Optimizing temperature & C/N ratios of newspaper and green vegetable waste for methane yield through manual optimization and response surface methodology

By

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206292

Session 2017-19

Supervised by

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MASTERS of SCIENCE in
ENERGY SYSTEMS ENGINEERING

US-Pakistan Center for Advanced Studies in Energy (USPCAS-E)

National University of Sciences and Technology (NUST)

H-12, Islamabad 44000, Pakistan

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August 2020

THESIS ACCEPTANCE CERTIFICATE

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DEDICATION

"Dedicated to my beloved *PARENTS* because whatever I am today could never be possible without their continuous guidance, effort, sincerity and prayers.

I am heartily thankful to them…"

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All praises to **Almighty Allah,** The Light of Heavens and Earths, The One Who put good thoughts in one's mind, turn them into determinations and then makes the way towards their fulfillments showering all His Blessings throughout the journey. After all thankful notations in the Royal court of Allah, the Beneficent, the merciful, without whose blessing I would not been able to achieve such a milestone.
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Abstract

Newspaper and green vegetable waste can be a noteworthy energy source for the production of renewable energy in the form of bio-methane. This research is focused on the optimization of temperature and carbon to nitrogen (C/N) ratio to enhance bio-methane yield from anaerobic co-digestion of newspaper and green vegetable waste. Manual optimization through hit and trial method and response surface methodology (RSM) using central composite design (CCD) were carried out in order to determine the optimum conditions of temperature and carbon to nitrogen (C/N) ratio for bio-methane yield. Both manual and CCD experiments were performed at organic loading of $10gVSL^{-1}$ and volatile solid (VS) substrate to inoculum ratio of 1:1. Results from the CCD experiments revealed that maximum specific bio-methane yield, 342 L/kgVS, is 12.87% more than the predicted value, obtained from reactor CCD-7 having C/N 30:1 and temperature 30.86 ^{o}C, while results from manual optimization experiments illustrated that maximum specific biomethane yield 401.8L/kgVS was obtained from rector MR3 having 48.3 % more than methane content than thermophilic reactor TR3 operated at C/N ratio of 30 and temperature 37^{o}C having 93.45% green vegetable waste and 6.54 % of newspaper waste. A significant synergistic effect in co-digestion of newspaper and green vegetable waste was observed at mesophilic temperature rather than thermophilic. Verification experiments confirmed that temperature and C/N ratio have influential effect on bio-methane yield.

Key words: *anaerobic co-digestion, response surface methodology, central composite design, newspaper waste, inoculum, substrate, green vegetable waste, optimization*

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List of Journal/Conference Papers

1. **N. Farooq**, R. Liaquat, Zeshan, N. Iqbal. “Optimizing temperature & C/N ratios of newspaper and green vegetable waste for methane yield through response surface methodology.” **Biomass and Bio energy** (under review)
2. **N. Farooq**, R. Liaquat, Zeshan. “Effect of C/N ratios and temperature on co-digestion of newspaper and green vegetable waste for bio-methane yield.” **1st National Symposium on Advanced Energy Materials 2019,** (Presented)
3. **N. Farooq**, R. Liaquat, Zeshan, N. Iqbal. “Optimizing temperature & C/N ratios of newspaper and green vegetable waste for methane yield through response surface methodology.” **Biomass and Waste Valorization**

List of Abbreviations

Anaerobic Digestion	AD
American Public Health Association	APHA
Analysis of Variance	ANOVA
Carbon to Nitrogen Ratio	C/N
Central Composite Design	CCD
Clean Development Mechanism	CDM
Feed to Inoculum	F/I
Gas Chromatography	GC
Green Vegetable Waste	GVW
Hydraulic Retention Time	HRT
Hydrogen Sulphide	H_2S
Mega Joule	MJ
Megatonnes Per Annum	Mtpa
Mesophilic Control	MC
Methane Gas	CH_4
Mili-Liter	mL
Moisture Content	MC
Mesophilic Reactor	MR
Newspaper Waste	NPW
Organic Loading Rate	OLR
Percentage	%
Response Surface Methodology	RSM
Thermophilic Reactor	TR
Thermophilic Control	TC
Total Kjeldahl Nitrogen	TKN
Total Organic Carbon	TOC
Total Solid	TS
Volatile Fatty Acids	VFAs
Volatile Solids	VS

Chapter # 1 Introduction

Energy has become an important prerequisite for the economic development of a country. Pakistan is facing a serious energy crisis. The number of inhabitants in the country are increasing rapidly, they need energy and other resources to fulfill their basic needs. Currently Pakistan fulfilled its energy requirement mostly from non-renewable resources. Non-renewable resources are the fossil fuels which includes coal, oil and natural gas. Constant burning of fossil fuels for energy needs causing reduction in its number, as well as increase in greenhouse gas emission and global warming [1]. This shortage of energy resources in the country has led to increase in fossil fuel prices. Therefore, research for developing alternative energy resources has become increasingly important. Renewable energy resources can provide a better alternative options to produce energy. Renewable energy resources comprises of solar energy, bioenergy, wind energy, geothermal energy, fuel cell and batteries [2]. Energy obtained from renewable sources is clean and have zero emission of air pollutants. In global energy mix fossil fuels contribute 79.7%, nuclear energy contribute 2.2%, traditional biomass contribute 7.5%, modern renewables contribute 10.6% out which hydropower contribute 3.6%, biomass/solar /geothermal heat contributes 4.2% and wind/ocean power contributes 2% [3].The share of the renewable energy consumption has been increased mostly used renewable resources are solar energy, biofuels, wind energy and hydropower. Advantages of renewables includes enhance diversity in energy supply markets, creates new employment opportunities, provides sustainable energy supplies, reduces atmospheric emissions [4].

Bio-energy is one of an interesting alternative to fulfill the energy necessities of the country without extra economic burden and any significant environmental impacts.[5]. It plays a positive role in establishment of sustainable developmental objectives because it reduces greenhouse gas emissions as compared to the fossil fuels in applications where it is used. In developing countries conventional use of biomass (for cooking and heating) accounts 8% of this, heat demands in building accounts 4%, transport needs accounts 3%, global electricity generation accounts 2% and 6% in industry [6]. Bio-energy can provide a central role for encouraging renewable alternatives because energy obtained from this source is cheap, clean and economical.

1.1 Biomass

Biomass is considered to be the fourth largest energy resource in the world because of its renewable, abundant, and widely applicable characteristics which makes it a nearly replacement for fossil fuels [7]. Fortunately, Pakistan has an massive amount of biomass resource in the form of agricultural waste in the form of vegetables, food waste, sugarcane bagasse, wood, municipal solid waste in the form of newspaper, cardboard and animal waste in the form of poultry litter dung and feces etc. [8]. In Pakistan approximately 6.5 million tons of fruits and vegetables are grown each year, almost 30% of these grown vegetables and fruits are dumped due to negligence and absence of processing facility[9]. Pakistan generates nearly 48.5 million tons of solid waste in a year which is increasing more than 2% annually. Government of Pakistan estimates that 87000 tons of solid waste is produced per day, mostly from metropolitan areas. Card board waste is 7%, paper waste is 6 %, yard waste is 14 % and food waste is 30 %. According to Pakistan renewable-energy society 2013, more than 20 Mtpa (megatonnes per annum) of solid waste is generated from the metropolitan areas of Pakistan produce, out of which 82 Mtpa is crop residue and 365 Mtpa is animal manure. In Pakistan waste production of about 0.28-0.61kg/capita/day occurs which have the capacity of 12 millionm/day biogas production. To cope with energy shortage, biomass will provide an alternative energy resource.

In this study newspaper and green vegetable waste have been selected as a source of biomass, selection have been made on the basis of low cost, easy availability, abundant in supply and potential to produce energy. As newspaper waste can only be recycled through limited amount of times because of its quality specifications. Various methods for newspaper waste disposal includes land filling, dumping and incineration. These methods have low economic benefits and significant environmental impacts [10]. Therefore, there is a need for developing various techniques for newspaper waste disposal. Newspaper waste contain high content of carbon that can be converted in to energy intensive fuel i.e., biogas which can be used for energy production. Co-digestion of newspaper waste with high nitrogen content containing substrates such as green vegetable waste have been carried out to improve the digestion performance. Anaerobic digestion and co-digestion have various advantages which includes waste utilization, waste management and waste volume reduction by the use of microorganisms producing energy intensive biogas. Anaerobic digestion and co-digestion can provide a better alternative option to manage the waste as well as to produce renewable source of energy. Energy obtained through this process is clean and environment friendly.

1.2 Anaerobic digestion

Anaerobic digestion technology provides a useful mean for the management of solid and agricultural waste by designing the bioreactors [11]. This technology has gained massive attention especially in rural areas of developing countries because it provides waste disposal and reduction in energy cost. People living in rural areas use animal manure, vegetable and fruit waste as a prime source of energy, animal feed and fertilizers.

Designing of the bioreactors influence the performance and efficiency of the anaerobic digestion process. Different types of anaerobic processes has been applied for the waste management i.e. batch system in which waste material is added once, until it is completely metabolized into products, continuous one-stage system which maintains environmental conditions to perform biochemical reactions in one digester , and continuous two-stage systems in which different phases of the digestion processes i.e., acidogenesis and methanogenesis phases occurs at two various digesters [12].

1.3 Anaerobic co-digestion

The concept of anaerobic co-digestion has been gaining popularity as we move towards a friendlier, more renewable economy. Anaerobic co-digestion involves mixing of two or more substrates to improve C/N ratio, macro and micro nutrients, biogas production and dilution of complex and inhibitory substances. [13]. The efficiency of methanogens increases because of the additional nutrients and pH regulation. Variety of substrates can be utilized, which generally improves the economic factors of a digestion plant. The search for and use of more unique substrates should base on a careful assessment protocol to define biodegradability. There are numerous factors that affect the functioning of anaerobic digestion and co-digestion process, among all the factors temperature, pH, organic loading rate (OLR) and carbon to nitrogen (C/N) ratio are major factors that have significant effect on biogas production process. Biogas production can occur at three different temperatures i.e. psychrophilic 15-20^{0}C, mesophilic 25- 45 ^{0}C and thermophilic 50-60 ^{0}C. At psychrophilic temperature methane production is very low [14]. Mesophilic and thermophilic temperature provides active environment to anaerobic bacteria for the digestion. For the stable biogas production pH value of the input mixture in the digester should lie between 6 and 7. Organic loading rate is very essential for the efficiency of anaerobic digestion as it provides the means of material stabilization. Overfed of the digester accumulate the acids and methane production will be inhibited, whereas under fed condition of the digester gas production will be low. Bacteria need a suitable ratio of carbon to nitrogen for their metabolic activities. The maximum yield of biogas production is achieved in the range of C/N ratio of 20-30:1 [15]. Carbon to nitrogen (C/N) ratio is also an effective parameter and it is necessary to optimize it for the various substrates to improve the co-digestion process in order to achieve higher efficiency, greater carbon content in the substrate will cause amplification of carbon dioxide formation and reduction in the pH of the reactor while, elevated content of nitrogen give rise to the improved production of the ammonia gas raising the pH which would cause damage to the micro-organisms that are effective for the degradation of volatile fatty acids [5].

1.4 Biogas

Biogas is a byproduct of the anaerobic digestion process of organic matter under anaerobic conditions [16].Major portion of biogas is consisted of methane along with trace amounts of other gases [17]. Biogas production process provides sustainable management of organic waste to recover the energy and also caused reduction in the odor, sludge and pathogens [18]. The remaining material i.e. digestate of anaerobic digester have significant fertilizing properties because of high nutrient content of nitrogen, phosphorus and potassium (N, P, and K) present in it, can be used as a soil fertilizer and is a very good option to replace inorganic fertilizers [19].

1.5 Response surface methodology

Response surface methodology (RSM) is a software that is utilized for making design for the experiments to fit an suitable mathematical function, check the quality of making models, analyzing the effect of several factors and optimizing the desired response, while optimization is used for determining conditions at which to apply a process that generates the best possible response [20] [21]. Many researchers have reported that RSM is a useful tool for optimizing the bio methane potential. Jiayu feng et al. (2017) used RSM and CCD to enhance methane production from vinegar residue, results of the experiment confirmed that RSM and CCD was useful for optimizing the anaerobic digestion parameters [22]. In order to use RSM, the choice of experimental design is essential to fit an suitable mathematical function and to evaluate the quality of the model used along with its accuracy, to setup a system in relation to the experimental data obtained [21]. In this study central composite design (CCD) of response surface methodology(RSM) have been used to optimize the parameters, which is the mostly utilized experimental design for the development of analytical procedures [23]. CCD consist of fractional design, central points and axial points, central point is often replicated in order to improve the precision of the experiment. [21].

1.6 Significance of study

Newspaper waste is considered as one of the significant municipal solid waste, having limited number of recycling. This study provides a suitable option for the reduction in the amount of newspaper waste by optimizing the parameters such as temperature and C/N ratio in order to enhance the bio-methane yield through the use of anaerobic co-digestion technique. Newspaper has very high C/N ratio which effects the anaerobic digestion process and makes it time consuming. That’s why newspaper waste is co-digested with green vegetable waste in order to improve the digestion efficiency. Biogas is produced during this process, is one of the reliable, clean renewable source of energy that can be used for cooking, heating and lightning. This technology can provide an alternative source of energy to meet the energy demands of the country, having various advantages which includes management of municipal solid waste, reduction of CO_2 emissions which helps to reduce greenhouse effect, provides health benefits from reduced indoor pollution and improvement of livelihood, slurry from the digester can be used as fertilizer which can replaced the use of chemical fertilizers, it can also provide an opportunity for carbon trading under the clean development mechanism(CDM). By using statistical software different parameters were analyzed to optimize the desire response by providing the advantage of reducing the number of experiments.

1.7 Aims and objective

The objective of the study was to investigate the possibility of improving bio-methane yield from anaerobic co-digestion of newspaper and green vegetable waste by optimizing temperature and carbon to nitrogen (C/N) ratios through response surface methodology (RSM) while central composite design (CCD) was used to optimize the operating parameters. To achieve this aim certain research objectives were designed as stated below:

1.7.1 Research objectives

1. Examine the effect of temperature and C/N ratios on anaerobic co-digestion and to evaluate the performance efficiency of the reactors
2. Identify the optimized operating conditions of temperature and C/N ratios for methane yield.
3. Using analysis of variance (ANOVA) of RSM to validate statistical significance of the quadratic model for dependent variable (methane yield)

Chapter # 02 Literature Review

2.1 Bioenergy conversion technologies

Bioenergy conversion technologies deals with the organic materials which can be highly variable in mass and energy density, easily availability of the material, size and moisture content present in it. Three technologies are commonly reported in the literature for the conversion of biomass into bioenergy [24]. These technologies are direct combustion, Thermochemical conversion and biochemical conversion. Figure 2.1 illustrates the biomass to bioenergy conversion technologies.

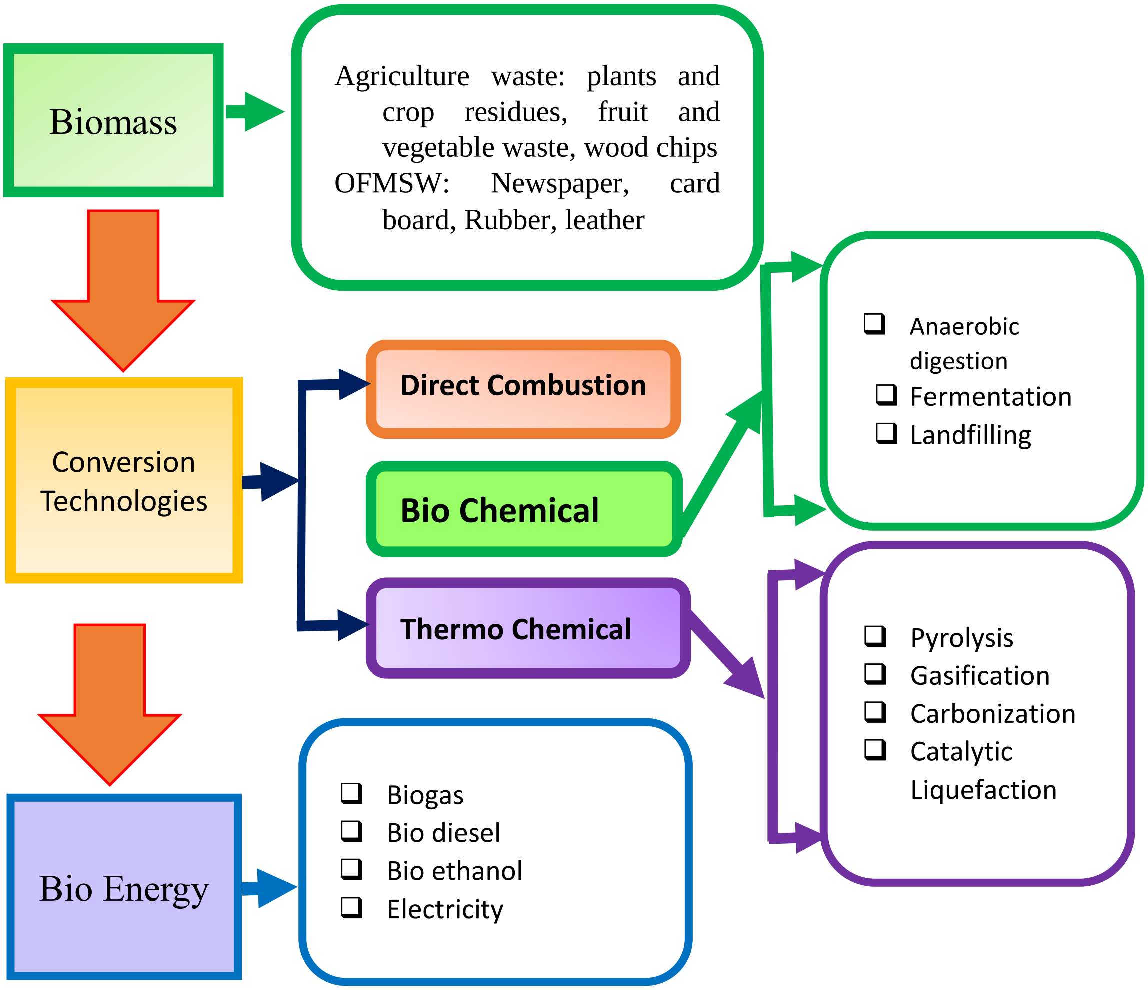


Figure 2.1: Bioenergy conversion technologies [24] [25].

First one is the combustion which is a commonly available commercial technology, in which direct burning of biomass in the presence of oxygen takes place to produce heat and electricity [25]. Such systems are alike to the most of the fossil fuel fired power plants for the heat and electricity production in which biomass fuel is burned in a boiler to produce high-pressure steam then introduced into a steam turbine, where it flows over a series of turbine blades, causing the turbine to rotate which is connected to an electric generator in which steam flows over and turns the turbine which electric generator rotates, producing electricity [26].

Second one is the thermochemical conversion, in which pyrolysis, gasification, carbonization and catalytic liquefaction processes are used to convert biomass into bioenergy[25]. These processes convert solid materials into less costly to handle, store and transport gases. In pyrolysis long chain molecules are broken down in to short chain molecules at elevated temperatures to produce heat in the absence of oxygen [27]. Such type of process can also be used to produce syngas which is composed of hydrogen, carbon monoxide and volatile organic compounds. Factors affecting pyrolysis process includes temperature, residence time, particle size and physical structure and material composition with increase in temperature greater quantity of non-condensable gases like Syngas will be produced whereas at lower temperature solid products like charcoal, bio coal will be produced [28]. Carbonization is process of the conversion of organic matter in to carbon through destructive distillation. It is considered as a complex process in which various reactions takes place simultaneously such as dehydrogenation, condensation, isomerization and hydrogen transfer. Gasification converts the carbonaceous material in to hydrogen, carbon monoxide and carbon dioxide attained through partial amount of oxygen or steam which reacts with the material at temperature more than 700°C, produce a gas mixture known as syngas (synthesis gas) which is a fuel itself, it may be converted in to synthetic fuel through the process fischer-tropsch, it can also be burned directly in the gas engines, and may be used to produce methanol and hydrogen [29]**.** Catalytic liquefaction is a type of thermochemical conversion process carried out in liquid phase at low temperature and high pressure, either requires a catalyst or high hydrogen partial pressure [30].

Third is the biochemical conversion technologies to convert biomass into bio energy, which includes anaerobic digestion, land filling and fermentation processes. Fermentation in terms of bio-chemistry is defined as the energy obtained from carbohydrates in the absence of oxygen which produces chemical changes by the action of enzymes to produce energy and useful products [31]. Landfill is the oldest form of waste disposal in which waste material is buried, produces a significant amount of methane which is powerful greenhouse gas, because of the anaerobic digestion carried out by the microbial decomposition of organic material [32]**.** Anaerobic digestion is a naturally occurring process in which anaerobic microorganism decompose the organic material in to simpler chemical components in the absence of oxygen to produce methane and traces of other gases as an end product [33].

2.2 Biomass and its types

Biomass is a renewable source of energy obtained from organic materials that comes from plants and animal and can be burned directly or transformed into liquid biofuels or biogas that can be used as fuel. Plants absorbs the sun energy through the process called photosynthesis and when it is burned, chemical energy stored in biomass is released as heat. Biomass resources include primary, secondary, and tertiary sources [25]. Figure 2.2 shows the different types of biomass.

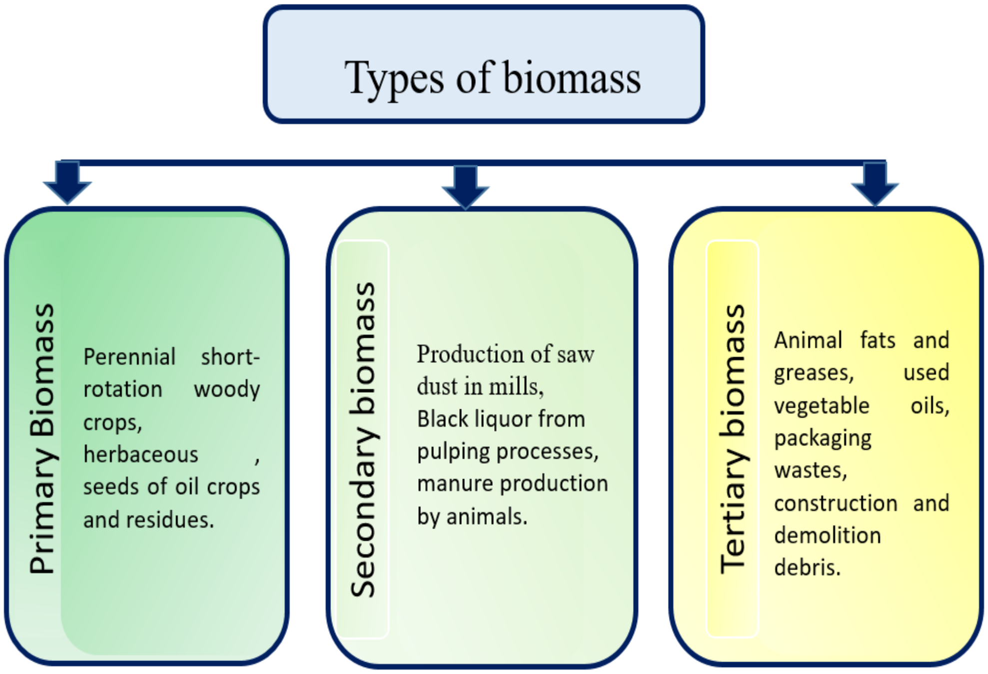


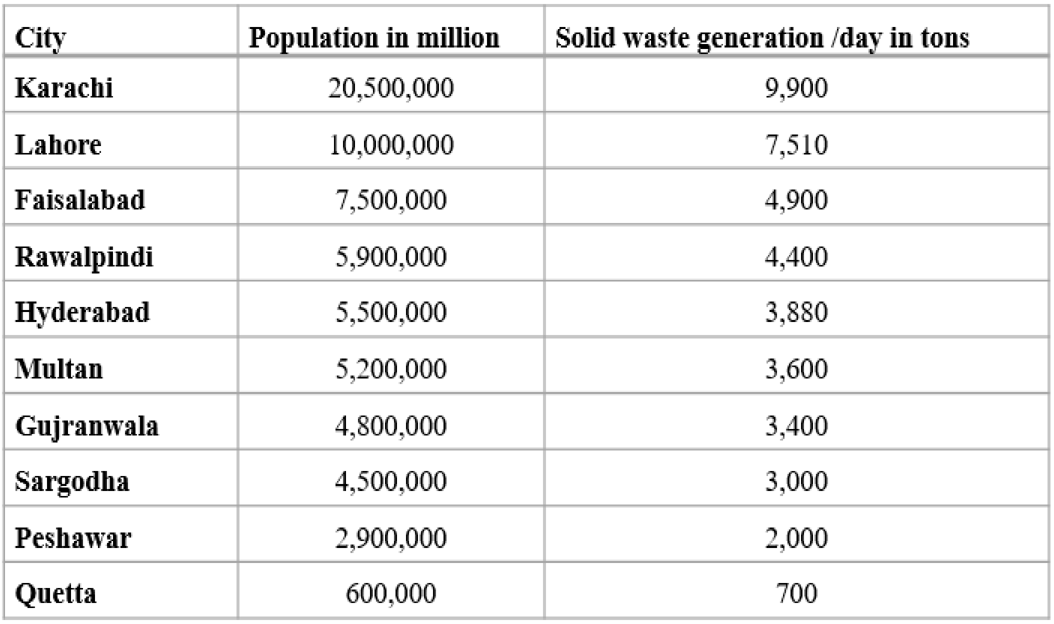
Figure 2.2: Types of biomass [25].

