INVESTIGATION ON ANAEROBIC CO-DIGESTION AND PRE-TREATMENT OF RICE STRAW FOR REDUCING GREENHOUSE GAS EMISSIONS

A Thesis
Submitted in partial fulfillment of the requirements
for the award of the degree of
Doctor of Philosophy

by

Mothe Sagarika
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Department of Civil Engineering
National Institute of Technology
Warangal-506004, India
April 2023

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Declaration

This is to certify that the work presented in the thesis entitled **“INVESTIGATION ON ANAEROBIC CO-DIGESTION AND PRE-TREATMENT OF RICE STRAW FOR REDUCING GREENHOUSE GAS EMISSIONS”** is a bonafide work done by me under the supervision of **Dr. P. Venkateswara Rao** and was not submitted elsewhere for the award of any degree. I declare that this written submission represents my ideas in my own words and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the institute and can also evoke penal action from the sources which have thus not been properly cited or from whom proper permission has not been taken when needed.

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Warangal,

Date:

Approval sheet

This thesis entitled **“INVESTIGATION ON ANAEROBIC CO-DIGESTION AND PRE-TREATMENT OF RICE STRAW FOR REDUCING GREENHOUSE GAS EMISSIONS”** submitted by Mrs. Mothe Sagarika is approved for the degree of Doctor of Philosophy.

Examiners

Supervisor

Chairman

Date:

Certificate

This is to certify that the thesis entitled **"INVESTIGATION ON ANAEROBIC CO-DIGESTION AND PRE-TREATMENT OF RICE STRAW FOR REDUCING GREENHOUSE GAS EMISSIONS"** submitted by Mrs. Mothe Sagarika to the National Institute of Technology, Warangal, for the award of the degree of Doctor of Philosophy in Civil Engineering is a bonafide research work carried out by her under my supervision and guidance. The results contained in this thesis have not been submitted in part or full to any other University or Institute for award of any degree or diploma.

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Abstract

RS has been burned in open fields in several countries, which is a significant environmental concern and has led to significant environmental damage and health hazards. Anaerobic digestion (AD) is one of the environmentally favourable processes for converting RS into methane, carbon dioxide, and digestate. The current study looks at the characteristics, ideas, and process elements (temperature, volatile fatty acids and pH, carbon to nitrogen ratio, metal elements, and rate of organic loading (OLR)). Co-digesting RS with nitrogen-rich foods can efficiently balance the carbon to nitrogen ratio. Pre-treatment is another practical approach. The current study analyses physical, chemical, and biological pre-treatments that improved digester performance. The use of RS in conjunction with other co-substrates and appropriate pre-treatment is suggested as a sustainable method for preventing dangers to the environment and human health. The current research work intends to improve biogas production by using locally accessible co-substrates for RS digestion. This goal is achieved through four aspects.

Aspect I: Food scraps, cow manure, sewage sludge, and chicken manure—all locally accessible co-substrates in Warangal that are compatible with anaerobic co-digestion of RS—were selected to balance the high C/N ratio of RS. Four TS contents, 15%, 20%, 25%, and 30%, were used in the experiments to test the potential binary and ternary combinations. For the substrates, proximate and final analyses have been undertaken. The ternary mixtures outperformed the binary mixtures with maximum production from combination of RS, SS, and ChM and RS, CM, and ChM at TS 20% of 408 mL/g-VS with significant reduction in volatile solids after digestion. The order of adaptability for choosing a co-substrate for RS can be listed as ChM>CM>SS>FW. The Modified Gompertz model provided a good fit for the experimental results, with $R^2 > 0.90$. According to the study's findings, co-digestion is a systematic way for boosting biogas production.

Aspect II: A total of four pretreatment techniques (thermal, hydrogen peroxide, thermal + hydrogen peroxide, and hydrogen peroxide + thermal) were used with four sizes of RS, namely, 3-5 cm, 1-2 cm, 5-10 mm, and 300 µm. For digestion of RS, the outcome of aspect I (combined RS, SS, and ChM at TS 20%) was chosen. Among the four pre-treatment methods studied, thermal pre-treatment was found to be the most effective for RS at 1-2 cm RS size with 389 mL/g-VS. A size reduction has improved bacterial activity's basic morphology and dissolution capabilities. The order of adaptability for choosing a RS size is in the following order 1-2 cm > 5-10 mm > 3-5 cm > 300 µm. However, pre-treatment could not produce more biogas than

control (combination of RS, SS, and ChM at TS 20%). It is recommended to choose co-digestion over pre-treatment in AD of RS.

Aspect III: Optimised results from aspects I and II were scaled up in pilot batch and semi-continuous reactors (500 L) using RS, SS, and ChM at TS 20% (co-digestion mixture from aspect I). The batch pilot scale study of anaerobic co-digestion of RS showed significant performance with 42% biogas production as well as reduced volatile solids compared to a lab scale study. A semi-continuous anaerobic co-digestion of RS on a pilot scale has demonstrated stable performance, generating 271.67 mL/g-VS of biogas, containing 48.55 % methane. In general, it was feasible to conduct pilot scale experiments on AD of RS using suitable co-substrates (ChM and SS).

Aspect IV: An analysis of the production of RS, usage trends, open field burning emissions, AD of RS, and other sources of emissions from rice cultivation in India is presented. AD is chosen over open combustion of RS, it can reduce the emissions of CH_4, N_2O, CO_2, and NO_x. Nevertheless, AD can produce CH_4, a substitute for natural gas that depicts AD as a circular economy that may reduce air pollution in Delhi caused by the burning of RS in Punjab and Haryana. The Global Warming Potential of RS would be 4,93,873 times more than that of AD if it were burned in a field. Sustainable development and circular economies are accomplished through reducing GHG emissions and generating sustainable energy from waste.

