BIOGAS AND ORGANIC FERTILIZER PRODUCTION FROM *AZOLLA PINNATA* R. BROWN

By

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CERTIFICATE

This is to certify that the thesis entitled "Biogas and Organic Fertilizer Production from *Azolla pinnata* R. Brown" prepared by Mrs. Monira Begum for the degree of Doctor of Philosophy to the University of Dhaka is a record of original research carried out in the National Professor A.K.M. Nurul Islam Laboratory, Department of Botany, University of Dhaka, Dhaka under my supervision. It is further certified that the entire work presented in this thesis based on the results of author's own investigations, and no part of this thesis has been submitted before in substance for any degree. The thesis is hereby approved as to the style and contents for submitting to the University of Dhaka.

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ABSTRACT

Biogas and organic fertilizer production from *Azolla pinnata* R. Brown supplementing cow dung and poultry droppings were studied in the laboratory batch digester (reactor) and in the outdoor "Mini-Biogas Plant". In the laboratory studies, treatments considered using 20:1 carbon-nitrogen ratio revealed that 50% *Azolla* supplementing 50% cow dung produced highest amount of biogas (22 L) followed by *Azolla* supplementing poultry droppings (20 L) and cow dung alone (16.5 L) after 45 days. When *Azolla* and cow dung alone were compared, *Azolla* produced lower amount of biogas because of longer time required to decompose than cow dung. *Azolla* appeared to have decomposed slower also when mixed with poultry droppings. When biogas from different treatments were compared in terms of percentage contributions by methane, *Azolla* supplemented cow dung always produced higher amount (63-68%) followed by cow dung (51-66%), and *Azolla* supplemented poultry droppings (64-65%), later two are not significantly different from each other. Again *Azolla* alone or in combination contributed lowest amount even after 14 days. To obtain maximum biogas with high percentage of methane, *Azolla* plants were crushed manually and mixed thoroughly with cow dung and was used in the "Mini-Biogas Plant" developed. On an average 18.63 ± 1.92 L biogas was produced per day from day nine to 45 days of semi-continuous digester. Using about 20 L biogas produced in the "Mini-Biogas Plant", 250 g rice was cooked. The Mini-Biogas Plant developed may be further refined in future for performing better.

Analysis of organic fertilizer produced from biogas residue (slurry) of laboratory digester revealed that N, P, K and S concentrations were significantly higher in *Azolla* supplemented cow dung compared to cow dung alone. Heavy metals were present but the concentrations were much lower than the acceptable limit. Using a mixture of 50% organic fertilizer obtained from *Azolla* supplemented cow dung residue with 50% recommended fertilizer doses (RFD) a much higher number of filled grains per pot and significantly higher 1000 grain weight (by 4.79%) were obtained in BRRI 28 rice variety compared to100% chemical fertilizer alone

It appeared that *Azolla* supplemented cow dung mixture produced higher amount of biogas with higher concentration of methane and that the organic fertilizer produced from the residue mixed with 50% RFD was better than the 100% RFD of chemical fertilizers.

Dedicated to my parents
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INTRODUCTION

1 INTRODUCTION

1.1 Introductory remarks

Natural gas extracted in Bangladesh for the last over half a century is being used for household cooking in cities and towns, for industries including electricity production. With the increasing population and demand the supply is gradually declining. Over half of the populations in villages are dependent on jute sticks, straw, dry cow dung and fuel wood for cooking. Use of trees as fuel wood is decreasing the tree plants of home-yards and forests. Under the prevailing situation alternative sources of gas production are being introduced in Bangladesh in a limited scale, e.g. biogas from cow dung using anaerobic digester (Eusuf *et al.*1983). Cow dung is becoming limited and thus some substitution by poultry dropping and water hyacinth, have been attempted, to increase the quantity biogas production and quality of biogas. Aziz *et al.* (2003) observed higher concentration of methane production from *Azolla pinnata* R. Brown, an aquatic fern in the laboratory studies. *A. pinnata* a multipurpose crop, may be used as biofertilizer in rice crop and as feed for fish, cattle and poultry. A sustainable method of larger scale cultivation (one ton per ha per day) of *A. pinnata* var. *pinnata* round the year has been developed (Aziz 2012). The present study has been undertaken to substitute a part of cow dung by *A. pinnata* for producing biogas and organic fertilizer developing a "Mini-Biogas Plant" for a small family.

1.2 History of biogas

It has been known for several centuries that combustible gas is generated when organic waste is allowed to rot in huge piles. As back as in the seventeenth century, Van Helmont (Abbasi *et al.* 2012) recorded that decaying organic material produced flammable gases. In 1776, Volta (Abbasi *et al.* 2012) resolved that there was a direct connection between how much organic material was used and how much gas the material produced. That the combustible gas is methane was established by the work conducted independently by John Dalton and Humphrey Davy during 1804–1808 (Tietjen 1975). Bechamp in 1868, reported that the formation of methane during the decomposition of organic matter was through microbiological processes. Omelianski 1890 (Abbasi *et al.* 2012) isolated microbes and reported that fermentation of complex materials occurs through oxidation-reduction reactions to form hydrogen, carbon dioxide, and acetic acid. He also demonstrated that methane is formed due to microorganism-mediated reaction between hydrogen and carbon dioxide (McCarty *et al.* 1982). Later, Sohngen (1910) seconded Omelianski's findings. He also assumed that acetic acid through decarboxylation forms methane. This assumption remained highly controversial for decades but is now known to be essentially correct (McCarty *et al.* 1982).

Biogas, the metabolic product of anaerobic digestion, is a mixture of methane and carbondioxide with small quantities of other gases such as hydrogen sulfide (McKendry 2002, Hiremath *et al.* 2009). Methane, the desired component of biogas, is a colorless, blue burning gas used for cooking, heating, and lighting (Itodo *et al.* 2007). Biogas is a clean, efficient, and renewable source of energy, which can be used as a substitute for other fuels like tree plants in rural areas (Yu *et al.* 2008).

1.2.1 How methane is formed?

Methane formation in anaerobic digestion involves four different steps-hydrolysis, acidogenesis, acetogenesis and methanogenesis (Karthik *et al.* 2012). Different bacterial/archaeal communities work in a syntrophic relationship with each other to form methane. In hydrolysis, complex carbohydrates, fats and proteins are first hydrolyzed to their monomeric forms by exoenzymes and bacterial cellulosome (multi-enzyme complexes). In the second phase (acidogenesis), monomers are further degraded into short-chain acids such as: acetic acid, propionic acid, butyric acid, isobutyric acid, valeric acid, isovaleric acid, caprionic acid, alcohols, hydrogen and carbondioxide. During acetogenesis, the short-chain acids are converted into acetate, hydrogen and carbondioxide. In the last phase, methanogens convert the intermediates product into methane and carbondioxide. Almost one-third of methane formation is due to reduction of carbondioxide by hydrogen (Deublein and Steinhauser 2008).

1.2.2 Biogas production with the use of mini digester

Using Mini biogas digester consisting of 12 digesters Vindis *et al.* (2008) obtained highest Biogas and methane yield using 75% sugar beet with 25% maize but lowest when used in 50% ratio.

1.2.3 Biogas production in Bangladesh

Biogas technology was introduced in Bangladesh by Dr. M A Karim, a professor of Bangladesh Agricultural University (BAU), Mymensingh, in the University campus in 1972. During 1982-1984, government through Environment Pollution Control Department (EPCD) constructed about 250 floating dome model and 119 fixed dome model biogas plants in the country. The floating dome plants initially worked well, but stopped within a few years due to leakage formed in the steel dome. The fixed dome model did not work due to design fault.

The biogas Pilot Plant Project of BCSIR, Dhaka, is effectively installing cow dung/poultry dropping-based biogas plants in villages (rural areas). But dependence only on cattle dung would be a limiting factor and hence to look for an alternative biomass/substrate along with cattle dung would be a better solution. This would be achieved by addition of residue of high biogas potential substrate in combination with cattle dung. Various suitable biomasses have been identified for their potential as a supplement to the cattle dung digesters for enhancing gas production. In Bangladesh alone, animal dung available from 24.48 million cattle and buffalo are nearly 185.67 million kg per day. One kg of dung can produce 0.037 m^3 of biogas. According to an estimate 29.7 billion m^3 of biogas can be obtained from the livestock of the country which is equivalent to 1.5 million tons of kerosene which is the principal fuel in the rural areas (Islam *et al.*2006). Besides, a substantial amount of biogas can be produced from various cellulose biomasses (agricultural wastes, marine plants, garbage, water hyacinth and *Azolla*) are available in plenty. There are reports on use of *Azolla* in biogas production (Wagner 1997). During 1992-1996 engineers of Local Government Engineering Department (LGED) and Scientists of Bangladesh Council for Scientific and Industrial Research (BCSIR) constructed about 300 fixed dome model biogas plants following Chinese design at the cost of the owners.

Grameen Shakti (GS) established as a private social business company in 1996 at the initiatives of Nobel Laureate Professor Muhammad Yunus to promote renewable energy technologies in Bangladesh and started working on solar. In 2005 they launched their biogas program. By now they constructed about 25,000 biogas plants, of which about 15,000 with the support from Infrastructure Development Company Ltd (IDCOL). GS has two systems for the biogas extension program. For small family size biogas plants, i.e. 1.6m^3 - 4.8 m^3 gas production per day, they give subsidy of Tk.7,000 and for bigger size plants they do not give any subsidy.

Although biogas technology is getting increasing attention all over the world, the use of bio-slurry is still neglected. Giving priority to the bio-slurry, IDCOL renamed their project title, and GS appointed one expert exclusively for the promotion of bio-slurry. But still, in most cases, bio-slurry is not properly used. It is mainly because of the ignorance of the farmers. Recently, GS has taken a decision to demonstrate use of slurry in different districts, so that the farmers become interested in using bio-slurry. Biogas has potentiality, but highly neglected in Bangladesh.

Bangladesh has a wonderful climate for biogas production. The ideal temperature for biogas is around 35^{0} C. The temperature in Bangladesh usually varies from 6 to 40^{0} C. But the inside temperature of a biogas digester remains at 22 to 30^{0} C, which is very near to the optimum requirement.

1.2.4 Biogas status in other countries

In developing countries, where energy is in short supply and expensive (on per capita and purchasing power basis, respectively), unlike the West, anaerobic digestion has a far greater relevance than it has to developed countries. Thus, anaerobic digestion in these countries has been primarily focused on energy production via biogas plants (Abbasi *et al.* 2012).

1.2.4.1 India

India is credited for having built the first-ever anaerobic digester, in 1897, when the Matunga Leper Asylum in Bombay (Mumbai) utilized human waste to generate gas to meet its lighting needs (Khanal 2008).

The first-ever attempt to build a plant to produce biogas from manure was also made in India, at Bombay, in 1900, but it was not very successful. The first successful attempt came in 1937, when S.V. Desai–a microbiologist of the Indian Agricultural Research Institute (IARI), (then the Imperial Agricultural Research Institute) conducted studies leading to the commissioning of a plant which worked satisfactorily for several years (Abbasi *et al*. 2012).

India is implementing one of the world's largest renewable-energy programs with different scales of technologies. India have 3.8 million households biogas digesters. One of the strategies is to promote biogas plants (Khoiyangbam 2011, Sarkar 1982). India began the project half a century ago, and was further supported by the National Project on Biogas Development in 1982 (NDRC 2007).Public toilets incorporating biogas units has been an attractive option, especially in semi-urban areas and small towns in India which are not covered by proper waste treatment facilities and where extra energy in the form of biogas is welcome.

1.2.4.2 China

China has the largest biogas programme in the world. Over twenty five million households in China are using biogas by now, which accounts for over 10% of all rural households. By the end of 2005 there were 2,492 medium and large-scale biogas digesters in livestock and poultry farms, while 137,000 biogas digesters had been constructed for the purification of household wastewater. In Sichuan Province alone, close to five million domestic biogas plants have been constructed by 2010. There is substantial government subsidy on biogas plants (Abbasi *et al*. 2012).

There are currently more than 30 million household digesters in China (Jiang *et al* 2011, Thien *et al.* 2012, Austin and Morris 2012). China has increased its investments in biogas infrastructure very rapidly. By 2020, 80 million households in China are expected to have biogas digesters serving 300 million people (NDRC 2007).

Hongzhi Alcohol Corporation Limited, which is the largest alcohol factory in south-western China, runs a service using industrial organic wastewater, sewage, and dregs to produce biogas. The service is paid for by the industry and the residents in cities, but is provided free to the farmers. The company has also built a biogas power plant generating seven million kilowatts per hour. The city of Mianzhu treats 98% of municipal sewage including wastewater from hospitals through digesters with a total capacity of 10,000 m^3. The treated water reaches national discharge standards, greatly improving the environment.

1.2.4.3 Nepal

In Nepal during 2004–2005, 17,803 domestic biogas plants were installed, bringing the total number installed since 1992 to over 140,000. In recent years, as many as 62 biogas construction companies have been established in Nepal and they installed 0.2 million biogas digester, along with 15 workshops for the manufacturing of biogas appliances. About 140 micro-finance institutes are involved in financing biogas plants in rural areas. These units have improved the social and environmental conditions of about 800,000 people (Abbasi *et al.* 2012).

1.2.4.4 Vietnam

Vietnam has a large and expanding animal husbandry sector with high potential of biogas generation. In Vietnam, as in other developing countries Colombia, Ethiopia, Tanzania, Cambodia, and Bangladesh the polyethylene tubular digester was promoted to reduce production cost by using local materials and simplifying installation and operation. The resulting low-cost digester has been well received by poor farmers, especially when farmers participate fully in the necessary maintenance and repair work. Within 10 years, more than 20,000 polyethylene digesters were installed and mainly paid by the farmers themselves (Abbasi *et al.* 2012).

1.2.4.5 Sri Lanka

Biogas digesters have been introduced in Sri Lanka in the 1970s, poor design, lack of maintenance skills and insufficient capacity to deal with the problems. The Intermediate Technology Development Group (ITDG) started a project in 1996 to improve the success rate of the units on a national level by setting up demonstration units to help spread information, restore abandoned units and train users to operate and maintain them (Abbasi *et al.* 2012).

1.2.4.6 Japan

In Japan, anaerobic digestion has received considerable attention during the last few years from the point of view of pollution control, and for the treatment of livestock, industrial, and urban waste. Japan is the only country in the region which has adopted thermophilic (high temperature) digestion of some wastes (Abbasi *et al.* 2012).

1.2.4.7 USA/Canada

In the USA, Canada anaerobic digestion has been used mainly for processing animal manure till the mid-1970s. The advancements in high rate anaerobic digesters began with the introduction of anaerobic filter in 1967 (Abbasi *et al.* 2012) In America, 162 farm scale plants were in operation by 2010, providing energy for 41,000 homes; in addition, 17 plants were operating in Canada.

1.2.4.8 Europe

Anaerobic digestion and aerobic composting of waste originating from kitchens, food processing units, and gardens is well established in Europe. By the end of 2006, there were some 124 anaerobic digestion plants with capacity greater than 3,000 tonne/year treating feedstock composed of at least 10% municipal solid waste (MSW). At the end of 2011, the number of these digesters was more than 4000 in Germany, 350 in Austria, 72 in Switzerland, 65 in the United Kingdom followed by Denmark with 20 community type and 35 farm scale plants, and Sweden had 12 plants (Wilkinson 2011, Raven and Gregersen 2007).

Spain treats about 10% of its organic waste using anaerobic digesters (Lesage *et al.* 1952). In West Germany most of the new biogas plants have an electrical capacity between 400–800 kW. The first industrial biogas energy park, Klarsee, with 40 biogas plants (total capacity 20 MW) has come into operation. Maize, corn, and wheat are the main substrates, manure constitutes less than 50%. This has given rise to the criticism that food crops are being diverted to energy production in developed countries even as millions in the developing world do not have adequate food to eat (Abbasi *et al.* 2012).

