (Claasen *et al*., 1999). In biogas synthesis selection of biomass is not only helpful in maintaining the microbial growth but also leads to the positive synergism (Mata–Alvarez *et al*., 2000). Cellulosic biomass, present excessively in nature, has high potential to cope up with the increasing energy demand but it cannot fulfill all the requirements to meet the energy demands. However, organic waste such as animal manure has been extensively demonstrated and practiced for such biogas production (El–Mashad and Zhang, 2010).

The biological anaerobic conversion of organic material is basically done in three evident steps. The first step i.e. hydrolysis, involves the transformation of the complex insoluble organic matter like fats, lipids, polysaccharides, nucleic acids, proteins etc. into easily soluble organic material i.e., fatty acids, monosaccharides and amino acids etc. This step is conducted by strict anaerobes including *Bacterioides* and *Clostridia* and facultative anaerobic bacteria like *Streptococci* etc. The second step is acidogenesis which includes microbial consortia which help in the breakdown of these simple, soluble organic materials into hydrogen, acetic acid, carbon dioxide and other lower weight simple volatile organic acids like butyric acid and propionic acid which later are converted into more simple form such as acetic acid (Yadvika *et al*., 2004). The acetotrophic archea is responsible for converting acetate into methane. They are another category of obligate anaerobes (Ferry, 1992). The third step called "methanogenesis" includes some strict anaerobes like *Methanosarcina* spp., *Methanococcus* spp., *Methanothrix* spp. and *Methanobacterium* spp. which convert the products of second step into a mixture of methane and carbon dioxide and some amount of energy (Yadvika *et al*., 2004). Hydrogen might be proved as a limiting factor for the growth of methanogens when produced during the reaction (Bagi *et al*., 2007).

In the process of anaerobic digestion of the biomass only little information is available for the activity of hydrogenotrophic and acetogenotrophic methanogens (Demirel & Scherer, 2008). When the microbial populations were determined by composition, biomass and number in an anaerobic digester for one and two–stage processes under

continuous process, the concentrations of both acidogenic and methanogenic strains came out to be 99% and 26%, respectively (Solera *et al*., 2001). However the data on the composition and equilibrium between different strains of microbial strata isn't well understood in this two–stage anaerobic process (Lozano *et al*., 2009).

The optimization of the conditions (pH, temperature, oxygen concentration, humidity etc.), use of different chemical and biochemical additives, controlling the nutritional requirements of the microorganisms and by changing the feeding proportions are of keen interest while dealing with the production processes (Lettinga *et al*., 1980; Santosh *et al*., 2004; Azbar *et al*., 2008; Li *et al*., 2010; Wei *et al*., 2010). The use of biological agents (microorganisms) is one of the best methods used for enhancing the yield of biogas. Some fungal and bacterial strains have been found to increase the production of biogas by the range of 8.4–44% form cattle dung (Attar, 1998; Tirumale and Nand, 1994; Potivichayanon *et al*., 2011). Temperature plays a very important role in the yield of biogas by these plants. In a recent study, the biogas yield was found out to be relatively decreased in the month of December at 24°C as compared to the yield observed in summer at 36°C in the month of April. This decreased ambient temperature led to the shift of microorganisms, as relatively a very diverse range of microbial community occupies these digesters (Rastogi *et al*., 2007).

Many countries are actively engaged in the fruitful improvement in this technology. Units for the methane gas production, using biomass had been employed in the rural areas of India and China, in order to meet their energy requirements (Levis, 1983) and most recently in Vietnam for the production of biogas efficiently.

MATERIALS AND METHODS

Isolation of the Desired Strain

The methanogens were collected and isolated from fresh manure samples collected from the PCSIR, Laboratories Complex Lahore, Pakistan. As the fresh manure is a reservoir of a number of bacterial species so for isolation purpose the sample was serially diluted and the cells were allowed to grow on nutrient agar medium and on MRS agar medium. After the incubation period the bacterial growth was observed on the nutrient agar plates and not on the MRS agar medium. The incubation was provided in anaerobic gas chamber at 35°C for 48–72 hours. The culture was preserved at 35°C in Fluid thioglycolate medium.

The medium used for the purification of the culture was "Fluid thioglycolate medium" set at the pH of 6.9–7.3. The cells were allowed to grow for 24–48 hours at 37°C.