Based on the results of the present study, the yield of biogas is enhanced from RS by using compatible co-substrates combined with ternary combinations for AD. Anaerobic co-digestion of RS in batch and semi-continuous scales at ambient temperature has performed significantly, suggesting that real-time plants could be used for managing RS and also for generating energy. The AD of RS is a promising sustainable method, which is recommended to reduce GHG emissions instead of open burning of RS, since open burning of RS results in a large amount of GHG emissions; however, AD of RS results in a lower level of emissions. RS can be managed in a circular economy in which energy is generated, and emissions are reduced.

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List of Acronyms

AD	Anaerobic Digestion
A-co-D	Anaerobic co-digestion
C	Carbon
CH_4	Methane
ChM	Chicken manure
CM	Cow manure
C/N	Carbon to Nitrogen ratio
CO	Carbon monoxide
CO_2	Carbon dioxide
COD	Chemical oxygen demand
e	Euler's constant
FESEM	Field emission scanning electron microscopy
FTIR	Fourier Transform Infrared Spectroscopy
FW	Food waste
GHG	Global House Gase
GWP	Global warming potential
H	Hydrogen
H_2O_2	Hydrogen peroxide
L-AD	Liquid anaerobic digestion
N	Nitrogen
N_2O	Nitrous oxide
NO_x	Oxides of Nitrogen
OLR	Organic loading rate
R	Biogas production potential
R^2	Coefficient of determination
RS	Rice straw
PM	Pig manure
P_{max}	Maximum methane potential
S	Sulphur
SS	Sewage sludge

SS-AD	Solid state anaerobic digestion
TS	Total solids
VFA	Volatile fatty acids
VS	Volatile solids
Y(t)	Cumulative biogas production at time t
ƛ	Lag phase time

Chapter 1 Introduction

The aim of this chapter is to describe the background of the topic, motivation, and objectives of the study. It concludes with an outline of the thesis structure.

1.1 Background

The majority of India's land is used for agriculture, and rice is the second most important crop. Rice cultivation has spread over many countries, with a total harvested area of close to 160 million hectares and an average yield of 760 Mt (metric tons) per year (FAO 2018). During rice crop processing, two residues are produced, i.e., rice straw (RS) and rice husk, with RS being the primary fraction of the rice field. It has been approximated that 60.8 Mt of RS residues were produced per year in India (Sarnklong et al. 2010). Mainly, RS is used as animal food and roof thatching in India (Meshram, 2002), in addition to other utilizations (e.g., mechanical collection, composting, mulching, power production, biogas production, ruminant feed, and composite materials). Nevertheless, RS is susceptible to open field burning, a practice common in north India and in many places around the world (Meshram, 2002; Pal et al., 2022). Paddy cultivation is a major contributor to greenhouse gas emissions accounting for 10% of global emissions from agriculture (FAO 2015). This number is even higher for Southeast Asia where 90% of the world's rice is produced, making up 10–20% of the region's total anthropogenic emissions and 40–60% of its agricultural emissions (UNFCC 2019). Burning of RS has become a common phenomenon across the globe, specifically in some parts of India. RS combustion has positive impacts on farm activities but has negative impacts on the environment (Romasanta et al. 2017). Farmers still opt to burn RS because of its lower cost, the convenience of tillage handling, and reduced weed development despite the detrimental effects it has on the environment, human health, and soil quality. Also, growers receive a limited amount of time between two rice crops (Sahai et al., 2011).

Nonetheless, RS burning is a major contributor to air pollution in many places as well as soil fertility deficits brought on by the loss of organic matter (Athira et al., 2019; Kumar et al., 2013). The open burning of RS generates carbon dioxide (CO_2), methane (CH_4), carbon monoxide (CO), nitrous oxide (N_2O), oxides of nitrogen (NO_x), hydrocarbons, etc. The release of these gases and particulates (when their concentration exceeds the threshold limit in the environment) has adverse effects on the ecosystem, ecology, and human well-being and also contributes to tropospheric ozone and the formation of Atmospheric Brown Cloud (ABC) - a cause of severe human health concern (Cheng et al., 2000). Among the above-mentioned gases

CO_2, CH_4 and N_2O are the principal greenhouse gases (GHGs) (Levine, 2003). Nevertheless, extensive work on GHG emissions by the open burning of RS in India is not available (Sahai et al., 2011). The burning of biomass is not regarded as a source of CO_2 emissions (Intergovernmental Panel on Climate Change (IPCC) 1995) because the next growing season plants reabsorb the CO_2 released (Levine, 2003; Houghton, 1991). Open burning produces a considerable amount of residues that can be used as resources. Moreover, initiatives are needed to take into account using the residue as a resource, which will implement the sustainability of the agroecosystem (Sahai et al., 2011).

One suitable method for turning wastes, such as RS, crop straw, or manure, into a potential energy source is the anaerobic digestion (AD) of RS (Forster et al., 2008; Kaur et al., 2016). Two important technologies that enhance the AD process and stability are co-digestion and pre-treatment (Mothe et al., 2020). Anaerobic co-digestion of RS with several co-substrates such as cow manure, sewage sludge, food waste, municipal solid waste, pig manure, chicken manure, etc. was conducted to enhance the biogas production. Along with co-digestion, pre-treatment technologies were also evaluated, which resulted in improved digestibility and a stable process (may balance the instability caused by sensitivity of microorganisms). The various pre-treatment technologies include physical (milling, shredding, grinding), chemical (acids, alkalis, heavy metals, oxidants), biological, and combined pre-treatment. To reduce the field burning of RS, the Indian government has called for bio-compressed natural gas (bio-CNG) produced from RS at a price of 46 rupees per kilogram, with subsidies of 700 rupees for projects using 70 tons of paddy per day (Krar et al., 2018). Support from the government in terms of policy and financing will encourage the industries to set up more AD plants to use RS produced by the agricultural sector. Therefore, the country can expect a higher demand for AD of RS and justifiable compensation for peasants in the upcoming years. Despite these efforts, there are insufficient data on the emissions from combustion and the use of RS as a resource for converting to bioenergy via AD processes.