Primary biomass resources comprises of perennial short rotation woody and herbaceous crops, seeds of oil crops, and residues resulting from the harvesting of agricultural crops and forest trees are produced directly by photosynthesis and are taken directly from the land. Secondary biomass resources are the outcome of the processing of primary biomass resources either physically (e.g., the production of saw dust in mills), chemically (e.g., black liquor from pulping processes), or biologically (e.g., manure production by animals), whereas tertiary biomass resources are post-consumer residue streams including animal fats and greases, used vegetable oils, packaging wastes, and construction and demolition debris.

Because of various factors like increase in urbanization, industrialization, economic growth and improved standard of living solid waste generation is increasing and due to lake of waste management infrastructure caused serious environmental problems [34]. Most of the solid waste is either burned, dumped or buried on vacant lots are the main reasons for the blockage of drains, breeding of flies and spread of epidemic diseases which affects the health and welfare of the people [35] [36]. Government of Pakistan estimates that 77,000 tons of solid waste is generated per day mostly from metropolitan areas.

Remote areas of Pakistan are mostly ignored and the collection efficiency is very low. Solid waste generation in major cities of Pakistan is shown in table 2.1.

Table 2.1: Solid waste generation in major cities of Pakistan [37]



City	Population in million	Solid waste generation /day in tons
Karachi	20,500,000	9,900
Lahore	10,000,000	7,510
Faisalabad	7,500,000	4,900
Rawalpindi	5,900,000	4,400
Hyderabad	5,500,000	3,880
Multan	5,200,000	3,600
Gujranwala	4,800,000	3,400
Sargodha	4,500,000	3,000
Peshawar	2,900,000	2,000
Quetta	600,000	700

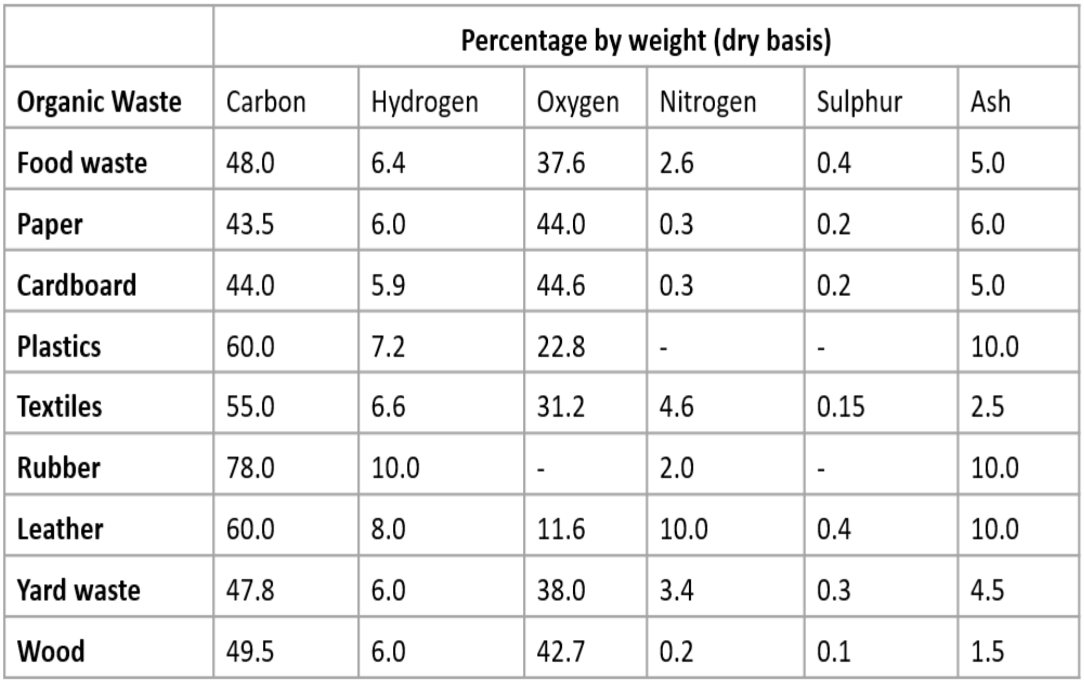
Solid waste is comprised of three categories i.e. biodegradable, non-biodegradable and recyclable. Biodegradables includes food waste, leaves, grass, animal waste, wood and straw and are being decomposed rapidly by the action of microorganisms. Non biodegradables includes plastics, rubber, textile waste, fines, metal and stones, do not degrade easily naturally or by the action of natural agents cause pollution and are also harmful to the living beings. Recyclable material includes paper, cardboard, rags and bones whereas recycling is the process of converting waste materials in to new materials and objects, it will prevent the potential of the useful materials and reduce the consumption of fresh raw materials caused reducing energy usage, air pollution and water pollution. [38]. The physical composition of solid waste in Pakistan are given in table 2.2.

Table 2.2: Physical composition of solid waste in Pakistan [38].

Type of the waste	% weight
Food waste	17
Paper	3
Animal waste	3
Card board	2
Leaves and grass	14
Wood	1
Fines	41
Rags	5
Plastic and Rubber	5

Despite of huge solid waste generation in Pakistan there is no any practice of using the solid waste for power generation in the country. Solid waste is becoming one of the reason for the environmental degradation causing contamination of surface and ground water through leachate, soil contamination through direct waste contact or leachate, air pollution by burning of waste and the spreading of diseases by different vectors like birds, insects and rodents [39]. Therefore there is an urgent need to look for waste to energy generation technologies that would be able to fulfil the energy requirements and manage the waste as well. Anaerobic digestion technology can provide a better alternative solution for the waste utilization along with energy production because it deals with biodegradable component of the solid waste in which microorganisms digest the organic component and release gas during the process which is known as biogas and can be used as a fuel for heat and electricity production. Ultimate analysis of the combustible components of solid waste depicts that it has a huge potential for energy production [40]. Ultimate analysis of the combustible components in municipal solid waste is shown in table 2.3.

Table 2.3: Ultimate analysis of the combustible components of the municipal solid waste.



	Percentage by weight (dry basis)					
Organic Waste	Carbon	Hydrogen	Oxygen	Nitrogen	Sulphur	Ash
Food waste	48.0	6.4	37.6	2.6	0.4	5.0
Paper	43.5	6.0	44.0	0.3	0.2	6.0
Cardboard	44.0	5.9	44.6	0.3	0.2	5.0
Plastics	60.0	7.2	22.8	-	-	10.0
Textiles	55.0	6.6	31.2	4.6	0.15	2.5
Rubber	78.0	10.0	-	2.0	-	10.0
Leather	60.0	8.0	11.6	10.0	0.4	10.0
Yard waste	47.8	6.0	38.0	3.4	0.3	4.5
Wood	49.5	6.0	42.7	0.2	0.1	1.5

Pulp and paper industry is linked with the development of the society and plays a fundamental role in the world wide economy. It is predicted that by 2020 global manufacturing of the pulp and paper industry is increased by 77% [41]. Paper mill sludge is produced after handling of the waste matter, dry sludge constitute 40-50 Kg which is a mixture of primary (70%) and secondary (30%)sludge generated by per ton of paper manufacture. Sludge has the potential for energy production as it contains micro and macro contaminants which makes it appropriate for the agricultural use as well. Newspaper waste is considered as one of the significant solid waste comprises 3% by weight, can be recycled through limited amount of times because of quality specification. The calorific values of the solid waste is shown in table 2.4. Anaerobic digestion technology can provide a suitable option for the reduction of the size of newspaper waste as well utilization for heat and electricity production.

Many researchers have used paper waste for methane production. Hong-Wei yen et al. (2005) used paper waste and algal sludge to produce methane through anaerobic co-digestion technique. In this study they found that adding 50 % of paper waste based on volatile solid (VS) with algal sludge enhance the methane production rate to 1170±75 ml/lday as compared to 573±28 ml/lday in which algal digestion alone, both digestions were operated at 4 g VS/L day, 35^{0}C and HRT of 10 days [42].

Table 2.4: Calorific values of the solid waste [43].

Name of the waste	Calorific value (MJ/Kg)
Newspaper	46.80
Normal Paper	24.24
Textile	15.70
Leather	15.70
Garden waste	12.90
Vegetable waste	30.7
Plastic	40

Food waste generation in Pakistan is approximately 36 million tons per year which includes food loss during supply chain in production, post-harvest, handling, agro-processing, distribution and consumption and about 40% at wedding different other ceremonies. Food waste effects the natural resources in terms of land and soil degradation and fertilizers, pesticides, transportation means, storage facilities go wasted when food is wasted. Green vegetable waste is the major component of the food waste contains carbon and high content of nitrogen which can be used by the microorganisms through anaerobic digestion process and will generate methane rich fuel that can be used as a source of heat and electricity production. Vegetable waste have the calorific value of 30.7 MJ/Kg as shown in table 2.4 means that it have notable potential for the heat generation.

F.J.Callaghan et al. (2001) used cattle slurry with chicken manure, fruit waste and vegetable waste through continuous co-digestion for the methane production. In this study they found that increasing portion of fruit vegetable waste from 20 to 50 % improved the methane yield from 0.23 to 0.45 m^3 CH$_4$/kg VS added, caused slightly decrease in the VS reduction [44].

2.3 History of anaerobic digestion

Jan Baptist van Helmont found some inflammable gases from the decaying organic material in the 17th century forms an explosive mixture with air. In 1804-1810 Dalton, Henry and Davy made the chemical composition of methane, revealed that methane was produced from the decaying cattle manure and confirmed that coal gas was similar to Volta's marsh gas. In 1884 a student Pasteur named Gayon fermented manure at 35 ^{0}C extracting 100 liters of methane and concluded that fermentation could be a source of fuel in the form of gas which can be used for heat and lighting. By the end of 19th century it was found that methanogenesis is related with microbial activity. Bechamp named the organisms responsible for methane production and was able to predict that depending upon substrates different types of products would be formed. In 1896 a gas from sewage was used for street lighting in Exteter England.

Anaerobic digestion is a process in which organic matter under anaerobic conditions is broken down into simpler compounds taken up by microorganisms to produce energy rich fuel and digestate as a byproduct. The process is divided into four main phases i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis [45]. In all these phases break down of organic matter takes place requires several conditions and variables to enhance the microbial activity, operating parameters like temperature, organic loading rate (OLR), pH, hydraulic retention time and carbon to nitrogen ratio.

2.4 Anaerobic co-digestion

It is the simultaneous treatment of two or more biodegradable organic waste for the production of bio-methane [46]. Pretreatment of feed stock can be done through screening, mechanical comminution, separation, ultrasonic and thermal pretreatments which involves removal of non-biodegradable substances which may cause decline in digestion, and take excessive space. Various factors like pH, C/N ratio, and temperature etc., slight change in the conditions strongly affect the performance of the anaerobic co-digestion [47]. Different substrate mixture provides the better utilization of waste as well reduction of its size. Advantages of co-digestion includes balancing of the nutrient in the substrates by co-digesting nitrogen rich substrates with carbon rich substrates [11].

2.5 Ingredients for anaerobic digestion and co-digestion

Four ingredients are needed for the biogas production includes heat, bacteria, organic matter and anaerobic condition [48]. Bacteria digest the organic matter and release biogas which is a source of renewable energy in the presence of heat and absence of oxygen.

2.6 Biochemical process of anaerobic digestion and co-digestion

Anaerobic digestion and co-digestion process is divided into four major phases in which different microorganism's breakout the organic matter into simpler ones. Figure 2.3 shows the phases for anaerobic digestion and co digestion.

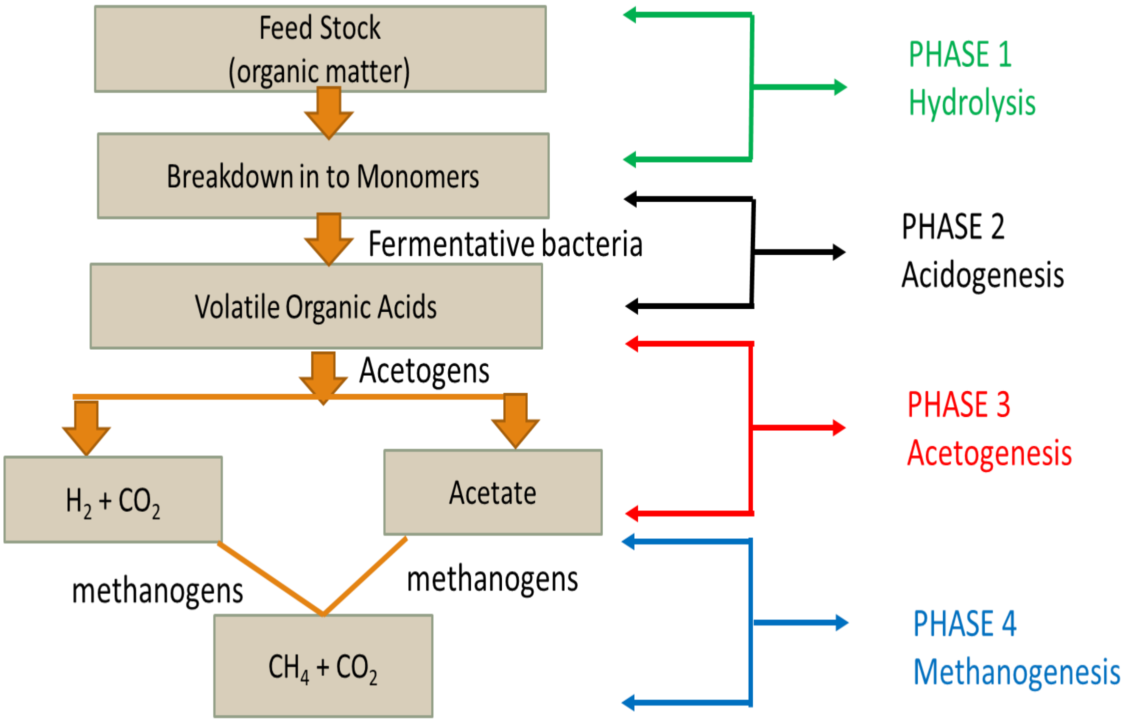


Figure 2.3: Anaerobic digestion processes [46][49][50].

2.6.1 Hydrolysis

Large molecule of feed stock containing carbohydrates, proteins and lipids is broken down in the presence of waste in to smaller components like sugars, fatty acids and amino acids by the help of extra-cellular enzymes secreted by microorganisms [49]. Substrate availability, bacterial population density, temperature and pH are the factors which affect the rate of hydrolysis.

2.6.2 Acidogenesis

In this phase , end product of the hydrolysis i.e., fatty acids, amino acid and sugars are converted into organic acids like acetic, propionic, butyric and small chain fatty acids, which includes butyrate, acetate, formate, lactate and succinate alcohols, hydrogen and carbon dioxide which is the result of fermentative acidogenesis [7] [50].

2.6.3 Acetogenesis

Acetate is the main end product of this phase which is produced by acetogenic bacteria in which the bacteria decompose the acids for the biogas production. This phase is important because electron sinks are not utilized by methane producing microorganisms.

2.6.4 Methanogenesis

The end products of the acetogenesis are transformed into methane, carbon dioxide and water by the methanogens which are the methane forming bacteria. The process is carried out by the obligate anaerobes which have slow growth rate as compared to other stages. Hydrogenotrophic methanogens yield approximately 30% of the methane by the conversion of carbon dioxide, hydrogen and other substances like methanol and methylamines, acetoclastic methanogens yield about 60% of the total amount of methane.

2.7 Factors affecting anaerobic co-digestion

Performance of anaerobic digestion and co-digestion are influenced by several factors which are illustrated in figure 2.4. pH effects the enzyme activity of the microorganisms for their metabolism and is a function of retention time.[51] . In the initial phases of fermentation due to the formation of organic acids caused the pH decrease to below 5 which sometimes inhibits the fermentation process. Optimum pH for biogas production is between 6 and 7, methanogenic bacteria are sensitive toward pH and cannot survive below pH 6.5 [52].

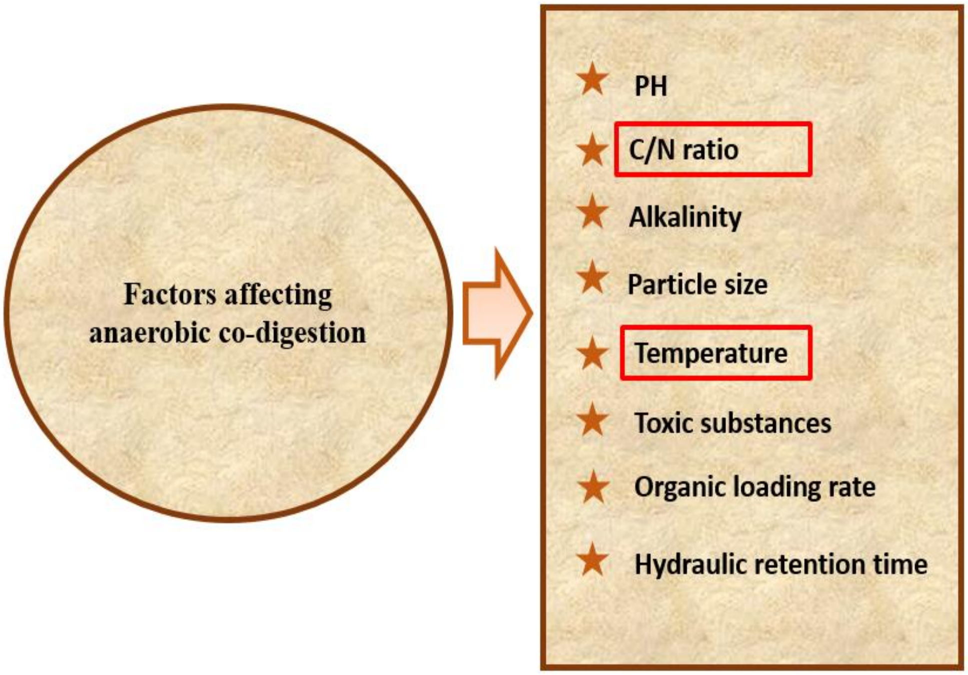


Figure 2.4: Factors affecting anaerobic co-digestion [51] [53] [7].

Organic loading rate is defined as the amount of raw materials fed per unit volume of digester capacity per day and is an important factor for defining the efficiency of anaerobic digestion process which depends upon various parameters like input characteristics of waste ,digester design, volume and retention time [54]. Overfeeding to the digester caused accumulation of the acids whereas underfed condition gas production will be low.

Hydraulic retention time (HRT) is the average time of the input substrate material spent in the digester for the biogas production before it comes out, varies from climate to climate, in tropical areas HRT varies from 30 to 50 days while in colder climate it may reached up to 100 days. Retention time depends upon various parameters includes temperature, characteristics of the substrate etc.[55]. HRT controls the biogas yield as it is estimated from the generation times of the microbes involved in the digestion of organic matter [53].

Particle size of the feed stock is important for the smooth process of the anaerobic digestion, large size of the feed stock caused clogging of the digester which created difficulty for the microbes to carry out digestion whereas smaller size of the feed stock provides large surface area for the microbes to digest the feed stock, increase the microbial activity which increase the biogas production. Particle size can reduced with the help of choppers, shredder and grinders which can reduce the size of the feed stock and volume of the digester.

2.7.1 C: N ratio

An optimal amount of feed stock is required that is capable for providing an optimal amount of C: N ratio for high level working of biogas reactor. Suitable range of C: N ratio are required to the microorganisms to carry out their metabolic activities, higher carbon content will release more carbon dioxide and lower the pH of the system whereas high nitrogen content will enhance the ammonia production which cause increase in pH and is harmful to the microorganisms [56] . Nitrogen in the feed stock is required for creating the anaerobic conditions and it is the essential element for the synthesis of amino acids, proteins and nucleic acids, is effectively utilized by the microbes present in the digester. Many researchers reported that optimum range of C: N is 20-30:1 is a function of feed stock characteristics and operational parameters for the digestion.

2.7.2 Temperature

Temperature is a significant factor for the microbial activity and its optimum range is required for the digestion process. Three ranges of temperature are reported by many researchers i.e. psychrophilic 15-20^{0}C, mesophilic 25- 45^{0}C and thermophilic 50-60^{0}C. Growth of microorganisms is low in psychrophilic range of temperature compared to mesophilic and thermophilic range of temperature [7].

Xiaojiao Wang et al. (2014) investigated the influence of temperature and C/N ratio by using dairy manure, rice straw and chicken dung as a substrate on the efficiency of anaerobic co-digestion process. They found that increased temperature enhanced the methane potential but the rate was decreased from mesophilic to thermophilic conditions because of accumulation of ammonium nitrogen and free ammonia. With increase in temperature an increased in the C/N ratio was required in order to condense the risk of ammonia inhibition.

2.8 Modes of feeding to the digester

An enclosed or air tight tank is designed for processing organic material through the process of anaerobic decomposition is known as the digester. Digesters are also known as biogas reactors. It uses variety of the organics along with organisms needed to convert the organic material into biogas which can be used as an energy alternative. It is the central part of the anaerobic biogas plant. Designing of the digester highly depends upon the material used for the construction of the digester, type of the organic waste to be used, temperature to be used for the digestion [57]. There are two different types of feeding batch feeding and continuous feeding.

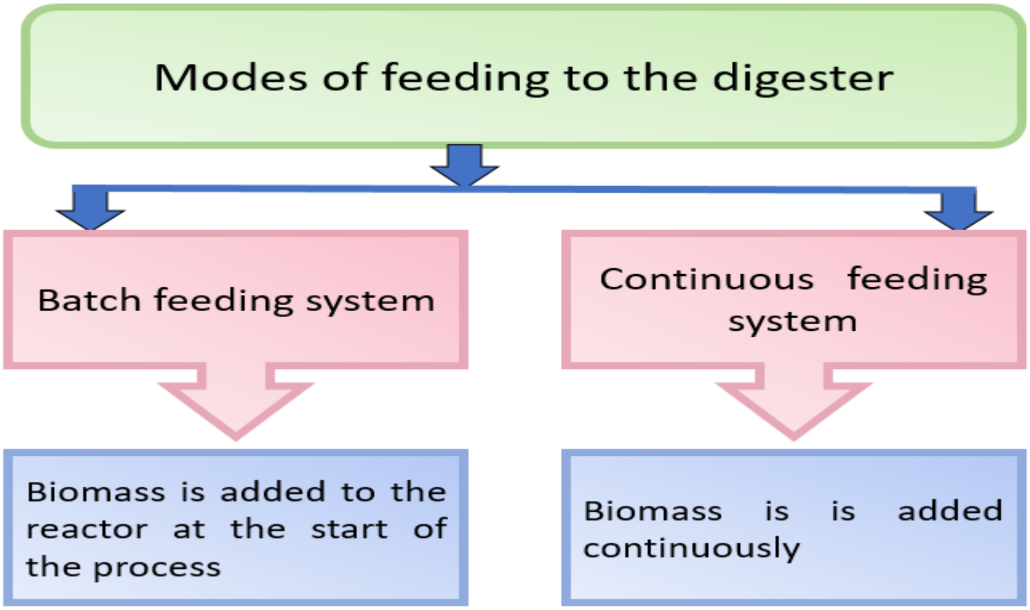


Figure 2.5: Modes of feeding to the digester [58] [59].

In batch feeding system, biomass is added to the reactor at the start of the process. The reactor is then air tight for the duration of the process. It is the simplest and cheapest form of digestion as it requires less equipment and lower level of design work. Depending upon waste material and operating temperature batch digester starts slowly producing biogas, therefore batch reactors are operated in groups so that at least one is always producing useful quantities of gas [58]. There can be severe odor issues if a batch reactor is opened and emptied before the process is well completed. A more advanced type of batch approach has limited the odor issues by integrating anaerobic digestion with in vessel composting. In this approach inoculation takes place through the use of recirculated degasified percolate. After the completion of anaerobic digestion, biomass is kept in the reactor which is then used for in-vessel composting before it is opened.