1.2.4.9 Africa

The level of biogas technology for household purposes is very low in many African countries (Amigun and Sigamoney 2008). Kenya has 1884 household biogas plants and Ethiopia has more than 1140 plants (Africa Biogas 2012). Small-scale biogas plants are located throughout Africa, but only a few are working.

1.3 Substrates for biogas production

Various types of feedstock can be used for the production of biogas: animal manure and slurries, crop residues, organic wastes from dairy production, food industries and agroindustries, wastewater sludge, organic fraction of municipal solid wastes, organic wastes from households as well as energy crops. Biogas can also be collected, with special installations, from landfill sites. One main advantage of biogas production is the ability to use "wet biomass" types as feedstock, all characterised by moisture content higher than 60–70% (e.g. sewage sludge, animal slurries, flotation sludge from food processing etc.). In recent years, a number of energy crops (grains, maize, rapeseed) have been largely used as feedstock for biogas production in countries like Austria or Germany. Besides energy crops, all kinds of agricultural residues, damaged crops, unsuitable for food or resulting from unfavorable growing and weather conditions, can be used to produce biogas and fertilizer. A number of animal by-products, not suitable for human consumption, can also be processed in biogas plants (https://algaebiogas.eu/biogas_substrates).

1.3.1 Cow dung

The biogas Pilot Plant Project of BCSIR, Dhaka, is effectively installing cow dung/poultry dropping-based biogas plant in rural areas. But dependence only on cattle dung would be a limiting factor and hence to look for an alternative biomass/substrate along with cattle dung would be a better solution. Various suitable biomass have been identified for their potential as a supplement to the cattle dung digesters for enhancing gas production.

Cow dung gas contains 55-65% methane and 30-35% carbondioxide of gas may be generated from one pound of cow manure at around 28° C. In Bangladesh alone, animal dung available from 24.48 million cattle and buffalo are nearly 185.67 million kg per day. One kg of cow dung can produce 0.037 m^3 of biogas. According to an estimate available cattle dung can produce 2.50 billion m^3 of gas that is equivalent to 1.28 million tons of kerosene or 2.56 million tons of coal.

Availability of cow dung is again limiting the widespread use of this system. An alternative of the cow dung or raw material parallel to this or as a supplement is thus needed. There are reports on use of *Azolla* in biogas production (Wagner 1997).

1.3.2 Cattle dung

Field study on a 2 m^3 biogas plant run on a 1:4 mixture of horse and cattle dung and a 1 m^3 biogas plant run on pure cattle dung gave average daily gas productions of 21.9 m^3 and 22.6 m^3 biogas kg^{-1} wet substrate fed, respectively, showing that 20% replacement of cattle dung can be made by horse dung for operating family-size biogas plants without much reduction in their gas production or encountering any operational problem (Kanwar and Kalia 1992).

Batch experiments with 1.750 L of medium containing 1.760 kg of cattle manure and 70–140 ml of crude glycerin were incubated under mesophilic and thermophilic condition in stirred tank reactors. Under mesophilic conditions, the addition of 4% glycerin to screened manure increased biogas production by up to 400% (Castrillon *et al.* 2011).

1.3.3 Human excreta

Treating human waste through Anaerobic Digestion is an incredibly ethical sanitation technology. This can cause a host of environmental problems that can lead to ecosystem collapse such as rendering a water body uninhabitable for many organisms. Untreated sewage causes algal blooms, red tide, and so called dead zones. Humans also suffer from untreated sewage (also called black water). Waterborne disease transmitted through human excrement is a leading cause of death worldwide, especially in the so-called developing world.

Production of biogas using human excreta in closed systems of limited population like passengers of long route buses are in practice in UK. The biogas is converted into electricity and used for lights needed in buses (www.appropedia.org/Biogas from human waste, 2015).

1.3.4 Poultry droppings

Poultry droppings are the feces of chickens used as an organic fertilizer, especially for soil low in nitrogen (Telkamp, Mick, 2015). Of all animal manures, it has the highest amount of nitrogen, phosphorus, and potassium (Deborah 1992). Chicken manure is sometimes pelletized for use as a fertilizer, and this product may have additional phosphorus, potassium or nitrogen added (Barrett, J.2008). Optimal storage conditions for chicken manure include it being kept in a covered area and retaining its liquid, because a significant amount of nitrogen exists in the urine (Pullin, R *et al.* 1980). Fresh chicken manure contains approximately 1.5% nitrogen. One chicken produces approximately 8-11 pounds of manure monthly (Foreman *et al.* 2015). Chicken manure can be used to create homemade plant fertilizer.

1.3.5 Municipal wastes

The experiments were conducted to investigate the production of biogas from municipal solid waste (MSW) and domestic sewage by using anaerobic digestion process. The batch type of reactor was operated at room temperature varying from 26 to 36 °C with a fixed hydraulic retention time (HRT) of 25 days. The digester was operated at different organic feeding rates of 0.5, 1.0, 2.3, 2.9, 3.5 and 4.3 kg of volatile solids (VS)/m^3 of digester slurry per day. Biogas generation was enhanced by the addition of domestic sewage to MSW. The maximum biogas production of 0.36 m^3/kg of VS added per day occurred at the optimum organic feeding rate of 2.9 kg of VS/m^3/day. The maximum reduction of total solids (TS) (87.6%), VS (88.1%) and chemical oxygen demand (COD) (89.3%) occurred at the optimum organic loading rate of 2.9 kg of VS/m^3/day. The quality of biogas produced during anaerobic digestion process was 68-72% methane (Elango *et al.* 2007).

1.3.6 Plant and plant wastes

Potentials of water hyacinth (*Eichhornia crassipes*), but a notorious weed and water chestnut (*Trapa bispinnosa*) employed for phytoremediation of toxic metal rich brass and electroplating industry effluent, were examined in terms of biogas generation (Verma *et al.* 2007). Inability of the plants to grow in undiluted effluent directed to select 20%, 40% and 60% effluent concentrations (with deionized water) for phytoremediation experiments. Slurry of both the plants used for phytoremediation produced significantly more biogas than that by the control plants grown in unpolluted water; the effect being more pronounced with plants used for phytoremediation of 20% effluent.

Maximum cumulative production of biogas (2.430 L/100 g dry matter of water hyacinth and 1.940 L/100 g dry matter of water chest nut) and percent methane content (63.82% for water hyacinth and 57.04% for water chestnut) was observed at

5 mm particle size and 1:1 substrate/inoculum ratio, after twenty days incubation (Verma *et al.* 2007). Maximum biogas production occurred in 8-12 days in water hyacinth whereas 12-16 days in water chestnut.

1.3.7 *Azolla* use as a substrate

Organic fertilizer from *Azolla* could be an alternative to cow dung manures and chemical fertilizers because it is environment friendly, increases soil organic matter, and farmers could produce it (Aziz 1999). *Azolla* has been found to supplement about 50% urea fertilizer and can control weed production in rice crop fields (Watanabe 1987, Aziz & Rahman 2000). A recent study showed that application of wet *Azolla* compost at the rate of 5 to 7.5 ton/ha could supplement all chemical fertilizers by about 50% (Aziz 2001). *Azolla* could also be effectively used for fish, poultry and cattle, and the whole system could be integrated by a farmer and held in poverty alleviation (Aziz 1999). Soil pH, organic matter, N, P, K, Ca, Mg and Na increased with rate of *Azolla*. Inorganic nitrogen fertilizers are expensive and supplies remain inadequate and uncertain for the majority of farmers in the tropics. Nitrogen-fixing crops and trees, composted crop wastes and livestock manures are least-cost alternatives source of nitrogen which have been adopted by farmers on a wide range of situations. Another option, specifically for those growing rice in flooded or irrigated land, is the use of *Azolla*. Addition of extra carbohydrate like *Azolla* to rich nitrogen containing substrate could improve the biogas production. Organic fertilizer from *Azolla* could be an alternative to cow dung manures and chemical fertilizers because it is environment friendly by increasing soil organic matter and sustainable because farmers could produce it. *Azolla* has been found to supplement about 50% urea fertilizer and can control weed production in rice crop fields. *Azolla* is expected to supply fixed nitrogen and increase the uptake of some nutrient elements such as calcium, magnesium, and potassium. The objective of present study, therefore, was to produce biogas in the laboratory and then in the outdoor in mini scale and fertilizer from *Azolla pinnata* R. Brown with cow dung.

1.3.8 Biogas production by plant biomass

A batch anaerobic test was conducted to evaluate the effects of adding high carbon content of corn straw to the digestion of Taihu blue algae to attain an optimal C/N ratio for higher methane yield. The addition of corn straw in algae at a C/N ratio of 20/1 increased methane yield by 61.69% at 325 mL g^{-1} VS^{-1} (compared with 201 mL $g^{-1}VS^{-1}$ of algae digestion alone), followed by C/N ratios of 16/1 and 25/1, all operated at 20 g VS L^{-1} and 35 °C. The results suggest the optimal C/N ratio for co-digestion of algae with corn straw is 20/1 (Zhong *et al.* 2012)

1.3.9 Biogas production from agricultural waste

The amount of solid wastes generated in developing countries increased over population explosion and continuous growth of industries and agricultural practices. In agriculture, particularly cattle rearing, in addition a large number of families generate heavy wastes in the kitchen on a daily basis, which could be converted to economic benefits. The biogas production from one kg of poultry droppings and kitchen wastes are about 0.03186m^3 and 0.0143m^3 per day respectively. It is conducted that the wastes can be managed through conversion into biogas, which is a source of income generation for the society. Organic olive cake in addition to animal wastes (sheep and goat waste) can be used as a source of fermentable organic matter in biomass technologies for gas production (Osman *et al.* 2006).

1.4 Energy status in Bangladesh

In Bangladesh, all the people fulfill their primary energy requirements from fossil fuels. In this modern era, the need for energy in every sector is growing rapidly. The energy demand in Bangladesh mainly includes cooking, lighting, heating, productive uses, motive power, leisure, and so on. These energy requirements are satisfied from various non-renewable and renewable energy sources, it is depicted that the main sources of commercial energy in Bangladesh are natural gas and oil. However, it is a matter of hope that the share of natural gas in commercial energy consumption is declining gradually and attention has been given to utilize renewable sources like biomass. In year 2009, the natural gas consumption was about 50% of total commercial energy has been reduced to 42% in year 2012. On the other hand, biomass energy consumption has been increased from 33.3% to 34.1% during this fiscal year as presented (FDMOF 2014).

1.4.1 Sector wise use of natural gas in Bangladesh

In rural areas, the majority of people primarily rely on biomass and kerosene for cooking, lighting, feeding, and heating. In Bangladesh, about 95% of households gather or purchase biomass energy for cooking whereas in rural areas almost 99% use wood, cow dung in the form of cake or stick, jute sticks or other agricultural wastes for cooking. In Bangladesh it shows that only firewood accounts for almost half of the total consumption (Energypedia). In addition, about 70% of the rural population uses kerosene for lighting purpose. An agriculture based country like Bangladesh has huge potentials for utilizing biogas technologies. According to an estimate 29.7 billion m^3 of biogas can be obtained from the livestock of the country which is equivalent to 1.5 million tons of kerosene (which is the principal fuel in the rural areas). Apart from this, it is also possible to get biogas from human excreta, poultry dropping, waste, marine plants, etc. If each family of Bangladesh can be associated with a biogas plant, then only human excreta will give about 10 billion cubic meter biogas.

1.5 Biogas residue or organic fertilizer

Fermented slurry or biogas residue, sometimes called bio-slurry, as a product of anerobic fermentation of animal excrements in the biogas digester, is an excellent organic fertilizer which can make an important contribution to better crop yields and lasting soil fertility. The fermented slurry which contains relatively high percentage of readily available nutrients, can be directly applied in liquid form to the plants both for basal and top-dressing, in a dried form and also for compost preparation together with other organic material (Sanmaneechai *et al*. 1992).

1.5.1 Chemical composition of biogas residue or organic fertilizer

Nitrogen is an important element for crop growth. The nitrogen in animal manure is normally available in an organic form but after passing through the fermentation process in a biogas digester it is changed (by bacteria) to inorganic form mostly ammonia nitrogen (NH_4^+) which is easily soluble and utilized by crop plants. The chemical composition of fermented slurry from cattle manure in a liquid form is shown in Table 1.

Table 1. Chemical composition (% fresh wt. basis) of fermented slurry from cattle manure (Sanmaneechai *et al.* 1992)

Parameters	% fresh wt.
pH	7.8
Water	86.2
Dry matter	13.8
Nitrogen (N)	0.37
Phosphorus (P)	0.35
Potassium (K)	0.21

1.5.2 Biogas residue as fertilizer for crops

Fermented slurry over-flowed from the outlet of the biogas digester can be readily utilized and applied directly to crop plants in a liquid form. This is the best method to apply fermented slurry to crops such as fodder grass, fruit trees or vegetables. Nevertheless, in most cases the fermented slurry (FS) could be dried up through sand bed filter and sold out from the farms in dry form.

1.5.3 Biogas residue from cereal crops

The studies of Sanmaneechai *et al.* (1992) at the Chiang Mai University Farm showed that in the treatments by application of FS alone or FS plus chemical fertilizer could increase the organic matter and phosphorus contents in the soil. In the treatment that only applied FS, the rice yield was 3,881 kg/ha which was 24.4 percent higher than the no fertilizer plot. The chemical fertilizer plot gave 1,081 kg more than the FS only plots in the first year of the experiment. In the same experiment, it was also shown that the application of FS plus chemical fertilizer at the ratios of 50:50 and 25: 75 to the rice plots could produce the same yield as the solely chemical fertilizer plot. In the following rice cropping without any application of fertilizer, the residual effects from the first cropping showed that in the FS plot still gave about 79 percent higher yield than no fertilizer plot but lower than the chemical fertilizer plot.

1.5.4 Biogas residue from vegetables

The same group of researchers (Sanmaneechai *et al.* 1992) also studied using a mixture of FS and chemical fertilizer at different ratios with chinese cabbage and kale. It was shown that in the no fertilizer plot, chinese cabbage could not grow well and produced only about 3,556 kg/ha which was about 4.8 times less yield than that of the FS plot but still less yield than the only chemical fertilizer plots. For kale, the FS plot gave about 4.7 times more yield than the no fertilizer plot and even more than the only chemical fertilizer plot.

1.5.5 Biogas residue from baby corn and Napier grass

Mikled *et al.* (2002) studied use of fermented slurry (0.09±0.04 % N) as liquid fertilizer for baby corn (field crops) and Napier grass (forage crops) at different rates as compared to chemical fertilizer. The measurements were concentrated mainly on baby corn yields, corn-stover yields and nutritive value. In Thailand, the advantages for the farmers to grow baby corn are to harvest the young ear corn and sell to the market as vegetable and to cut the stover and feed to the cattle as roughage. It can be seen that baby corn yields and baby corn stover from chemical fertilizer was higher than only the first two crops but, towards the end of the first year and throughout the second year, all FS treatments plants could produce at about the same yield or even higher at the higher levels of FS. The average baby corn and stover yields from both years were substantially similar to other reports (Lekhakul 1988, Sompong 1988). However, in the long run by application of FS the tendency of higher yield of baby corn would be more prominent without any detrimental effect on soil fertility (see later in the part of soil properties). The types and rates of N fertilizer affected the chemical compositions of the baby corn stover specially the crude protein content, as at the higher rate of N fertilizer showed the tendency of higher crude protein value. Generally cattle dung is deficient in both phosphorous and nitrogen. If urine is added, then protein source increases the nitrogen content of the raw materials, causes fertilizer value to the optimum conditions. Good quality compost contains (%) (Islam 1996): Nitrogen 1.0 ~ 1.5, Phosphorous 0.3 ~ 0.4,

Potassium 1.0 ~ 1.3.