1.2 Motivation

Open burning of RS in several places in India has increased GHG emissions drastically over the decade (Athira et al., 2019). To manage the RS, landfilling, incineration, gasification, composting, pyrolysis, and anaerobic digestion (AD) are commonly used organic waste management techniques in India. Among the above AD has several positive outcomes as it generates biogas which can be a substitute for natural gas and nutrient-rich digestate. Moreover, renewable energy generation from AD minimises fossil fuel use and controls GHG emissions

(Tonini et al., 2016). Because of these advantages, the proposed research work is motivated to adopt AD for RS management.

Plate. 1.1 Open field burning of RS in neighbouring states of Delhi, India

Source: Times news paper

1.3 Anaerobic digestion process

Anaerobic digestion (AD) is biogas production through the lignocellulosic substrate by microorganisms under anaerobic conditions leaving carbon dioxide and nutrient-rich digestate (Pore et al. 2015). Digestate obtained from AD can be used as an organic fertiliser, containing phosphorus and remineralized nitrogen (Chen et al., 2008). Since the twentieth century, AD has been used to treat animal wastes, municipal sludge, and individual disposals containing high organic content (Cooney and Wise, 1975). AD process achieved the greatest importance in the last 3 decades (Cooney and Wise, 1975) as a large number of wastes were used as potential substrates for digestion. When an organic substrate is degraded anaerobically, the end products are CO_2, and CH_4, where carbon dioxide is the most oxidised form and methane is the most reduced form, as they cannot be oxidised and reduced further, respectively (Meng et al., 2018). Among all the fermentation processes (composting, vermi-composting, gasification, pyrolysis, incineration, land filling), AD is the most complete process (Meng et al., 2018). Proportions of CO_2 and CH_4, in the end, products rely on the degree of oxidation of carbon in an organic substrate. Biomethane productivity from the process depends on the reduced amount of organic carbon (Rajagopal et al., 2013). There is an increasing demand for the anaerobic conversion of lignocellulosic residues as more substrates are available for their utilization (Riya et al., 2018). Among the variety of agricultural residues available, RS has a great capability to be converted to biomethane (Pore et al., 2015). RS is the most preferred lignocellulosic substrate to make the most of it for bioenergy production throughout the world (Mustafa et al., 2017). AD is an appropriate method for producing renewable energy using crop straw and manure, which can convert waste into a resource (Forster et al., 2008).

The process can be described in four sequential stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Plate. 1.2). The first stage is hydrolysis in which complex polymeric substances like carbohydrates, proteins, and lipids are hydrolysed to monomers such as water-soluble sugars, amino acids, and long chain fatty acids. The second stage is called acidogenesis in which water-soluble monomers are converted to acids, alcohols, carbon dioxide, and hydrogen. The third stage is acetogenesis in which products of acidogenesis are converted to acetic acid. The last stage is the most crucial stage called methanogenesis during which the formed intermediate compounds like acetic acid, hydrogen, and CO_2 are converted to biogas. The overall process efficiency depends on the balanced equilibrium of these four stages.

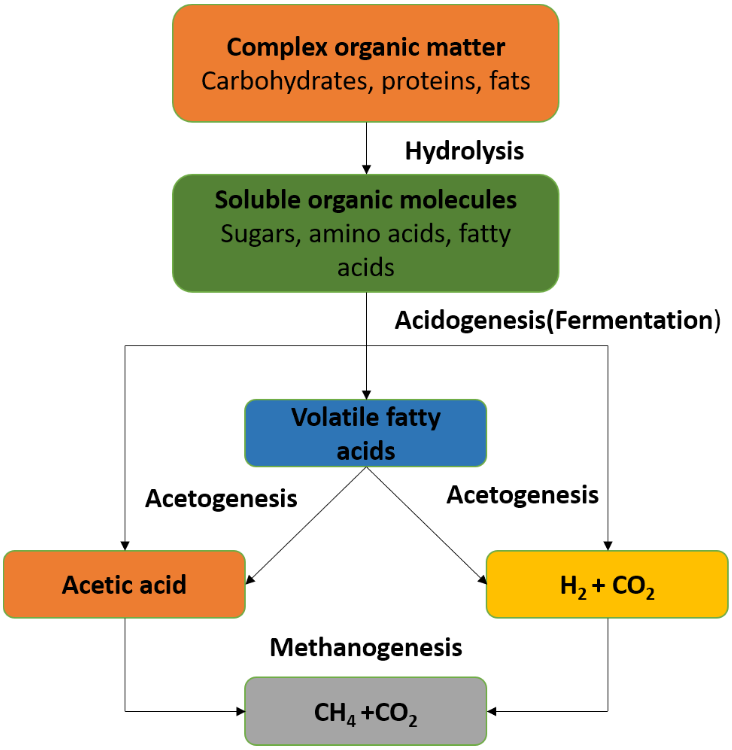


Plate. 1.2 Flow chart of anaerobic process steps

1.4 Limitations of the AD process

The efficiency and steadiness of the AD system are significantly controlled by the source and characteristics of the substrate, quality of inoculums, feedstock to inoculum ratio, organic loading rate (OLR), type of AD process, trace elements, VFAs production, C/N ratio, feeding methods, temperature, pH, hydraulic retention time, redox potential and mixing patterns (Luo et al., 2018; Terashima et al., 2009). There are challenges in AD that have a significant influence on the performance of the AD on RS, which are high C/N ratio of RS; elements of

RS (lignin, hemicellulose, and cellulose) make the substrate recalcitrant to the microbes in turn may make the first step in AD, i.e., difficult hydrolysis, as it is the rate-limiting step in slowly degradable feed. Even though the barriers of the AD of RS can be faded through some strategies namely co-digestion with high nitrogen, substrates (manures) can equalise the C/N ratio, and several pre-treatments can alleviate the complexity of RS, making it easy for the rate-limiting step. These limitations need to be addressed for the efficient utilization of RS in AD.