In continuous type of feeding organic matter is added continuously (continuous complete mixed) or added in stages to the reactor. In this type of reactor end products are periodically removed, resulting in constant production of biogas [59]. Examples of this type of reactors includes up flow anaerobic sludge blankets, internal circulation reactors, continuous stirred-tank reactors and internal circulation reactors. It is essential to ensure that the reactor is large enough to contain all the material that will be fed through in a whole digestion cycle. One solution is to use a double digester, consuming the waste in two stages, with the main part of the biogas being produced in the first stage and the second stage finishing the digestion at a slower rate, but still producing another 20 % or so of the total biogas.

2.9 By products of anaerobic co-digestion

Anaerobic digestion and co-digestion is a meaningful way to utilize the biodegradable waste producing biogas and digestate as a byproduct which can be used as a source of renewable energy and sale of both can provide economic benefits. In order to obtain the maximum value from these products, further processing may be required. By products of anaerobic co-digestion process were illustrated in figure 2.6.

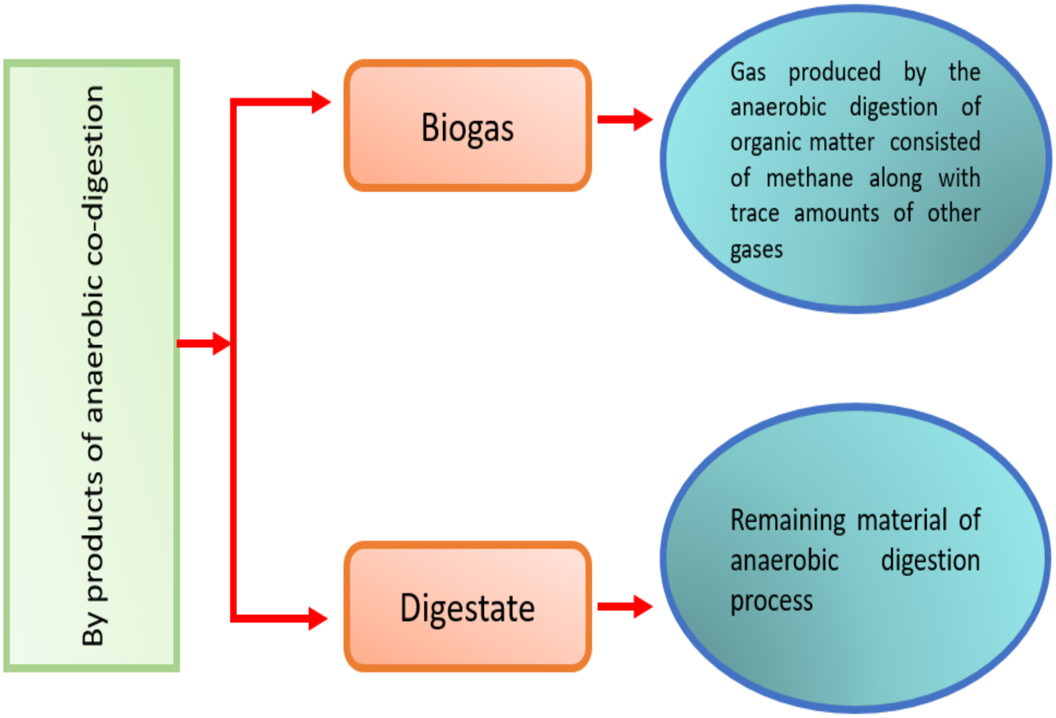


Figure 2.6: By products of anaerobic co-digestion [60] [17] [61].

2.9.1 Biogas

Biogas is formed during anaerobic digestion of organic matter which includes manure, sewage sludge, municipal solid waste, biodegradable waste or any other biodegradable feedstock, under anaerobic conditions and is an essential part of biogeochemical carbon cycle [60]. Methanogens (methane producing bacteria) comes in the last stage of anaerobic co-digestion process and are the micro-organisms which decompose the organic material and return the disintegrated products in to the environment releasing biogas during the process which is a source of renewable energy comprised primarily of methane and carbon dioxide, also contains smaller amounts of hydrogen sulphide, nitrogen, hydrogen and methylmercaptans. [62]. Biogas is about 20 percent lighter than air and is an odorless and colorless gas that burns with a clear blue flame, quality of biogas may be upgraded by filtering it through limewater to remove carbon dioxide, iron filings to absorb corrosive hydrogen sulphide.

2.9.2 Composition of biogas

The composition and properties of biogas varies to some degree depending on feedstock types, digestion systems, temperature, retention time etc. Composition of biogas have been given in Table 2.1 and is majorly composed of methane, carbon dioxide and traces of other compounds which includes carbon monoxide, hydrogen sulphide, nitrogen, ammonia, hydrogen.[17]

Table 2.5 Composition of biogas.

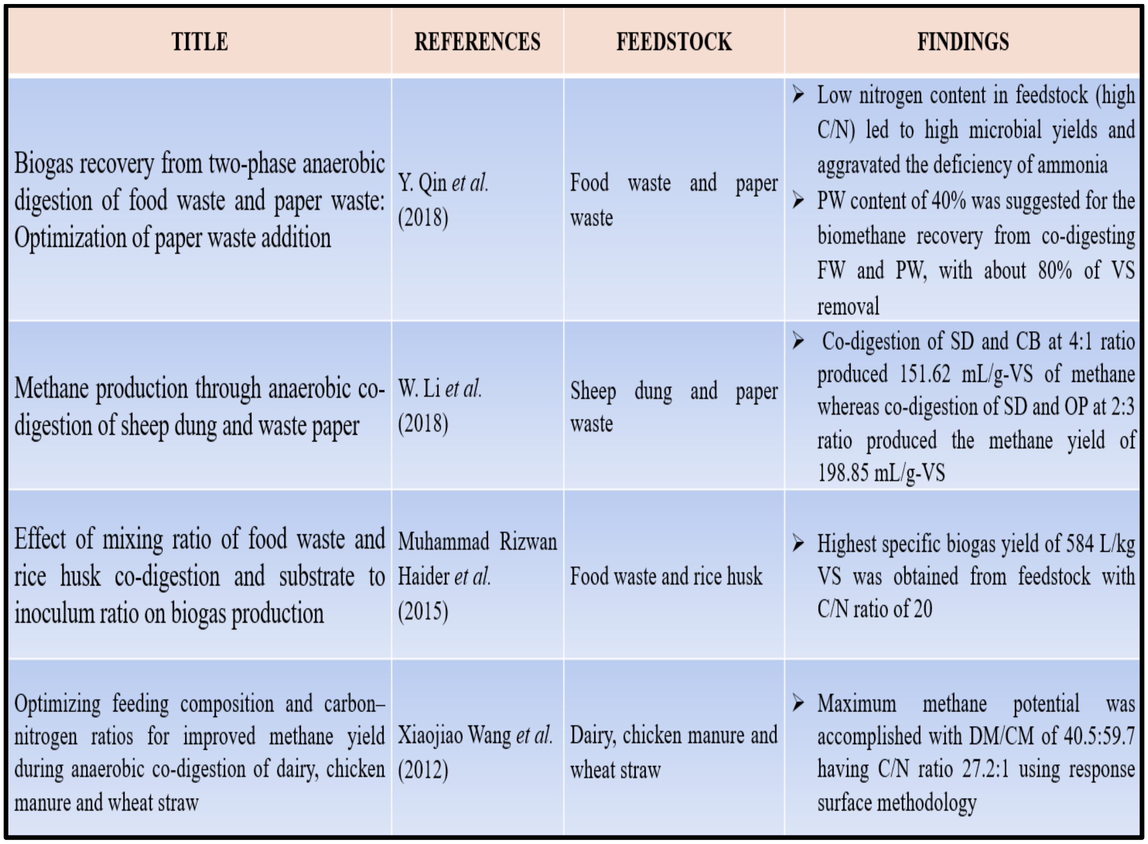
Chemical compound	Content (%)
Methane CH_4	50-70%
Carbon dioxide CO_2	25-45%
Hydrogen H_2	<1
Nitrogen N_2	<2
Ammonia NH_3	<1
Hydrogen Sulphide H_2S	<1
Water vapors	2(20°C)-7(40°C)

2.9.3 Digestate

It is the remaining material of the anaerobic digestion process of a biodegradable feed stock, having essential plant nutrients nitrogen, phosphorus and potassium (N, P and K) that plays an important role for the plant growth [61]. Digestate has 25% more inorganic nitrogen and a higher pH value than untreated liquid manure [48]. It reduces the odor nuisance by about 80%, can be processed in to compost also be used as fertilizer, soil amendment in agriculture and landscaping [63]. Such use permits the creation of a nutrient cycle and improves soil structure due to the application of organic matter. It would ensure a complete breakdown of the organic components as well as fixing the mineral nitrogen on to humus-like fraction, which would reduce nitrogen loss.

2.10 Response surface methodology (RSM)

Response surface methodology (RSM) is a significant tool to evaluate the relationship and interaction between different independent variables [64]. George E. P. Box and K. B. Wilson introduced this method in 1951, to use a sequence of designed experiments to obtain an optimal response. The term was originated from the graphical perspective generated after fitness of the mathematical model. Various operational parameters have successfully been optimized by RSM in a number of studies. Many researchers have also used RSM and central composite design (CCD) for estimating the optimum conditions for biogas production. Some previous work related with biomass for methane potential were shown in figure 2.7.



TITLE	REFERENCES	FEEDSTOCK	FINDINGS
Biogas recovery from two-phase anaerobic digestion of food waste and paper waste: Optimization of paper waste addition	Y. Qin *et al.* (2018)	Food waste and paper waste	➢ Low nitrogen content in feedstock (high C/N) led to high microbial yields and aggravated the deficiency of ammonia ➢ PW content of 40% was suggested for the biomethane recovery from co-digesting FW and PW, with about 80% of VS removal
Methane production through anaerobic co-digestion of sheep dung and waste paper	W. Li *et al.* (2018)	Sheep dung and paper waste	➢ Co-digestion of SD and CB at 4:1 ratio produced 151.62 mL/g-VS of methane whereas co-digestion of SD and OP at 2:3 ratio produced the methane yield of 198.85 mL/g-VS
Effect of mixing ratio of food waste and rice husk co-digestion and substrate to inoculum ratio on biogas production	Muhammad Rizwan Haider *et al.* (2015)	Food waste and rice husk	➢ Highest specific biogas yield of 584 L/kg VS was obtained from feedstock with C/N ratio of 20
Optimizing feeding composition and carbon–nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw	Xiaojiao Wang *et al.* (2012)	Dairy, chicken manure and wheat straw	➢ Maximum methane potential was accomplished with DM/CM of 40.5:59.7 having C/N ratio 27.2:1 using response surface methodology

Figure 2.7: Previous work related with biomass for methane potential.

Many researchers have reported that RSM is a useful tool for optimizing the bio methane potential. Jiayu feng et al. (2017) used RSM and CCD to enhance methane production from vinegar residue, results of the experiment confirmed that RSM and CCD was useful for optimizing the anaerobic digestion parameters. The results of the study revealed that maximum methane yield of 203.91 mL/gVS and biodegradability of 46.99% were attained at feed to inoculum ratio of 0.5, organic loading of 31.49gVS/L and initial pH of 7.29 were the best conditions[22].

CCD provides the number of experiments which further evaluated for the purpose of optimizing various variables and responses. The minimum, intermediate and maximum values of each variables are given as -1, 0 and +1, central point is the at the center of the design space while, axial points are positioned on the axes of the coordinates system with respect to the central point at a distance α from the design center.

Xiaojiao et al. (2012) used CCD for designing the experiments for the methane yield from co-digestion of chicken manure, wheat straw and dairy leftover, results of the study showed that maximum methane potential was achieved with dairy manure / chicken manure of 40.3: 59.7 and C/N ratio of 27.1:1 after optimization by using response surface methodology (RSM) [65].

Chapter # 03 Materials and Methods

3.1 Methodology of research

The methodology of research was divided into six phases mentioned in figure below. Substrate and inoculum were selected on the basis of cheap, easy availability and potential for the bio-methane production. Analytical and statistical analysis of the material were performed, reactor was setup according to the analytical analysis and statistical analysis. At the end of the process performance efficiency of the bio-reactors were checked.

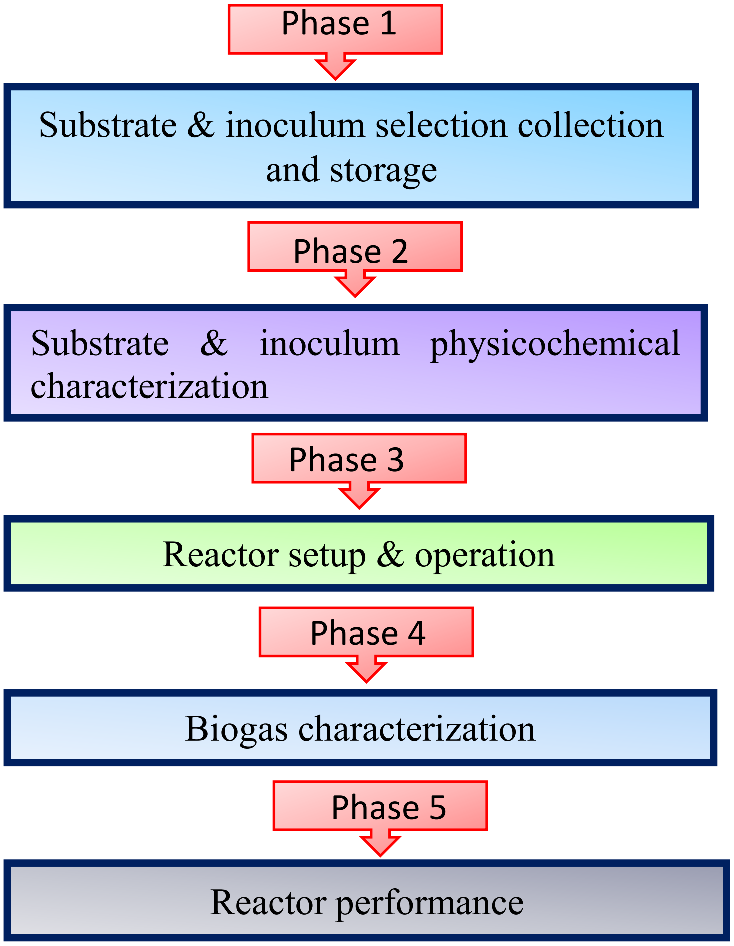


Figure 3.1: Methodology of research.

3.2 Substrate and inoculum

The newspaper and green vegetable waste were used as substrate, newspaper waste was collected from the library of National University of Science and Technology, Islamabad while green vegetable waste including cabbage, spinach and lettuce, was collected from the cash and carry market near National University of Science and Technology. The substrate and inoculum were selected on the basis of cheap and easily availability of the material. Furthermore, cow manure, collected from native livestock farm shed Fateh Jang, Punjab, Pakistan, was used as inoculum. Newspaper waste was shredded using shredder to reduce the particle size up to <5mm and green vegetable waste was cut manually by choppers then grinded to reduce their particle size <5 mm by using lab grinder and stored at 4^0C prior to conducting the lab scale testing.

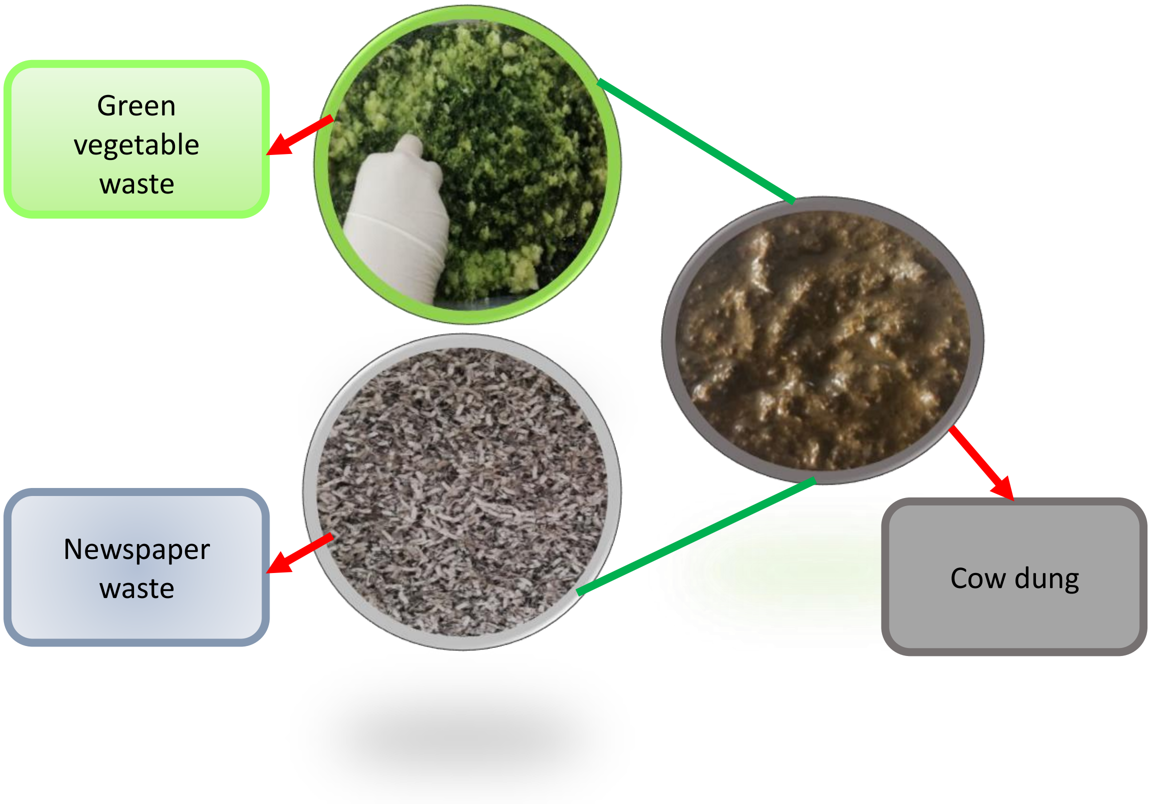


Figure 3.2: Substrate and Inoculum.

3.3 Physicochemical analysis of substrate and inoculum

The physicochemical properties of substrate and inoculum tested in this study are given in table 3.1. Fresh samples of inoculum, newspaper and green vegetable waste were investigated for their pH, total solids (TS), volatile solids (VS), total kjeldahl nitrogen (TKN) and total organic carbon. All these examination were performed in accordance with APHA standard methods [66]. All the samples were collected in triplicates and average of the three measurements are illustrated.

Table 3.1: Physicochemical properties of substrate and inoculum.

Parameters	**Newspaper waste**	**Green vegetable waste**	**Inoculum**
TS (%)	94.25	9.85	12.09
VS (%)	92.5	8.416	10.084
VS(%of TS)	98.14	85.44	83.40
TOC (%)	54.522	47.4667	46.33
TKN (%)	0.0224	2.78	5.4
Moisture content (%)	5.75	90.15	87.91

TS=total solids, VS=volatile solids TKN= total kjeldahl nitrogen, TOC= total organic carbon

Figure 3.3: Total solids characterization of newspaper (left) and green vegetable waste (right)

3.3.1 Method for calculation of total solids

Firstly determined the weight of the empty china dish by putting the china dish on to the digital balance scale. Samples were added in to the china dish and sample was weighed and then placed in to the drying oven at the temperature of 105^{o}C, after that sample was placed in the desiccator for cooling and was weighed again.

Table 3.2: Measurements for calculating total and volatile solids

Sample	**Weight of empty china dish(W_1) (g)**	**Weight of empty China dish + sample (W_2) (g)**	**Weight after dry oven 105°C (W_3) (g)**	**Weight after furnace 550°C (W_4) (g)**
Newspaper waste	63.676	63.676+2 =65.676	65.561	63.720
Green vegetable waste	62.6002	62.6002+3 =65.6002	62.8957	62.6432
Inoculum	81.5898	81.5898+10 =91.5898	82.7988	81.7904

Formula for calculating total solids (TS) = $\left\{ \frac{W_3 - W_1}{W_2 - W_1} \right\} * 100$ (3.1)

For Newspaper waste:

$$\text{Total solids} = \left\{ \frac{65.561 - 63.676}{65.676 - 63.676} \right\} * 100$$

Total solids = 94.25%

For Green vegetable waste:

$$\text{Total solids} = \left\{ \frac{62.8957 - 62.6002}{65.6002 - 62.6002} \right\} * 100$$

Total solids = 9.85%

For Inoculum:

$$\text{Total Solids} = \left\{ \frac{82.7988 - 81.5898}{91.5898 - 81.5898} \right\} * 100$$

Total solids = 12.09%

3.3.2 Method for calculation of volatile solids

Firstly determined the weight of the empty china dish. Samples were added in to the china dish and were weighed, then placed in to the drying oven at the temperature of 105°C. After drying samples were placed in the desiccator for cooling and then weighed again. Samples were then ignited in the furnace at 550°C and were placed in the desiccator for cooling and then weighed again.

Formula for calculating volatile solids (VS) =

$$\left\{\frac{W_3-W_4}{W_2-W_1}\right\}*100 \tag{3.2}$$

For Newspaper waste:

$$\text{Volatile solids} = \left\{\frac{65.561 - 63.676}{65.676 - 63.676}\right\}*100$$

Volatile solids = 94.25%

For Green vegetable waste:

$$\text{Volatile solids} = \left\{\frac{62.8957-62.6432}{65.6002 - 62.6002}\right\}*100$$

Volatile solids = 8.416%

For Inoculum:

$$\text{Volatile solids} = \left\{\frac{82.7988 - 81.7904}{91.5898 - 81.5898}\right\}*100$$

Volatile solids = 10.084%

Where

W_1= Weight of empty china dish

W_2= Weight of empty china dish + sample

W_3= Weight after dry oven 105°C

W_4= Weight after furnace 550°C

3.3.3 Method for calculation of total kjeldahl nitrogen

The total nitrogen present in the substrate and inoculum was examined by Kjeldahl method [67]. Total nitrogen includes organic, ammonium and nitrate nitrogen. The sample was digested in sulfuric acid, using $CuSO_4/TiO_2$ as catalysts, to raise the boiling temperature and to promote the conversion from organic-N to ammonium-N. Ammonium-N from the digest was obtained by steam distillation, using excess NaOH to raise the pH. The distillate was collected in saturated H_3BO_3 and then titrated with dilute H_2SO_4 to pH 5.

3.3.4 Method for calculation of moisture content

Moisture content was calculated by subtracting total solids from 100. Formula for calculating moisture content is MC= 100-TS

Moisture content of newspaper waste = 100- 94.25

MC = 5.75%

Moisture content of green vegetable waste = 100-9.85

MC = 90.15%

Moisture content of inoculum = 100-12.09

MC = 87.91

3.3.5 Method for calculation of total organic carbon

Total organic carbon was obtained by dividing volatile solids (% of total solids) with 1.8.

$$\text{Total organic carbon (TOC)} = \frac{\text{volatile solids (\% of TS)}}{1.8} \qquad (3.3)$$

TOC of newspaper waste was 54.5222%, green vegetable waste was 47.4667% and inoculum was 46.333%

3.4 Reactor setup and operation

Anaerobic batch digestion experiments were performed by two methods. First was hit and trial method in which different trials were performed to find the optimizing parameters for co-digestion of green vegetable and newspaper waste for bio-methane potential, whereas second method was through response surface methodology in which different runs were made by using the Design Expert Version 12.0.3.0 software to find the optimizing parameters for bio methane yield.