Production of Biogas

The production of biogas was studied first in the laboratory by setting a small practice which included 3 flasks with different media under specified conditions. Flask 1 was fed with a synthetic media which had the ingredients in the concentration of g/L, respectively. The ingredients include glucose 4.8688, yeast extract 0.05, NH_4Cl 0.955, K_2HPO_4 0.0636, KH_2PO_4 0.123, NaCl 0.6, KCl 0.185, $MgSO_4$ 0.1236, CaCl 0.02, $FeCl_2$ 0.0001, $MnCl_2$ 0.0009, H_3BO_3 0.0002, $CoCl_2$ 0.0015, $CaCl_2$ 0.0022, NiCl 0.0012, Na_2SeO_3 0.0006, $ZnCl_2$ 0.0009, citric acid 0.105, nitrotriacetic acid (NTA) 0.04. The pH of this medium was set to be at 6.5–7.0.

Flask 2 was fed with the synthetic medium+ Sodium sulfide. Flask 3 contained only Fluid thioglycolate medium which served as the control. All the three flasks were inoculated with the isolated strain Methanosarcina WS1. Sterilized balloons were tied up with the flask assembly to study the formation of gas in the closed flask environment. All the openings were sealed with wax and then with parafilm tape to avoid the entrance of air in the flasks. The flasks were then incubated for 7 days at 35°C.

Measurement of Biogas Fractions

Gas chromatography and mass spectrometry (GCMS) was performed for the measurement of the gas fractions produced within the flasks. The injection temperature of the equipment was 200°C and the pressure maintained was at 60.1 kPa.

Anaerobic Digester

The biogas production was carried out in a digester (15' in height and 4.5' width). The inoulum was prepared in synthetic medium. For feeding the experimental digester 300 ml of the inoculum was prepared and then diluted with sterilized distilled water making the final volume up to 1L. Thirty kg of the animal manure was added in the digester followed by addition of inoculums for the sake of producing biogas efficiently. The incubation was carried out for 7–10 days. Animal manure (30 kg) was fed daily whereas inoculum was added on alternate days. The temperature, pressure, pH and humidity were monitored on daily bases. Two digesters were used in the present work. One served as control with only animal manure and no inoculum, while the other as experimental which was fed with both.

RESULTS

The strain obtained was referred as WS1 strain in the laboratory. The strain was gram positive cocci, non–motile and non–spore forming. Creamy yellowish and smooth colonies were obtained. The strain was catalase and indole positive.

Table 1: Effect of temperature on the growth of Methanosarcina WS1 strain

Temperature	OD_{600}	Observation
Control	0.00	––
25°C	1.23	Growth
30°C	1.26	Growth
35°C	1.90	Optimum growth
37°C	1.85	Growth
40°C	1.43	Growth
45°C	1.2	Growth
50°C	0.21	Mild growth
55°C	0.14	Mild growth
60°C	––	No growth

Table 2: Effect of salt concentration on Methanosarcina WS1 strain

Salt concentration M	Absorbance OD_{600}	Observation
Control	0	–
0.1	1.23	Growth
0.2	1.48	Growth
0.3	1.87	Optimum growth
0.4	1.43	Growth
0.5	1.09	Growth
0.6	0.64	Mild growth
0.7	0.51	Mild growth
0.8	0.47	Mild growth
0.9	0.39	Minute growth
1	0.23	No growth

WS1 had the tendency to grow within the range of 25–60°C but the optimum growth was observed at 35°C (Table 1). The salt

range of the strain WS1 was observed between 0.1–0.5 M NaCl but the strain showed optimum growth in terms of turbidity of the medium at 0.3 M concentration of the NaCl (Table 2). Under these set of conditions the organism was termed as *Methanosarcina* WS1 according to Bergey's Manual of Determinative Bacteriology.

Figure 1: Gas production by *Methanosarcina* WS1 Purple balloons: synthetic media–maximum Red balloon: fluid thioglycolate media Green balloon: synthetic+ NaS medium conditions

Synthetic medium alone showed biogas production in term of expansion of balloon within 5 days of incubation while fluid sodium thioglycolate medium showed gas formation (balloon swelling) within 8 days of incubation. The flask containing synthetic medium+NaS showed very minute swelling of the balloon indicating very less gas formation. This is illustrated in Figure 1.

Gas produced in the distillation flasks was analyzed by GCMS. The graph obtained after running the samples indicated the presence of CO_2, Fluoroacetylene, tricholoromethane and benzene. Methane could not be analyzed because GCMS system used in present study was not operational at 16°C which is the temperature equivalent to molecular weight of methane and is required for its analysis of CH_4. The peaks and relative concentration has been shown in the chromatogram in Figure 2.