1.5 Aim and objectives of the thesis

The main objective of this research is to reduce GHG emissions by open burning of RS through thermo-chemical pre-treatments of anaerobic co-digestion of RS with locally available substrates.

The specific objectives of the present work are:

i) To find the suitable binary and ternary combinations of co-substrates and optimal operating conditions for better utilization of RS for improved biogas generation.

ii) To assess the performance of thermo-chemical pre-treatment for the selected co-digestion mixture at various substrate sizes for maximising the biodegradability of the substrate.

iii) To assess the performance of the scaling options for the optimised conditions.

iv) To compute GHG emissions from open burning of RS and AD of RS, analysis for the optimized conditions.

1.6 Organization of the thesis

The present thesis is detailed in eight chapters with the first being the introduction and the last conclusions.

Chapter 1 presents a brief overview of the need to control GHG emissions from the open burning of RS and AD, being one of the appropriate technologies to utilise RS, and states the scope of research. The motive of the thesis is to reduce GHG emissions through AD of RS with locally available organic wastes.

Chapter 2 presents a literature review on influencing factors such as temperature, VFA and pH, C/N ratio, Metal elements, organic loading, and total solids. The chapter also presents strategies that can be considered for enhancing the process performance. An overview of recent research on co-digestion and pre-treatment has been presented.

Chapter 3 presents the preparation of substrates for AD analytical and experimental methods used in the study.

Chapter 4 presents experimental investigations on AD of RS with binary and ternary combinations with chosen four co-substrates at four various total solids content for maximum biogas production.

Chapter 5 presents experimental investigations on four pre-treatment combinations and four different sizes of RS on a better-performed mixture obtained from chapter 4 for maximum biogas production.

Chapter 6 presents the experimental feasibility of a pilot-scale reactor for a better-performed combination obtained from the previous objectives with batch and semi-continuous study.

Chapter 7 presents the computation and comparison of GHG emissions from the open burning of RS and AD of RS.

Chapter 8 presents the significant conclusions drawn from the study and perspectives for future research work.

Chapter 2 Review of Literature

The present chapter aims to describe the operational and design factors that influence the AD process of RS and strategies to improve the process performance. The chapter also explains the importance of co-digestion and pre-treatment in AD of RS. The summary is presented at the end of the Chapter.

2.1 Key parameters

AD is a sensitive process, and several operational and design factors influence its efficiency. The following section thoroughly reviews the influence of temperature, VFA and pH, C/N ratio, metal elements, organic loading rate, total solids (TS %) and operational strategies on AD.

2.1.1 Temperature

Temperature is a significant parameter of reaction velocity, physical diffusion and chemical dissociation influencing the efficiency of the AD process (Hagos et al., 2017; Atelge et al., 2018). Optimal temperature is the basic need for reducing the vulnerability of the anaerobic system and its sustainability (Shetty et al., 2017). In general, temperature ranges for AD microorganisms are psychrophilic (10–20°C), mesophilic (30–40°C) and thermophilic (50–60°C) temperatures (Kwietniewska and Tys, 2014; Schnürer and Nordberg, 2008). The microbe’s growth rate is best at mesophilic and thermophilic temperature ranges (Hagos et al., 2017; Cooney and Wise, 1975), which also inactivates most of the pathogenic population (Takdastan et al., 2005). Full-scale AD systems mostly operated at mesophilic temperature conditions around 35 °C (Cooney and Wise, 1975). In general, the temperature increase in the AD system improves the metabolic rate of microbes and improves the digestion procedure. Still, the thermophilic system is severe to manage and needs additional energy to support the consistent temperature of the reactor (Hagos et al., 2017). Whereas mesophilic temperatures involve a wide range of microorganisms and are more stable than thermophilic temperature systems (Appels et al., 2008). The disadvantages of thermophilic temperatures are deficient process stability and inferior supernatant quality (Angelidaki and Sanders, 2004).

In general, methanogenic microorganisms are more vulnerable than hydrolytic and acidogenic bacteria (Atelge et al., 2018). Methanogenic microorganisms at mesophilic conditions can indulge ± 3 °C without a bit of notable change in biomethane productivity. In contrast, at thermophilic temperature, they are very sensitive to changes and are vulnerable to even ± 1 °C variation, and they require additional time to adjust to the latest condition (Seadi et al., 2008).

In the AD of RS, total biomethane production improved with the temperature increased from ambient to mesophilic temperature, whereas in thermophilic temperature, acidification occurred, which led to the inhibition of the reactor with poor biogas production (Lianhua et al., 2010). SS-AD of RS obtained from post-composting produced the best results at 35.6°C temperature with 20% initial substrate concentration and a C/N ratio of 29.6 (Yan et al., 2015). $Ca(OH)_2$ pre-treatment, a chemical method applied at 35°C temperature, produced maximum methane yield at 8% dosage (Gu et al., 2015). In a similar mesophilic temperature range, for alkali-pre-treated RS, the highest cumulative biogas production was observed at 37 °C, proceeded by 42 °C and lowest at 30 °C (Shetty et al., 2017). At 38 °C mesophilic temperature, different pre-treatments of NaOH, HCl and $CO(NH_2)_2$ were operated on RS (Dai et al., 2018). At 37 °C, diluted sulphuric acid-pre-treated RS achieved 94% COD removal efficiency (Cheng et al., 2016). At 37 ± 2 °C, sodium carbonate pre-treated RS shows significant enhancement in indigestibility (Kaur and Phutela, 2016). The thermophilic system has advantages over a mesophilic system for attaining excessive rates of digestion, large conversion of waste organics to biogas, rapid solid-liquid separation and reduction of bacterial and viral pathogen accumulation (Ward et al., 2008). As a result of high loading rates and high energy density of the substrate, self-heating effects have increased working temperatures from mesophilic to sub-thermophilic and thermophilic conditions (Moody et al., 2009). The solubility of various gases, such as CH_4, H_2S and $NH_{3,}$ changes with temperature (Atelge et al., 2018). Low-temperature water has more solubility than higher water, so it can influence the inhibitory material in the digester (Atelge et al., 2018). Most AD processes employ mesophilic conditions due to the simple management of methanogenic microorganisms and the need for low energy demand (Atelge et al., 2018) despite the advantages associated with thermophilic systems.