3.5 Manual optimization

Previous studies showed a wide range of C/N ratios (10-90) for methane fermentation [68]. The suitable C: N ratio for the effective metabolic processes of microbial group falls within the range of 20-30, which is sufficient to maintain system stability and meet expected energy and nutrient requirements for cell growth [69]. Feed stocks having higher C: N ratios such as newspaper, rice and wheat straw, sea wheat, corn stalks and algae can be co digested by the feed stock of lower C: N ratios for instance green vegetable waste to achieve nutrient balance and to avoid the inhibition that leads to system instability and reduced biogas production as a result of an unsuited C: N ratio. By understanding all these studies reactors were designed having C/N ratios of 20, 25, 30 and 35 at mesophilic (37^oC) and thermophilic (55^oC) temperatures for 50 days. Reactors having C/N ratio of 20 at 37^oC named as MR1 (mesophilic reactor 1), C/N ratio of 25 at 37^oC named as MR2 (mesophilic reactor 2), C/N ratio of 30 at 37^oC named as MR3 (mesophilic reactor 3), C/N ratio of 35 at 37^oC named as MR4 (mesophilic reactor 4). Those reactors that were designed having C/N ratio of 20 at 55^oC named as TR1(thermophilic reactor 1), C/N ratio of 25 at 55^oC named as TR2 (thermophilic reactor 2), C/N ratio of 30 at 55^oC named as TR3 (thermophilic reactor 3), C/N ratio of 35 at 55^oC named as TR4 (thermophilic reactor 4),while control for 37^oC named as MC (mesophilic control), at 55^oC named as TC (thermophilic control). Anaerobic digestion experiments were carried out in laboratory glass bottles of 300mL volume which act as anaerobic reactor for 50 days. All the digestion experiments were carried out in triplicate. Each reactor have 20.8g of inoculum. Reactor had a working volume of 210ml and head space was kept at 90ml. For all the experimental setup volatile solid (VS) ratio of substrate to inoculum was kept at 1:1 and organic loading rate was kept at 10gVSL^{-1}. Operational parameters for the experimental setup are shown in table 3.3.

Table 3.3: Operational parameters for the batch test.

Parameters	**Quantity**
Reactor bottle volume	300mL
Working volume	210mL
Head space	90mL
Organic loading rate	10gVS/L
Temperature	37 & 55^{o}C
pH	6.8-7.2
Feed to inoculum ratio	1:1
Hydraulic retention time	50 days

For making C/N ratio's following formula was used [5]

$$\frac{C}{N} = \frac{\text{NPW (TS x TOC) + GVW (TS x TOC)}}{\text{NPW (TS x TKN) + GVW (TS x TKN)}} \qquad (3.4)$$

The quantity of newspaper waste was kept at 1g whereas the quantities of green vegetable waste were varied for making the required ratios. Organic loading rate was kept at 10gVS/L, while pH was in the range of 6.8-7.2. Feed to inoculum ratio was adjusted on the basis of volatile solids was kept at 1:1. For equating organic loading rate on the basis of volatile solids following formula was used.

$$\text{Organic loading rate of 10gVS/L} = \left\{ \frac{10}{1000} \right\} {*210} \qquad (3.5)$$

Organic loading rate of 10gVS/L = 2.1gVS

Thus 2.1gVS of co-substrate and inoculum was equal to the organic loading of 10gVS/L for the reactors.

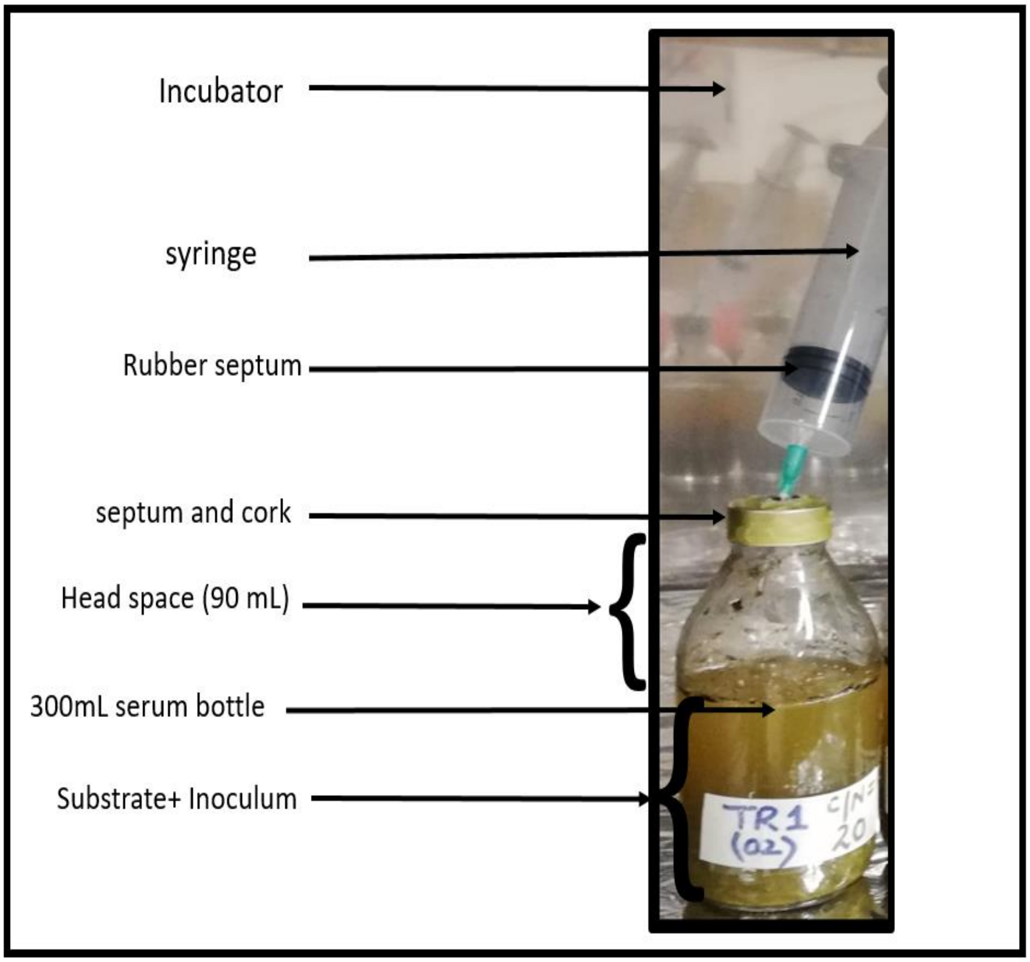


Figure 3.4: Lab scale batch type digester set-up

3.5.1 Making C: N 20

For making C: N 20 newspaper and green vegetable waste were required in the ratio of 1:61.8 which depicts that 1.5923 % of newspaper and 98.4076% of green vegetable waste were used for making that C: N 20. Total solids (TS) of 11.19389 % while 85.642% of volatile solids (VS) were required for making C: N 20. Total solids (TS) of the mixture containing newspaper and green vegetable waste were calculated by multiplying percentage of newspaper used with the TS of the newspaper adding with the percentage of green vegetable waste used with the TS of the green vegetable. 21.9054 g of co-substrate mixture containing newspaper and green vegetable waste were required for making the organic loading of 2.1g VS. By using equation (3.2) C: N 20 were calculated.

$$\frac{C}{N} = \frac{NPW\ (94.25 \times 54.522) + GVW\ (9.85 \times 46.33)}{NPW\ (94.25 \times 0.0224) + GVW\ (9.85 \times 2.78)}$$

$$= \frac{1\ (5138.7174) + 61.8(467.5470)}{1\ (2.1112) + 61.8\ (27.383)}$$

$$\frac{C}{N} = 20$$

3.5.2 Making C: N 25

For making C: N 25 newspaper and green vegetable waste were required in the ratio of 1: 23.4 which depicts that 4.09836% of newspaper and 95.9016% of green vegetable waste were used for making that C: N 20. Total solids (TS) of 13.3090% while 85.9604% of volatile solids (VS) were required for making C: N 25. 18.35588 g of co- substrate mixture containing newspaper and green vegetable waste were required for making the organic loading of 2.1g VS. By using equation (3.2) C: N 25 were calculated.

$$\frac{C}{N} = \frac{NPW\ (94.25 \times 54.522) + GVW\ (9.85 \times 46.33)}{NPW\ (94.25 \times 0.0224) + GVW\ (9.85 \times 2.78)}$$

$$= \frac{1\ (5138.7174) + 23.4\ (467.5470)}{1\ (2.1112) + 23.4\ (27.383)}$$

$$\frac{C}{N} = 25$$

3.5.3 Making C: N 30

For making C: N 30 newspaper and green vegetable waste were required in the ratio of 1: 14.3 which depicts that 6.5359% of newspaper and 93.4640% of green vegetable waste were used for making that C: N 30. Total solids (TS) of 15.366% while 86.2699% of volatile solids (VS) were required for making C: N 30. 15.8416 g of co- substrate mixture containing newspaper and green vegetable waste were required for making the organic loading of 2.1g VS. By using equation (3.2) C: N 30 were calculated.

$$\frac{C}{N} = \frac{NPW\,(94.25 \times 54.522) + GVW\,(9.85 \times 46.33)}{NPW\,(94.25 \times 0.0224) + GVW\,(9.85 \times 2.78)}$$

$$= \frac{1\,(5138.7174) + 14.3\,(467.5470)}{1\,(2.1112) + 14.3\,(27.383)}$$

$$\frac{C}{N} = 30$$

3.5.4 Making C: N 35

For making C: N 35 newspaper and green vegetable waste were required in the ratio of 1: 10.30 which depicts that 8.8495% of newspaper and 91.1504% of green vegetable waste were used for making that C: N 35. Total solids (TS) of 17.3189% while 86.8659% of volatile solids (VS) were required for making C: N 35. 13.9588 g of co-substrate mixture containing newspaper and green vegetable waste were required for making the organic loading of 2.1g VS. By using equation (3.2) C: N 35 were calculated.

$$\frac{C}{N} = \frac{NPW\,(94.25 \times 54.522) + GVW\,(9.85 \times 46.33)}{NPW\,(94.25 \times 0.0224) + GVW\,(9.85 \times 2.78)}$$

$$= \frac{1\,(5138.7174) + 10.30\,(467.5470)}{1\,(2.1112) + 10.30\,(27.383)}$$

$$\frac{C}{N} = 35$$

Figure 3.5: Lab scale batch type reactors setup at mesophilic temperature.

Figure 3.6: Lab scale batch type reactors setup at thermophilic temperature.

3.6 Optimization through response surface methodology

3.6.1 Central composite design (CCD)

Optimization of studied parameters were carried out through response surface methodology (RSM) and central composite design (CCD) was obtained by using Design Expert Version 12.0.3.0 software. Batch experiments were performed based on CCD with two factors, carbon to nitrogen (C/N) ratio and temperature, to find their effect on methane yield which was a dependent output response. The level of factors studied for optimization are presented in table 3.4. Four axial points coded in ± α and replication of three central point's provides estimation for the experimental error and measure of lack of fit factor resulting in a total number of 11 runs [21]. The response (specific bio-methane yield) was fitted using polynomial quadratic equation in order to correlate the response variable to the independent variable while second order polynomial coefficients were evaluated using the analysis of variance (ANOVA). Codified and real values for both factors are presented in table 3.4 Through determination coefficient, R^2 quality fit of the model equation was expressed and its statistical significance was determined by F test [23].

Table 3.4: Levels of factors selected for the central composite design.

Variables	Label	Level				
		-1.414 (-α)	-1	0	1	1.414 (+α)
X	C/N Ratio	15.8579	20	30	40	44.1421
Y	Temperature(degree Celsius)	30.8579	35	45	55	59.1421

3.6.2 Experimental setup

CCD experiments were performed at organic loading rate of $10gVSL^{-1}$ keeping the volatile solid (VS) substrate to inoculum ratio of 1:1 for 50 days in triplicate according to the method described by Wang et al. (2012) [70] and the experiment was conducted in laboratory glass bottle of 300mL capacity containing 20.8g of inoculum and 210 ml of working solution with a head space of 90 ml, head space was flushed with N_2 gas for creating anaerobic condition. To calculate the desired C/N ratios, mixing ratios of newspaper to green vegetable waste, 1:500,1:61.8, 1:14.3, 1:8, 1:6.74, were required to obtain C/N ratios of 15.86, 20, 30, 40, and 44.4 respectively. C/N ratios were theoretically calculated from the formula used for the manual optimization using TOC, TS and TKN of newspaper and green vegetable waste obtained previously from chemical analysis for preparing reactors [5]. On the basis of volatile solids (VS), reactors were loaded with respective amount of 35.19g, 21.91g, 15.85g, 12.58g and 11.62g respectively of mixing ratios of newspaper to green vegetable waste to obtain the total feed of $10gVSL^{-1}$. Reactors were tightly closed with rubber septa and screw caps and were shaken manually to stir the mixture present in the reactor for about 1 minute once a day prior to the measurement of biomethane.

Table 3.5: Codified and real values for C/N ratio & temperature in co-digestion.

Reactors	**Coded values**		**Real values**		**Specific methane yield (L/kgVS)**	
	C/N	**Temp.**	**C/N**	**Temp. (°C)**	**Actual**	**Predicted**
CCD-1	0	0	30	45	262.2	243
CCD-2	-1.414	0	15.86	45	130.2	109.49
CCD-3	1	-1	40	35	120.286	162.73
CCD-4	0	0	30	45	262.2	243
CCD-5	1.414	0	44.14	45	55.2	23.99
CCD-6	-1	-1	20	35	204.43	239.45
CCD-7	0	-1.414	30	30.86	342	297.98
CCD-8	1	1	40	55	34.2	51.10
CCD-9	0	1.414	30	59.14	125	117.10
CCD-10	-1	1	20	55	85.184	95.29
CCD-11	0	0	30	45	204.6	243

CCD= Central composite design

3.7 Biogas characterization

Volume of the biogas on daily basis were measured with the help of gas syringe [67]. Methane content in terms of percentage was analyzed with gas chromatograph (SHIMADZU GC-2010 Plus) with RT-Sieve 5A column (length of 30 m, diameter of 0.32mm) with thermal conductivity detector (TCD) [20].The temperature of injector, detector and column were Kept at 250,250 and 50°C. Injection volume was 1μL. Helium was used as a carrier gas with a linear velocity of 40cm/sec. For gas chromatography calibration gas standard consisting of 99 (V/V) % methane (CH_4) was used.

Figure 3.7: Gas chromatography used for biogas characterization.

Chapter # 04 Results and discussion

4.1 Manual optimization of selected parameters

C/N ratio and temperature are the significant factors in anaerobic co-digestion, because of its importance it is vital to optimize it for various substrates in order to achieve higher productivity and positive synergistic effect on the degradation of the individual substrates in the reactor [71]. Many previous studies suggested that optimum C/N ratios in anaerobic digesters lies between 20 and 30 [65] [72]. As shown in table 4.7 and 4.8 mesophilic reactors having C/N ratio 20:1 and 30:1 had highest specific bio-methane yield about fourfold higher than that from thermophilic reactors having C/N ratio of 20:1 and 30:1. Specific bio-methane yield was calculated by dividing specific biogas yield with the percentage of methane content. Table 4.1 and 4.2 illustrates the the cumulative biogas in terms of mL for the mesophilic and thermophilic reactors operated for 50 days, specific biogas yield of the reactors expressed in terms of L/kgVS are shown in table 4.3 and 4.4, table 4.5 and 4.6 depicts the methane content in terms of percentage of the reactors operating at mesophilic and thermophilic temperature, while table 4.7 and 4.8 described the specific bio-methane yield expressed in the unit of L/kgVS.

Many researchers conveyed that co-digestion of various organic waste caused improved biogas yield. Yeqing Li et al. (2019) revealed that methane yield of co-digestion of kitchen waste, corn stover and chicken manure was high compared to the individual digestion. A large synergistic effect was establish in the co-digestion process which is because of proper C/N ratio and minimum volatile fatty acids resulted in enhanced buffering capacity and boosting microorganisms for the degradation of the substrate to produce more methane yield [73].

Co-digestion of newspaper and green vegetable waste effectively balance C/N ratio of feed stock that benefits the methane yield. Mesophilic condition involves diversity of microorganisms which boosts the process of degradation and provide stability and kinetic advantage to the reactors for the co-digestion of newspaper and green vegetable waste. Elevated temperature caused reduction in the value of pH, which in turn, resulted in inhibition of methanogenesis leading towards the low specific bio-methane yield. As shown in table 4.7 mesophilic reactors having C/N ratio of 30:1 comprising of 93.45% of green vegetable waste and 6.54% of newspaper waste had highest specific bio-methane yield i.e., 401.8 L/kgVS as compared to thermophilic reactors.

Table 4.1: Cumulative biogas (mL) of reactor at mesophilic temperature.

Cumulative biogas (mL) of reactor at mesophilic temperature					
Days	**MR1 C/N=20**	**MR2 C/N=25**	**MR3 C/N=30**	**MR4 C/N=35**	**MC Control**
1	0	0	0	0	0
2	4	5	10	7	8
3	14	18	20	15	12
4	18	22	28	20	18
5	20	29	38	25	25
6	22	33	46	30	28
7	28	38	52	40	32
8	30	44	59	45	37
9	33	49	64	50	42
10	38	55	72	56	45
11	40	65	85	65	48
12	45	78	96	75	52
13	48	86	120	86	55
14	50	99	137	94	65
15	76	105	148	99	74
16	85	120	163	110	82
17	110	148	182	130	105
18	139	190	230	150	130
19	159	220	280	160	150
20	188	240	320	200	180
21	229	288	360	230	210
22	280	310	380	260	250

23	300	340	400	290	280
24	340	373	430	320	310
25	350	398	450	350	330
26	380	440	490	379	360
27	440	480	520	410	390
28	480	500	550	430	420
29	500	540	590	460	440
30	530	580	630	498	480
31	550	595	668	520	509
32	576	609	699	530	525
33	589	625	720	560	550
34	610	650	740	574	560
35	620	680	770	598	580
36	640	699	799	613	610
37	660	720	810	639	626
38	683	738	829	659	648
39	699	749	845	679	663
40	719	765	889	690	675
41	726	785	919	710	683
42	743	796	929	720	690
43	756	810	940	732	700
44	780	820	955	744	710
45	790	840	964	752	720
46	807	855	980	769	740
47	829	875	989	770	760
48	850	889	995	789	777
49	865	914	1010	800	784
50	880	935	1029	810	790

Table 4.2: Cumulative biogas of the reactors at thermophilic temperature.

Cumulative biogas of the reactors at thermophilic temperature					
Days	**TR1 C/N=20**	**TR2 C/N=25**	**TR3 C/N=30**	**TR4 C/N=35**	**TC control**
1	0	0	0	0	0
2	8	10	19	20	14
3	25	39	40	29	20
4	34	48	55	32	29
5	49	58	63	44	32
6	58	64	77	53	39
7	69	72	82	59	48
8	73	78	88	63	55
9	79	82	91	68	69
10	81	85	94	71	72
11	83	88	99	73	88
12	88	94	103	75	93
13	91	95	110	81	99
14	99	103	111	89	110
15	104	109	119	91	115
16	113	118	121	102	119
17	119	121	124	109	124
18	121	127	129	114	129
19	129	133	139	121	134
20	132	141	144	129	142
21	139	144	147	132	149
22	146	149	151	139	154
23	151	155	171	145	161
24	159	161	179	149	169
25	165	169	181	156	179
26	172	175	189	166	186
27	182	182	191	169	199
28	189	192	199	171	220
29	194	198	205	180	260
30	205	213	220	189	280
31	212	219	228	193	300
32	229	232	232	201	320

33	240	258	269	220	350
34	250	263	278	229	390
35	270	298	310	249	420
36	300	340	370	269	440
37	320	369	380	280	460
38	350	380	420	319	480
39	368	394	444	340	510
40	379	400	450	370	530
41	387	450	469	398	550
42	410	489	497	420	570
43	450	504	520	460	581
44	478	530	540	480	593
45	489	540	569	505	615
46	510	559	589	515	629
47	540	567	593	513	637
48	563	572	610	524	648
49	589	599	622	534	660
50	596	610	630	548	675

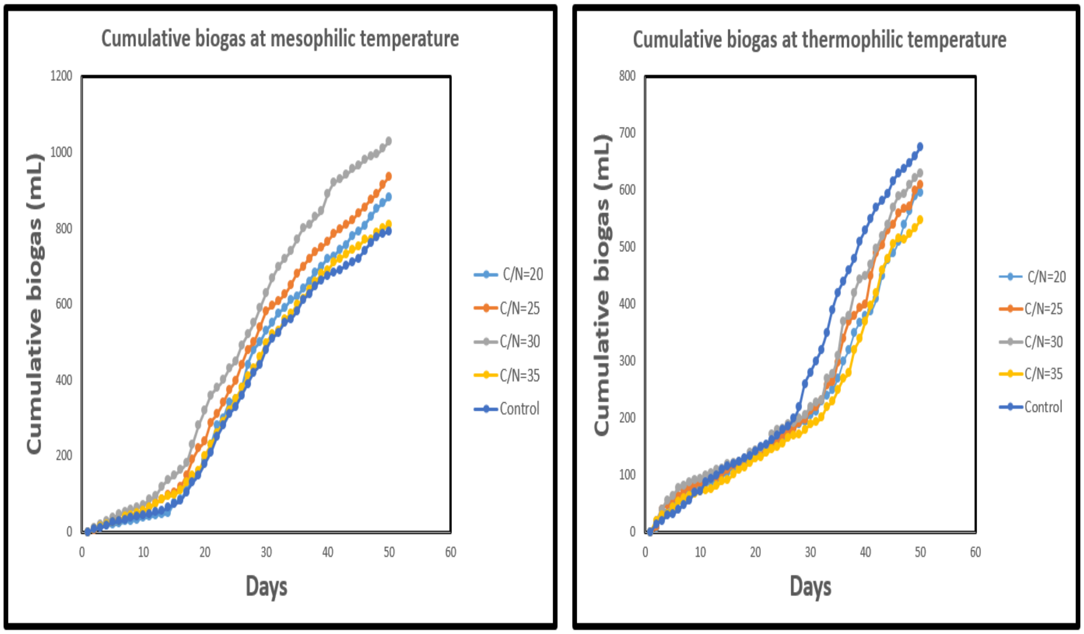


Figure 4.1: Cumulative biogas yield (mL) for mesophilic reactors (left) and thermophilic reactors (right).

Figure 4.1 explained cumulative biogas yield measured in mL of all the mesophilic and thermophilic reactors operated at various C/N ratios over a period of 50 days. Cumulative biogas yield for mesophilic reactors was high compared to thermophilic and was measured by plunger displacement method on daily basis. Initially rate of biogas production was low because microbes were not active to degrade the organic content to produce biogas but with the increase in number of days a smooth increasing trend of biogas were observed. Reactor MR3 having C/N 30 had highest degradation of organic content by microorganisms resulted in highest biogas yield while, in thermophilic rectors biogas production rate was high in initial days but with increase in number of days synergistic effect of microorganisms decreased caused low yield of biogas.

Table 4.3: Specific biogas yield (L/kgVS) of reactors at mesophilic temperature.

Specific biogas yield(L/kgVS) of reactors at mesophilic temperature					
Days	**MR1 C/N=20**	**MR2 C/N=25**	**MR3 C/N=30**	**MR4 C/N=35**	**MC control**
1	0	0	0	0	0
2	0.907048	2.380952	4.7619	3.333333	3.809524
3	6.666667	8.571429	9.52381	7.142857	5.714286
4	8.571429	10.47619	13.3333	9.52381	8.571429
5	9.52381	13.80952	18.0952	11.90476	11.90476
6	10.47619	15.71429	21.9048	14.28571	13.33333
7	13.33333	18.09524	24.7619	19.04762	15.2381
8	14.28571	20.95238	28.0952	21.42857	17.61905
9	15.71429	23.33333	30.4762	23.80952	20
10	18.09524	26.19048	34.2857	26.66667	21.42857
11	19.04762	30.95238	40.4762	30.95238	22.85714
12	21.42857	37.14286	45.7143	35.71429	24.7619
13	22.85714	40.95238	57.1429	40.95238	26.19048
14	23.80952	47.14286	65.2381	44.7619	30.95238

15	36.19048	50	70.4762	47.14286	35.2381
16	40.47619	57.14286	77.619	52.38095	39.04762
17	52.38095	70.47619	86.6667	61.90476	50
18	66.19048	90.47619	109.524	71.42857	61.90476
19	75.71429	104.7619	133.333	76.19048	71.42857
20	89.52381	114.2857	152.381	95.2381	85.71429
21	109.0476	137.1429	171.429	109.5238	100
22	133.3333	147.619	180.952	123.8095	119.0476
23	142.8571	161.9048	190.476	138.0952	133.3333
24	161.9048	177.619	204.762	152.381	147.619
25	166.6667	189.5238	214.286	166.6667	157.1429
26	180.9524	209.5238	233.333	180.4762	171.4286
27	209.5238	228.5714	247.619	195.2381	185.7143
28	228.5714	238.0952	261.905	204.7619	200
29	238.0952	257.1429	280.952	219.0476	209.5238
30	252.381	276.1905	300	237.1429	228.5714
31	261.9048	283.3333	318.095	247.619	242.381
32	274.2857	290	332.857	252.381	250
33	280.4762	297.619	342.857	266.6667	261.9048
34	290.4762	309.5238	352.381	273.3333	266.6667
35	295.2381	323.8095	366.667	284.7619	276.1905
36	304.7619	332.8571	380.476	291.9048	290.4762
37	314.2857	342.8571	385.714	304.2857	298.0952
38	325.2381	351.4286	394.762	313.8095	308.5714
39	332.8571	356.6667	402.381	323.3333	315.7143
40	342.381	364.2857	423.333	328.5714	321.4286
41	345.7143	373.8095	437.619	338.0952	325.2381

42	353.8095	379.0476	442.381	342.8571	328.5714
43	360	385.7143	447.619	348.5714	333.3333
44	371.4286	390.4762	454.762	354.2857	338.0952
45	376.1905	400	459.048	358.0952	342.8571
46	384.2857	407.1429	466.667	366.1905	352.381
47	394.7619	416.6667	470.952	366.6667	361.9048
48	404.7619	423.3333	473.81	375.7143	370
49	411.9048	435.2381	480.952	380.9524	373.3333
50	419.0476	445.2381	490	385.7143	376.1905

Table 4.4: Specific biogas yield (L/kgVS) of reactors at thermophilic temperature.