Table 1.3 depicts different parameters i.e. temperature, humidity, pressure, pH and digester height that were monitored daily for period of nine days. The variations in temperature and percentage humidity of the digester were observed within range of 23–30°C and 42–49%, respectively. Maximum temperature was observed on 6th day after the inoculation. The pH within the digester remained the same throughout the whole period. There was a proportional increase in the pressure within the digester and in the digester height. Both the parameters increased with the incubation period and maximum values were recorded on the last day.

Biogas produced in both control and experimental digester was used for burning the individual burners (Table 4). The flame of burner was attached to experimental digester and it continued to burn for 3 hr compared to that of control which burnt for only 1 hr and 24 min. The demonstration of both the control and that of experimental digester has been provided in Figure. 3. The elevated level of the digesters indicates the formation of the biogas within the digester.

Table 3: study of different parameters for biogas production

Days	Feed Kg	Temperature °C	pH	Humidity %	Inoculums ml	Pressure Psi	Digester height Cm
1	30	23	8	40	1000	0	12.2
2	30	24	8	43	–	2.5	15.2
3	30	26	8	45	1000	4	19.7
4	30	25	8	48	–	5	25
5	30	26	8	47	1000	6	30
6	30	30	8	46	–	8.5	34.5
7	30	26	8	49	1000	10	36.8

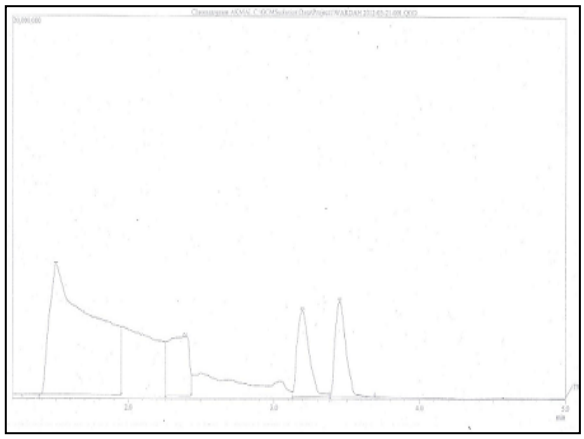


Figure.2: Gas chromatogram. First peak shows the occurrence of CO_2, second shows Fluoroacetylene, third being Tricholoromethane and fourth is of benzene. The details of these gases has been discussed and compared with respect to methane

Figure 3: Formation of biogas in control and experimental digesters. The right being control and the left served as the experimental digester.

Table 4: Comparison between the control and experimental digesters

Parameters	Control	Experimental
Inoculum	No	Yes
Time interval	11:21am–12:45pm	11:00am–02:00pm
Duration of flame burning	1hr 24 min	3 hours

DISCUSSION

As a matter of fact, the production of biogas can be accomplished cozily, economically and with no harms. But despite of its practical implications, in a developing country like Pakistan, the masses are not much aware of its beneficial aspects. Keeping this thing in consideration, the present study was conducted that aimed at the isolation of the mesophilic strains which were isolated from animal muck. Serial dilution method was employed for the isolation of methanogens followed by spread plate. A single colony was selected and designated as isolate WS1. Fluid thioglycolate medium was used for the growth of WS1 to confirm its anaerobic nature. The presence of thioglycolate provides a complete oxygen free environment, as the oxygen present in the head space of the vessel was reduced completely. Moreover, this medium contained all the necessary nutrients and a resazurin dye, the color of which varies from red–dark pink (in presence of oxygen), pale yellow (in the absence of oxygen) and is an indicator for the anaerobic environment. When selected isolate WS1 was grown in this medium, it turned to pale yellow. Furthermore, growth was observed at the bottom of the tube which is a characteristic of strict anaerobic growth.