2.1.2 VFA and pH

VFAs are the most crucial intermediaries produced in AD, indicating their stability and potential (Luo et al., 2018). The predominant volatile acids in AD were acetic acid, propionic acid, butyric acid, valeric acid, lactic acid and formic acid (Luo et al., 2018, Cai et al., 2017). When acids are created during hydrolysis but cannot be absorbed by methanogenic bacteria, VFA accumulation occurs, which lowers pH and destabilises the system (Cai et al., 2017; Pore et al., 2015). VFA build-up drastically lowers pH, restricts methanogenic bacteria, and disrupts AD (Song et al., 2013). The accumulation of VFAs and the subsequent lowering of reactor pH is caused by bacteria with high rates of hydrogenic, acidogenic, and acetogenic metabolism. (Pore et al., 2015). Methanogenic and acidogenic microbial activities differ from optimal pH and optimum nutrient requirements (Ye et al., 2013). The favourable pH range for AD is 6.8–7.2, whereas optimal pH for hydrolysis and acidogenesis is in the field of 5.5–6.5, while it is around 7.0 for methanogenesis. However methanogens get inhibited with a pH less than 6.6 (Shetty et al., 2017; Schnürer and Nordberg, 2008). In general, pH fall is buffered by bicarbonates, which are produced from methanogenesis, and ammonia formed controls the excess volatile acid accumulation (Daiem et al., 2018). When the reactor becomes sour, pH can be balanced by adding $NaHCO_3$ and sodium hydroxide (Ye et al., 2013). pH dropped from 7.0 to 5.4 due to VFA accumulation in the co-digestion of RS and cow manure (Li et al., 2015). Methane production was inhibited in the AD of RS due to the acid cumulation when it was more than 7000 mg/L at day 12 under wet thermophilic conditions (Lianhua et al., 2010). Due to the cumulation of VFAs, especially propionate and acetate, in a high C/N ratio, biomasses like RS are not performing well in AD, which leads to a reduction in biomethane production (Li et al., 2018). Accumulation of acetate and propionate started from day 14 at total solids, 42% in co-digestion of RS and pig manure (PM) (Riya et al., 2018).

Digesters failed for feeding frequency once in 14 days and once in 21 days at day 112 and day 56 with VFA levels 1730 ± 336 and 3470 ± 355 ppm, respectively, in AD of RS (Zealand et al., 2017). At OLR 12 kg VS/(m^3d), co-digestion is restrained due to serious cumulation of VFAs, with butyrate (689 – 1618 mg/L), propionate (1820 – 2761 mg/L) and overall VFA concentration is 8030 – 12,443 mg/L in co-digestion of RS and cow manure (CM) (Li et al., 2015). Whereas in co-digestion of RS and the cumulation PM, of VFA 8293 mg/L occurred, in which propionate and butyrate were 1300 mg/L and 530 mg/L, respectively, when OLR was raised to 12 kg VS/(m^3d) and propionate is the strongest inhibitor of biomethane production among several VFAs (Li et al., 2015). VFA inhibition has not occurred in SS-AD of RS with co-digestion of pig urine at a F/I ratio ranging from 0.5 to 3 (Meng et al., 2018). The addition of trace elements reduced the VFA's inhibition and improved the stability of co-digestion of RS and food waste; from the changing trends in VFAs and pH and at the same concentration, nickel is shown to better acetic acid utilisation than cobalt (Zhang et al., 2018). With the addition of trace elements, the common acids in VFAs were acetate and propionic acid (Cai et al., 2017). At high OLR rates, VFA hinders the AD process of RS; however, in several co-digestion methods, the VFA accumulation effect is balanced by the buffering capacity of the substrates.

2.1.3 C/N ratio

C/N ratio is another major parameter in AD as the favourable nutrient balance is needed by anaerobic bacteria for their evolution and maintaining a steady environment (Li et al., 2018) and is an important factor for the characterisation of a substrate (Atelge et al., 2018). Generally, a C/N ratio of 20–30 is recommended to be favourable for AD (Chandra et al., 2012; Kim et al., 2013; Kwietniewska and Tys, 2014), whereas it is recommended that the C/N ratio range for hydrolysis is 16–45 and for methanogenesis is 20–30 (Atelge et al., 2018). Various authors have reported that the C/N ratio of the RS varies between 25 and 75, indicating the large variation in the substrate produced at different places (Chandra et al., 2012; Ye et al., 2013; Gao et al., 2013; Meng et al., 2018). Too high a C/N ratio leads to ammonia cumulation, which eventually may lead to inhibition of the bacterial microorganisms. Insufficient nitrogen in the substrate may cause inadequate utilisation of carbon sources (Resch et al., 2011).

In general, the C/N ratio of RS is balanced by co-digesting with substrates that are rich in nitrogen, i.e., pig urine (Meng et al., 2018), cow manure (Li et al., 2015) and food waste (Zhang et al., 2018), which improved the system stability and enhanced the methane production significantly. Sometimes, it is also observed that urea can be added to RS to balance the C/N ratio between 20 and 30 (Chandra et al., 2012). AD of a substrate with high nitrogen content may result in a high concentration of TAN (ammonia and ammonium ion) and NH_3, which, linked with high pH and temperature, may hinder methane production (Hansen et al., 1998). An imbalance of the C/N ratio may result in inhibitions of high ammonia nitrogen and accumulation of VFAs (Meng et al., 2018) if the C/N ratio is less, representing the substrate is a protein-rich, and vice versa (Kwietniewska and Tys, 2014). It is vital to balance a favourable C/N ratio for better performance of an anaerobic system, as a high concentration of nitrogen, which is a low C/N ratio, leads to toxicity, and a low concentration of nitrogen, which is a high C/N ratio, leads to inhibition (Shetty et al., 2017). Even though RS is a high C/N ratio substrate in which the C/N ratio is not in the permissible range, it can be balanced by co-digesting with low C/N ratio substrates.