Specific biogas yield(L/kgVS) of reactors at thermophilic temperature					
Days	**TR1 C/N=20**	**TR2 C/N=25**	**TR3 C/N=30**	**TR4 C/N=35**	**TC control**
1	0	0	0	0	0
2	3.80952381	4.761904762	9.047619048	9.523809524	6.666666667
3	11.9047619	18.57142857	19.04761905	13.80952381	9.523809524
4	16.19047619	22.85714286	26.19047619	15.23809524	13.80952381
5	23.33333333	27.61904762	30	20.95238095	15.23809524
6	27.61904762	30.47619048	36.66666667	25.23809524	18.57142857
7	32.85714286	34.28571429	39.04761905	28.0952381	22.85714286
8	34.76190476	37.14285714	41.9047619	30	26.19047619
9	37.61904762	39.04761905	43.33333333	32.38095238	32.85714286
10	38.57142857	40.47619048	44.76190476	33.80952381	34.28571429
11	39.52380952	41.9047619	47.14285714	34.76190476	41.9047619
12	41.9047619	44.76190476	49.04761905	35.71428571	44.28571429
13	43.33333333	45.23809524	52.38095238	38.57142857	47.14285714
14	47.14285714	49.04761905	52.85714286	42.38095238	52.38095238
15	49.52380952	51.9047619	56.66666667	43.33333333	54.76190476

16	53.80952381	56.19047619	57.61904762	48.57142857	56.66666667
17	56.66666667	57.61904762	59.04761905	51.9047619	59.04761905
18	57.61904762	60.47619048	61.42857143	54.28571429	61.42857143
19	61.42857143	63.33333333	66.19047619	57.61904762	63.80952381
20	62.85714286	67.14285714	68.57142857	61.42857143	67.61904762
21	66.19047619	68.57142857	70	62.85714286	70.95238095
22	69.52380952	70.95238095	71.9047619	66.19047619	73.33333333
23	71.9047619	73.80952381	81.42857143	69.04761905	76.66666667
24	75.71428571	76.66666667	85.23809524	70.95238095	80.47619048
25	78.57142857	80.47619048	86.19047619	74.28571429	85.23809524
26	81.9047619	83.33333333	90	79.04761905	88.57142857
27	86.66666667	86.66666667	90.95238095	80.47619048	94.76190476
28	90	91.42857143	94.76190476	81.42857143	104.7619048
29	92.38095238	94.28571429	97.61904762	85.71428571	123.8095238
30	97.61904762	101.4285714	104.7619048	90	133.3333333
31	100.952381	104.2857143	108.5714286	91.9047619	142.8571429
32	109.047619	110.4761905	110.4761905	95.71428571	152.3809524
33	114.2857143	122.8571429	128.0952381	104.7619048	166.6666667
34	119.047619	125.2380952	132.3809524	109.047619	185.7142857
35	128.5714286	141.9047619	147.6190476	118.5714286	200
36	142.8571429	161.9047619	176.1904762	128.0952381	209.5238095
37	152.3809524	175.7142857	180.952381	133.3333333	219.047619
38	166.6666667	180.952381	200	151.9047619	228.5714286
39	175.2380952	187.6190476	211.4285714	161.9047619	242.8571429
40	180.4761905	190.4761905	214.2857143	176.1904762	252.3809524
41	184.2857143	214.2857143	223.3333333	189.5238095	261.9047619
42	195.2380952	232.8571429	236.6666667	200	271.4285714
43	214.2857143	240	247.6190476	219.047619	276.6666667
44	227.6190476	252.3809524	257.1428571	228.5714286	282.3809524
45	232.8571429	257.1428571	270.952381	240.4761905	292.8571429

46	242.8571429	266.1904762	280.4761905	245.2380952	299.5238095
47	257.1428571	270	282.3809524	244.2857143	303.3333333
48	268.0952381	272.3809524	290.4761905	249.5238095	308.5714286
49	280.4761905	285.2380952	296.1904762	254.2857143	314.2857143
50	283.8095238	290.4761905	300	260.952381	321.4285714

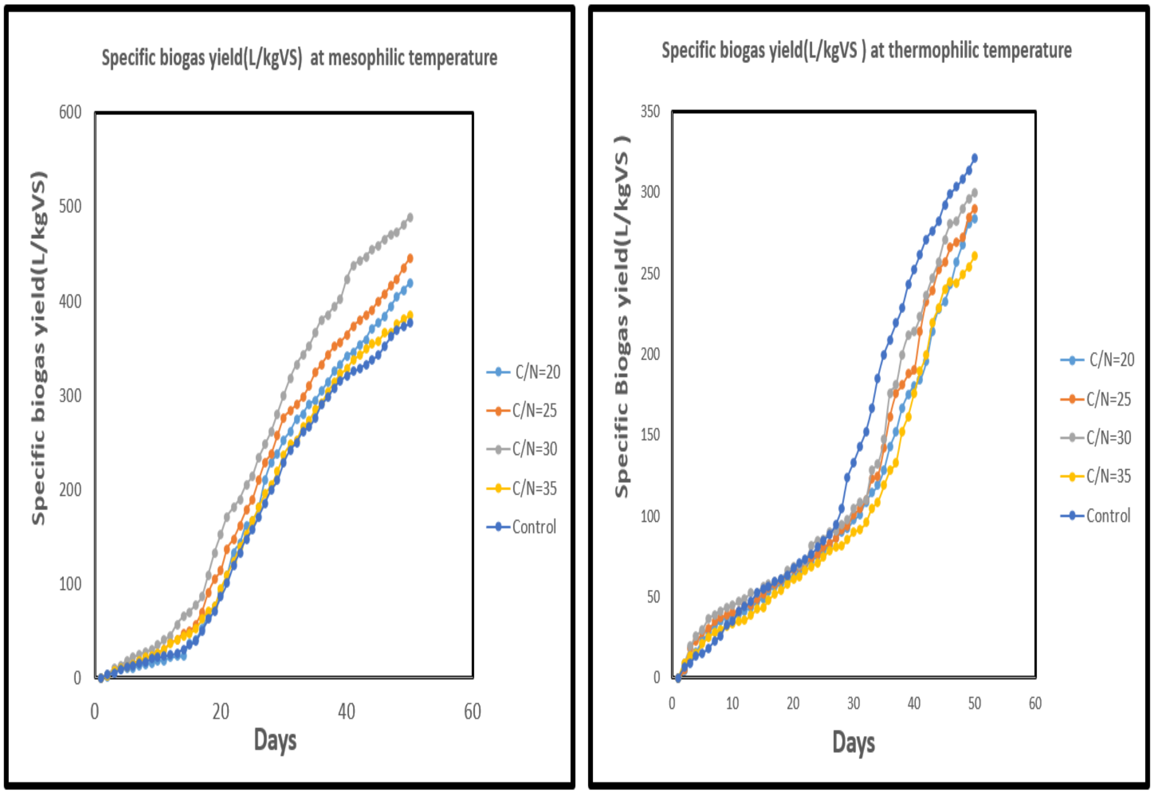


Figure 4.2: Specific biogas yield (mL) for mesophilic reactors (left) and thermophilic reactors (right).

Figure 4.2 illustrates specific biogas yield and was calculated by dividing cumulative biogas yield with volatile solid added to the reactor. In mesophilic reactor highest specific biogas yield of 490 mL was obtained from MR3 while lowest specific biogas yield of 385.7143 mL was obtained from MR4, while 376.1905 mL was obtained from control having inoculum only. Reactor MR3 had 30% more specific biogas yield while reactor MR4 had 2.5316% more specific biogas yield in comparison with control which showed that as the C/N ratio increased specific biogas yield increased then start decreasing. In thermophilic rector control had high specific biogas yield as compared to thermophilic reactors. Highest specific biogas yield of 300mL was obtained from thermophilic reactor TR3 which was 6.667 % less than specific biogas yield obtained from control depicts that thermophilic rector had unfavorable environment for the microorganisms for the degradation of substrate resulted in unstable biogas production.

Table 4.5: Methane content (%) of the reactors at mesophilic temperature.

Methane content (%) of reactors at mesophilic temperature					
Days	MR1 C/N=20	MR2 C/N=25	MR3 C/N=30	MR4 C/N=35	MC control
1	0	0	0	0	0
2	0.9	0.85	0.95	0.55	0.19
3	0.95	0.9	0.98	0.7	0.25
4	0.95	0.95	1.1	0.85	0.5
5	1.5	1.8	2	0.93	0.7
6	1.9	2	2.5	1	0.9
7	2.2	2.2	3	1.5	1
8	2.9	3.1	4.5	1.7	1.3
9	3.5	3.9	6	1.9	1.7
10	3.9	4.6	8	2.5	2.6
11	5	5.5	10	3	2.9
12	6.5	6.9	12	3.6	3.2
13	7.2	7.9	13	4	3.4
14	8.9	9	14.5	5	4.9
15	9.7	10	16	5.5	5
16	10.5	12	18	6	6
17	11.8	12.9	20	7	6.5
18	12.8	13.6	22	8	7.2
19	14	15	25	9	8
20	18	19	28	12	8.9
21	22	24	30	14	10

22	25	28	34	16	14
23	28	32	38	17	15
24	32	37	44	19	18
25	36	39.9	48	22	20
26	38	43	52	24	22
27	40	45	54	28	25
28	42	47	58	31	28
29	42.9	48	62.7	34	30
30	43.8	48.9	65	36	31
31	45	51	66	38	32
32	47	51.9	66.7	40	33
33	48	52.7	67.6	41.9	34
34	48.7	54	68.3	42.4	35.5
35	49	56	69	43.9	36.6
36	50	56.8	71	44.2	37
37	51	57.8	72	45	37.9
38	51.9	59	73.6	46	38.8
39	52.7	61	74.9	47	39.2
40	53	61.6	75.8	47.5	40
41	54	62.8	76.3	48.8	41
42	55	63.4	77	49.4	42.9
43	56	64.9	78	50.1	44
44	58	65.4	78.5	51.2	45
45	59	66.5	79	52.5	46
46	59.9	66.9	79.4	53.9	47
47	60.3	67	79.9	54.4	48
48	61.8	68.2	80.5	54.9	49
49	62	69.8	81.6	55.9	50
50	63	70	82	56	51

Table 4.6: Methane content (%) of reactors at thermophilic temperature.

Methane content (%) of reactors at thermophilic temperature					
Days	**TR1 C/N=20**	**TR2 C/N=25**	**TR3 C/N=30**	**TR4 C/N=35**	**TC control**
1	0	0	0	0	0
2	0.2	0.4	0.9	0.1	0.5
3	0.3	0.62	1.3	0.16	0.6
4	0.5	0.95	1.4	0.85	1
5	1.5	1.8	2	0.93	1.6
6	1.9	2	2.5	1	2
7	2.2	2.9	3	1.5	2.6
8	2.9	3.6	4.5	1.9	2.9
9	3.2	3.9	5.3	2.3	3.5
10	3.9	4.6	5.9	2.5	3.9
11	4.4	4.9	6.3	3.1	4.2
12	4.6	5.3	6.8	3.7	4.9
13	5.7	6.3	7.3	4.6	5.2
14	6.4	6.9	7.8	4.9	5.5
15	6.6	7.3	8.1	5.2	5.9
16	6.8	7.3	8.8	5.3	6.1
17	6.9	7.4	9.3	5.9	6.3
18	7.2	7.6	9.7	5.9	6.7
19	7.4	7.9	10.1	6.2	6.9
20	8	8.2	10.4	6.7	7.2
21	8.1	8.5	10.7	6.9	9
22	8.7	8.9	11.2	7.4	10
23	9.8	10	12	8.2	11.6
24	10.5	10.9	12.5	8.9	12.2
25	11.2	11.4	12.9	9.3	13.6
26	11.8	12	13.4	10.5	14.7

27	12.6	13	14.5	11	15.8
28	13	13.6	14.9	11.5	16.5
29	13.7	14.7	15.4	12.7	18.4
30	14.3	15.4	16.3	13.2	19.6
31	14.9	16.3	17.6	13.9	20.8
32	15.4	17.1	18.5	14.4	21.6
33	16.2	17.7	19.3	15.5	22.9
34	17.2	18.3	19.8	16.2	24.3
35	18.1	19.5	20.4	16.9	25.3
36	18.5	20.3	21.3	17.4	26
37	19.3	21.1	22.4	18.2	26.5
38	20.5	22.4	23.6	19.1	27.4
39	21.6	22.9	24.6	20.3	27.9
40	22.7	23.7	25.7	20.9	28.4
41	23.4	24.8	26.3	21.5	29
42	24.1	25.2	27.1	21.9	29.4
43	25.1	25.9	27.7	22.4	30.2
44	26.4	26.6	28.8	23.5	31.1
45	27.1	28.1	29.6	24.1	32.1
46	27.9	28.7	30.7	24.6	32.9
47	28.7	29.4	31.4	25.5	33.5
48	29	30.1	31.9	26.8	34.6
49	29.6	30.8	32.6	27.4	35.3
50	30.4	31.7	33.7	28	36

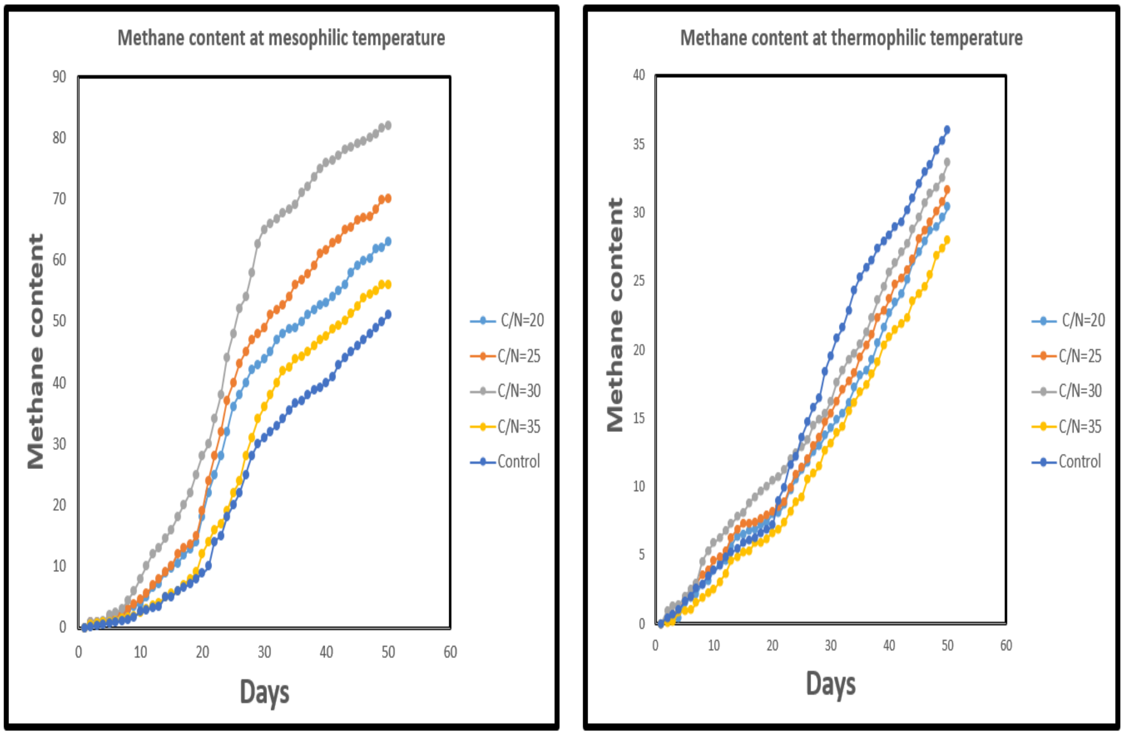


Figure. 4.3 Methane content (%) for mesophilic reactors (left) and thermophilic reactors (right)

Figure. 4.3 showed the methane content in terms of percentage for mesophilic and thermophilic reactors and was analyzed with gas chromatograph and for gas chromatography calibration gas standard consisting of 99 (V/V) % methane (CH_4) was used. Methane content was very low in the starting days because of hydrolysis, acidogenesis and acetogenesis phase, hydrolysis is the slowest phase of anaerobic co-digestion in which fermentative bacteria break down the substrate in to monomers that could be digestible for the microbes to produce hydrogen, methanogen are the microorganisms that produce methane and comes in the last phase of anaerobic co-digestion i.e., methanogenesis. Methane content of reactor MR3 was highest among all the mesophilic and thermophilic reactors which means bacterial microflora of the reactor MR3 was better for the degradation of the substrate.

Table 4.7: Specific biomethane yield (L/kgVS) of the reactors at mesophilic temperature.

Specific biomethane yield (L/kgVS) of reactors at mesophilic temperature					
Days	**MR1 C/N=20**	**MR2 C/N=25**	**MR3 C/N=30**	**MR4 C/N=35**	**MC control**
1	0	0	0	0	0
2	0.008163429	0.020238095	0.045238095	0.018333333	0.007238095
3	0.063333333	0.077142857	0.093333333	0.05	0.014285714
4	0.081428571	0.09952381	0.146666667	0.080952381	0.042857143
5	0.142857143	0.248571429	0.361904762	0.110714286	0.083333333
6	0.199047619	0.314285714	0.547619048	0.142857143	0.12
7	0.293333333	0.398095238	0.742857143	0.285714286	0.152380952
8	0.414285714	0.64952381	1.264285714	0.364285714	0.229047619
9	0.55	0.91	1.828571429	0.452380952	0.34
10	0.705714286	1.204761905	2.742857143	0.666666667	0.557142857
11	0.952380952	1.702380952	4.047619048	0.928571429	0.662857143
12	1.392857143	2.562857143	5.485714286	1.285714286	0.792380952
13	1.645714286	3.235238095	7.428571429	1.638095238	0.89047619
14	2.119047619	4.242857143	9.45952381	2.238095238	1.516666667
15	3.51047619	5	11.27619048	2.592857143	1.761904762
16	4.25	6.857142857	13.97142857	3.142857143	2.342857143
17	6.180952381	9.091428571	17.33333333	4.333333333	3.25
18	8.472380952	12.3047619	24.0952381	5.714285714	4.457142857
19	10.6	15.71428571	33.33333333	6.857142857	5.714285714
20	16.11428571	21.71428571	42.66666667	11.42857143	7.628571429
21	23.99047619	32.91428571	51.42857143	15.33333333	10
22	33.33333333	41.33333333	61.52380952	19.80952381	16.66666667
23	40	51.80952381	72.38095238	23.47619048	20

24	51.80952381	65.71904762	90.0952381	28.95238095	26.57142857
25	60	75.62	102.8571429	36.66666667	31.42857143
26	68.76190476	90.0952381	121.3333333	43.31428571	37.71428571
27	83.80952381	102.8571429	133.7142857	54.66666667	46.42857143
28	96	111.9047619	151.9047619	63.47619048	56
29	102.1428571	123.4285714	176.1571429	74.47619048	62.85714286
30	110.5428571	135.0571429	195	85.37142857	70.85714286
31	117.8571429	144.5	209.9428571	94.0952381	77.56190476
32	128.9142857	150.51	222.0157143	100.952381	82.5
33	134.6285714	156.8452381	231.7714286	111.7333333	89.04761905
34	141.4619048	167.1428571	240.6761905	115.8933333	94.66666667
35	144.6666667	181.3333333	253	125.0104762	101.0857143
36	152.3809524	189.0628571	270.1380952	129.0219048	107.4761905
37	160.2857143	198.1714286	277.7142857	136.9285714	112.9780952
38	168.7985714	207.3428571	290.5447619	144.352381	119.7257143
39	175.4157143	217.5666667	301.3833333	151.9666667	123.76
40	181.4619048	224.4	320.8866667	156.0714286	128.5714286
41	186.6857143	234.752381	333.9033333	164.9904762	133.347619
42	194.5952381	240.3161905	340.6333333	169.3714286	140.9571429
43	201.6	250.3285714	349.1428571	174.6342857	146.6666667
44	215.4285714	255.3714286	356.9880952	181.3942857	152.1428571
45	221.952381	266	362.647619	188	157.7142857
46	230.1871429	272.3785714	370.5333333	197.3766667	165.6190476
47	238.0414286	279.1666667	376.2909524	199.4666667	173.7142857
48	250.1428571	288.7133333	381.4166667	206.2671429	181.3
49	255.3809524	303.7961905	392.4571429	212.952381	186.6666667
50	264	311.6666667	401.8	216	191.8571429

Table 4.8: Specific biomethane yield (L/kgVS) of the reactors at thermophilic temperature.