A simple experiment using distillation flasks with attached balloons containing synthetic medium was carried out to analyze the production of gas by the *Methanosarcina* WS1. This medium contained all the macro and micro nutrients essential for the growth of methanogens. After incubation of 7–8 days, the balloons swelled to show the presence of the gas formation inside the flask. The gas produced was analyzed by Gas Chromatography and Mass Spectrometry (GCMS). Three peaks were obtained. The first peak showed similarity with carbon dioxide (CO_2), which is basic component of biogas or natural gas (Berglund, 2006). The second peak obtained showed similarity with fluoroacetylene. Fluoroacetylene when combined with air it becomes highly explosive with explosion limits in air of 2.4 to 13%. This compound has auto–ignition temperature of 365°C and the flash point of 6°C. It is highly heat sensitive having flammability of 3. (Pohanish and Greene 2009) (ChemSpider, the free chemical database). The specifications of methane gas include the auto–ignition temperature of 537°C and explosive limits in air being 5–15%. It's highly flammable gas. (IPCS, 2000) The third peak obtained showed 23.19% similarities with trichloromethane (chloroform). This however isn't flammable when in liquid state. The fourth peak obtained showed 25.77% similarities with benzene (C_6H_6). This is highly flammable and explosive as well having a flash point of −11°C and the auto–ignition temperature of 498°C. Its flammability is 3. (Hook *et al.*, 2006)

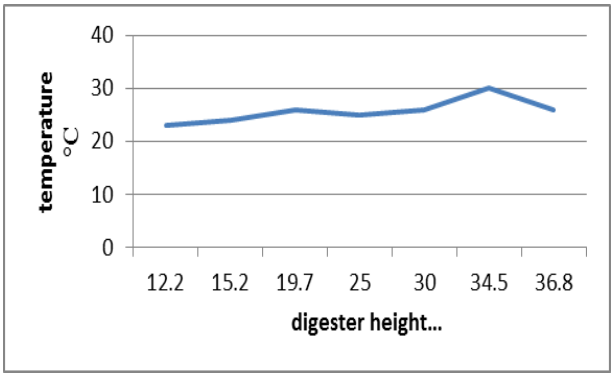
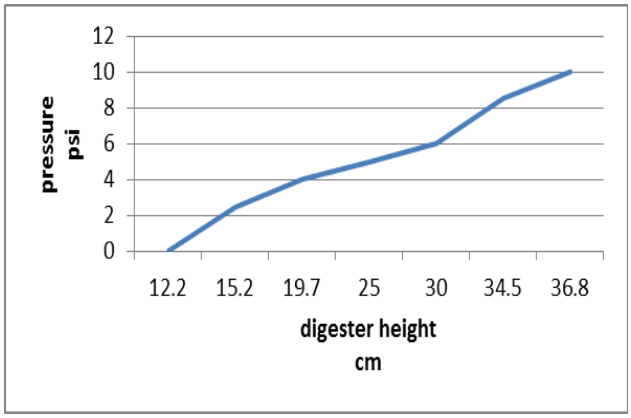


Figure 4: Effect of temperature and digester height

Figure 5: Effect of pressure on the digester height



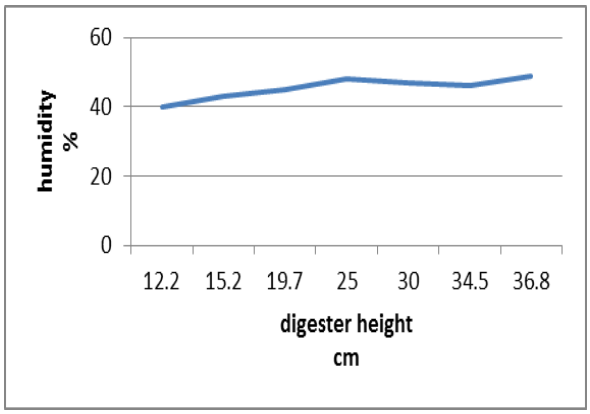


Figure 6: Effect of humidity on the height of the digester

Later, the biogas was produced in a large digester located in PCSIR Laboratories, Lahore. The digester was fed daily with 30 kg of animal manure and with the prepared inoculums at alternate days. The assembly was given 9 days of incubation. The prescribed parameters like temperature, pressure, pH, humidity, digester height etc were determined on daily basis. The external environment was proved favorable for mesophilic conditions at that time so the research was conducted with quite ease. After the given incubation time, when the height of the digester was considerably high, the flame was burnt to study the duration to which it can withstand. Both the control and experimental digesters were burnt at the time. The control digester was the one, which lacked the inoculum, while the experimental was the one which contained inoculum diluted to a great extent. The burner from the control digester was burnt for almost 1 hr and 24 min while, the burner of the experimental digester was kept on burning for 3 hrs approx. This indicated that the added inoculum served the purpose of enhancing the effect of the biogas which usually too was produced, but by the addition of prepared inoculums it went far away in context of burning.