1.6 Carbon nitrogen ratio

Carbon-nitrogen ratio of raw material is an important factor for biogas production. It is commonly recognized that a C/N ratio of 20-30:1 (Biogas Technology 1983). Co-digesting swine manure with three agricultural residues, like corn stalks, oat straw, and wheat straw, to enhance biogas productivity showed that all crop residues (grounded to 40 mesh size) significantly increased biogas production and net CH_4 volume at all C/N ratios, among which corn stalks performed the best with increase in daily maximum biogas volume by 11.4-fold as compared to the control, followed by oat straw (8.45-fold) and wheat straw (6.12-fold) at the C/N ratio of 20/1, which was found to be the optimal C/N ratio for co-digestion (Wu *et al.* 2010). In addition, corn stalks achieved the highest CH_4 content in the biogas (68%), which was about 11% higher than that of oat straw (57%), whereas wheat straw and the control both had produced biogas with 47% CH_4 content. Wheat straw demonstrated lower biogas productivity than corn stalks and oat straw even it had higher carbon content (46%) than the latter two residues (39%) (Wu *et al.* 2010)

Vascular plants from terrestrial sources tend to have C/N ratios greater than 20. The lack of cellulose, which has a chemical formula of $(C_6H_{10}O_5)_n$ and greater amount of proteins in algae versus vascular plants causes this significant difference in the C/N ratio (Meyers *et al.* 1999, Muller 1977).

When composting, microbial activity utilizes a C/N ratio of 30-35:1 and a higher ratio will result in slower composting rates (Prahl 1994). However, this assumes that carbon is completely consumed, which is often not the case. Thus, for practical agricultural purposes, a compost should have an initial C/N ratio of 20-30:1 (Dahlem 1988). Carbon-nitrogen ratio of cattle and human wastes are shown below (Biogas Technology 1983).

Constituents	**Cattle waste**	**Human waste**
Carbon content, %	7.30	2.50
Nitrogen content, %	0.29	0.85
C - N ratio	25:1	3:1

1.7 Applications of biogas from household digesters

1.7.1 Cooking and heating

Biogas produced from the household digesters is mainly used for cooking (Gautam *et al.* 2009, Ferrer *et al.* 2009). The amount of biogas used for cooking purposes usually varies between 30 and 45 m^3 per month. This number can be compared with other commonly used fuels such as kerosene where the consumption is between 15 and 20 L, and Liquefied Petroleum Gas (LPG) between 11 and 15 kg per month, respectively. The energy equivalent was around 300, 200, and 150 kWh for biogas, kerosene, and LPG, respectively (Gosling 1982, UNDP 2003). The surplus biogas in the domestic digester could be used for water and space heating.

1.7.2 Biogas stoves

Biogas burning is not possible in commercial butane and propane burners because of its physiochemical properties. However, it is possible to use these burners after some modifications (Bond *et al.* 2011). Burners are changed in the gas injector, its cross-section, and mixing chambers. The biogas burners are designed to meet a mixture of bio-gas and air in the ratio of 1:10 (Subramanian 1977). Different burners like vertical flame diffuser, horizontal flame diffuser, and no diffuser with biogas have been examined. A vertical flame diffuser had a high heat transfer efficiency compared to other diffusers (Ferrer *et al.* 2011). The efficiency is obtained by calculating the heat gained by the water subjected for heating and the amount of fuel consumed during this process. The efficiency of the heat entering the vessel from the stove was high for biogas with 57.4%, followed by LPG, kerosene, and wood with 53.6%, 49.5%, and 22.8%, respectively (CES 2001). The biogas consumption and the thermal efficiency in the biogas stoves varied between 0.340–0.450 m^3/h and 59–68% (Singh and Gupta 1990, Laichena and Wafula 1997, Kurchania *et al.* 2011).

The Institute of Fuel Research and Development (IFRD) has developed a number of improved stoves. These stoves save 50-70% fuel as compared to the traditional ones. So far, more than 100,000 stoves have been installed in the user's kitchens. The potential of biogas technology is immense. According to an official estimates there is a cattle population of 24 million and poultry population of 75 million. This can produce about 3 billion m^3 biogas. The Local Government Engineering Department (LGED) and IFRD are working to install the biogas plants in the rural areas. So far, a total of 19,596 biogas plants have been installed.

1.7.3 Lighting and power generation

The other major application of household biogas is for lighting and power generation. In many developed countries, biogas from the digesters is sent to a combustion engine to convert it into electrical and mechanical energy (Mohammad 1991). Biogas requires a liquid fuel to start ignition (Luijten *et al.* 2011). Diesel fuel can also be combined with biogas for power generation (Duc and Wattanavichien 2007, Bari 1996, Henham and Makkar 1998). For instance, in Pura (India), a well-studied community biogas digester can fuel a modified diesel engine and run an electric generator (Reddy 2004). Bari (1996) reported that carbon dioxide up to 40% will not decrease the engine performance using biogas as a fuel. Biogas can also be used to power engines when mixed with petrol or diesel, and it can also help in pumping water for irrigation (Gosling 1982, Jawurek *et al.* 1987). Cottage/small scale industries use biogas for pumping, milling, and for some other production activities (Vijay *et al.* 1996).

For a medium-sized farm in Jordan, the monthly energy consumption for various purposes is about 1282 kWh. The biogas required for producing 982 kWh is around 6.7 m^3/day, and for water-heating 2 m^3/day. The use of 1 kW generator proved that half of the energy needed could be met by using a domestic digester. Satisfactory results were observed when tested for water-heating and electricity generation from biogas (Aburas *et al.* 1996). In Earth University (USA), electricity from biogas is used for milking operations (Ciotola *et al.* 2011). Biogas is blended with jatropha oil in a

12 kW diesel engine generator to act as a dual fuel for rural electrification. Jatropha seeds remain as a waste product after oil production. This waste gets converted into biogas. The oil and biogas is combined in a duel fuel engine for electricity generation (Luijten *et al.* 2011). The fertilizer from biogas is used for jatropha plantation. Hence, the nutrients are in the closed cycle, which can act as a bio-refinery (Butterworth 2066). Biogas conversion into electricity using fuel cells is a hot research topic nowadays. However, it is not commercially affordable due to the requirement of clean gas and the cost of fuel cells (Bond and Templeton 2011).

Biogas lamps are more efficient than the kerosene powered lamps, but the efficiency is quite low compared to electric-powered lamps. However, the light intensity of the biogas lamp compared to a kerosene lamp or an electric light bulb, was in the power range of 25–75W (Laichena and Wafula 1997). One cubic meter of biogas is equal to lighting 60–100 watt bulb for 6 h, or cooking three meals a day for 5–6 persons. In contrast, 0.7 kg of petrol can run 1 hp motor for 2 h or generate 1.25 KW for electricity (Kristoferson and Bokalders 1991). To provide electricity for a home with a family of five, about 0.25 to 0.5 m^3 biogas is needed (Gosling 1982). Until recently, many of the rural areas in India depended on kerosene lamps for lighting due to the energy shortage. Using these kerosene-powered lamps was inefficient as well costly. Battery-operated solar panels were also an expensive means for lighting. This resulted in research to design a digester, which could provide lighting to a home. A mini-biogas digester developed especially for lighting purposes. his digester could produce 0.5 m3/day biogas which is enough for 4 h of use (Jash and Basu 1999).

1.7.4 Other applications

Domestic biogas is also utilized for other purposes gas-powered refrigerators or chicken incubator, which is a well known application in Kenya (Sibisi and Green 2005, Laichena and Wafula 1997). In India, around 4600 public toilets are connected to biogas digesters by a local NGO to improve social living conditions of the people. Similarly, in Nepal, public toilets are connected to biogas digesters to light these toilets (Sibisi and Green 2005).

1.8 Advantages of the present study

Well-functioning biogas production systems can yield following benefits for users, the society and the environment in general:

- The biogas is a "Green" energy.
- Use of cattle dung, poultry droppings, municipal wastes, cellulosic wastes and also hydrophytes would help in reducing global warming factor.
- Biogas residue could be used as an organic fertilizer that improves soil structure, resulting in a crumb-like structure that increases the water retention capacity enhancing soil fertility.
- Elements of organic fertilizers are released slowly there by no chances of eutrophication and wastage like chemical fertilizers.
- The organic fertilizers produced from agricultural wastes of small farmers can save in part the cost of crop production.
- Biogas is convertible to electricity.
- The whole process is non-hazardous, environment friendly, cost effective and simple for villagers.

1.9 Aim of the present study

Biogas production from cow dung is an economically viable and eco-friendly process. But dependence on only cow dung has become a limiting factor. Addition of *Azolla* as a source of extra carbohydrate having high nitrogen containing substrate with cow dung or poultry droppings could increase biogas production and improve quality with increased Methane concentration.

Therefore, in the present study for optimizing biogas production from cow dung and also in a limited scale from poultry droppings, *Azolla pinnata* R. Brown, an indigenous species has been used. Established method using 20:1 ratio of carbon-nitrogen in substrates will be applied for producing quality biogas and organic fertilizer by developing a "Mini-Biogas Plant". The aims of the research are:

(i) Using *A. pinnata* as a supplement to cow dung and poultry droppings for biogas production and developing recommendations.

(ii) Designing commercially viable "Mini-Biogas Plant" for a small family.

(iii) Using biogas residue as nutrient-rich organic fertilizer.

CHAPTER 2
MATERIALS AND METHODS

2 MATERIALS AND METHODS

2.1 Substrates for biogas production

Variety of substrates may be used in the production of biogas as an alternative source of energy and its by-product the digested residue as organic fertilizer through anaerobic digester. This is a process where controlled degradation of organic substances occurs in the absence of oxygen and presence of anaerobic microorganisms. Cow dung and recently poultry-based biogas production is in practice in Bangladesh and elsewhere (Eusuf 1983, Hill 1983, Ojolo *et al.* 2007). Some attempts for using plant material such as water hyacinth, duckweeds, straw of various crops, etc. have been made in large-scale biogas production using "dome type" digester for large size families in villages as integrated farming (Fig. 2.1).

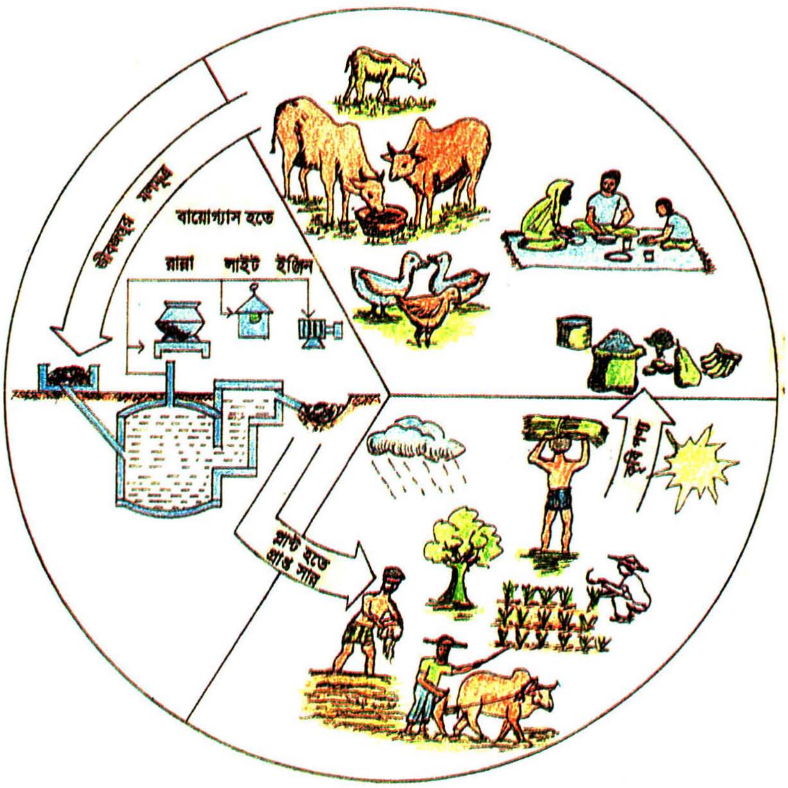

Fig. 2.1. Integrated farming system for producing biogas and organic fertilizer using "Sthir-dome" Biogas Plant (Source: Sthir Dome Poddhatite Biogas Utpadan, IFRD, Bangladesh Council of Scientific and Industrial Research, Dhaka)

Large number of floating and submerged hydrophytes grows during flood period in Bangladesh (Whitton *et al.* 1988). *Azolla pinnata* R. Brown also called "red duckweed" a small floating fern was also recorded. The plant is soft and easily decomposable and has relatively high nitrogen. After 3-4 months of flood period huge amount of hydrophytes under natural conditions decomposed releasing global warming gases CH_4 and CO_2. *A. pinnata* also grows in road side ditches and small water bodies forming red cover during winter and spring seasons. In the laboratory-scale *Azolla* plant has been found to produce biogas with 68% methane (Aziz *et al.* 2003). The plant has been designated as a multipurpose crop that may be used as poultry, fish and cattle feed and as biofertilizer directly or making compost through integrated farming system (Aziz 1999, 2001). For making compost in an open system by dumping CH_4 and CO_2 are released but using closed system like an anaerobic digester may hold these gases and used as an alternative of natural gas. Method of large-scale production of *A. pinnata* var. *pinnata* round a year has been developed (Aziz 2012) and integrated system may be developed.

In the present study *A. pinnata* has been used as a supplement and amended with cow dung/poultry droppings for determining the ratio of substrates and producing good quality biogas (Section 2.3.1) in the laboratory scale and then use the know-how for producing biogas using "Mini-Biogas Plant" for cooking rice (Section 3.1.4) and the biogas residue (slurry) as organic fertilizer (Section 2.4) for growing crops.