2.1.4 Metal elements

Trace elements play a crucial role in AD, and any shortage may lead to reactor upset (Choong et al., 2016). The requirements of these elements are different for different groups of species found in the AD process. Methanogenic microorganisms require Co, Ni, Fe, Se, Mo and Wo, whereas Cu, Zn and Mn are essential for hydrolytic species (Choong et al., 2016). In general, enough trace elements will be available during the co-digestion process and may fall short in the case of the mono-digestion of substrates (Atelge et al., 2018). Trace elements added to the anaerobic digester are one of the ways to improve methane yield (Cai et al., 2017); certain metals are used as a fraction of the enzyme structure of the microorganisms (Chen et al., 2008). Phosphorus (P), nitrogen (N), sulphur (S) etc., are the macronutrients which are vital constituents of biomass and play a crucial role as buffering agents (Lo et al., 2012b; Schattauer et al., 2011). However, some heavy metals, microelements and macro elements may have a toxic impact on anaerobic bacteria in the system if they present in higher concentrations (Kwietniewska and Tys, 2014; Lo et al., 2012a). Alkali metals like sodium (Na), potassium (K), calcium (Ca) and magnesium (Mg) in high concentrations can be toxic to anaerobic microorganisms (Schnürer and Nordberg, 2008). High amounts of calcium lead to the precipitation of carbonate and phosphate, which affects the reactors in sealing and microbial cells; sealed biomass activity reduces due to mass transfer limitations (Kwietniewska and Tys, 2014). High potassium results in the neutralisation of membrane potential and lead to a passive influx of K-ions (Pore et al., 2015).

There is no statistically significant increase in biomethane yield on adding Co, Ni and Se in the AD of RS (Mancini et al. 2018; Cai et al., 2017). In addition, to trace elements, Mn, Se and Mo to AD of RS have enhanced the methane yield at low, medium and high concentrations with a maximum increase of 144%, 137.5% and 140.8%, respectively, and with Zn dosage at high concentration of 200 mg/L inhibited methane productivity; on the contrary, methane yield from high Zn concentration is more than that of digester without Zn dosage (Cai et al., 2017). Trace element dosage in the AD of RS can modify the potential of AD by altering the diversity and structure of bacteria and archaea (Cai et al., 2017). Adding Ni ions to alkali-pretreated RS can improve the acetate utilisation rate of methanogenic bacteria, and there is no increase in biogas yield with the addition of tungsten (W) (Shetty et al., 2017). There is no notable improvement in biogas production with the addition of trace elements Fe, Mo, Zn, Co and Ni at the dosages where the system observed maximum biogas yield of alkali-pretreated RS (Shetty et al., 2017). The addition of trace elements cut down the necessary time to attain a pH steady-state and enhanced the VFAs acid-generating ability in co-digestion of RS and food waste, and the combination of cobalt and nickel has shown better operating performance like maintaining relatively consistent pH (Zhang et al., 2018). At the same concentrations in co-digestion of RS and food waste, Ni plays a major role in inhibiting acid accumulation, high methane production, volatile solids removal rate, high cumulative biogas yield and better acetic acid utilisation than

Co (Zhang et al., 2018). Adding trace elements to the AD of RS has improved methane productivity, whereas some did not show significant results.

2.1.5 Organic loading rate

The amount of VS to be fed into the reactor in intervals per unit volume is known as the organic loading rate (OLR), which is a significant design component (Kwietniewska and Tys, 2014; Tanimu et al., 2014; Yavini et al., 2014; Nair, 2013). Overloading the feedstock could result in digester failure in a short amount of time, hence this component is crucial. Many experiments have been conducted to determine how OLR affects digester performance (Tanimu et al. 2014, Yavini et al. 2014, Nair 2013). The volumetric biogas production rate may be increased by a high organic loading rate, but the stability of the anaerobic system is decreased (Li et al., 2013). Biomethane yield increases as the organic loading rate increases to the optimum value, but above the optimum biomethane yield and volatile solids degradation rate decrease due to overloading (Hashimoto, 1986). With up to 2 kg VS/(m^3d) in the AD of RS, biogas productivity was raised as the OLR improved (Zhou et al., 2017). Higher specific methane yields and stable operations were observed at an organic loading rate of 1 g VS/L/d (at lower OLR), and higher methane volumes were seen at OLR 2 g VS/L/d when the AD system was not overloaded (at higher OLRs) in AD of RS (Zealand et al., 2017). A low organic loading rate and less frequent feeding may be a better option for enhancing the RS AD system performance (Zealand et al., 2017). Biomethane productivity decreased at OLR 8 kg VS/(m^3d), and more due to severe foaming in co-digestion of RS and cow manure (CM), and suggested OLR is 6 kg VS/(m^3d) for co-digestion of RS and cow manure at 1:1 ratio (based on VS) at mesophilic temperature (Li et al., 2015). In co-digestion of RS and PM at OLR of 12 kg VS/(m^3d), biogas production reduced along with average methane content of 34.3% (in general, 50 to 60%) (Li et al., 2015). Luo et al. (2018) suggested that further research is needed on the optimisation of main parameters like OLR to enhance methane yield and for treating liquid digestate in a comprehensive two-stage AD system. For AD of RS, low OLR is suggestible as high OLR is making the system fail or overloading or serious foaming which reduces the methane productivity.