Figure7: Flame intensity

CONCLUSION

In present work mesophilic methanogen was isolated from animal manure on nutrient agar using anaerobic jar. The isolate was gram positive, non–spore former, non–motile, spheroid in shape, indole and catalse positive with optimum growth temperature 35°C and NaCl concentration of 0.3M. Thus it was identified as *Methanosarcina* sp. following the *Bergey's Manual* and was designated as *Methanosarcina* WS1. This isolate was used for the production of biogas in a digester of 15 feet that was fed with 30 kg animal manure on daily basis and 1000 ml of inoculum (5 days old) on alternate days. Incubation was carried out for 9 days and gas produced was used to burn the Bunsen burner. The flame of the burner attached with the experimental digester burned for 3 hrs 1 hr and 36 min more compared to the burner attached with control digester (without the addition of inoculum). This study would help to find new solutions of energy crises in Pakistan.

ACKNOWLEDGMENTS

This work was supported by the keen interest and thought provoking ideas of Prof. Dr. Ikram–ul–Haq. The authors would like to thank Dr. Sakhawat, Dr. Uzma Hameed and Dr. Qurat–ul–Ain Syed for many thoughtful discussions and critical reading of the manuscript.

REFERENCES

Abbasi T, Tauseef SM and Abbasi SA (2012). Biogas capture from animal manure. Springer briefs in environmental science, (2): 41–62.

Amon T, Amon B, Kryvoruchkoa V, Zollitsch W, Mayer K. and Gruber L (2007). Biogas production from maize and dairy cattle manure–influence of the biomass on the composition of methane yield. Agriculture, ecosystem and environment, 114(1–4): 173–182.

Amon T, Hackl E, Jeremic D, Amon B, Boxberger J (2001). Biogas production from animal wastes, energy plants and organic wastes. In: van Velsen, A., W. Verstraete (Eds). Proc. 9th World Congress on Anaerobic Digestion: 381–386.

Amon T, Amon B, Kryvoruchko V, Bodiroza V, Potsch E and Zollitsch W (2006). Optimising methane yield from anaerobic digestion of manure: Effects of dairy systems and of glycerine supplementation. International congress series, 1293: 217–220.

Anjelidaki I and Ahring BK (2000). Methods for increasing the biogas potential from the recalcitrant organic matter contained in manure. Water Science and Technology, 41(3): 189–194.

Anjelidarki I and Ellegaard L (2003). Codigestion of manure and organic wastes in centralized biogas plants–status and future trends. Applied biochemistry and biotechnology, 109(1–3): 95–105.

Attar Y, Mhetre ST and Shawale MD (1998). Biogas production enhancement by cellulytic strains of Actinomycetes. Biogas Forum I, (72): 11–15.

Azbar N, Keskin T and Yuruyen A (2008). Enhancement of biogas production from olive mill effluent (OME) by co–digestion. Biomass Bioenerg., (32): 1195–1201.