2.2 Production of *Azolla pinnata* R. Brown

The *Azolla pinnata* R. Brown used in the present experiment has later been identified as *A. pinnata* var. *pinnata* R. Brown (Aziz 2012). Fertilizer recommendation dose used for large-scale cultivation of *A. pinnata* in a pond round

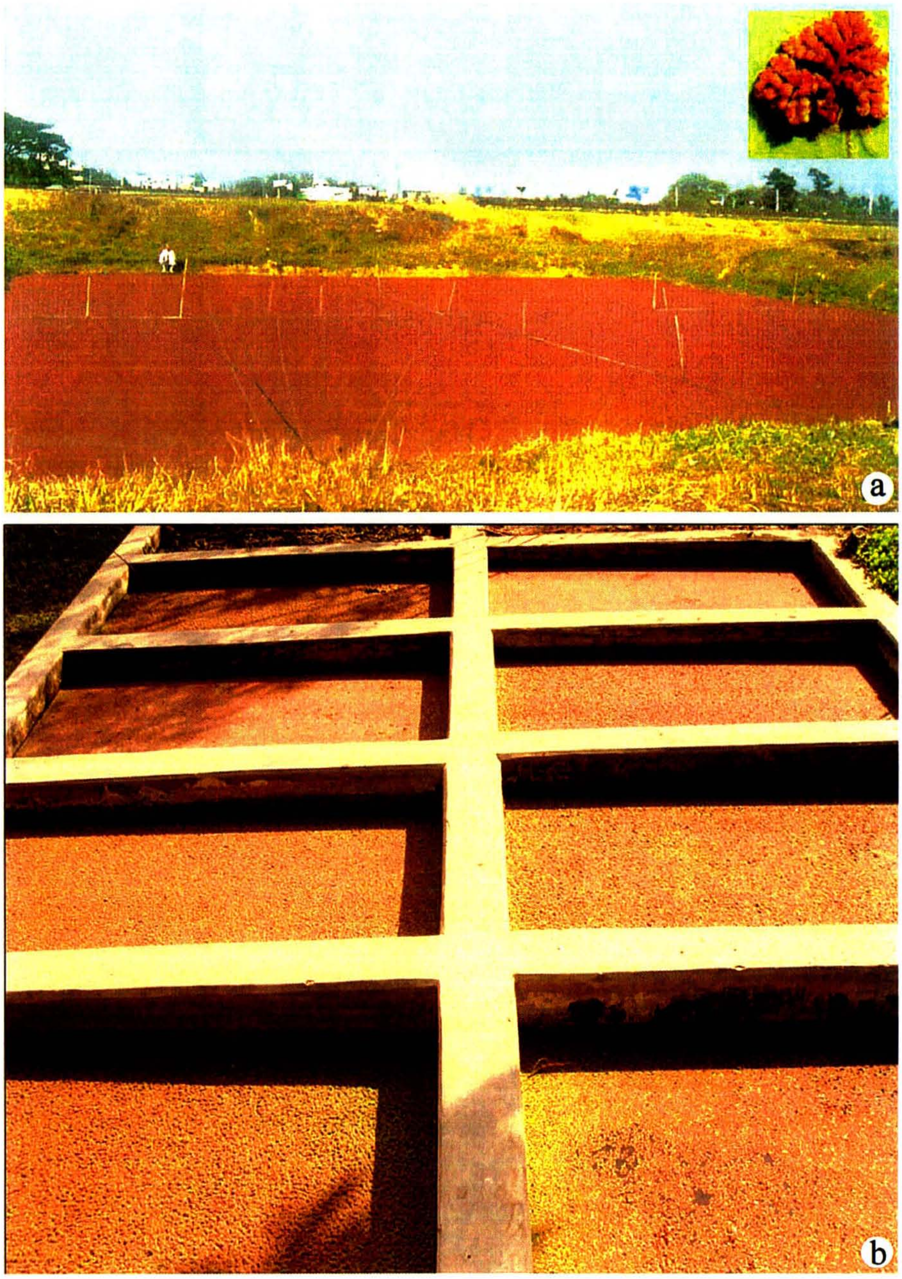

Fig. 2.2a-b. Production of *Azolla pinnata* var. *pinnata*. (a) Large-scale production in a deepwater rice pond of Bangladesh Rice Research Institute (BRRI) at Gazipur, during late February. A whole plant is in the inset (picture courtesy of Prof. A. Aziz, DU). (b) Cultivation of *Azolla* in cemented pits at Botanical garden, University of Dhaka (picture was taken by the end of December giving lighter colour).

the year developed by Aziz (2012) has been used. Using the method on an average 1 ton fresh biomass was produced per ha per day (Fig. 2.2a). For the present study, small-scale *A. pinnata* production was carried out in cemented pits of one square meter area having a depth of 0.35 meter (Fig. 2.2b).

2.2.1 Preparation of fertilizer solution and application in pits

Fertilizers used, were 1 g TSP and 0.5 g MP per one square meter pit per day (equivalent to 10 kg TSP and 5 kg MP per ha per day). *Azolla* plant has symbiotic cyanobacterium *Anabaena azollae* (some bacteria have also been reported to be present with the cyanobacterium) in pockets of leaves that fixes atmospheric N_2 and thus no nitrogen fertilizer is needed for their growth, an advantage for large-scale cultivation (Aziz 2012). The method of every day harvest and adding fertilizer every day after harvest was adopted. After full cover of pits and with little aggregation at around noon *Azolla* plants were harvested to an amount so that no empty water surface remains in pits. Fertilizers were added immediately after harvesting. The fertilizers were soaked overnight in a plastic pot with tap water, grounded in to a solution. The fertilizer solution was then thoroughly mixed with pit water making turbulence with a plastic pot. Relatively low production occurred during winter with the red colour due to abundant anthocyanin formation (Figs. 2.2a-b). Full sunlight is recommended for *Azolla* production even during hot summer. High temperature is not a problem if sufficient fertilizer is applied. One square meter pit can produce about 100 g fresh *Azolla* per day. Therefore, construction of seven pits of one square meter size (10 square meter land) can serve required amount of *Azolla* per day for the digester (Fig. 2.2b).

2.2.2 Management

Pyralid insects (*Nymphula* sp.) are used to infest the *Azolla* crop and produce larvae which feed on the plant. The "cocoons" (a larva within 2-3 *Azolla* plant) produced were removed manually. Snails may also eat up *Azolla* and were removed. Care should be taken so that at least 15 cm water always remains in the pits (Aziz 2012). Harvesting should be done without keeping any empty space that limited lights resulting limited submerged or plankton algae to grow.

2.2.3 How to get *Azolla* seeds?

Azolla plants propagate vegetatively round a year. Reproductive structures mega and microsporangia develop in early February when the vegetative growth was not affected (Aziz 2002). The zygotes formed function in overcoming the adverse hot summer months. Whole plant of *Azolla* is used as seeds for cultivation. As the plant grows apically the lateral proximal branches develop into young plants and get separated as independent plants and the process continues. *A. pinnata* grows in almost all districts of Bangladesh from November to April along road side ditches and small water bodies. Transfer of 100 g fresh *Azolla* to a fertilized one square meter pit would cover whole water surface in about 11-12 days (doubling time 3.5 days).

2.3 Biogas production

The biogas production experiments were carried out in the laboratory scale (indoor) and in mini-scale using "Mini-Biogas Plant" (outdoor) in the Department of Botany, University of Dhaka.

2.3.1 Laboratory scale biogas production

Laboratory scale (indoor) biogas production was carried out at National Professor AKM Nurul Islam Laboratory, Department of Botany, University of Dhaka. The biogas production unit used is shown in Fig. 2.3. The unit is composed of a batch digester or batch reactor, here a conical flasks which had a total capacity of 3.5 liter with a side outlet. The digester was connected with a 5 liter gas collecting glass aspirator using a 3 necked glass tube. The gas collector was graduated to measure the amount of gas accumulated through water displacement method. The displaced water was collected in a plastic aspirator which acts as a water reservoir (Fig. 2.3). The ambient room temperature was 20-25° C in winter to 30-35° C in other months and had diffuse light (<5-10$\mu Em^{-2}s^{-1}$). Literature review revealed that carbon:nitrogen ratio of 20:1 to 30:1 is important for producing optimum biogas. Total solids of cow dung, *Azolla* and poultry dropping were 7-8%, 4-5% and 10-11%, respectively. Nitrogen content in the substrates also varies the C:N ratio was about 20:1 in the cowdung alone where in *Azolla* it was 23:1 and in poultry droppings it was 16:1. (becous of high amount of ammonia). Therefore, carbon and nitrogen contents in determine Methods of determination of carbon and nitrogen in cow dung, poultry droppings and *Azolla* are given in Section 2.5.2. The composition of various treatments for 3.5 L digesters were as follows:

T_0= Cowdung 400 g

T_1= *Azolla* 665 g

T_2= Cowdung 225 g + *Azolla* 665 g

T_3= Poultry droppings 120 g + *Azolla* 665 g

In all the above treatments 100 ml biogas slurry (as starter with methanogenic bacteria) and one liter tap water were added during charging batch digesters.

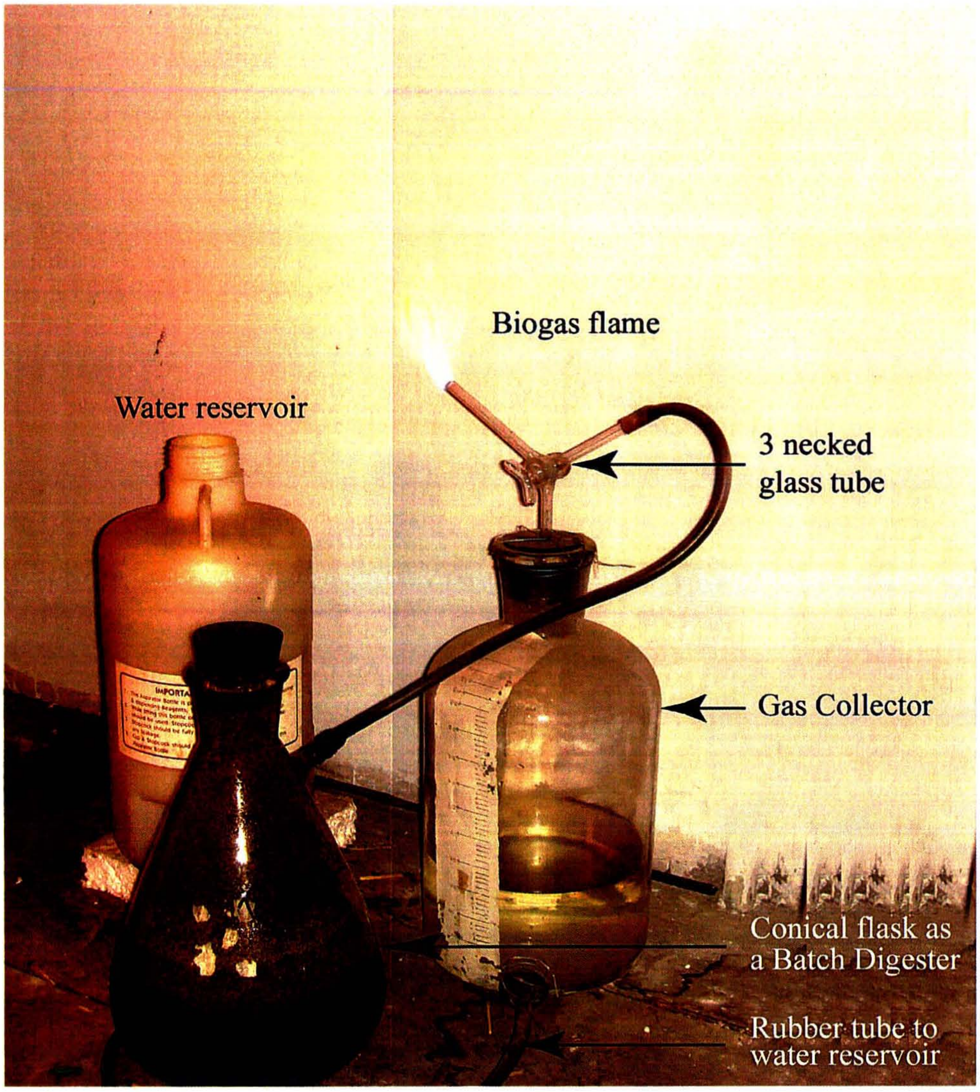

Fig. 2.3. An anaerobic batch digester (reactor) for producing biogas in the Laboratory.

2.3.2 Preparation of "Mini-Biogas Plant"

A "Mini-Biogas plant" (Outdoor) was developed following the principles of integrated "Sthir-dome" Biogas Plant (Fig. 2.1) of the Institute of Fuel Research and Development (IFRD), BCSIR, Dhaka using locally available materials. The volume of the digester was about 15.7 m^3 which can produce 3 m^3 gas every day and can support cooking for a family of 5-7 members. At the start of the operation, the digester is charged with 50 kg cow dung and or 50 litre water in 1:1 (w/v) ratio and kept undisturbed for seven days (IFRD 2003) For the production of 3000 liter gas every day, 50 kg cow dung and 50 liter water were added. The biogas residue is used as organic manure in villages.

The "Mini-Biogas plant" prepared for the present experiment is shown in Figs. 2.4 and 2.5. The "Mini-Biogas plant" was composed of a digester made of plastic drum of 200 L capacity, which was about 0.75 m tall and less than 0.40 m average diameter having total volume of about 0.10 m^3. It has an inlet fixed on the side of digester at about 10-12 cm from bottom for introducing *Azolla* and cow dung mixture and three outlets: (i) biogas outlet for connecting with a gas burner fixed with sealed cover of the digester, (ii) outlet to release biogas residue at certain interval fixed at about 30 cm above the ground and (iii) water pipe fixed at about 30 cm above ground level connecting the digester with a graduated water reservoir and fixed at 2.5 cm from its bottom to measure the amount of biogas produced through water displacement (Figs. 2.4 and 2.5).

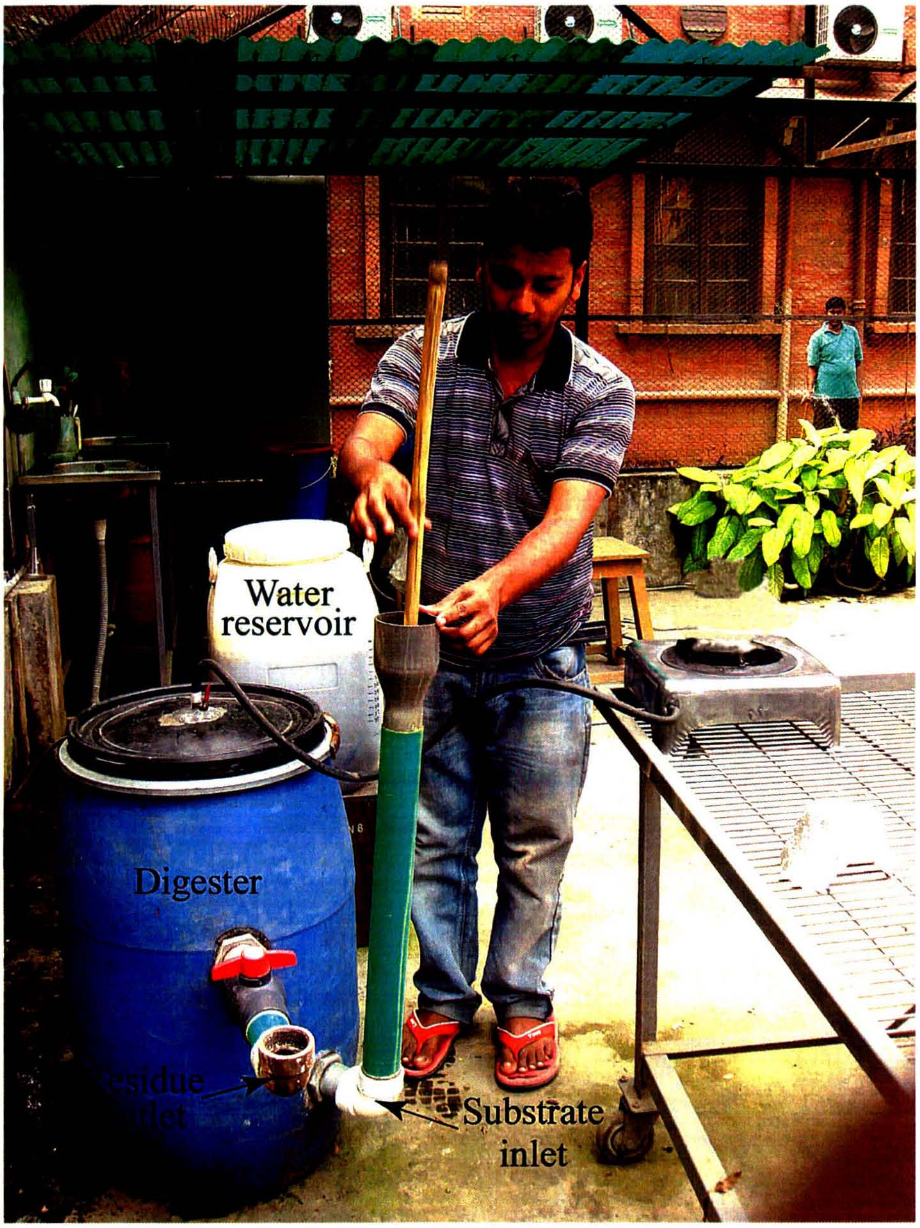

Fig. 2.4. Side-view of the "Mini-Biogas Plant" used in the present experiment. The digester is being feed with *Azolla* and cow dung mixture.