Specific biomethane yield(L/kgVS) of reactors at thermophilic temperature					
Days	**TR1 C/N=20**	**TR2 C/N=25**	**TR3 C/N=30**	**TR4 C/N=35**	**TC control**
1	0	0	0	0	0
2	0.007619048	0.019047619	0.081428571	0.00952381	0.033333333
3	0.035714286	0.115142857	0.247619048	0.022095238	0.057142857
4	0.080952381	0.217142857	0.366666667	0.12952381	0.138095238
5	0.35	0.497142857	0.6	0.194857143	0.243809524
6	0.524761905	0.60952381	0.916666667	0.252380952	0.371428571
7	0.722857143	0.994285714	1.171428571	0.421428571	0.594285714
8	1.008095238	1.337142857	1.885714286	0.57	0.75952381
9	1.203809524	1.522857143	2.296666667	0.744761905	1.15
10	1.504285714	1.861904762	2.640952381	0.845238095	1.337142857
11	1.739047619	2.053333333	2.97	1.077619048	1.76
12	1.927619048	2.372380952	3.335238095	1.321428571	2.17
13	2.47	2.85	3.823809524	1.774285714	2.451428571
14	3.017142857	3.384285714	4.122857143	2.076666667	2.880952381
15	3.268571429	3.789047619	4.59	2.253333333	3.230952381
16	3.659047619	4.101904762	5.07047619	2.574285714	3.456666667
17	3.91	4.263809524	5.491428571	3.062380952	3.72
18	4.148571429	4.596190476	5.958571429	3.202857143	4.115714286
19	4.545714286	5.003333333	6.685238095	3.572380952	4.402857143
20	5.028571429	5.505714286	7.131428571	4.115714286	4.868571429
21	5.361428571	5.828571429	7.49	4.337142857	6.385714286
22	6.048571429	6.314761905	8.053333333	4.898095238	7.333333333
23	7.046666667	7.380952381	9.771428571	5.661904762	8.893333333
24	7.95	8.356666667	10.6547619	6.314761905	9.818095238

25	8.8	9.174285714	11.11857143	6.908571429	11.59238095
26	9.664761905	10	12.06	8.3	13.02
27	10.92	11.26666667	13.18809524	8.852380952	14.97238095
28	11.7	12.43428571	14.11952381	9.364285714	17.28571429
29	12.65619048	13.86	15.03333333	10.88571429	22.78095238
30	13.95952381	15.62	17.07619048	11.88	26.13333333
31	15.04190476	16.99857143	19.10857143	12.7747619	29.71428571
32	16.79333333	18.89142857	20.43809524	13.78285714	32.91428571
33	18.51428571	21.74571429	24.72238095	16.23809524	38.16666667
34	20.47619048	22.91857143	26.21142857	17.66571429	45.12857143
35	23.27142857	27.67142857	30.11428571	20.03857143	50.6
36	26.42857143	32.86666667	37.52857143	22.28857143	54.47619048
37	29.40952381	37.07571429	40.53333333	24.26666667	58.04761905
38	34.16666667	40.53333333	47.2	29.01380952	62.62857143
39	37.85142857	42.9647619	52.01142857	32.86666667	67.75714286
40	40.96809524	45.14285714	55.07142857	36.82380952	71.67619048
41	43.12285714	53.14285714	58.73666667	40.74761905	75.95238095
42	47.05238095	58.68	64.13666667	43.8	79.8
43	53.78571429	62.16	68.59047619	49.06666667	83.55333333
44	60.09142857	67.13333333	74.05714286	53.71428571	87.82047619
45	63.10428571	72.25714286	80.20190476	57.9547619	94.00714286
46	67.75714286	76.39666667	86.10619048	60.32857143	98.54333333
47	73.8	79.38	88.66761905	62.29285714	101.6166667
48	77.74761905	81.98666667	92.66190476	66.87238095	106.7657143
49	83.02095238	87.85333333	96.55809524	69.67428571	110.9428571
50	86.27809524	92.08095238	101.1	73.06666667	115.7142857

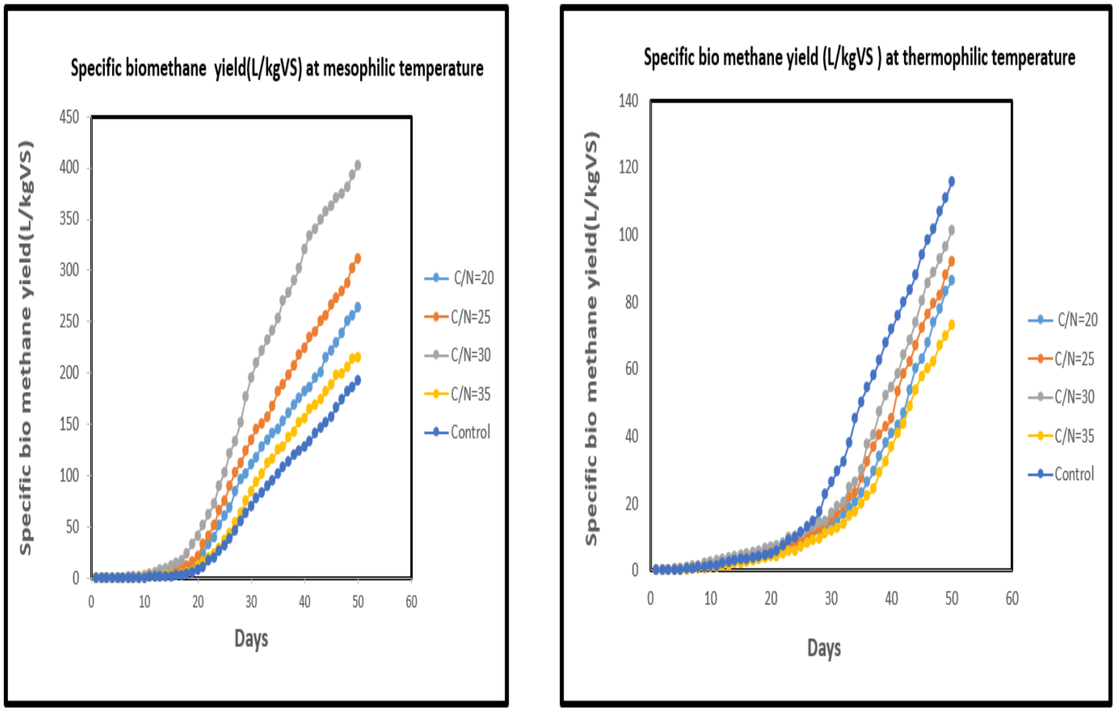


Figure: 4.4 Specific bio-methane yield (L/kgVS) for mesophilic reactors (left) and thermophilic reactors (right).

Figure 4.4 depicts the specific biomethane yield expressed in L/kgVS and was calculated by multiplying specific biogas yield with percentage of methane content. Sustainable operation of anaerobic co-digestion requires effective temperature for microorganisms to decompose the organic material to produce bio-methane. Microbial growth can be affected with temperature variation that will reduce the bio-methane yield in a considerable way[74]. Mesophilic condition involves diversity of microorganisms which boosts the process of degradation and provide stability and kinetic advantage to the reactors for the co-digestion of newspaper and green vegetable waste. Maximum specific bio-methane yield i.e., 401.8 L/kgVS was obtained from the reactor MR3 operated at 37^{o}C, while lowest specific bio-methane 73.0666 L/kgVS was obtained from the reactor TR4 operated at 55^{o}C which confirmed that methanogenic activity was more favorable at mesophilic temperature.

Related work has been carried out by many researchers. K.komemoto et al. (2009) studied the effect of temperature on solubilization and acidogenesis of food waste. Highest solubilization rate of food waste 70.0% and 72.7% based on suspended solid removal was obtained at 35^{o}C and 45^{o}C while biogas production was 64.7 and 62.7 mL/gVS at 35^{o}C and 45^{o}C depicting solubilization of food waste was higher at mesophilic temperature rather than thermophilic. [75].

Table 4.9: Performance of digesters at different C/N ratios and temperature

Reactors	Specific biogas yield (L/KgVS)	Specific bio methane yield (L/KgVS)	Initial pH	Final pH	TS Reduction (%)	VS Reduction (%)
MR1	419.048	264	7.33	7.64	52	65
MR2	445.238	311.667	7.34	7.22	58	68
MR3	490	401.8	7.22	7.36	72	85
MR4	385.714	216	7.2	7.12	65	72
MC	376.191	191.8571	7.11	7.23	49	59
TR1	283.81	86.278	7.10	6.555	24	31
TR2	290.476	92.081	7.16	6.345	36	39
TR3	300	101.1	7.04	6.186	44	50
TR4	260.952	73.066	6.75	6.289	42	47
TC	321.4285	115.714	6.97	6.925	46	52

Overall performance of the reactors of manual optimization were shown in table 4.9. The reactors with higher C/N ratios having higher fraction of newspaper waste resulted less specific bio-methane yield in the present study. Higher specific bio-methane yield at C/N 30 may be credited to higher proportion of green vegetable and low proportion of newspaper waste. These results are in accordance with other studies where synergistic effect for co-digestion of substrates increased because of improved C/N ratio [71]. At higher C/N ratios methanogens rapidly consumed the nitrogen, making unfavorable conditions for the methanogens resulted in lower yield of biomethane.

E. Saanchez et al. (2000) studied the influence of temperature and pH on the kinetics of methane production, organic nitrogen and phosphorus removal from cattle manure. They found that at mesophilic temperature methane production 2.3 times improved when the pH of the influent was elevated from 7.0 to 7.6, while increase in operating temperature from 35°C to 60°C caused decrease in kinetic constants of methane production. Major inhibition factors such as increased organic nitrogen removal and ammonia nitrogen production was reported at thermophilic temperature (60^0C) [76].

4.2 Results of response surface methodology

4.2.1 ANOVA of the fitted Model

Experimental and predicted results of specific bio-methane yield were illustrated in table 3.5. Specific bio-methane was calculated by dividing specific biogas yield with the percentage of methane content and were subjected to the response analysis to evaluate the effect of the operating parameters. Analysis of variance (ANOVA) determines the significance of the quadratic model and by applying multiple regression analysis, the results were fitted to a second - order polynomial equation. Summary of the ANOVA is shown in the table 4.10, whereas second order polynomial equation for bio-methane yield fitted in terms of coded factors was obtained as follow:

$$Y=243-30.23A-63.95B+8.31AB-88.13A^2-17.73B^2 \qquad (4.1)$$

Where Y was bio-methane yield, A was (C/N) ratio, B was temperature.

Table 4.10: Analysis of variance of the model.

Source	Df	Sum of squares	Mean squares	F-Value	p-Value
Model	5	84563.74	16912.75	9.38	0.0141
A	1	7309.86	7309.86	4.05	0.1002
B	1	32715.07	32715.07	18.14	0.0080
AB	1	264.55	264.55	0.1467	0.7175
A^2	1	43859.50	43859.50	24.32	0.0044
B^2	1	1775.04	1775.04	0.9842	0.3667
R^2	-	0.9036	-	-	-
Adjusted R^2	-	0.8073	-	-	-
CV	-	25.58	-	-	-
Lack of fit	3	6806.21	2268.74	2.05	0.3443

Many researchers have also used RSM and CCD model for estimating the optimum conditions for bio-gas production. Wang et al. (2007) used RSM and CCD for designing the experiments, in order to determine the optimum conditions for methane fermentation for evaluating the individual effect and interactive effects of substrate concentration, ratio of inoculum to substrate and Ca^{++2} concentration of effluent of bio-hydrogen fermentation of food waste. Results of the experiment based on projected optimum conditions ratified that RSM was useful for optimizing the methane yield from effluent of bio hydrogen fermentation of food waste [20].

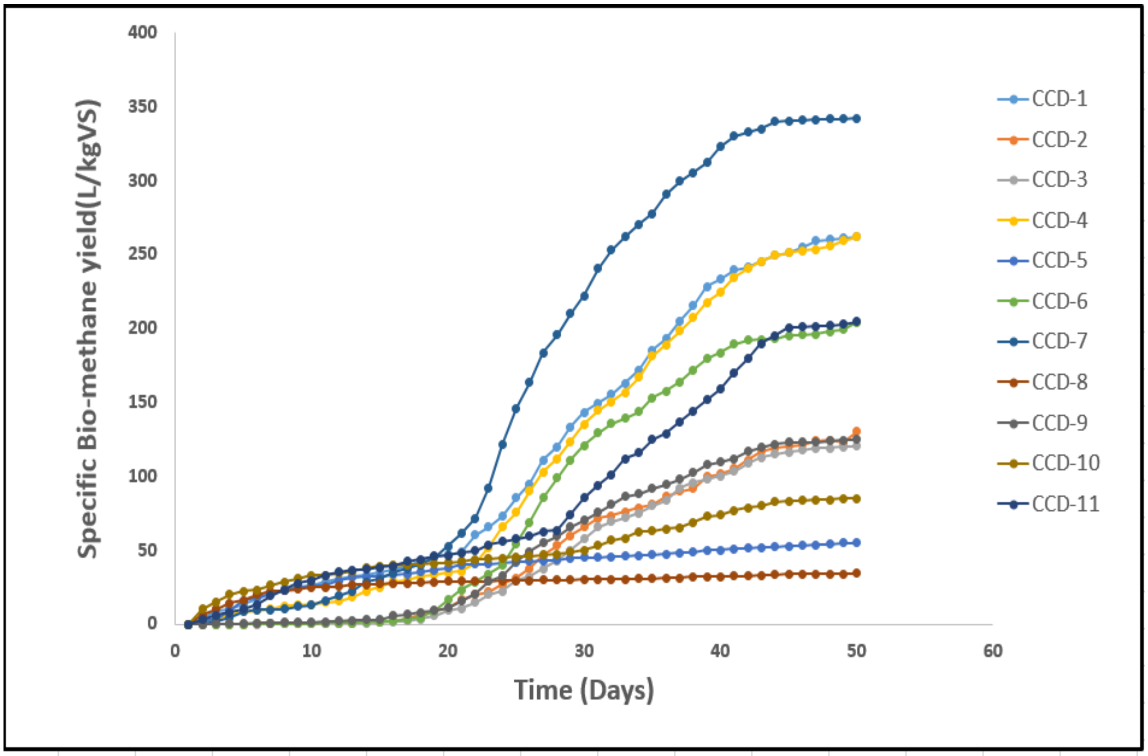


Figure 4.5: Specific bio-methane yield (L/kgVS) of CCD reactors.

Specific bio-methane yield for all the CCD-reactors are presented in figure. 4.5. Specific bio-methane for the CCD-rectors are 262.2, 130.2, 120.286, 262.2, 55.2, 204.3, 342, 34.2, 125, 85.184 and 204.6 L /kgVS. In the first 20 days organic content undergoes acetogenesis phase which decreases the pH of the reactors as shown in Fig.5 after that methanogens rapidly consume those acids produced in the acetogenesis phase increasing the pH of the reactors and stabilizing the reactor performance. Specific bio-methane yield of CCD-7 was higher from all the reactors having C/N ratio 30 at 30.86. Specific bio-methane yield decreased with increase in temperature and C/N ratio, which indicates that microbial activity to degrade the organic content was lowest, at high temperature the growth of microorganisms becomes stagnant which inhibits the degradation process resulting in lesser specific biomethane yield.

Actual and predicted values presented in figure 4.6 confirmed that second order polynomial model fitted the experimental results corresponding to the methane yield quite well. The model F-value of 9.38 implies that model was significant and there was only a 1.41% chance that a 'model F- value' could occur due to noise. p-values less than 0.05 indicated the significant model terms. Regression analysis of the experimental design illustrates that the linear model term B is 0.0080 that is less than 0.05 which means that linear model term B is significant. It may be considered coefficients were extremely significant and had a comparatively high impact on dependent variables. The p-value of interactive model term AB is 0.7175 which is greater than 0.05 that means model term AB is insignificant. The value of the R^2 coefficient obtained 0.9036 showed that the majority of data are explained by the model. The 'lack of fit F-value' of 2.05 indicates the lack of fit is not significant relative to the pure error having s 34.43% of chance that a lack of fit F-value could occur due to noise. Non-significant lack of fit is good which means model is fit.

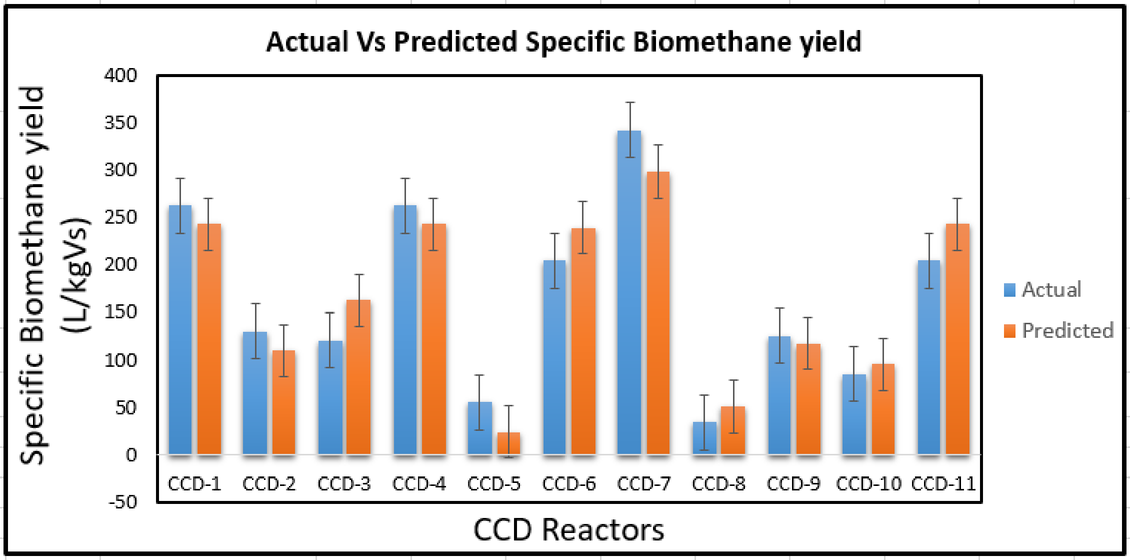


Figure 4.6: Actual and predicted specific bio-methane yield for CCD (Central composite design) reactors.

Figure 4.7 shows the relationship between the two independent variables C/N ratio and temperature on bio-methane yield in the form of three dimensional response surface plot depicting significant effect of C/N ratio on methane potential occurs at mesophilic temperature range (20-41^{o}C) as compared to thermophilic temperature range (41-60^{o}C). As the C/N ratio increased from 20 to 30, bio-methane yield improved, after that yield was not stable, it started declining. The potential reason for this reduction in the yield of bio-methane may be that at higher C/N ratios methanogens rapidly consumed nitrogen which distrubs the anaerobic environment which became the reason for low yield of bio-methane [42]. Methanogens have favouable conditions at mesophlic temperature range that leads to high bio-methane yield. Methane yield was high and to some extent remained consistent when C/N ratio was 30 and temperature was 30.86^{o}C. Highest specific bio-methane yield was estimated as 342 L/kgVS.

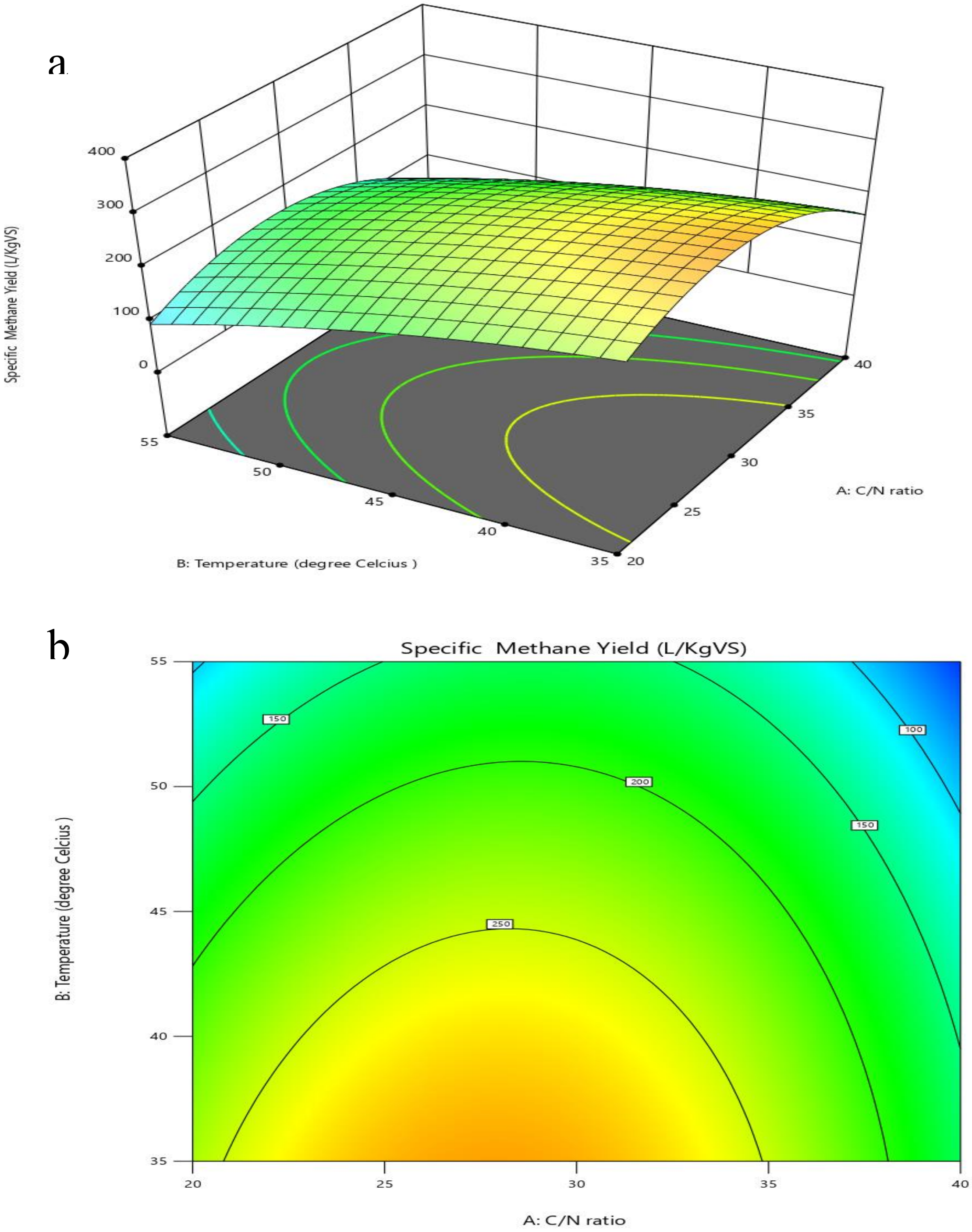


Figure 4.7: Response surface (a) and contour plot (b) for specific bio-methane yield depending on variation of temperature and C/N ratios.

As shown in table 3.5 CCD reactors having C/N ratio 20:1 and 30:1 had highest specific bio-methane yield about tenfold higher than that from CCD reactors having C/N ratio of 15:1 and 40:1. Related work has been carried out by many researchers. Xiaojiao et al. (2012) studied optimizing feeding composition and C/N ratio of multiple substrates which includes dairy manure (DM), chicken manure (CM) and wheat straw (WS) for the methane potential. They found that maximum methane potential was accomplished with DM/CM of 40.5:59.7 having C/N ratio 27.2:1 using response surface methodology, C/N ratios of 25:1 and 30:1 had enhanced digestion performance with stable pH and low concentrations of total ammonium nitrogen and free NH_3[65].

Co-digestion of newspaper and green vegetable waste effectively balance C/N ratio of feed stock that benefits the bio-methane yield. As shown in table 3.5 CCD-7 having C/N ratio of 30:1 comprising of 93.45% of green vegetable waste and 6.54% of newspaper waste had highest specific biomethane yield i.e., 342 L/kgVS whereas CCD-8 having C/N of 40:1 comprising 88.88% of green vegetable waste and 11.11% of newspaper waste has lowest specific bio-methane yield i.e., 34.2 L/kgVS. Newspaper waste have less biodegradability and nitrogen content because of having lingocellulosic content. Methanogens difficulty digest lingocellulosic content which makes the digestion process lengthy and time consuming [42]. Bio-methane potential of such waste enhanced when it was mixed with waste having high nitrogen content like green vegetable waste, which is supported by the result of this study.

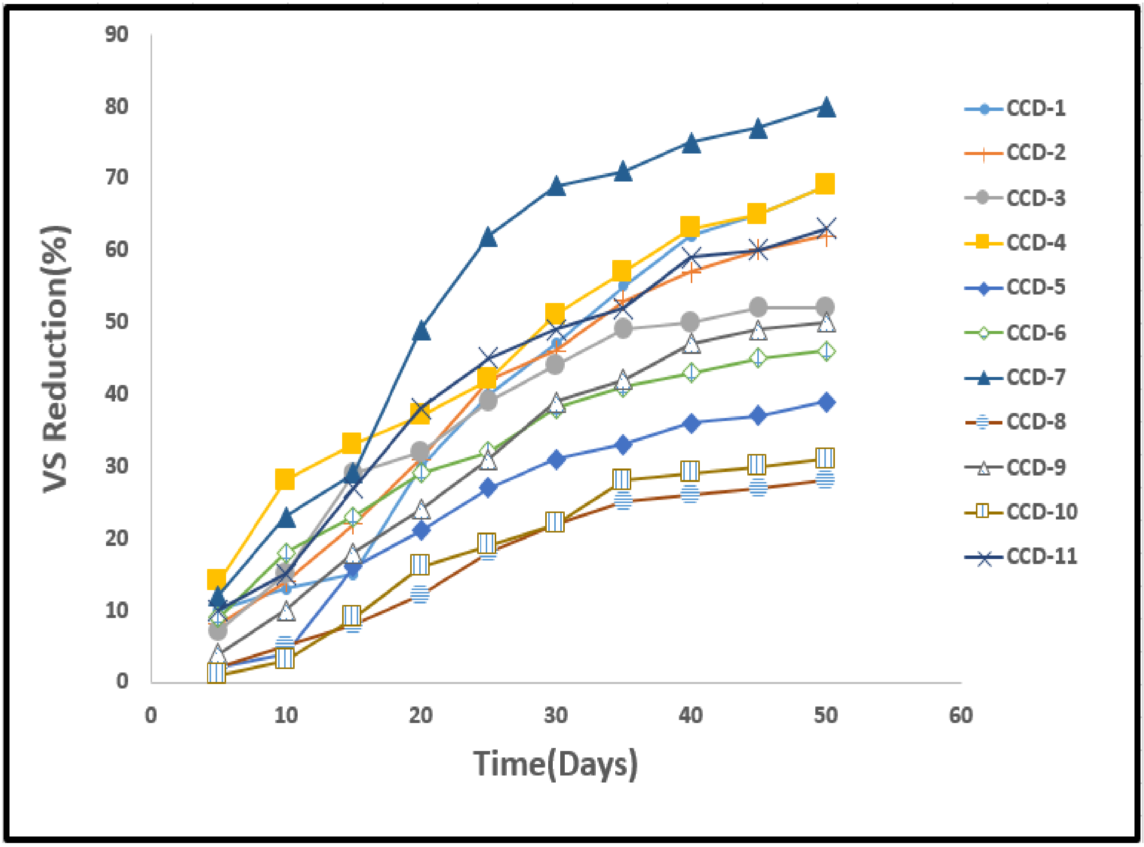


Figure 4.8: VS reduction (%) for the CCD-reactors operated at different C/N ratio and temperature.

Momoh et al. (2011) reported that addition of paper waste by co-digesting fixed amount of cow dung and water hyacinth in digesters B-E at room temperature improves the biogas yield while digester A acting as the control. The maximum biogas production for digesters B, C, D and E was estimated to be 0.282, 0.262, 0.233 and 0.2176 Lg^{-1}VS fed respectively. They observed that addition of paper waste to fixed amount of cow dung and water hyacinth improve biogas production [77].