Bagi Z, Acs N, Balint B, Horvath L, Dobo K, Perei KR, Rakhely G and Kovacs KL (2007). Biotechnological intensification of biogas production. Appl Microbiol Biotechnol., (76): 473–482.
Barnett A, Pyle L and Subramanian SK (1978). Biogas technology in the third world–a multidisciplinary review. Energy citations database.
Berglund M (2006). Biogas Production from a Systems Analytical Perspective. Biomass and bioenergy. 30(3): 254–266.
Braun R (2007). Anaerobic digestion: a multi–faceted process for energy, environmental management and rural development. Biomedical and life sciences: 335–416.
Braun R, Drosg B, Bochmann G, Weiß S and Kirchmayr R (2010). Recent developments in bio–energy recovery through fermentation. Biomedical and life sciences: 35–58.
Bryant MP and Boone DR (1987). Isolation and characterization of Methanobacterium formicicum MF. International journal of systematic bacteriology, 37(2): 171.
Chem. Spider, the free chemical database. (http://www.chemspider.com/Chemical–Structure.30363.html)
Cheremisinoff NP and Cheremisinoff PNJA. 1980. Biomass : applications, technology, and production. Vol. 5. M. Dekker., New York.
Claassen PAM, Lier JBV, Contreras AML, Neil EWJV, Sijtsma L, Stams AJM, de Vries SS and Weusthuis RA (1999). Utilization of biomass for the supply of energy carriers. Appl Microb Biotechnol., (52): 741–745.
Demirbas MF and Balat M (2006). Recent advances on the production and utilization trends of bio–fuels: A global perspective. Energy conservation and management, (47): 2371–2381.
Demirel B and Scherer P (2008). The roles of acetotrophic and hydrogenotrophic methanogens during anaerobic conversion of biomass to methane: a review. Rev. Environ. Sci. Biotechnol., (7): 173–190.
Doddema HJ and Vogels GD (1978). Improved identification of methanogenic bacteria by fluorescence microscopy. Applied and environmental microbiology, 36(5): 752–754.
Edwards T and McBride BC (1975). New method for the isolation and identification of methanogenic bacteria. Appl. Microbiology, 29(4): 540–545.
El–Mashad HM, Zhang R (2010). Biogas production from co–digestion of dairy manure and food waste. Bioresource technology, (101): 4021–4028.
Engler CR, Jordan ER, McFarland MJ and Lacewell RD (1999). Economics and environmental impact of biogas as a manure management strategy. pp. 109–140.
EurObserv'er Report (2008). The state of renewable energies in Europe: 47–51.
Fachverband B (2009). Biogas dezentral erzeugen, regional profitieren, international gewinnen. In. Proc. 18. Jahrestagung des Fachverbandes Biogas, Hannover.
Fehrenbach H, Giegrich J, Reinhardt G, Sayer U, Gretz M, Lanje K and Schmitz J (2008). Kriterien einer nachhaltigen Bioenergienutzung im globalen Maßstab. UBA–Forschungsbericht., 206: 41–112.
Feist AM, Scholten JCM, Palsson BO, Brockman FJ and Ideker T (2006). Modeling methanogenesis with a genome–scale metabolic reconstruction of Methanosarcina barkeri. Molecular systems biology: 1–14.
Ferry JG (1992). Methane from acetate. J. Bacteriol., (174): 5489–5495.
Ghosh, S., M.P. Henry, A. Sajjad, M.C. Mensinger and J.L. Arora. 2000. Pilot–scale gasification of municipal solid wastes by high–rate and two–phase anaerobic digestion (TPAD). Water Sci Technol., 41(3): 101–110.
Goel B, Pant DC and Kishore VVN (2001). Two–phase anaerobic digestion of spent tea leaves for biogas and manure generation. Biores Technol., 80(2): 153–156.
Hai–Lou X, Jing–Yuan W and Joo–Hwa T (2002). A hybrid anaerobic solid–liquid bioreactor for food waste digestion. Biotechnol Lett., (24): 757–761.
Hermann M, Noll KM and Wolfe RS (1986). Improved agar bottle plate for isolation or other anaerobes in a defined gas atmosphere. Applied and environmental microbiology, 51(5): 1124–1126.
Hickey AJ, Macario AJL and de Macario EC (2000). Identification of genes in the genome of the archaeon Methanosarcina mezeii that code for homologs of nuclear eukaryotic molecules involved in RNA processing. GENE–an international journal on genes, genome and evolution, (253): 77–85.
Hook PA, Westville NJ and Toledo OH (2006). Benzene–achieving HES excellence. Sunoco chemicals: 2.
Holt JG, Krieg NR, Sneath PHA, Staley JT and Williams ST (2000). Bergey's Manual of determinative bacteriology, 9th edition. Lippincott Williams & Wilkins. Maryland, USA. pp.724.
Hossain MZ (2001). Farmers view on soil organic matter depletion and its management in Bangladesh. Nutrient cycling in Agroecosystems., (61): 197–204.
IEA (2006). World Energy Outlook. International Energy Agency.
IPCC (2000). Special report on emission scenarios, Intergovernmental Panel on Climate Change.