Fig. 2.5. Front view (partially) of the "Mini-Biogas Plant". The raise of the water level in the translucent plastic reservoir from 16 L up to 52 L mark indicates 36 L of biogas stored in the digester. The 16 L level corresponds to the upper level of substrates in the digester without any gas.

2.3.2.1 Biogas production using the "Mini-Biogas Plant"

Biogas production using mini-scale plant (outdoor) prepared from locally available materials named as "Mini-Biogas Plant" was carried out in the Net House of the Department of Botany, University of Dhaka (Fig. 2.4). Laboratory studies showed that cow dung mixed with *Azolla* at 20:1 ratio of C and N produced maximum biogas (Section 3.1.1) and highest methane concentration compared to using mixture of *Azolla* with poultry droppings (Section 3.1.2). Therefore, only *Azolla* mixed with cow dung was used for producing biogas in the "Mini-Biogas Plant" (Figs. 2.4 and 2.5). The digester (blue drum) was first charged with 19.75 kg *Azolla* and 5.25 kg cow dung and mixed well into a paste. The paste was placed into the digester covered with air tight plastic lid. Then 25 liter tap water was added through inlet and kept as it is for 7 days as an incubation period.

After incubation period, the digester was recharged every day with 375 g cow dung mixed with 925 g *Azolla*, made in to a paste as before and kept in an air tight polythene bag. The paste was poured in to the digester through inlet with 1.3 liter tap water. As they will produce a mixture of biogas and air was present in the gas phase. The water level will rise from initial 16 L mark. Biogas sample was collected every day using gas bladder. The raise of the water level in the water reservoir from 16 L mark up to about 52 L indicated total biogas amount of about 36 L in about 36 hours (Fig. 2.5). The bio gas was used for cooking 250 g rice. (Fig. 3.8 a-d).

2.3.2.2 Biogas analysis

Biogas analysis was carried out in the IFRD, BCSIR, Dhaka using Gas Chromatograph Model Trace GC Ultra, Thermo Scientific (USA), where peak time for CO_2 was after 31.73 min of injecting biogas and for CH_4 after 39.70 min.

2.4 Making organic fertilizer from the biogas residue of Mini-Biogas Plant

Biogas production in the Mini-Biogas Plant was done by using mixture of *Azolla* and cow dung. To produce organic fertilizer from biogas residue (slurry) of

Mini-Biogas Plant the outlet was opened every day at around noon, just before charging the digester. About 2.5 L residue was allowed to come out of the digester. The residue had watery consistency and was dried at 100- 101° C for 24 hours in the oven. A solid mass of organic fertilizer was produced after drying. This was grounded to coarse powder (not over 1 mm diameter particles) for using in pot culture of rice crop (Section 2.5). Chemical analysis of nutrient elements the residue was carried out by various methods (Section 2.4.1). Drying the residue would be time consuming. The upper fluide part may be separated by decanting and may be used directly or after dilution on crop plants as foliar spray.

2.4.1 Chemical composition of *Azolla*, cow dung, poultry droppings and organic fertilizer

For producing biogas C:N ratio is an important factor and optimum ratio for fermentation has been found to be approximately 20:1 to 30:1 (Haque *et al.* 2006). Therefore, chemical analyses of *Azolla*, cow dung and poultry droppings were carried out in the Department of Soil Water Environment, University of Dhaka to determine the amount of substrates to be taken.

Azolla, cow dung and poultry droppings were dried in the electric oven at 80° C until fully dried. The biogas residue had watery consistency and was dried at 100-101° C for 24 hours. The quantity of C, N, P, K and S was determined by the following methods:

(i) C by Tyurin's method

(ii) N by Kjeldahl method

(iii) S was determined by Turbidimetric method (Hunt 1980)

(iv) P by molybdenum-blue method (Murphy and Reilly 1962) and

(v) K by Flame photometer.

The content of Pb, Cd, Cr, Ni and Zn were determined by Atomic Absorption Spectrophotometer (AAS) after digestion of samples with nitric-perchloric acid mixture (2:1).

2.4.2 Percentage composition of N, P and K in soil and organic fertilizer

Percentage composition of N, P and K in soil and organic fertilizers produced from biogas slurry were determined. The values were as follows:

Soil: Nitrogen= 0.091-0.180% (low)

Available P= 17.63 ppm (medium)

Available K= 68.38 ppm (medium)

Organic fertilizer: Nitrogen= 3.45%

Phosphorus= 0.54%

Potassium= 1.51%

2.5 Pot culture of rice plants using organic fertilizer

Late rice (*Oryza sativa* L.) variety BRRI 28 seedlings supplied by Dr. Partha, PSO, BRRI were used. Seedlings of 40 day old were planted on 18 March 2015.

The fertilizer experiment was carried out in 30 cm diameter earthen pots. The garden soil was dried and grounded and 10.5 kg was used in each pot. Fertilizer doses for the rice cultivation in pot culture were calculated considering Fertilizer Recommendation Doses (2012) of NPK for BRRI 28, presence of 46% N in Urea, 18% P in TSP, 50% K in Murate of potash and percentage of NPK of soil and organic fertilizer mentioned in Section 2.4.2. For each treatments three replicates were taken and set in direct sunlight at Botanical garden, University of Dhaka. The soil in the pot was fully watered using tap water. The amount of chemical and organic fertilizers added was as follows:

Urea= 120 kg/ha (100%) or 1.3660 g/pot in three splits (at the beginning, after tillering and flowering) in T_1 and 50% (60 kg/ha or 0.6830 g/pot in T_3).

TSP= 10 kg/ha or 0.0525 g/pot as above.

MP= 50 kg/ha or 0.5250 g/pot as above.

Org. fertilizer= 41.444 g/pot in T_2 (100%) and 50% of it (20.722 g/pot) in T_3 in two splits (at the beginning and after tiller formation).

Four treatments were considered as follows:

T_0= Control (no fertilizer)

T_1= Chemical fertilizers, recommended doses of N, P and K (100%)

T_2= Organic fertilizer (100%)

T_3= Chemical Fertilizers (50%) + organic fertilizer (50%)

Three seedlings together were placed in the centre of each pot. Chemical fertilizers were added where necessary as Urea-N, TSP-P and MP-K at the beginning, after tiller formation (34 days) and flowering of rice plant cycle. Required amount of organic fertilizers were added in two splits, at the beginning and after tiller formation. All types of fertilizers depending on treatments were spread on the top of pot soil and mixed thoroughly with upper 10-15 cm soil. Pots were watered every day keeping about 5 cm water. The crop was harvested after 78 day. Yield attributes considered for determining the effects of organic fertilizers were: plant height, straw weight per hill, number of filled and unfilled grains and weight of 1000 grains.

CHAPTER 3
RESULTS

3 Results

3.1 Biogas production

Experiments for the production of biogas and organic fertilizer (biogas slurry) were carried out in the laboratory and outdoor in mini-scale using *Azolla* as a supplement with cow dung or poultry droppings. Results are presented.

3.1.1 Biogas production in the laboratory batch digester

Biogas production from cow dung, *Azolla* and *Azolla* mixing with cow dung, poultry droppings is shown Fig. 3.1. There was a lag period of two days for cow dung and *Azolla* mixed with cow dung (T_0 and T_2) treatments were as, *Azolla* and *Azolla* mixed with poultry droppings (T_1 and T_3) had lag phage for five days. Gas production

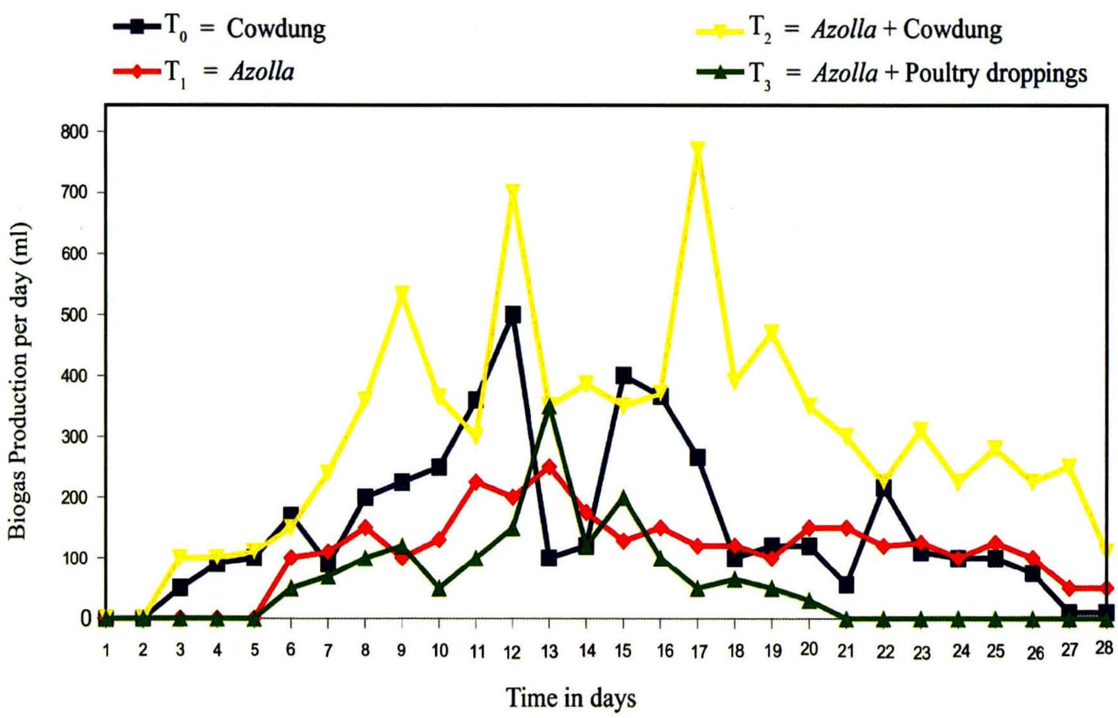

Fig. 3.1. Daily biogas production in the laboratory batch digester from four treatments (1st experiment). Ambient temperature was between 32° C to 35° C.

increased exponentially in *Azolla* mixed with cow dung substrate (T_2) after six days and for cow dung alone (T_0) after eight days. In all treatments except T_2 there were ups and downs in gas production with maxima between eight and twenty two days (Fig. 3.1). However, quite good peaks were formed up to 27 days in *Azolla* with cow dung. Total gas production in the T_0, T_1, T_2 and T_3 were 4.6, 4.0, 9.1 and 2.0 liters studied over 28 days (Fig. 3.2). Maximum gas production occurred in *Azolla* and cow dung mixture at 1:1 ratio in the laboratory batch digester and this amounts to 9.1 L followed by cow dung (4.6 L), *Azolla* and *Azolla* supplement with poultry droppings (Fig. 3.2).

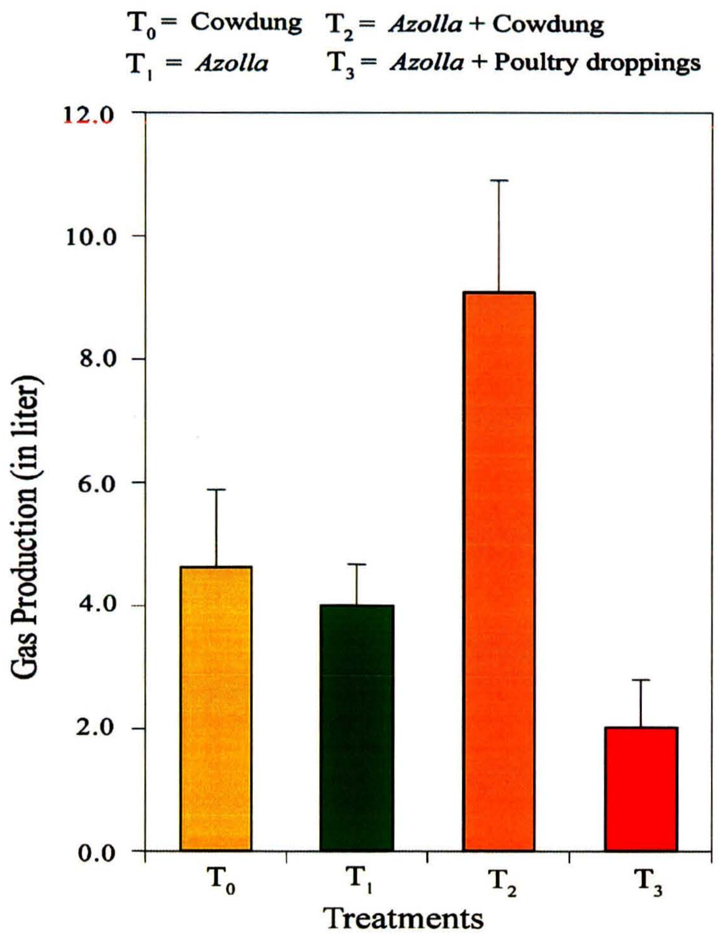

Fig. 3.2. Total biogas production after 28 days in laboratory batch digester (derived from the data in Fig. 3.1). Ambient temperature was between 32° C and 35° C.

In another set of experiment biogas production from cow dung, *Azolla* mixing with cow dung and poultry droppings were determined (Fig. 3.3). There was a lag period of about six days for all treatments. After that biogas production increased exponentially in all treatments showing ups and downs with maxima between seven to 30 day by *Azolla* supplement with cow dung, and between seven to twenty five day by cow dung and *Azolla* supplement with poultry droppings (Fig. 3.3). Total biogas produced was about 13, 17 and 15 L by T_0, T_1 and T_2, respectively. On the other hand when gas production continued for 45 days total gas produced by cow dung, *Azolla* supplement with cow dung and poultry droppings were 16.5, 22 and 20 liters (Fig. 3.4). Therefore, maximum gas production occurred in *Azolla* and cow dung mixture of 1:1 ratio in the laboratory batch digester and this was about 22 L followed by cow dung alone and *Azolla* supplement with poultry droppings (Fig. 3.4).

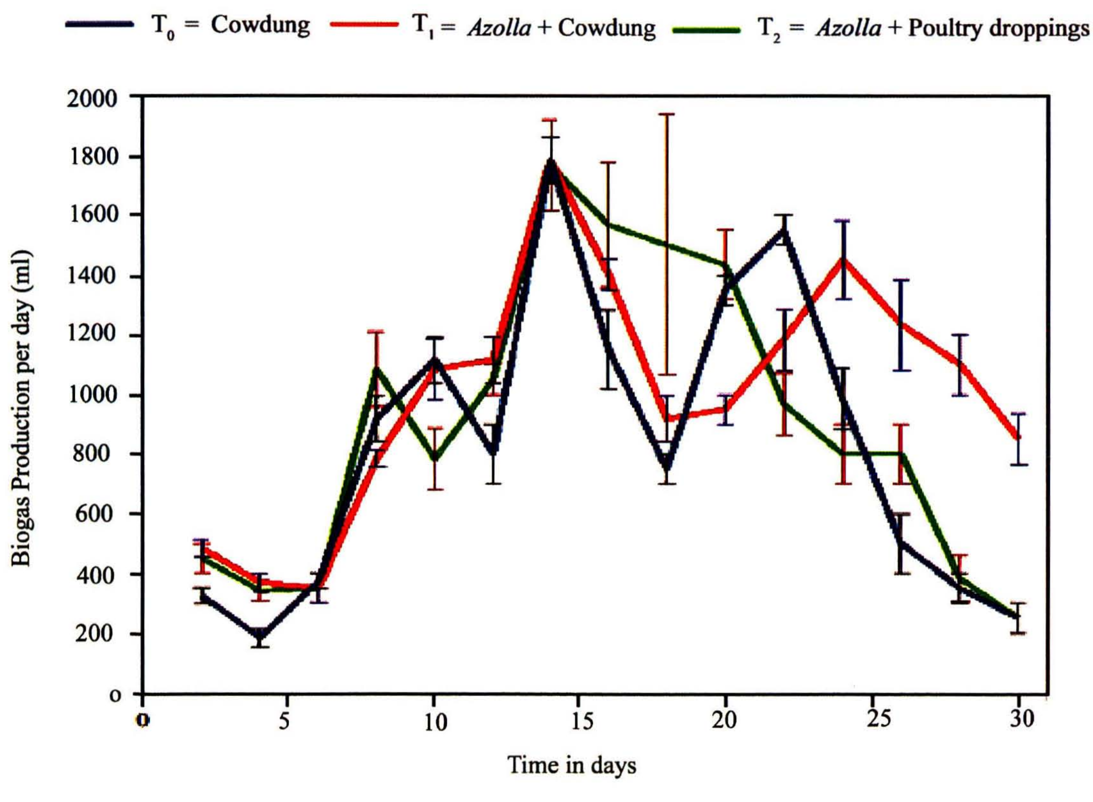

Fig. 3.3. Daily biogas production in the laboratory batch digester from three treatments (2nd experiment). Ambient temperature was between 32° C and 35° C.