2.1.6 L-AD and SS-AD

AD was distinguished into liquid and solid state based on the total solid content of the feedstock. If TS < 15% it falls under liquid or wet AD, and if TS > 15% it is solid-state or dry AD (Ge et al., 2016; Yan et al., 2015) which deals with high-solid biomass (Luo et al., 2018; Xua et al., 2015). In large-scale AD systems, L-AD has been broadly operated using animal manure and waste as raw materials (Brown et al., 2012). L-AD process has merits like easy maintenance and operation, but it needs a large amount of water and gives out high quantities of liquid digestate (Brown et al., 2012). L-AD is more feasible for treating high-moisture manures like animal manures, whereas SS-AD is more favourable for straw digestion (Luo et al., 2018). Besides, L-AD is a conventional technique operating in engineering applications; it is not efficient enough, and commercial-scale two-stage L-AD systems are limited (Brown et al., 2012). SS-AD features are smaller reactor volume, can handle high organic loading rates, needs low energy for heating, produce a high volume of methane and generate less wastewater (Li et al. 2011). In the SS-AD process, the F/I ratio is critical in operating the AD system (Luo et al., 2018). Digestate of SS-AD is convenient for transportation and land applications, as it has a low moisture content compared with L-AD effluent (Riya et al., 2018, Kim et al., 2013). L-AD needs short time to start up but forms floating scum easily, whereas SS-AD needs more time to get higher methane yields but is a relatively stable system (Lianhua et al., 2010). System instability in SS-AD may be due to nutrient imbalance and accumulation of ammonia and VFAs. At the same time, low methane production may be caused by slow mass transfer or recalcitrance of lignocellulosic feedstock (Li et al., 2011).

In the L-AD system of RS, the hydrogenotrophic pathway was the main biochemical pathway of methanogenesis (Zhou et al., 2017). In L-Co-AD of RS and PM, foaming can be minimised by mixing strength and optimum design of the reactor system (Li et al., 2015). Thermophilic and solid-state AD processes vary in their biochemical and biological processes from the usual liquid mesophilic AD process (Lay et al., 1997). Due to the supplement of nitrogen, moisture and balance of microbial community, L-Co-AD of RS and cow manure, digestate is a great inoculum for SS-AD (Ge et al., 2016; Luo et al., 2018). The L-SS-AD process is a feasible technology for treating various wastes like RS and OLR, and the F/I ratio has a notable effect on the operation of the L-SS-AD process (Luo et al., 2018). RS total solids vary from 90 to 96.1 (Albertson, 1961; Negi et al., 2018; Zealand et al., 2017; Yan et al., 2015; Mustafa et al., 2017), which is high in total solids content and, it is suggestible to operate SS-AD with L-AD digestate as inoculum.

2.2 Operational strategies

2.2.1 Pre-treatment

The primary aim of pre-treatment technology on lignocellulosic biomass is to change or alleviate the structural and compositional impediments to hydrolysis (Kaur & Phutela, 2016).

There are three types of pre-treatments: (1) physical pre-treatment (milling, grinding and chipping), (2) chemical pre-treatment (acids, alkalis and oxidants) and (3) biological pre-treatment. The pre-treatment technology results in chemical and physical changes in the lignocellulosic biomass (Mosier et al., 2005). Getting biomethane from lignocellulosic residue is complex, as it is recalcitrant to enzymic or microbial degradation because of its composition and structure (Hendriks & Zeeman, 2009). Pre-treatment is done to alleviate the recalcitrance of the biomass (Menardo et al., 2015; Gao et al., 2013).

In lignocellulosic biomass, biomethane production efficiency is limited by hydrolysis of the substrate due to lignin and hemicellulose, respectively, when OLR was raised to 12 kg VS/(m^3d) and propionate is the strongest inhibitor of biomethane production among several VFAs (Li et al., 2015). VFA inhibition not occurring in SS-AD of RS with co-digestion can be one of the feasible methods to raise the biodegradability of the substrate by breaking the ester bond linkages joining lignin and polysaccharides (Zhang et al., 2018). That is why the pre-treatment of lignocellulosic biomass is an important step in the conversion process (Mosier et al., 2005; Sun & Cheng, 2002). This procedure increases the cellulose's accessibility to the enzymes during the lignocellulosic structure's breakdown (Song et al., 2013). Grade of polymerization, crystallinity, solubility, surface area, and lignin content are some factors that affect the lignocellulosic biomass's capacity to degrade (Monlau et al., 2013). Selecting of pre-treatment method depends on the structure and characteristics of biomass, which should improve the biodegradable substrate formation without losing the matter throughout the process (Martínez-Gutiérrez, 2018).

2.2.2 Physical pre-treatment

Physical pre-treatment can raise the pore size and accessible surface area of the substrates and reduce the degree of polymerisation and crystallinity of the cellulose in substrates (Harmsen et al., 2010). Milling, extrusion, grinding, steam explosion and liquid hot water pre-treatments are generally used for physical pre-treatments. Extrusion improved cellulose and hemicellulose degradation of RS by around 9% and 6%, respectively, in the AD of RS (Menardo et al., 2015). West disk milling of RS is an economical, practical pre-treatment for agricultural biomass for enzymic hydrolysis compared with dry ball milling and hot compressed water pre-treatment (Hideno et al., 2009). Maximum gas production was 0.52 m^3/kg VS with a methane content of 60.29% at a straw particle size range of 0.45 to 0.6 mm (Yong et al., 2015). But physical pre-treatment is comparatively ineffective in improving biomass digestibility (Chang et al., 1998). In the AD process of RS, milling is the most used physical pre-treatment.