IPCS. INCHEM (2000).
Kaparaju P, Serrano R, Thomsen AB, Kongjan P and Angelidaki I (2009). Bioethanol, biohydrogen and biogas production from wheat straw in a biorefinery concept. Bioresource technology, (100): 2562–2568.
Kaparaju PLN and Rintala JA. (2003). Effects of temperature on post–methanation of digested dairy cow manure in a farm scale biogas production. Environmental technology, 24(10): 1315–1321.
Kapdi SS, Vijay VK, Rajesh SK and Prasad R (2005). Biogas scrubbing, compression and storage: perspective and prospectus in Indian context. Renew Energy, (30): 1195–202.
Kendall M M, Liu Y, Sieprawska–Lupa M, Stetter KO, Whitman WB and Boone DR (2006). Methanococcus aeolicus sp. nov., a mesophilic, methanogenic archaeon from shallow and deep marine sediments. International journal of systematic and evolutionary microbiology, (56): 1525–1529.
Kennedy KJ and Berg LVD (1982). Anaerobic digestion of pig gery waste using a stationary fixed film reactor. Agric. Wastes, 4: 151–158.
Lettinga G, Velson AFMV, Hobma SW, De Zeeuw W and Klapwijk A (1980). Use of upflow sludge blanket reactor for biological wastewater treatment, especially for anaerobic treatment. Biotechnol Bioeng., (4): 674–699.
Li J, Jha AK, He J, Ban Q, Chang S and Wang P (2011). Assessment of the effects of dry anaerobic codigestion of cow dung with waste water sludge on biogas yield and biodegradability. International journal of physical sciences, 6(15): 3679–3688.
Li R, Chen S and Li X (2010). Biogas production from anaerobic codigestion of food waste with dairy manure in a two–phase digestion system. Appl Biochem Biotechnol.,(160): 643–654.
Li R, Chen S, Li X, Lar JS, He Y and Zhu B (2009). Anaerobic codigestion of kitchen waste with cattle manure for biogas production. Energy and fuels, (23): 2225–2228.
Lier JBV, Tilche A, Ahring BK, Acarie H, Moletta R, Dohanyos M, Hulshoff Pol LW, Lens P and Verstraete W (2001). New perspective in anaerobic digestion. Water science and technology, 43(1): 1–18.
Lo KV, Liao PH, Bulley NR and Chieng ST (1984). A comparison of biogas production from dairy manure filtrate using conventional and fixed–film reactor. Can. Agric. Eng., (26): 73–78.
Lozano CJS, Mendoza MV, de Arango MC and Monroy EFC (2009). Microbiological characterization and specific methanogenic activity of anaerobe sludges used in urban solid waste treatment. Waste management, (29): 704–711.
Maestrajuan GM, Boone JE, Mah RA, Menaia JAGF, Sachs MS and Boone DR (1992). Taxonomy and halotolerance of mesophilic Methanosarcina strains, assignment from strains to species, and synonym of Methanosarcina mazei and Methanosarcina frisia. International journal of systematic bacteriology, 42(4): 561–567.
Maestrojuan GM and Boone DR (1991). Characterization of Methanosarcina barkeri MST and 227,Methanosarcina mazei S–6T, and Methanosarcina vacuolata Z–761T. International journal of systematic bacteriology, 41(2): 267–274.
Mata–Alvarez J, Macé S and Llabrés P (2000). Anaerobic digestion of organic solid wastes: an overview of research achievements and perspectives. Bioresource Technology, 74(1): 3–16.
McKendry P (2002). Energy production from biomass (part 1): overview of biomass. Bioresource technology, 83 (1): 37–46.
Mikucki JA, Liu Y, Delwiche M, Colwell FS and Boone DR (2003). Isolation of a methanogen from deep marine sediments that contain methane hydrates and description of Methanoculleus submarinus sp. nov. Applied and environmental microbiology, 69(6): 3311–3316.
Moller HB, Sommer SG and Ahring B (2004). Methane productivity of manure, straw and solid fractions of manure. Biomass Bioenergy, 26(5): 485–495.
Mshandete A, Bjornsson L, Kivaisi AK, Rubindamayugi MST and Matthiasson B (2006). Effect of particle size on biogas yield from sisal fibre waste. Renew energy, 31: 2385–2392
Murray PA and Zinder SH (1985). Nutritional requirements of Methanosarcina sp. strain TM–1. Applied and environmental microbiology, 50(1): 49.
Nagamani B and Ramasamy K (1999). Biogas production technology: an Indian perspective. Fermentation–science and technology: 44–56.
Narihiro T, Terada T, Ohashi A, Wu J, Liu W, Araki N, Kamagata Y, Nakamura K and Sekiguchi Y (2009). Quantitative detection of culturable methanogenic archaea abundance in anaerobic treatment systems using the sequence–specific rRNA cleavage method. The ISME journal, (3): 522–535.
Neves L, Oliveira R and Alves MM (2004). Influence of inoculums activity on the bio methanization of a kitchen waste under different waste/inoculum ratios. Proc Biochem., (39): 2019–2024.