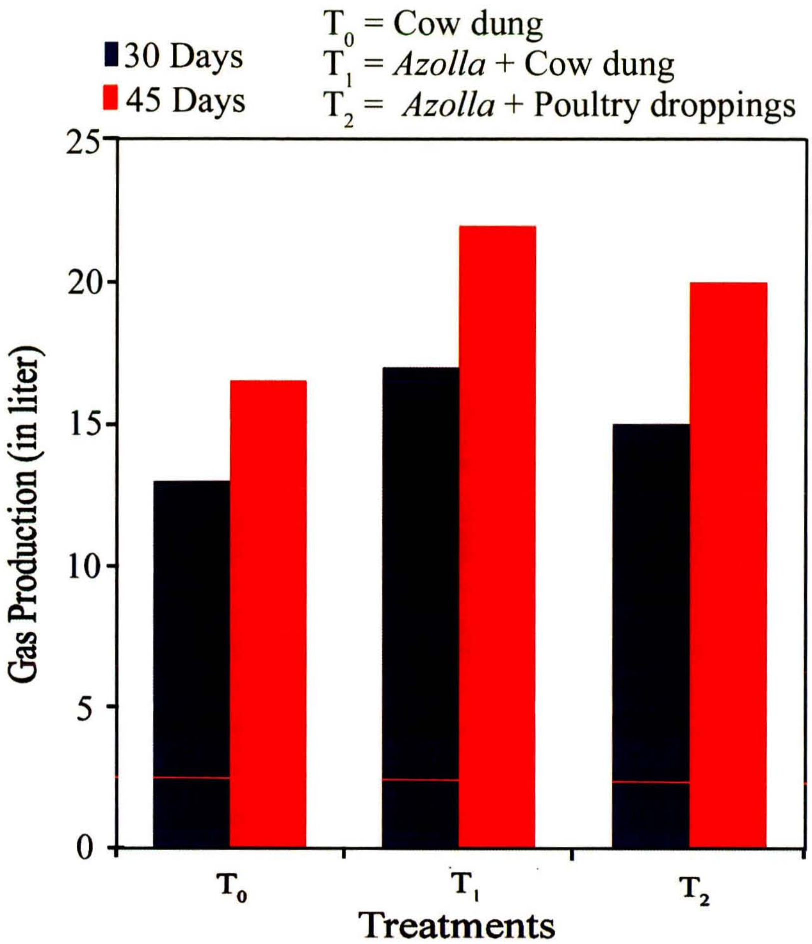

Fig. 3.4. Total biogas produced from three treatments after 30 and 45 days in laboratory batch digester (derived from data in Fig. 3.3). Ambient temperature was between 32° C and 35° C.

3.1.2 Percentage composition of biogas

Percentage of air, CH_4 and CO_2 in the biogas produced by cow dung, *Azolla* and *Azolla* + cow dung were determined taking samples from the first experiment shown in Fig. 3.1 (Table 3.1). A maximum of 63.55% CH_4 was produced by *Azolla* mixing with cow dung followed by cow dung alone and *Azolla*. *Azolla* (T_1) was found to be floating thus low decomposition, resulting no biogas production even after five days in the batch digester (Fig. 3.1). Percentage of methane produced over time in this set of experiment was plotted in Fig. 3.5. It revealed that *Azolla* + cow dung produced

Table 3.1. Percentage of the constituents of biogas produced in three treatments over time in the laboratory batch digester. Data were taken from the 1st experiment (Fig. 3.1.)

Treatments	Composition of biogas	Incubation period (days)			
		7	14	21	28
T_0 (cow dung only)	Air	48.0	2.5	24.97	24.80
	CH_4	31.2	53.23	**55.65**	52.14
	CO_2	20.8	20.16	19.30	22.06
T_1 (*Azolla* only)	Air	90.0	56.20	33.70	45.32
	CH_4	3.0	22.75	38.09	**44.36**
	CO_2	7.0	21.05	28.21	10.33
T_2 (*Azolla* +cow dung mixture)	Air	10.6	16.36	13.97	18.61
	CH_4	51.7	58.00	**63.55**	63.34
	CO_2	34.7	25.65	22.48	18.05

highest methane than cow dung alone and maximum of 63.55% was found after 21 days. Cow dung alone produced 55.65% during the same period. The higher concentration was due to amendment of *Azolla* + cow dung that enhanced decomposition of cow dung. Methane percentage in cow dung with *Azolla* (T_2) was over 51% from day seven, that increased afterwards and by 21 days increased over 63% (Fig. 3.5). The high concentration after seven days was due to quick decomposition of cow dung and even higher after 21 day due to contribution by decomposed *Azolla*. *Azolla* alone produced almost no methane after seven days. Percentage of methane produced was determined taking samples from 2nd experiment where, *Azolla* was excluded and mixture of *Azolla* + poultry droppings was included (Fig. 3.6). Highest percentage of methane (68%) was obtained in the mixture of

Azolla + cow dung (T_1) followed by cow dung alone and *Azolla* supplement poultry droppings. In all treatments highest methane concentration was found after 28 days. In

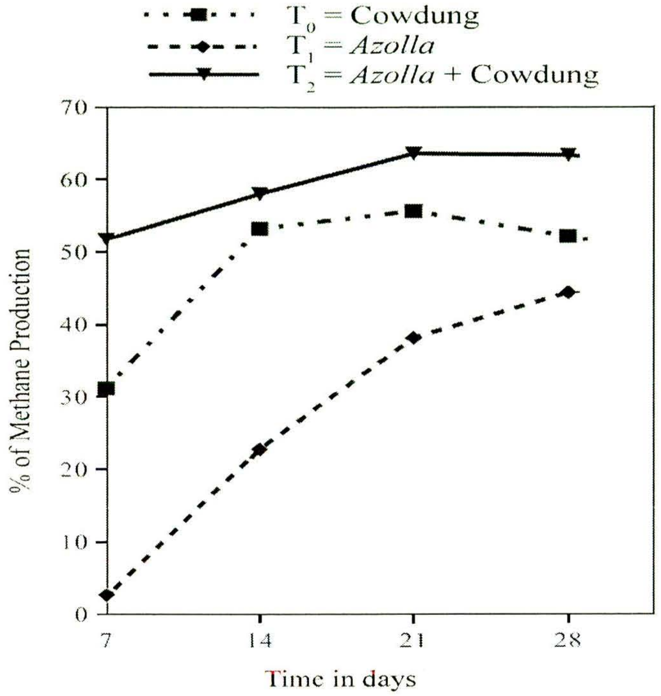

Fig. 3.5. Percentage of methane concentration over time in different treatments. Methane data were taken from Table 3.1. Ambient temperature was between 32° C and 35° C.

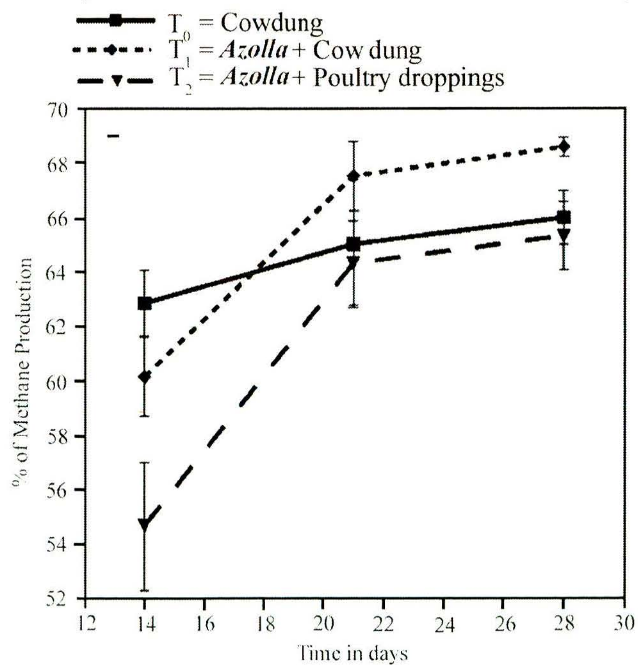

Fig. 3.6. Percentage of methane concentration in different treatments using *Azolla* and poultry droppings in addition. Methane data were taken from Fig. 3.3. Ambient temperature was between 32° C and 35° C.

treatments with *Azolla* mixture (T_1 and T_2) methane formation was lowest after 14 days (Fig. 3.6). This might be due to maximum decomposition of *Azolla* thus releasing hydrocarbon compounds (e.g. anthocyanin).

3.2 Biogas production using "Mini-Biogas Plant"

From the results obtained in the Laboratory batch digester it was revealed that *Azolla* and cow dung mixture is the best combination to obtain maximum biogas with highest percentage of CH_4. Therefore 1:1 ratio of *Azolla* and cow dung was used in the outdoor "Mini Biogas Plant" with the aim of using it by a small family (Section: 3.1.4). The daily gas production is shown in Fig. 3.7. The biogas production started after three days and exponentially approximately up to seven day. It further increased forming a plateau-like feature after about 15 to 41 days, the period of maximum biogas production. An average of over 18.6 3± 1.92 L biogas per day was produced in the outdoor system calculated using good quality biogas production from day 9 to day 45 using the Mini-Biogas Plant developed (Fig. 3.7).

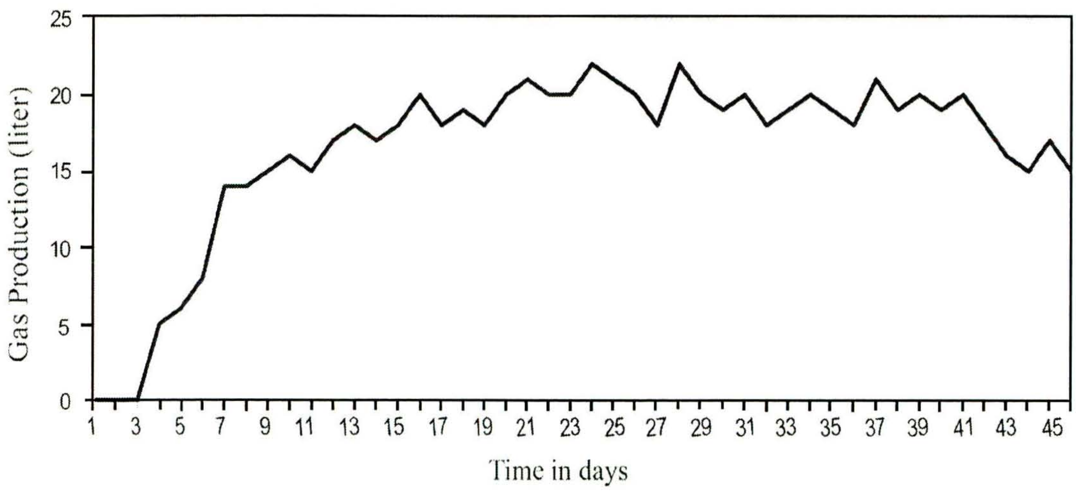

Fig. 3.7. Daily gas production by the Mini-Biogas Plant Using 50% *Azolla* (925 g) and 50% cow dung (375 g), using 20:1 carbon-nitrogen ratio.

3.2.1 Cooking rice with biogas from "Mini-Biogas Plant"

Biogas burner was connected with the digester of Mini-Biogas Plant by a synthetic pipe (Figs. 2.4-2.5). A total of 36 L biogas was stored for using in rice cooking. About 250 gm rice was taken into an aluminum utensil washed three times with water and allowed to soak in 500 ml water for 25-30 minutes (Fig. 3.8a). To create pressure on gas max sufficient heating 5.0 L tap water was added in to the water reservoir. Cooking of rice was completed within the next 30 minutes (Fig. 3.8 b-d). A total of about 20 L gas was needed for cooking 250 gm rice. The cooked rice was served to Professor Dr. Moniruzzaman Khondokar, Chairman, Department of Botany, Dhaka University and to my supervisor (Fig. 3.9).

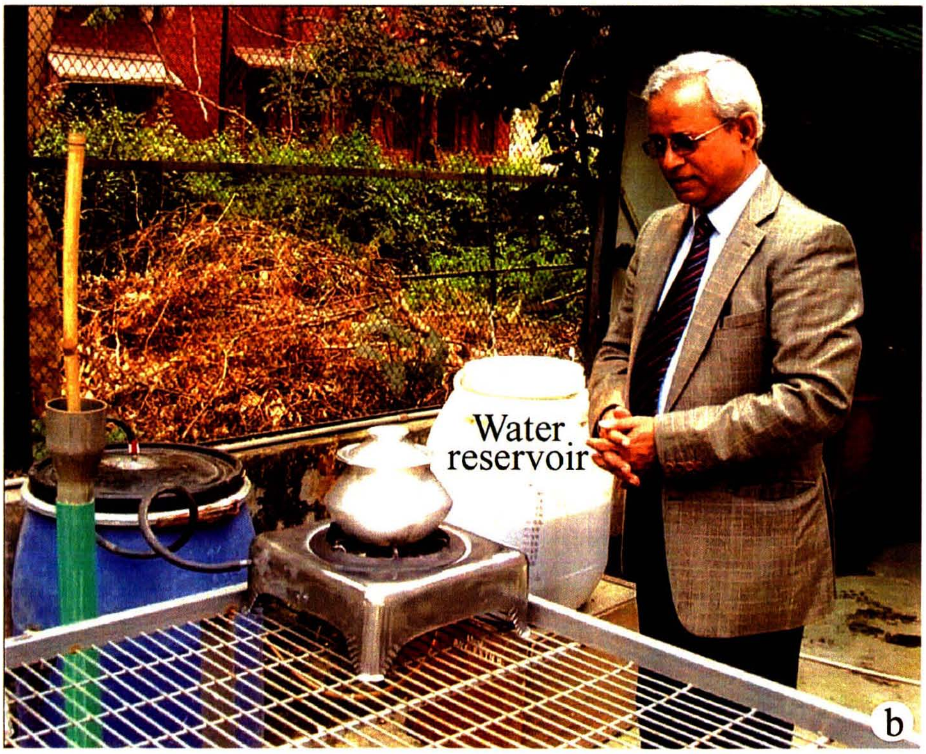

Fig. 3.8a-b. Cooking rice using biogas from Mini-Biogas Plant: (a) Rice utencil with rice and water for soaking. (b) Inspection of cooking rice with biogas produced by the Mini-Biogas Plant developed. Flame not visible due to blue colour. (contd)