Overall performance of the CCD reactors were shown in table 4.11. The maximum VS reduction (80%) was obtained from the reactor CCD-7 operated at 30.86^{o}C resulted in highest specific bio-methane yield of 342L/kgVS, while lowest VS reduction (28%) was obtained from the reactor CCD-8 operated at 55^{0}C resulted in lowest specific bio-methane of 34.2 L/kgVS as shown in figure 4.8. Elevated temperature caused reduction in the value of pH, which in turn, resulted in inhibition of methanogenesis leading towards the low specific bio-methane yield. Methanogenic activity occurs at pH values between 6.2-8, while the optimum range of pH lies between 7.0-7.2 [65].

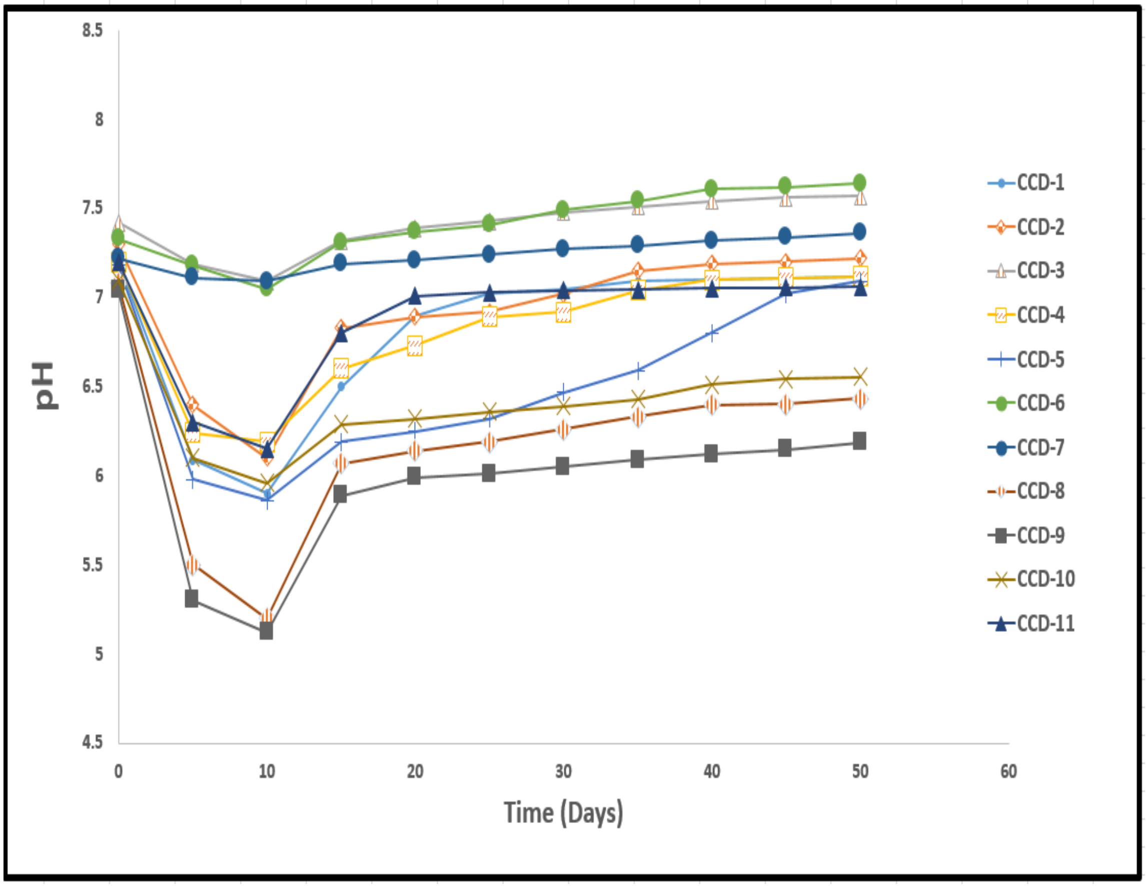


Figure 4.9: pH values of the CCD-reactors operated at different temperatures and C/N ratios.

Reactor CCD-7 having initial pH of 7.22 and final pH of 7.36 produced maximum specific bio-methane yield revealed that methanogenic activity was significant while reactor CCD-8 operated at thermophilic temperature of 55°C having initial pH of 7.08 drops to the value of 6.4 revealed that methanogenic activity was inhibited because of accumulation of volatile fatty acids as shown in figure 4.9.

Table 4.11: Performance of CCD reactors at different C/N ratios and temperature

Reactors	Specific bio methane yield (L/kgVS)	Initial pH	Final pH	TS Reduction (%)	VS Reduction (%)
CCD-1	262.2	7.2	7.12	60	69
CCD-2	130.2	7.3	7.22	55	62
CCD-3	120.286	7.42	7.57	49	52
CCD-4	262.2	7.2	7.12	60	69
CCD-5	55.2	7.18	7.09	36	39
CCD-6	204.3	7.33	7.64	42	46
CCD-7	342	7.22	7.36	72	80
CCD-8	34.2	7.08	6.432	20	28
CCD-9	125	7.04	6.186	44	50
CCD-10	85.184	7.10	6.555	24	31
CCD-11	204.6	7.2	7.06	52	63

CCD= central composite design, TS= total solids, VS= volatile solids

ANOVA of this study illustrates that temperature was a significant parameter which effect the co-digestion of newspaper and green vegetable waste, highest specific bio-methane yield of 342L/kgVS was obtained at mesophilic temperature i.e., 30.86^{o}C which implies that microbial activity was highest at this temperature as microorganisms degrade much of the co-substrate throughout the experiment, releasing bio-methane.

Chapter # 05 Conclusions and Recommendations

The following conclusion and recommendations were extracted from this study which has been compiled in the form of a thesis.

5.1 Conclusions

- Anaerobic digestion process not only manages the waste but also utilize it as a source of renewable energy in the form of bio-methane.
- Bio-methane potential of newspaper and green vegetable waste were analyzed through anaerobic co-digestion and C/N ratios and temperature were optimized through two methods i.e., manual optimization involving hit and trial method and through RSM by using central composite design.
- RSM was beneficial for predicating the specific bio-methane yield from the anaerobic co-digestion process and model verification experiment proved that specific bio methane yield is near to the estimated by using RSM.
- Maximum specific bio-methane yield of 401.8 L/kgVS was obtained from reactor MR3 operated at C/N ratio 30 and temperature 37^0C through manual optimization while, through RSM optimization, maximum specific bio-methane yield of 342 L/kgVS was achieved at C/N ratio of 30:1 and temperature 30.86^0C through the co-digestion of newspaper and green vegetable waste.
- Results from manual optimization and optimization through RSM illustrated that mesophilic temperature was more favorable for the anaerobic co-digestion of newspaper and green vegetable waste.

5.2 Recommendations

- The data obtained from this study could be used as a basis for designing large scale anaerobic digesters for treatment of newspaper, food, green wastes and their mixture.
- Digestate can also be used further in the production of methane or as a fertilizer.
- Various designs of response surface methodology like box behnken could be used for investigating biomethane yield of various substrates.

Chapter # 6

Research work at ASU

AOI Effect on Clean and Soiled PV Modules

6.1 Introduction and Literature review

When light travels from one medium to another medium, a refraction (or bending of light) take place at the interface. In case of solar photovoltaic (PV) module various interfaces occurs which affect the total amount of light reaching the PV cell that can be converted into power. In case of soiled PV modules, the incident light is influenced by two additional interfaces of air/soil and soil/superstrate [78]. The effect of AOI (angle of incident) on the performance of clean PV modules with air/glass interface has been extensively investigated for the crystalline silicon modules and for five different PV technologies of mono-Si, poly-Si, CdTe, CIGS and a-Si.

Soiling is the accumulation of snow, dust, and other particulates on the glass of PV modules. Increased soiling results in decreased short-circuit current (I_{sc}) and power (P_{max}) production [78]. Because of loss in short –circuit current (I_{sc}), non- uniform soil coverage or uneven shading can cause hot spots [79]. Soil particles at the surface of the module caused hurdles shadowing the cell. Soiling losses can changed based on environmental conditions, soiling type and installation as shown in figure 6.1. To mitigate soiling losses, methods such as physical (manual or automated) cleaning, and/or anti-soiling coatings are used. Work is being done by many research groups to standardize the replication of natural soiling in controlled indoor conditions to study the effectiveness of anti-soiling coatings developed by university researchers and industry stakeholders. The extent of transmittance of light from the surface of PV modules to the solar cell depends on the surface/air interface and angle of incidence. The module surface could be flat glass (clean module) or covered with dust/snow (other pollutants, pollens, algae etc).

There are two conditions AOI (angle of incident) effects the short circuit current of photovoltaic (PV) modules, first one is mechanical/ geometrical effect which occurs because of module's orientation with respect to the incident light. It is often called as the cosine effect. Second way is due to the optical effects or often known as surface characteristics of the superstrate and referred to as optical effect [80].

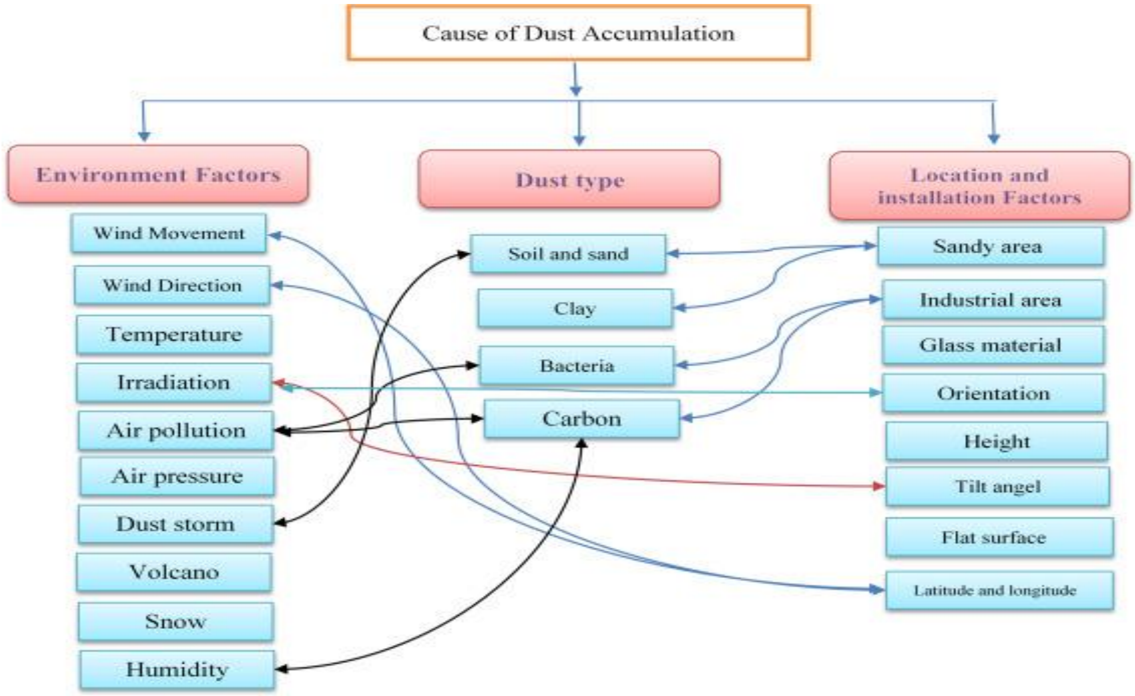


Figure 6.1: Schematic showing the potential causes of soiling [80].

6.2 Methodology

The effect of varying tilt angles and soiling on the cell I_{sc} have been done through this set of experiments as shown in figure 6.2. Each coupon has two cells. The right side of the cell is clean. Three data sets based on the experiments below will be collected. All the three data sets will be collected on three clear sunny days within a week from the start of the experiment. The clear sunny time is defined as the time at which the DNI (direct normal irradiance) exceeds 85%.

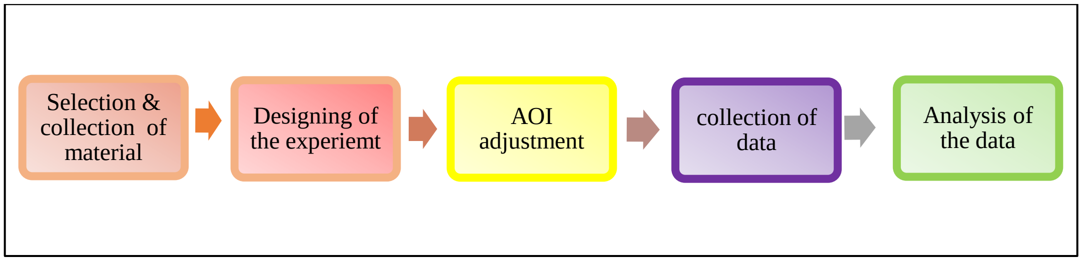


Figure 6.2: Methodology of the experiment.

6.2.1 Designing of the experiemt

The experimental setup conist of solar cells inclined at different angles, thermocouples, data acquisition system(DAS), irradiance sensors, pyranometer etc. Irradiance sensors are used to measure the global and direct irradiance levels, pyranometer for global irradiance while pyheliometer for the direct normal irradiance. Thermocouples sensors are used to measure the temperature of the solar coupons, data acquisition system (DAS) collects and stores the output of the thermal sensors and the short circuit current of the solar coupons.Experimental setup is shown in the figure 6.3.

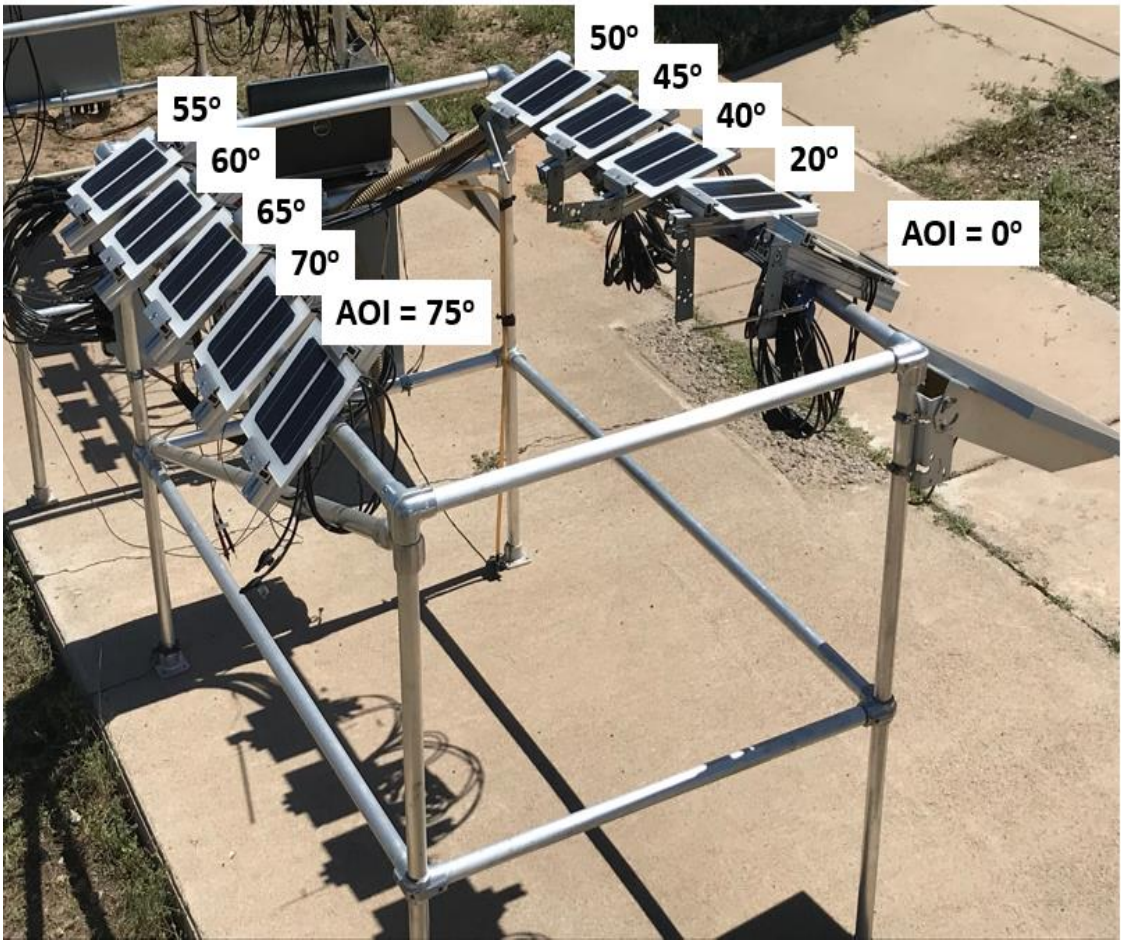


Figure 6.3: Experimental setup.

6.3 Results and discussion

At zero angle of incidence, the solar panel is directly facing the sun and has minimum losses [81]. The highest level of transmittance occurs at 0^{o} angle (normal to the module surface) of incidence of light. Transmittance decreases as the angle of incidence increases from 0^{o} to 90^{o} due to cosine loss and reflection loss. The cosine loss does not depend on the surface property of the plane/target as it is purely dictated by geometry (i.e. the cosine loss does not depend whether the flat surface is glass, piece of metal or wood). The reflection loss, however, depends on the surface property of the plane: clean glass, soil-covered glass or snow-covered glass [80]. The power or current loss between clean and soiled coupons is much higher at higher AOI as compared to lower AOI, caused excessive energy production loss of soiled coupons on cloudy days, early morning and late afternoon hours.

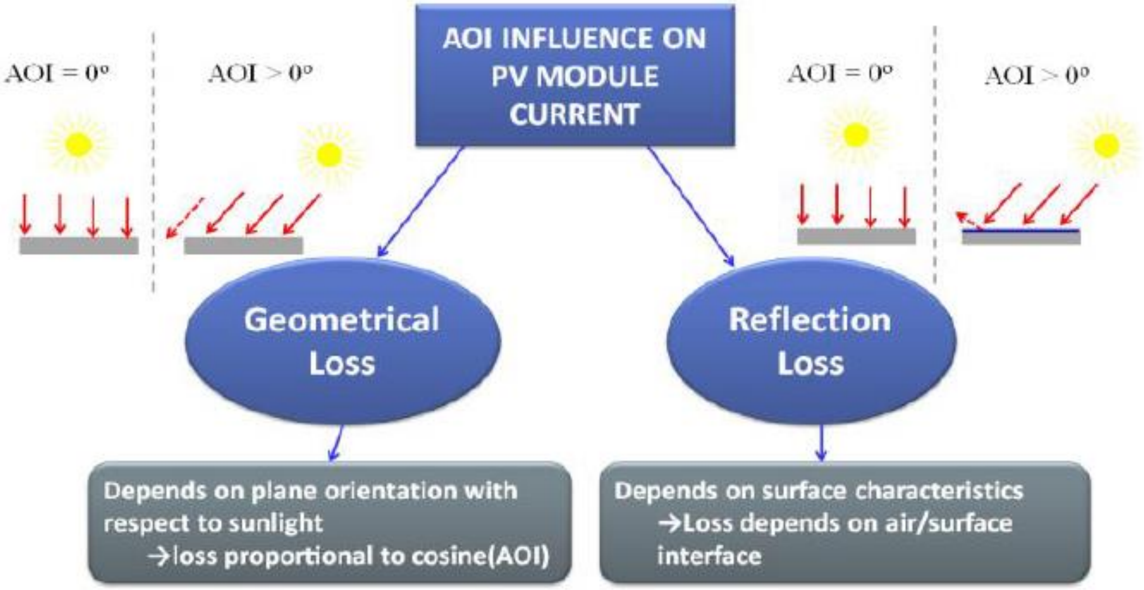


Figure 6.4: AOI influence on PV module current

Figure 6.5 depicts that the AOI curves are heavily influenced by the air/soil/superstrate layer and is dependent on the thickness of the soiling layer. The critical AOI for the air/glass interface is about 57° and it decreases dramatically as the soil gravimetric density (g/m^2) increases. The critical angle forms a good measure to indicate where the soiling loss turns out to be significant for a certain soil gravimetric density (g/m^2).

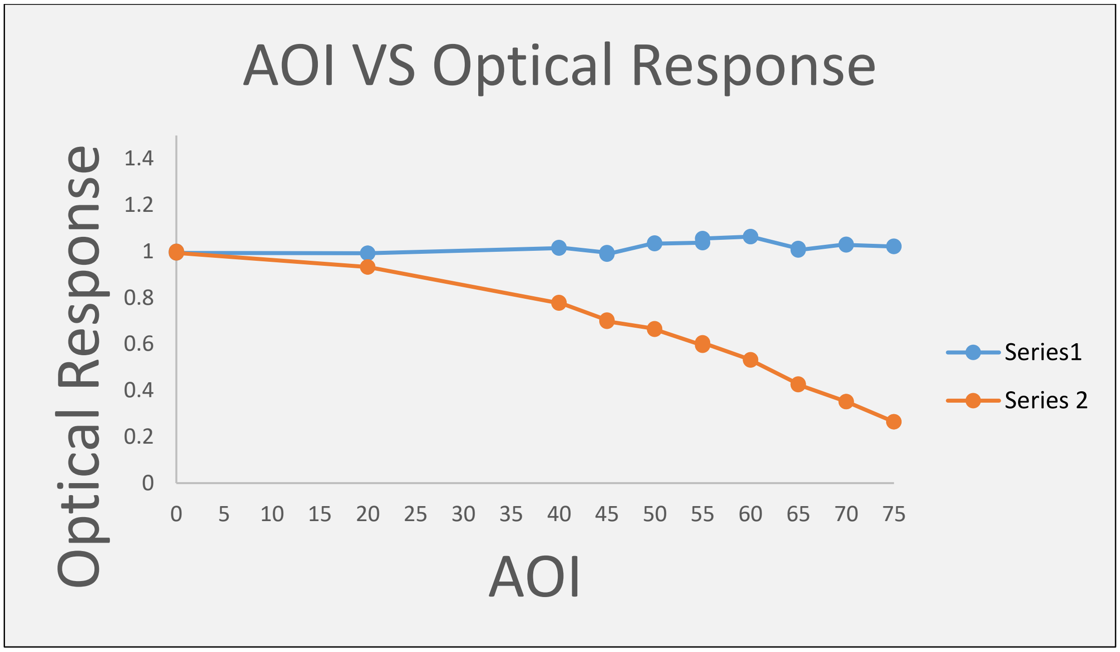


Figure 6.5: AOI VS optical response

6.4 Conclusion

- ❑ With increase in AOI the power and current loss increased between clean and soiled coupons.
- ❑ If there is an identical soil density on the PV modules, the soiling loss will be nearly identical irrespective of the PV technology type.

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Appendix – Research Article

Optimizing temperature & C/N ratios of newspaper and green vegetable waste for methane yield through response surface methodology

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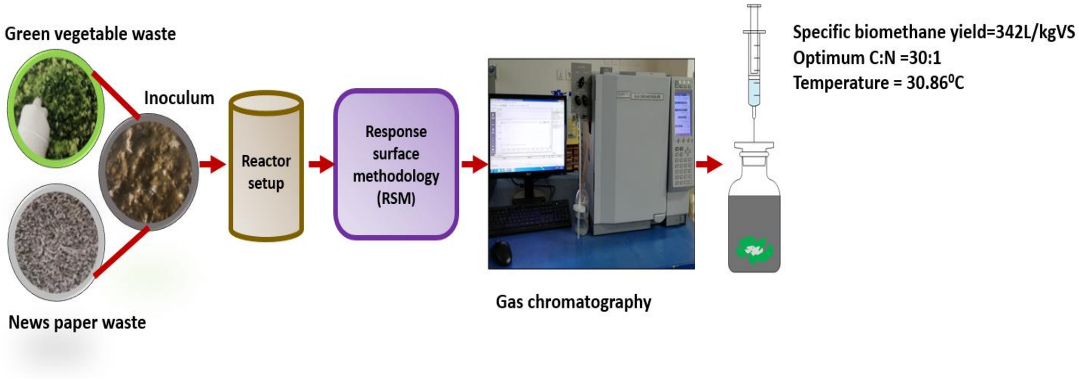
Abstract

Newspaper and green vegetable waste can be a noteworthy energy source for the production of renewable energy in the form of bio methane. This research is focused on the optimization of temperature and carbon to nitrogen (C/N) ratio to enhance bio-methane yield from anaerobic co-digestion of newspaper and green vegetable waste. Response surface methodology (RSM) and central composite design (CCD) were carried out in order to determine the optimum conditions of temperature and carbon to nitrogen (C/N) ratio for bio-methane yield. CCD experiments were performed at organic loading of 10gVSL^{-1} and volatile solid (VS) substrate to inoculum ratio of 1:1. Results from the CCD experiments revealed that maximum specific bio-methane yield, 342 L/kgVS, is 12.87% more than the predicted value, obtained from reactor CCD-7 having C/N 30:1 and temperature 30.86 ^{o}C. A significant synergistic effect in co-digestion of newspaper and green vegetable waste was observed at mesophilic temperature rather than thermophilic. Verification experiments confirmed that temperature and C/N ratio have influential effect on bio-methane yield.