Nicholson MJ, Evans PN and Joblin KN (2007). Analysis of methanogen diversity in the rumen using temporal temperature gradient gel electrophoresis: identification of uncultured methanogens. Microbial ecology: 141–150.

Oosterkamp VJ (2001). Enhancement of biogas production from straw and manure–An annotated bibliography. Waste and biomass valorization: 1–21.

Parawira W, Read JS, Mattiasson B and Björnsson L (2008). Energy production from agricultural residues: high methane yields in a pilot–scale two–stage anaerobic digestion. Biomass Bioenergy, 32: 44–50.

Peters V and Conrad R (1995). Methanogenic and strictly anaerobic bacteria in desert soil and other oxic soils. Applied and environmental microbiology, 61(4): 1673–1676.

Pohanish RP and Greene SA (2009). Wiley guide to chemical incompatibles. Wiley and sons. pp. 307.

Potivichayanon S, Sungmon T, Chaikongmao W and Kamvanin S (2011). Enhancement of biogas production from bakery waste by Pseudomonas aeruginosa. World academy of science: engineering and technology, (80): 529–532.

Rao MS, Singh SP, Singh AK and Sodha MS (2000). Bioenergy conversion studies of the organic fraction of MSW: assessment of ultimate bioenergy production potential of municipal garbage. Appl Energ., 66(1): 75–87.

Raskin L, Stromley JM, Rittmann BE and Stahl DA (1994). Group specific 16S rRNA hybridization probes to describe natural communities of methanogens. Applied and environmental microbiology, 60(4): 1232.

Rastogi G, Ranade D, Yeole TY, Patole MS and Shouche YS (2007). Investigation of methanogen population structure in biogas reactor by molecular characterization of methyl–coenzyme M reductase A (mcr A) genes. Biores Technol (in press)

Santosh Y, Sreekrishnan TR, Kohli S and Rana V (2004). Enhancement of biogas production from solid substrates using different techniques–a review. Bioresource Technol., (95): 1–10.

Scherer PA, Dobler S, Rohardt S, Loock R, Buttner B, Noldeke P and Brettschuh A (2003). Continuous biogas production from fodder beet silage as sole substrate. Water Sci Technol., 48(4): 229–233.

Seckiguchi Y, Kamagati Y, Nakamura K, Ohashi A and Harada H (1999). Fluorescence in situ hybridization using 16S rRNA–targeted oligonucleotides reveals localization of methanogens and selected uncultured bacteria in mesophilic and thermophilic sludge granules. Appl. Environ. Microbiol., 65(3): 1280.

Singh N, Kendall MM, Liu Y and Boone DR (2005). Isolation and characterization of ethylotrophic methanogens from anoxic marine sediments in Skan Bay, Alaska: description of Methanococcoides alaskense sp. nov., and emended description of Methanosarcina baltica. International journal of systematic and evolutionary microbiology, (55): 2531–2538.

Solera R, Romero LI and Sales D (2001). Determination of the microbial population in thermophilic anaerobic reactor: comparative analysis by different counting methods. Anaerobe, (07): 79–86.

Sowers KR and Ferry JG (1983). Isolation and characterization of a methylotrophic marine methanogen, Methanococcoides methylutens, gen. nov., sp. nov. Appl Environ Microbiol., (45): 684–690.

Stevens CV and Verhe R (2004). Renewable bio–resources: scope and modification for non–food application. John wiley & sons, ltd.: 107–108.

Stewart DJ, Bogue MJ and Badger DM (1984). Biogas production from crops and organic wastes–results of continuous digestion tests. New Zealand journal of science, 27(3): 285–294.

Taleghani G and Kia AS(2005). Technical–economical analysis of the Saveh biogas power plant. Renew Energy, (30): 441–446.

Tirumale S and Nand K (1994). Influence of anaerobic cellulolytic bacterial consortia in the anaerobic digesters on biogas production. Biogas Forum III, (58): 12–15.

Wei Q, Wei W, Cuiping Z and Zhongzhi Z (2010). Biogas recovery from microwave heated sludge by anaerobic digestion. Sci China Tech Sci., (53): 144–149.

Weiland P (2010). Biogas production: current state and perspectives. Appl Microbiol Biotechnol., (85): 849–860.

Wett B and Insam H (2010). Biogas technology–controlled gas flow for enhanced mixing, heating, and desulfurization. Biomedical and life sciences: 79–91.

Wilkie AC, Smith PH and Bordeaux FM (2004). An economical bioreactor for evaluating biogas potential of particulate biomass. Biores Technol., 92(1): 103–109.

Yadvika, Santosh, Sreekrishnan TR, Kohli S and Rana V (2004). Enhancement of biogas production from solid substrates using different techniques–a review. Bioresource technology: (95): 1–10.

Yu Z and Schanbacher FL (2010). Production of methane biogas as fuel through anaerobic digestion. Biomedical and life sciences: 105–127.

.