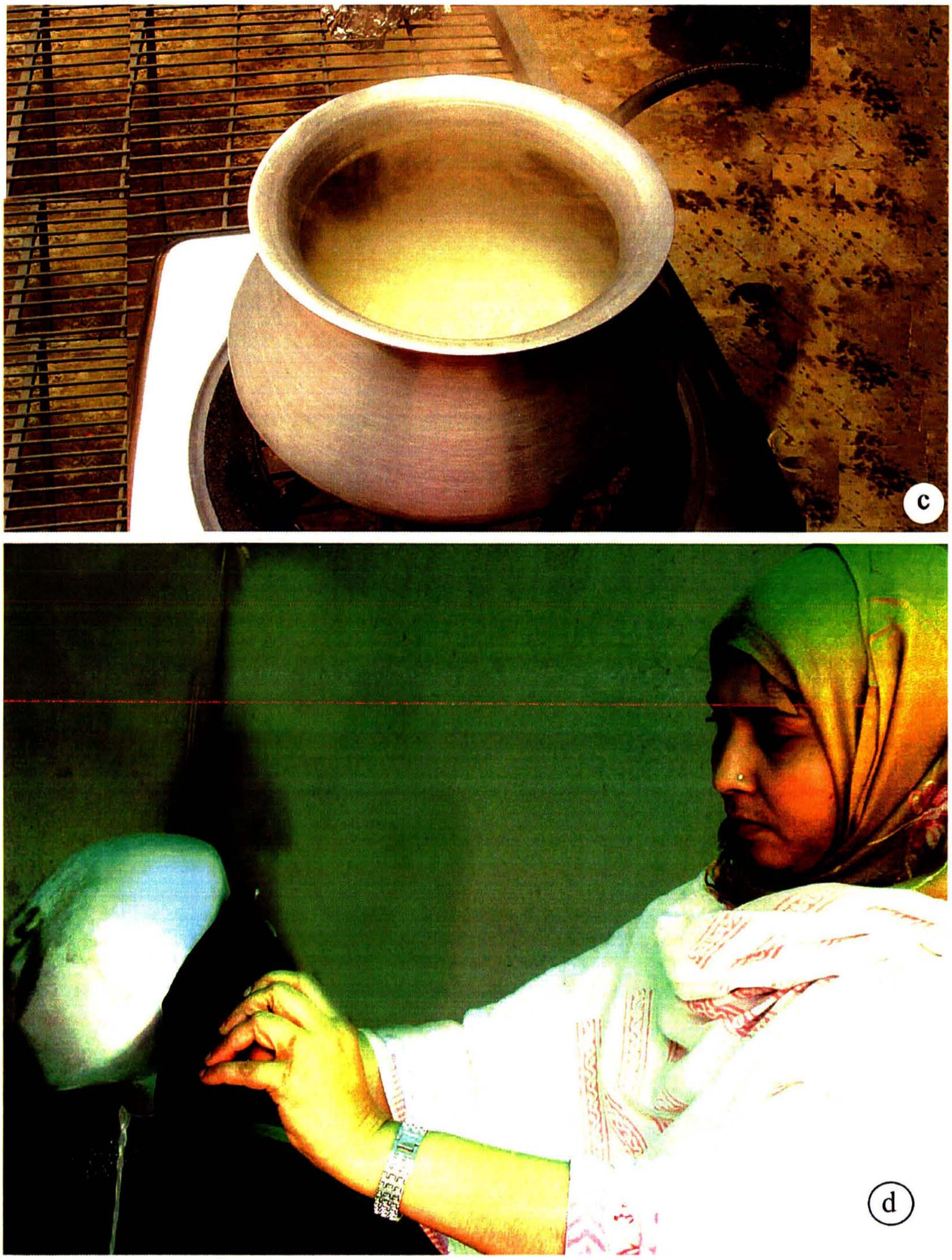

Fig. 3.8c-d. (c) Nearly cooked rice by biogas produced in the Mini-Biogas Plant. (d) Pouring off excess fluid ('Bhater maar')

Fig. 3.9. Rice cooked by biogas burner is being served to Professor Dr. Moniruzaman Khondkar, Chairman, Department of Botany and Professor Dr. Abdul Aziz, my Supervisor.

3.3 Biogas residue as Organic fertilizer

In the production of biogas cow dung, both the cow dung and poultry droppings were used separately with *Azolla* in the laboratory scale. The biogas residues or organic fertilizers produced were analyzed to determine which of the two substrates with *Azolla* produce best quality for cultivating rice crop compared to cow dung alone.

3.3.1 Chemical composition of the Organic fertilizer

Chemical composition of organic fertilizers (biogas slurry) obtained from laboratory batch digester charged with cow dung, mixtures of *Azolla* and cow dung and *Azolla* and poultry droppings are shown in (Fig. 3.10). Organic fertilizer obtained

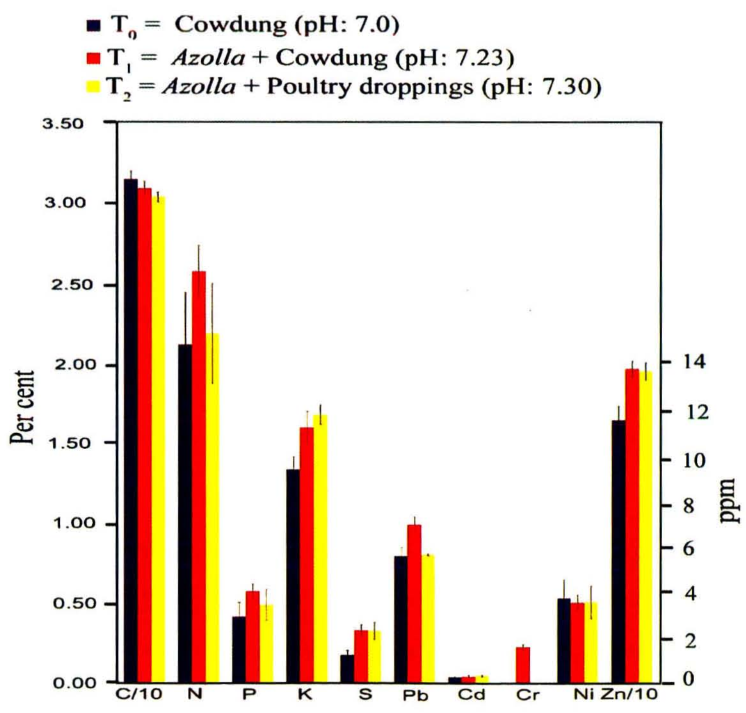

Fig. 3.10. Percentage composition of C, N, P, K and S in the *Azolla* and cow dung-based residues obtained from the Laboratory batch digester. Rest of the elements are in ppm.

from *Azolla* and cow dung substrate contained high concentration of N, P, K and S compared to organic fertilizer obtained from *Azolla* and poultry droppings. Both the organic fertilizers contained Pb, Cd, Cr, Ni and Zn heavy metals but the concentration was lower than the permissible limit (Fig. 3.10). Therefore, organic fertilizer from using *Azolla* mixed with cow dung was considered as the best organic fertilizer. It can be safely said that organic fertilizer that will be produced using *Azolla* mixed with cow dung in the Mini-Biogas Plant will be ideal.

3.3.2 Effects of organic fertilizer on rice cultivation

It is revealed from Section 3.2.1 that organic fertilizer obtained from *Azolla* mixed with cow dung was chemically best. Therefore, effects of organic fertilizer obtained from biogas slurry of "Mini-Biogas Plant" run by adding a mixture of *Azolla* and cow dung every day (Section 2.3) was used for studying the growth and yield attributes of rice. Results obtained (Fig. 3.11) indicated that all the amendments resulted better

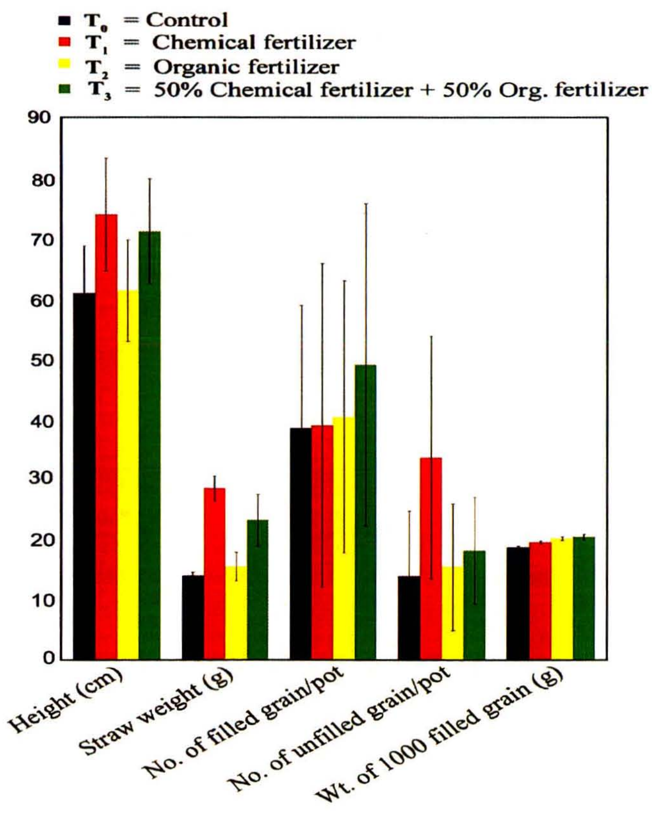

Fig. 3.11. Effects of organic fertilizer (*Azolla* and cow dung-based residue produced in the Mini biogas Plant) on the growth of rice plants and grains weight.

performance over the control treatment. Organic fertilizers produced better performance on the straw weight and number of filled grain/pot. Mixed organic and chemical fertilizers created best effect on the filled grains and on the grains production (weight). Combination of the organic and synthetic fertilizers also had considerable effects on other observed parameters such as plant height and straw weight/pot. On the other hand application of chemical fertilizers however resulted highest plant height and number of unfilled grains. The positive yield attributing factors obtained from mixing 50% organic fertilizer with 50% chemical fertilizers can be considered as best organic fertilizer.

CHAPTER 4

DISCUSSION

4 Discussion

4.1 Biogas production in the laboratory batch digester

A preliminary study was carried out in the laboratory batch digester to determine biogas production using *Azolla* as a supplement with cow dung and poultry droppings separately to determine the best possible combination for producing good quantity and quality of biogas. Carbon nitrogen ratio of 20:1 for the substrates was used. There were ups and downs of gas production more or less at the same time in all treatments (Figs. 3.1, 3.3). Moreover, the *Azolla* and cow dung mixture produced 98% or 1.98 times higher biogas than the cow dung alone (Fig. 3.2). Similar results were found by Ojolo *et al.* (2007) for cow dung, poultry droppings and kitchen wastes. Das *et al.* (1994) found 1.4 times higher biogas production when *Azolla* was mixed with cow dung compared to cow dung alone. *Azolla* alone has been found to produce 20 L biogas per kg in 30 days in batch digester (Aziz *et al.* 2003). A quality biogas contains >60% methane. In the present study it was found that cow dung alone and a mixture of *Azolla* with cow dung produced highest amount (22 L) of biogas with highest concentration of methane (69%) compared to *Azolla* mixed with poultry droppings where 20 L biogas with 64.5% methane was produced (Figs. 3.4-3.6). Khorashani *et al.* (2000) obtained 23.5 L biogas from a mixture of 40% water hyacinth with 60% poultry droppings.

A comparison of % composition of biogas obtained from various plant materials like straw, water hyacinth and plant biomass with cow dung and poultry droppings (Table 4.1) revealed that straw with cow dung produced only 51% methane while the rest of the plant materials produced 61.7% methane (Zhong *et al.* 2012).

There are reports of 68% methane by *Azolla* plant only (Aziz *et al.* 2003). It appeared that supplementing animal wastes with plant biomass keeping C:N to 20:1 produce good quantity and quality of biogas.

Table 4.1. Comparison of biogas composition (%) obtained by using different plant substrates alone or with cow dung/poultry droppings. nr = not recorded

Substrates	Composition (%) of gases			References
	CH_4	CO_2	H_2S	
Azolla+Cow dung (Lab. scale)	69	29.8	1.2	Present study
Azolla+Poultry droppings (Lab. scale)	66	32	2	Present study
Azolla+Cowdung (Mini biogas plant)	67	32	1	Present study
Cowdung+Straw	51	nr	nr	Moller *et al.* 2004
Plant and plant wastes (water hyacinth)	63.82	nr	nr	Kotpal and Bali 2003
Plant biomass	61.69	30	nr	Zhong *et al.* 2012
Azolla pinnata only	68	nr	nr	Aziz *et al.* 2003
Cow dung only	60-65	nr	nr	(Eusuf *et al.* 1983).

The carbon nitrogen (C/N) ratio is one of the important parameters influencing the digestion processes (Kumar *et al.* 2010). C/N ratio of the co-digestion of food waste and cattle manure was 15.8:1 (Zhang *et al.* 2010). The result indicate that the optimum C/N ratio was 15.8, in-line with previous findings of C/N-15 (Huang *et al.* 2004) or 13.9-19.6 (Kumar *et al.* 2010). Both the *Azolla* supplemented cow dung and poultry droppings at a ratio of 1:1 (w/w) or about 20:1 ratio of carbon and nitrogen produced highest amount of biogas with high concentration of methane in the present study (Figs. 3.5-3.6). It appears from the discussion that amendment of cow dung and poultry droppings with *Azolla pinnata*, attaining the Carbon and Nitrogen ratio of about 20:1 should be maintained for maximum biogas production.

Azolla addition increased biogas production with high percentage of methane possibly due to presence of anthocyanin pigment, a high molecule hydrocarbon compound. The high amount of biogas with high % of methane with *Azolla* and cow dung mixture was due to soft nature of the plant which decomposed quickly being embedded in the paste of mixture. The efficiency may further be increased for producing mini-scale biogas by partially crushing the *Azolla,* mixing with required amount of cow dung, make a paste and store it in a airtight polythene bag overnight before adding in to the digester. The concept is supported by the production of over 64 to about 68% methane after 21 days of incubation of *Azolla* with poultry droppings and with cow dung, respectively in the present study (Fig. 3.6). Similarly during 28 to 30 days incubation maximum biogas production was found between eight and 22 days. Lucas and Bamgboye (1998) observed high biogas production after 15 days. Therefore, 1:1 ratio of *Azolla* and cow dung mixture and amending the mixture as mentioned above could be a good substrate for producing good amount of good quality biogas every day.

4.2 Preparing "Mini-Biogas Plant" for biogas production

After confirming that the *Azolla* and cow dung mixture at 1:1 ratio can produce good amount of good quality biogas in the laboratory scale (Section 3.1.1, Figs. 3.4, 3.5, 3.6), an experiment was conducted outdoor by preparing a "Mini-Biogas Plant" from locally available materials (Figs. 2.4, 2.5).