Key words: anaerobic co-digestion, response surface methodology, central composite design, newspaper waste, inoculum, substrate, green vegetable waste, optimization

Graphical abstract:



Introduction

Energy has become an important prerequisite for the economic development of a country. To meet this need, combustion of fossil fuel which includes coal, oil and natural gas increased causing depletion of nonrenewable resources as well as increase in greenhouse gas emission and global warming.[1] There is a need to look for renewable, inexpensive and environmental friendly sources of energy. Advantages of renewables includes enhance diversity in energy supply markets, creates new employment opportunities, provides sustainable energy supplies and reduces atmospheric emissions [2].

Bioenergy can provide a suitable option to fulfill the energy requirements, which do not have any significant environmental impacts, utilizing waste for energy generation in addition to providing reduction in the volume of waste and energy cost. It plays a positive role in establishment of sustainable developmental objectives because it reduces greenhouse gas emissions as compared to the fossil fuels in applications where it is used [3]. Anaerobic digestion is a bio chemical conversion process for converting biomass existing naturally as agricultural residues, solid waste, animal manure, food waste and sewage in to methane rich biogas having various advantages which includes waste utilization, waste volume reduction by the use of microorganisms producing energy intensive biogas [2].Co-digestion deals with mixing of multiple substrates to improve C/N ratio, macro and micro nutrients, biogas production and dilution of complex and inhibitory substances which enhances the efficiency of methanogens by providing additional nutrients and pH regulation [4]. People living in rural areas of developing countries immensely use animal manure, vegetable and fruit waste as a prime source of energy, animal feed and fertilizers.

Newspaper waste is considered as one of the significant solid waste and have limitations on its number of recycling. Various methods for newspaper waste disposal includes land filling, dumping and incineration which have low economic benefits and significant environmental impacts [5]. Anaerobic co-digestion can provide a suitable option for reducing the volume of newspaper waste and utilizing it for the bioenergy production as it have high carbon content in it. Co-digestion of newspaper with green vegetable waste can improve the digestion performance and overcome the rapid acidification as newspaper waste have high (C/N) ratio which is a limiting factor for the anaerobic digestion process is adjusted with high nitrogen content containing substrates such as green vegetable waste. Many researchers conveyed that co-digestion of various organic waste caused improved biogas yield. Xiaojiao et al. (2012) revealed that methane yield of co-digestion of chicken manure, wheat straw and dairy leftover was high compared to individual digestion [3].

Various parameters affect the decomposition rate of organic waste and bio gas production which includes temperature, substrate concentration, pH, carbon to nitrogen (C/N) ratio and hydraulic retention time [6]. Anaerobic digestion can be performed at three different temperatures i.e. psychrophilic 15-20^{0}C, mesophilic 25- 45^{0}C, thermophilic 50-60 ^{0}C and is a significant factor for the microbial activity, a slight fluctuation in it can cause destabilization in the process resulted in decrease of the biogas production. [7]. Carbon to nitrogen (C/N) ratio is also an effective parameter and it is necessary to optimize it for the various substrates to improve the co-digestion process in order to achieve higher efficiency, greater carbon content in the substrate will cause amplification of carbon dioxide formation and reduction in the pH of the reactor while, elevated content of nitrogen give rise to the improved production of the ammonia gas raising the pH which would cause damage to the micro-organisms that are effective for the degradation of volatile fatty acids [8].

Response surface methodology (RSM) is a software that is utilized for making design for the experiments to fit an suitable mathematical function, check the quality of making models, analyzing the effect of several factors and optimizing the desired response [9]. Many researchers have reported that RSM is a useful tool for optimizing the bio methane potential. Jiayu feng et al. (2017) used RSM and CCD to enhance methane production from vinegar residue, results of the experiment confirmed that RSM and CCD was useful for optimizing the anaerobic digestion parameters [10]. In order to use RSM, the choice of experimental design is essential to fit an suitable mathematical function and to evaluate the quality of the model used along with its accuracy, to setup a system in relation to the experimental data obtained [11].

The objective of the study was to investigate the possibility of improving bio-methane yield from anaerobic co-digestion of newspaper and green vegetable waste by optimizing temperature and carbon to nitrogen (C/N) ratios through response surface methodology (RSM) while central composite design (CCD) was used to optimize the operating parameters.

Methods

Substrate and Inoculum

The newspaper and green vegetable waste were used as substrate, newspaper waste was collected from the library of National University of Science and Technology, Islamabad while green vegetable waste including cabbage, spinach and lettuce, was collected from the cash and carry market near National University of Science and Technology. Furthermore, cow manure, collected from native livestock farm shed Fateh Jang, Punjab, Pakistan, was used as inoculum. Newspaper waste was shredded using shredder to reduce the particle size up to <5mm and green vegetable waste was cut manually by choppers then grinded to reduce their particle size <5 mm by using lab grinder and stored at 4^0C prior to conducting the lab scale testing. The physicochemical properties of substrate and inoculum tested in this study are given in table 1. Fresh samples of inoculum, newspaper and green vegetable waste were investigated for their pH, total solids (TS), volatile solids (VS), total kjeldahl nitrogen (TKN) and total organic carbon. All these examination were performed in accordance with APHA Standard methods [12]. All the samples were collected in triplicates and average of the three measurements are illustrated.

Table 1: Physicochemical properties of substrate and inoculum used in the digestion experiments.

Parameters	Newspaper waste	Green vegetable waste	Inoculum
TS (%)	94.25	9.85	12.09
VS (%)	92.5	8.416	10.084
VS(%of TS)	98.14	85.44	83.40
TOC (%)	54.522	47.4667	46.33
TKN (%)	0.0224	2.78	5.4
Moisture Content (%)	5.75	90.15	87.91

TS= total solids, VS= volatile solids, TOC = total organic carbon, TKN= total kjeldahl nitrogen

Central composite design (CCD)

Optimization of studied parameters were carried out through response surface methodology (RSM) and central composite design (CCD) was obtained by using Design Expert Version 12.0.3.0 software. Batch experiments were performed based on CCD with two factors, carbon to nitrogen (C/N) ratio and temperature, to find their effect on methane yield which was a dependent output response. The level of factors studied for optimization are presented in Table 2. Four axial points coded in ± α and replication of three central point's provides estimation for the experimental error and measure of lack of fit factor resulting in a total number of 11 runs [11]. The response (specific bio-methane yield) was fitted using polynomial quadratic equation in order to correlate the response variable to the independent variable while second order polynomial coefficients were evaluated using the analysis of variance (ANOVA). Codified and real values for both factors are presented in Table 3. Through determination coefficient, R^2 quality fit of the model equation was expressed and its statistical significance was determined by F test [13].

Table2: Levels of factors selected for the central composite designs.

Variables	**Label**	**Level**				
		-1.414 (-α)	**-1**	**0**	**1**	**1.414 (+α)**
X	C/N Ratio	15.8579	20	30	40	44.1421
Y	Temperature(degree Celsius)	30.8579	35	45	55	59.1421

Experimental Setup

CCD experiments were performed at organic loading rate of $10gVSL^{-1}$ keeping the volatile solid (VS) substrate to inoculum ratio of 1:1 for 50 days in triplicate according to the method described by Wang et al. (2012) [14] and the experiment was conducted in laboratory glass bottle of 300mL capacity containing 20.8g of inoculum and 210 ml of working solution with a head space of 90 ml, head space was flushed with N_2 gas for creating anaerobic condition. To calculate the desired C/N ratios, mixing ratios of newspaper to green vegetable waste, 1:500,1:61.8, 1:14.3, 1:8, 1:6.74, were required to obtain C/N ratios of 15.86, 20, 30, 40, and 44.4 respectively. C/N ratios were theoretically calculated from the following formula using TOC, TS and TKN of newspaper and green vegetable waste obtained previously from chemical analysis for preparing reactors [8].

$$\frac{C}{N} = \frac{\text{NPW (TS x TOC) + GVW (TS x TOC)}}{\text{NPW (TS x TKN) + GVW (TS x TKN)}}$$

Where NPW is weight of newspaper waste fresh matter (g), GVW is weight of green vegetable waste fresh matter (g), TS is total solids, TOC is total organic carbon (% of TS) and TKN is total kjeldahl nitrogen.

On the basis of volatile solids (VS), reactors were loaded with respective amount of 35.19g, 21.91g, 15.85g, 12.58g and 11.62g respectively of mixing ratios of newspaper to green vegetable waste to obtain the total feed of $10gVSL^{-1}$. Reactors were tightly closed with rubber septa and screw caps and were shaken manually to stir the mixture present in the reactor for about 1 minute once a day prior to the measurement of biomethane.

Table 3 : Codified and real values for C/N ratio and temperature in co-digestion of GVW and NPW.

Reactors	Coded Values		Real Values		Specific Methane Yield (L/kgVS)	
	C/N	Temp	C/N	Temp.	Actual	Predicted
CCD-1	0	0	30	45	262.2	243
CCD-2	-1.414	0	15.86	45	130.2	109.49
CCD-3	1	-1	40	35	120.286	162.73
CCD-4	0	0	30	45	262.2	243
CCD-5	1.414	0	44.14	45	55.2	23.99
CCD-6	-1	-1	20	35	204.43	239.45
CCD-7	0	-1.414	30	30.86	342	297.98
CCD-8	1	1	40	55	34.2	51.10
CCD-9	0	1.414	30	59.14	125	117.10
CCD-10	-1	1	20	55	85.184	95.29
CCD-11	0	0	30	45	204.6	243

CCD= Central composite design

Biogas Characterization

Volume of the biogas on daily basis were measured with the help of gas syringe [15]. Methane content in terms of percentage was analyzed with gas chromatograph (SHIMADZU GC-2010 Plus) with RT-Sieve 5A Column (length of 30 m, diameter of 0.32mm) with thermal conductivity detector (TCD) [9].The temperature of injector, detector and column were Kept at 250,250 and 50^0C. Injection volume was 1µL. Helium was used as a carrier gas with a linear velocity of 40cm/sec. For gas chromatography calibration gas standard consisting of 99 (V/V) % methane (CH_4) was used.

Results and Discussion

ANOVA of the fitted Model

Experimental and predicted results of specific biomethane yield were illustrated in Table 3. Specific biomethane was calculated by dividing specific biogas yield with the percentage of methane content and were subjected to the response analysis to evaluate the effect of the operating parameters. Analysis of variance (ANOVA) determines the significance of the quadratic model and by applying multiple regression analysis, the results were fitted to a second - order polynomial equation. Summary of the ANOVA is shown in the Table 4, whereas second order polynomial equation for bio-methane yield fitted in terms of coded factors was obtained as follow:

$$Y=243-30.23A-63.95B+8.31AB-88.13A^2-17.73B^2$$

Where Y was bio-methane yield, A was (C/N) ratio, B was temperature.

Table 4: Analysis of Variance of the Model

Source	Df	Sum of squares	Mean squares	F-Value	p-Value
Model	5	84563.74	16912.75	9.38	0.0141
A	1	7309.86	7309.86	4.05	0.1002
B	1	32715.07	32715.07	18.14	0.0080
AB	1	264.55	264.55	0.1467	0.7175
A^2	1	43859.50	43859.50	24.32	0.0044
B^2	1	1775.04	1775.04	0.9842	0.3667
R^2	-	0.9036	-	-	-
Adjusted R^2	-	0.8073	-	-	-
CV	-	25.58	-	-	-
Lack of fit	3	6806.21	2268.74	2.05	0.3443

Many researchers have also used RSM and CCD model for estimating the optimum conditions for bio-gas production. Wang et al. (2007) used RSM and CCD for designing the experiments, in order to determine the optimum conditions for methane fermentation for evaluating the individual effect and interactive effects of substrate concentration, ratio of inoculum to substrate and Ca^{++2} concentration of effluent of bio hydrogen fermentation of food waste. Results of the experiment based on projected optimum conditions ratified that RSM was useful for optimizing the methane yield from effluent of bio hydrogen fermentation of food waste [9].

Actual and predicted values presented in table 3 confirmed that second order polynomial model fitted the experimental results corresponding to the methane yield quite well. The model F-value of 9.38 implies that model was significant and there was only a 1.41% chance that a ‘model F- value’ could occur due to noise. p-values less than 0.05 indicated the significant model terms. Regression analysis of the experimental design illustrates that the linear model term B is 0.0080 that is less than 0.05 which means that linear model term B is significant. It may be considered coefficients were extremely significant and had a comparatively high impact on dependent variables. The p-value of interactive model term AB is 0.7175 which is greater than 0.05 that means model term AB is insignificant. The value of the R^2 coefficient obtained 0.9036 showed that the majority of data are explained by the model. The ‘lack of fit F-value’ of 2.05 indicates the lack of fit is not significant relative to the pure error having s 34.43% of chance that a lack of fit F-value could occur due to noise. Non-significant lack of fit is good which means model is fit.

Fig.1 Shows the relationship between the two independent variables C/N ratio and temperature on bio-methane yield in the form of three dimensional response surface plot depicting significant effect of C/N ratio on methane potential occurs at mesophilic temperature range (20-41^{0}C) as compared to thermophilic temperature range (41-60^{0}C). As the C/N ratio increased from 20 to 30, bio-methane yield improved, after that yield was not stable, it started declining. The potential reason for this reduction in the yield of bio-methane may be that at higher C/N ratios methanogens rapidly consumed nitrogen which distrubs the anaerobic environment which became the reason for low yield of bio-methane [16]. Methanogens have favouable conditions at mesophlic temperature range that leads to high bio-methane yield. Methane yield was high and to some extent remained consistent when C/N ratio was 30 and temperature was 30.86. Highest specific bio-methane yield was estimated as 342 L/kgVS.

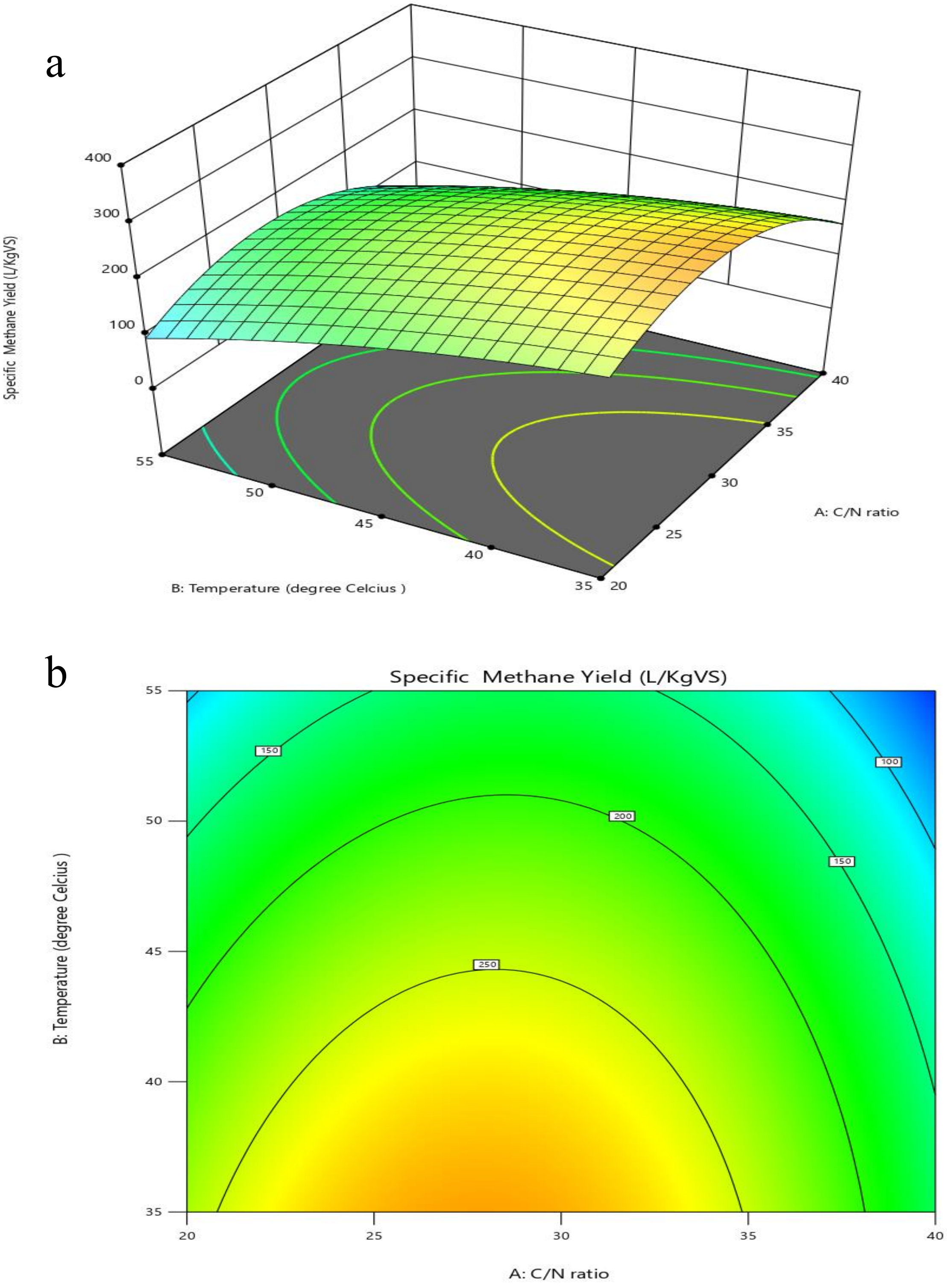


Figure 1: Response surface (a) and contour plot (b) for specific bio-methane yield depending on variation of temperature and C/N ratios.

Effect of the C/N ratio on co-digestion

C/N ratio is a significant factor in anaerobic co-digestion, because of its importance it is vital to optimize it for various substrates in order to achieve higher productivity and positive synergistic effect on the degradation of the individual substrates in the reactor [17]. Many previous studies suggested that optimum C/N ratios in anaerobic digesters lies between 20 and 30 [3] [18]. As shown in Table 3 CCD reactors having C/N ratio 20:1 and 30:1 had highest specific biomethane yield about tenfold higher than that from CCD reactors having C/N ratio of 15:1 and 40:1. Related work has been carried out by many researchers. Xiaojiao et al. (2012) studied optimizing feeding composition and C/N ratio of multiple substrates which includes dairy manure (DM), chicken manure (CM) and wheat straw (WS) for the methane potential. They found that maximum methane potential was accomplished with DM/CM of 40.5:59.7 having C/N ratio 27.2:1 using response surface methodology, C/N ratios of 25:1 and 30:1 had enhanced digestion performance with stable pH and low concentrations of total ammonium nitrogen and free NH_3[3].
Co-digestion of newspaper and green vegetable waste effectively balance C/N ratio of feed stock that benefits the methane yield. As shown in Table 3 CCD-7 having C/N ratio of 30:1 comprising of 93.45% of green vegetable waste and 6.54% of newspaper waste had highest specific biomethane yield i.e., 342 L/kgVS whereas CCD-8 having C/N of 40:1 comprising 88.88% of green vegetable waste and 11.11% of newspaper waste has lowest specific biomethane yield i.e., 34.2 L/kgVS. Newspaper waste have less biodegradability and nitrogen content because of having lingocellulosic content. Methanogens difficulty digest lingocellulosic content which makes the digestion process lengthy and time consuming [16]. Bio-methane potential of such waste enhanced when it was mixed with waste having high nitrogen content like green vegetable waste, which is supported by the result of this study.

Momoh et al. (2011) reported that addition of paper waste by co-digesting fixed amount of cow dung and water hyacinth in digesters B-E at room temperature improves the biogas yield while digester A acting as the control. The maximum biogas production for digesters B, C, D and E was estimated to be 0.282, 0.262, 0.233 and 0.2176 $Lg^{-1}VS$ fed respectively. They observed that addition of paper waste to fixed amount of cow dung and water hyacinth improve biogas production [19].

Overall performance of the reactors were shown in Table 5. The CCD reactors with higher C/N ratios having higher fraction of newspaper waste resulted less specific bio-methane yield in the present study. Higher specific bio-methane yield at C/N 30 may be credited to higher proportion of green vegetable and low proportion of newspaper waste. These results are in accordance with other studies where synergistic effect for co-digestion of substrates increased because of improved C/N ratio [17]. At higher C/N ratios methanogens rapidly consumed the nitrogen, making unfavorable conditions for the methanogens resulted in lower yield of biomethane.

Table 5: Performance of reactors at different C/N ratios and temperature

Reactors	**Specific bio methane yield (L/kgVS)**	**Initial pH**	**Final pH**	**TS Reduction (%)**	**VS Reduction (%)**
CCD-1	262.2	7.2	7.12	60	69
CCD-2	130.2	7.3	7.22	55	62
CCD-3	120.286	7.42	7.57	49	52
CCD-4	262.2	7.2	7.12	60	69
CCD-5	55.2	7.18	7.09	36	39
CCD-6	204.3	7.33	7.64	42	46
CCD-7	342	7.22	7.36	72	80
CCD-8	34.2	7.08	6.432	20	28
CCD-9	125	7.04	6.186	44	50
CCD-10	85.184	7.10	6.555	24	31
CCD-11	204.6	7.2	7.06	52	63

CCD= central composite design, TS= total solids, VS= volatile solids

Effect of the temperature on co-digestion

Sustainable operation of anaerobic co-digestion requires effective temperature for microorganisms to decompose the organic material to produce bio-methane. Microbial growth can be affected with temperature variation that will reduce the bio-methane yield in a considerable way[20]. ANOVA of this study illustrates that temperature was a significant parameter which effect the co-digestion of newspaper and green vegetable waste, highest specific biomethane yield of 342L/kgVS was obtained at mesophilic temperature i.e., 30.86 ^{0}C which implies that microbial activity was highest at this temperature as microorganisms degrade much of the co-substrate throughout the experiment, releasing bio methane.

Related work has been carried out by many researchers. K.komemoto et al. (2009) studied the effect of temperature on solubilization and acidogenesis of food waste. Highest solubilization rate of food waste 70.0% and 72.7% based on suspended solid removal was obtained at 35^{0}C and 45^{0}C while biogas production was 64.7 and 62.7 mL/gVS at 35^{0}C and 45^{0}C depicting solubilization of food waste was higher at mesophilic temperature rather than thermophilic. [6].

Mesophilic condition involves diversity of microorganisms which boosts the process of degradation and provide stability and kinetic advantage to the reactors for the co-digestion of newspaper and green vegetable waste. The maximum VS reduction (80%) was obtained from the reactor CCD-7 operated at 30.86^{0}C resulted in highest specific biomethane yield of 342L/kgVS, while lowest VS reduction (28%) was obtained from the reactor CCD-8 operated at 55^{0}C resulted in lowest specific biomethane of 34.2 L/kgVS. Elevated temperature caused reduction in the value of pH, which in turn, resulted in inhibition of methanogenesis leading towards the low specific biomethane yield. Methanogenic activity occurs at pH values between 6.2-8, while the optimum range of pH lies between 7.0-7.2 [3]. Reactor CCD-7 having initial pH of 7.22 and final pH of 7.36 produced maximum specific biomethane yield revealed that methanogenic activity was significant while reactor CCD- 8 operated at thermophilic temperature of 55^{0}C having initial pH of 7.08 drops to the value of 6.4 revealed that methanogenic activity was inhibited because of accumulation of volatile fatty acids.

E. Saanchez et al. (2000) studied the influence of temperature and pH on the kinetics of methane production, organic nitrogen and phosphorus removal from cattle manure. They found that at mesophilic temperature methane production 2.3 times improved when the pH of the influent was elevated from 7.0 to 7.6, while increase in operating temperature from 35^{0}C to 60^{0}C caused decrease in kinetic constants of methane production. Major inhibition factors such as increased organic nitrogen removal and ammonia nitrogen production was reported at thermophilic temperature (60^0C) [21].

Conclusion

Through RSM optimization, maximum specific biomethane yield of 342 L/kgVS was achieved at C/N ratio of 30:1 and temperature 30.86^0C through the co-digestion of newspaper and green vegetable waste. The results illustrated that RSM was beneficial for predicating the specific bio-methane yield from the anaerobic co-digestion process and model verification experiment proved that specific bio methane yield is near to the estimated by using RSM.

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