In the preparation of the "Mini-Biogas Plant" the principle used in constructing "Underground Biogas Plant" (digester) by IFRD, BCSIR, Dhaka, was followed. The plant is being used for the last two decades for large scale production of biogas from cow dung (Fig. 2.1). The "Mini-Biogas Plant" (Fig. 2.4, 2.5) developed is a continuous system and once charged could be used for over two months. The plant could produce on an average 18.63 ±1.92 liter biogas per day (Section 3.2, Fig. 3.7) using 375 gm cow dung mixed with 925 gm *Azolla* and adding 1.25 L tap water (Section 2.3.2). It was possible to cook 250 g of rice from the gas produced in a day (Section 3.2.1). Cost of preparing a "Mini-Biogas Plant" was about Tk. 3,000.00 (Table 4.2)

4.3 Designing "Mini-Biogas Plant"

Based on the results obtained in Section 3.2, a model has been designed (Fig 4.1) and described for using by a small family in villages, and also by a family living in buildings of towns and cities during gas crisis (Fig. 4.2). The digester should be a plastic air tight drum (black or blue colour body is preferred) at least 0.75 m tall and 0.41 m inside diameter having slightly narrowed upper and lower parts (total volume 200 L or 0.1 m^3) with a lid was used as digester (Fig. 4.1). A 65 cm long and 7.35 cm diameter PVC pipe with funnel-shaped mouth as an inlet for raw materials, *Azolla* and CD mixture was fixed with the cover so that the bottom end remained at about 12 cm above the bottom of the digester. To remove the digested residue an outlet of about 7.35 cm diameter fitted with a stopper was set at about 5 cm above the digester bottom. After full charging, about one third of the digester in the upper part remains as a gas phase. A gas valve was fixed with the lid of the digester and connected with a gas burner by a synthetic pipe. Every joint was made air tight using sealing paste. To measure the amount of gas produced and also to maintain pressure on gas while cooking, a transparent/translucent water reservoir of about 50 liter capacity (having a height of 0.4 to 0.5 m, the top of which should remain above the level of the digester)

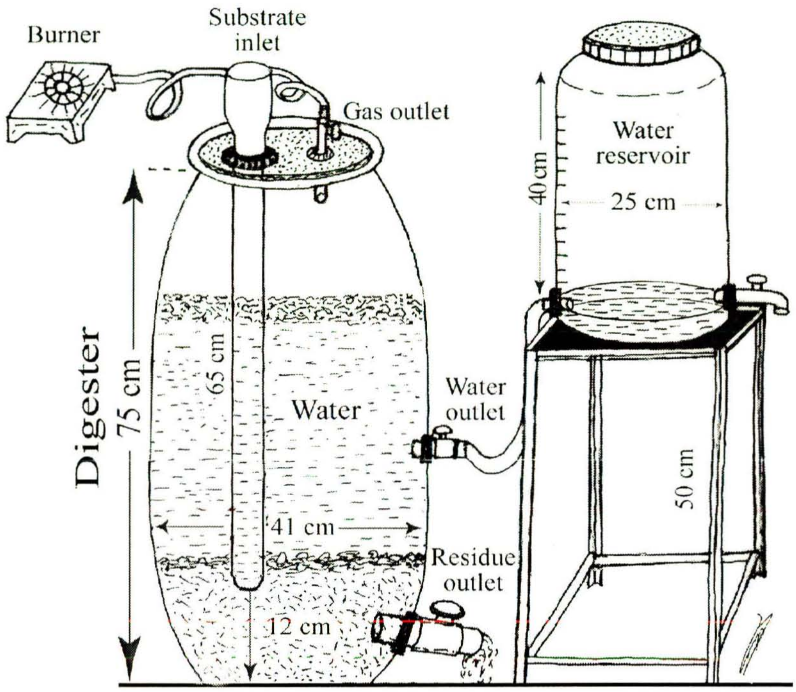

Fig. 4.1 Design of the Biogas digester developed.

was set beside the digester at a height of 40 to 50 cm. The water reservoir should be connected with the digester at its middle point (may be at about 30 cm from the bottom of the digester) by a synthetic tube of 3.5 cm diameter (Fig. 4.1). The lid of the reservoir is kept loose for ventilation. The reservoir was scaled to measure the volume of water increased which is equivalent to the amount of biogas produced in the digester. To create pressure on the gas during cooking, about 5 liter water is added and this excess amount of water is taken out after cooking and used again on the next day during cooking or 1.25 L water may be used while adding the substrate mixture into the digester. Every day at 8 to 9 a.m. about 2.5 liter biogas residue mostly deposited near the bottom of the digester should be removed before adding the required amount of mixed substrate.

Once charged the plant may be used for over two months. During operation period some semisolid partially digested material accumulate (residue) on the surface of digested water. Opening the lid, the semisolid material should be well stirred and replace the cover immediately and made airtight. Adding the substrate mixture into the digester is done on the next day to get biogas on a regular basis.

Fig. 4.2. A housewife is probably boiling water using fuel wood in an earthen burner at Dhaka city during crisis of gas supply. Source: The Daily Star.

Table 4.2. Costing for making a "Mini-biogas Plant" for a family of four members

Items	Price in BDT
1. Drum (300 litre)	1,000.00
2. Drum (75 litre)	500.00
3. 40 cm high table made of MS angle	550.00
4. PVC pipe ½´´ 30 cm	40.00
5. PVC pipe 2´´ (100 cm)	120.00
6. PVC Funnel 1X4X5´´ (1 piece)	50.00
7. Hose pipe ½´´ 60 cm	50.00
8. Ball bulb ½´´ (1 piece)	120.00
9. Sealing nut ½´´ (4 pieces)	60.00
10. Sealing nut 2" (4 pieces)	120.00
11. Bulb 2´´ (1 piece)	300.00
12. Gas valve (1 piece)	100.00
Total cost	3,000.00

4.4 Use of biogas residue

Biogas residue could be used in two ways: (i) separating liquid part and use as foliar spray and (ii) drying solid part that may produce granules by using with cow dung or poultry droppings.

4.4.1 Use as foliar spray

Everyday about 2.5 liter biogas slurry will be produced in a digester of 200 liter capacity. As mentioned before the slurry is of watery consistency and drying the whole amount everyday would be difficult. Therefore, it would be easy to separate the fluid part by decanting after about an hour of settling. The composition of elements in liquid part should determine and if concentrate it may be diluted and sprayed to all types of crops.

4.4.2 Use as Organic granules

Aquatic fern *Azolla pinnata* and cow dung were used for producing biogas in the "Min-Biogas Plant (Section 3.2, Fig. 3.7). In the process of decomposition some amount of residue called biogas residue was produced. The residue after drying produced organic granules which contain mineral nutrients like C, N, P, K, S, Pb, Cd, Cr, Ni and Zn (Fig. 3.10). There are reports that the organic fertilizer produced is more effective in plant growth than that of farm yard manure alone (Rajni *et al.* 2001).

In the present study, the organic fertilizer was found to contain 30.45% organic carbon, 2.57% N, 0.58% P, 1.60% K and 0.33% S compared to 13.02% organic carbon, 1.01% N, 1.78% P, 1.40% K and 0.05% S in other studies (Anonymous 2000). The very high organic carbon and nitrogen is indicative of best quality, where *Azolla* component contributed higher nitrogen in the slurry. Another important feature is that pH range at neutral to just above to it in contrast to the report by Ndegwa *et al.* (2000). The relatively low P (0.58%) was found compared to 1.78% reported by Anonymous (2000). This may be due to the fact that mineralization rate is determined by composting activities of associated microbes in the cow dung (Ndegwa *et al.* 2000). However, previous studies showed that the *Azolla* plant itself contains round the year 3.20-3.75% N, 0.52-0.65% P, 1.42-1.62% K and 0.235-0.148% S (Aziz 2001). The P and K present in the organic fertilizer of the present study were similar but N was low and S was high. It is difficult to say why the N percent was so low compared to inoculated *Azolla.*

The mean concentrations of Pb, Cd, Cr, Ni and Zn in the three treatments are shown in Fig. 3.10. The concentrations of heavy metals recorded in organic fertilizer are less the maximum permissible levels of Pb 100 µg/gm, Cd 3.0 µg/gm, Cr 100 µg/gm, Ni 50 µg/gm, and Zn 300 µg/gm in all types of soils. Therefore, application of organic fertilizer obtained by mixture of *Azolla* and cow dung will be of no harm from heavy metal point of view. Reduced urea application will reduce eutrophication in water bodies. The high amount of organic carbon will increase water holding capacity and produce ideal soil structure.

Azolla has been found to supplement about 50% urea fertilizer and can control weed production in rice crop fields (Watanabe 1987, Aziz & Rahman 2000). A recent study showed that application of wet *Azolla* compost at the rate of 5 to 7.5 ton/ha could supplement all chemical fertilizers by about 50% (Aziz 2001). Soil pH, organic matter, N, P, K, Ca, Mg and Na increased with rate of *Azolla* application. *Azolla* is expected to supply fixed nitrogen and increase the uptake of some nutrient elements such as Calcium, Magnesium and Potassium (Awodun 2008).

4.5 Effects on plant growth and yield attributes

Plant height was highest in the treatment with chemical fertilizers followed by treatments with 50% organic fertilizer + 50% chemical fertilizer. Similar was the case with straw weight. Rajput and Warsi (1992) and Singh (2001) reported that organic manure and chemical fertilizers increased straw yield. Babu *et al.* (2004) observed that the plant height was significantly influenced by the incorporation of organic manures with fertilizers.

Number of filled grains was much higher in mixed treatment than the chemical fertilizer only indicating that organic fertilizer contributed in the formation grain while the chemical fertilizer contributes to vegetative growth. The view is further substantiated by very high number of unfilled grains. The number of filled grains 432, 595, 481, 776 and number of unfilled grains 157, 582, 210, 295 in T_0, T_1, T_2, and T_3 respectively, were found. There are reports that 50% organic fertilizer and 50% urea nitrogen had similar effects on number of filled grains per panicle with recommended fertilizer dose of BRRI 29 (Dr. Masuda Begum, Soil Resource Development Institute, Krishi Khamar Sharak, Dhaka). Application of chemical fertilizers however resulted highest plant height and number of unfilled grains indicating diversion of plant nutrients for vegetative growth.

The 1000-grain weight of BRRI dhan 28 was significantly influenced by the different treatments. 1000-grain weight ranged from 18.59 to 20.36 g (Fig. 3.10). The highest was obtained in treatments having mixed organic and chemical fertilizers (treatment T_3). On the other hand 1000 grain weight in treatment with recommended fertilizer dose was only 19.43 g. Use of biogas slurry with urea at 50% ratio demonstrated significant effects on filled grains per panicle and 1000 grain weight (19.17 g) of BRRI 29 over recommended fertilizer dose (18.18 g) (Dr. Masuda Begum, Soil Resource Development Institute, Krishi Khamar Sharak, Dhaka 1215). Singh (2001) reported that application of chemical fertilizers with farmyard manure or wheat straw in alternate wetting and drying condition increased N, P, & K uptake by rice plants, increased 1000 grain weight and grain yield of rice. The increased filled grains and significantly higher grain weight in the mixed fertilizers might be due to the release of nutrients from sources in harmony to the requirements for yield attributing factors.

Application of cow dung, poultry manure and water hyacinth in combination with chemical fertilizers increased grain yield of BRRI dhan 28 (Haque *et al.* 2001, Rajni *et al.* 2001). Singh (2001) reported that the application of mixed organic manure and chemical fertilizers increased the grain and straw yields of rice.

Azolla and cow dung-based biogas residue had higher concentration of some elements (N, P, K and S) and low concentration of heavy metals. *Azolla* and cow dung-based biogas residue (organic fertilizer) may supplement chemical fertilizers of Recommended Fertilizer Dose by about 50% in rice production. *Azolla* and cow dung derived organic fertilizer can significantly offer an economically attractive alternative using 50% chemical fertilizers for increasing productivity and reducing environmental risks.

4.6 Concluding remarks

From the present study it can be safely said that *Azolla* supplemented cow dung mixture in the Mini-Biogas Plant produced higher amount of biogas with higher concentration of methane and that the organic fertilizer produced from the residue mixed with 50% RFD was better (4.79% higher weight of 1000 grain) than applying 100% RFD of chemical fertilizers in BRRI 28 rice variety.

Using the biogas produced by the Mini-Biogas Plant developed it was possible to cook 250 g rice per day. The Plant may be further refined for performing better.

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Appendix A

SUPPLEMENTARY DATA RELEVANT TO FIGURES IN THE TEXT

Table A1. Biogas production per day in different treatments (Fig. 3.1)

Day	T_1	T_2	T_3	T_4
1	0	0	0	0
2	0	0	0	0
3	50	0	100	0
4	90	0	100	0
5	100	0	110	0
6	170	100	150	50
7	90	110	240	70
8	200	150	360	100
9	225	100	533	120
10	250	130	365	50
11	360	225	300	100
12	500	200	700	150
13	100	250	350	350
14	120	175	386	120
15	400	128	350	200
16	366	150	370	100
17	266	120	770	50
18	100	120	390	65
19	120	100	470	50
20	120	150	350	30
21	56	150	300	0
22	216	120	225	0
23	110	125	310	0
24	100	100	225	0
25	100	125	280	0
26	75	100	225	0
27	10	50	250	0
28	10	50	110	0

Table A2. Gas production in 4 treatments (Fig. 3.2)

Treatments	**gas production (in Liter)**
T_0	4.6
T_1	4.0
T_2	9.1
T_3	2.0

Table A3. Composition of CH_4 in 4 treatments (Fig. 3.5)

Treatment	% during incubation periods (days)			
	7	14	21	28
T_1	31.17	53.23	55.65	52.14
T_2	0.00	22.75	38.09	44.36
T_3	51.71	58.00	63.55	63.34

Table A4. Daily gas production in the mini biogas digester (Fig. 3.7)

Day	Gas production (liter)	Day	Gas production (liter)
1	0	24	22
2	0	25	21
3	0	26	20
4	5	27	18
5	6	28	22
6	8	29	20
7	14	30	19
8	14	31	20
9	15	32	18
10	16	33	19
11	15	34	20
12	17	35	19
13	18	36	18
14	17	37	21
15	18	38	19
16	20	39	20
17	18	40	19
18	19	41	20
19	18	42	18
20	20	43	16
21	21	44	15
22	20	45	17
23	20	46	15

Table A5. Comparison of the chemistry of the Biogas slurry from laboratory experiments with cow dung, *Azolla* mixed with cow dung and poultry droppings (Fig. 3.10)

	pH	C	N	P	K	S	Pb	Cd	Cr	Ni	Zn
Cowdung	7.10 ±0.02	31.50 ±0.50	2.12 ±0.33	0.42 ±0.09	1.34 ±0.08	0.17 ±0.03	0.80 ±0.06	0.04 ±0.01	0.00 ±0.00	0.53 ±0.12	16.46 ±0.88
Azolla + cowdung	7.23 ±0.04	30.91 ±0.45	2.57 ±0.16	0.58 ±0.05	1.60 ±0.10	0.33 ±0.04	0.99 ±0.05	0.04 ±0.01	0.23 ±0.02	0.51 ±0.05	19.67 ±0.51
Azolla +poultry droppings	7.30 ±0.18	30.39 ±0.31	2.19 ±0.31	0.49 ±0.10	1.68 ±0.06	0.33 ±0.05	0.81 ±0.01	0.05 ±0.01	0.00 ±0.00	0.51 ±0.10	19.52 ±0.51

Table A6. Effects of organic fertilizer (from 50% *Azolla* and 50% cow dung-based residue from the Mini biogas Plant) on the growth of rice plants and production of grains. n= 3 (Fig. 3.11)

	Height (cm)	Straw weight/pot (gm)	No of filled grain/pot	No of unfilled grain/pot	Weight of 1000 filled grain (gm)
T_0	60.98 (±7.86)	13.88 (±0.57)	38.5 (±20.5)	13.85 (±10.83)	18.59 (±0.23)
T_1	74.09 (±9.37)	28.46 (±2.07)	38.96 (±26.96)	33.6 (±20.22)	19.43 (±0.24)
T_2	61.39 (±8.44)	15.4 (±2.39)	40.39 (±22.65)	15.37 (±10.53)	20.11 (±0.3)
T_3	71.28 (±8.69)	23.18 (±4.34)	49.02 (±26.83)	18.06 (±8.91)	20.36 (±0.5)