2.2.3 Chemical pre-treatment

Chemical pre-treatment that decreases lignin and hemicellulose improves cellulose's biodegradability while lowering the substrate's degree of polymerization and crystallinity (Behera et al., 2014). As chemical pre-treatment is clean and simple, it has received major attention (Song et al., 2013). The aim of chemical acid pre-treatment is hemicellulose solubilisation by breaking ether bonds in lignin and making the cellulose accessible. Alkali pre-treatment (NaOH), acidic pre-treatment (dilute sulphuric acid), calcium hydroxide pre-treatment, hydrogen peroxide and ionic pre-treatment are the general chemical pre-treatments used in the AD process. Alkaline pre-treatment enhanced the biomethane productivity of RS by 21.4% and is more effective than adding Co, Ni and Se (Mancini et al., 2018). Alkali pre-treatment degraded lignin by break down of ester and glucoside side chains which resulted in raising substrate porosity, which directly improves substrate accessibility to microbes. It also reduces acetyl and several uronic acid substitutes, which are inhibitors for sugar degradation, and it produced the best results at 1% of NaOH for 3 hours at ambient temperatures with a 34% increase in methane yield in AD of RS (Shetty et al., 2017).

Biomethane production from hydrothermal-pre-treated RS followed by 5% NaOH addition was twice the untreated RS, and 5% NaOH addition outperformed the 3% NaOH-pre-treated biomass (Chandra et al., 2012). Among 5%, 8%, 10%, 12% and 15% $Ca(OH)_2$ pre-treatment, 8% dosage is recommended for the best performance of the anaerobic system of extruded RS, whereas higher dosages and lower dosages lead to lower methane yields due to over dissolving of cellulose and hemicellulose and high lignin content, respectively (Gu et al., 2015). It is recommended the pre-treatment of low-concentrated acetic-propionic acid to improve the biodegradability of RS (Zhao et al., 2010). Ionic pre-treatment disrupts the 3D matrix network of RS and solubilises it (Gao et al., 2013). The total biomethane production was enhanced by 35.84% through acid pre-treatment on RS compared with untreated straw (Zhao et al., 2010). However, chemical pre-treatment requires high quantities of chemicals to balance the reaction conditions. After chemical and thermal pre-treatment, non-degradable biological and hindering compounds can be produced in the system (Fu et al. 2018). Alkaline pre-treatment (NaOH) is the most common chemical pre-treatment used in the AD of RS (Sabeeh et al., 2020; Khalid et al., 2019; Chuetor et al., 2021).

Table 2.1 Several pre-treatment approaches of RS

Pre-treatment method	**measures**	**Operating Conditions**	**Raw material(s)**	**results**	**mechanism**	**Reference**
physical	Wet disk milling	Grinders revolve at 1800 rpm, repeated 2-20 times with an operation time of 3 min.	RS	Produce no inhibitors of fermentation	Effectiveness in enzymatic hydrolysis	(Hideno et al., 2009)
	Hydrothermal	At a temperature of 200^0 C with a residence time of 10 min, saturated water vapour pressure 1.55MPa	RS	Biogas production increased by 225.6%	Accelerating the pre-hydrolysis	(Chandra et al., 2012)
	Ball milling	At 1700 rpm, milled for the required time (5,10,15,30,60,120 or 180 min)	RS	Increased monomeric sugar yields	Enzymatic hydrolysis with milling time	(Hideno et al., 2019)
	extrusion	Screw extruder at 74kW, at a temperature of 40^0 C	RS, maize silage and triticale silage	Methane production increased by 16%	Organic matter degradation	(Menardo et al., 2015)
	Hot-compressed water treatment	Autoclave required temperature 160-180^0C at 2-3^0 C/min heating rate maintained for 30min with nitrogen initial pressure 2.0 MPa	RS	Generation of phenolic and heterocyclic compounds	Solubilisation of lignin and hemicellulose	(Hideno et al., 2009)
chemical	NaOH	At temperature 37^0 C, 3% NaOH on a dry basis, residence time 120h	RS	Biogas production increased by 132.0%	Improvement of biodegradability	(Chandra et al., 2012)
	acid	0.075,0.15,0.3,0.75 mol/L acetic acid and propionic acid mixed solution in a 1:1 ratio	RS	34.19% lignin removed; methane production improved by 35.85%	Enhanced the biodegradability of RS	(Zhao et al., 2010)
	alkali	1% (w/v) NaOH for 3h at room temperature	RS	Methane yield increased by 34%, and the removal of lignin	Decrystallisation of cellulose and subsequent swelling (not direct degradation)	(Shetty et al., 2017)
	alkali	1.6% (w/w) NaOH for 24h at 30^0 C in a 500 mL bottle	RS	Methane yield increased by 21.4%	Increased hydrolysis	(Mancini et al., 2018)

	$Ca(OH)_2$ + extrusion	5%, 8%, 10%, 12% and 15% $Ca(OH)_2$ on dry basis and incubated for 72 h at 25^{o} C	RS	Increased biogas production by 36.7%	Increase fermentable sugar content and remove lignin	(Gu et al., 2015)
	Ionic liquid	[C_4mim]Cl at 120^{0} C	RS	Increased biogas productivity b 140% and reduced lignin percentage by 64.8%	Reduce the crystallinity and increase accessible surface area	(Gao et al., 2013)
	Hydrogen peroxide (H_2O_2)	1%, 2.5% and 4% H_2O_2 for 1, 4 and 7 days	RS	Reduced concentrations of lignin, cellulose and hemicellulose and improved methane yield by 88%	Increased accessibility to cellulose, hemicellulose, lignin and rupturing of cell wall	(Song et al., 2013)
biological	Biological solubilisation	Peptone-cellulose solution	RS+PM	Increased VMPR by 62.4% and methane yield by 37.8%	Breakdown complex structure into cellulose and hemicellulose	(Shen et al., 2018)
	fungal	Trichoderma reesei	RS	Removal of lignin by 23.6%, hemicellulose and cellulose	Increasing surface area and larger pore size	(Mustafa et al., 2016)
	fungal	Pleurotus ostreatus	RS	Improved methane yield by 92%	Removal of lignin	(Mustafa et al., 2017)
	fungal	Pleurous ostreatus	RS	Degradation of lignin, cellulose and hemicellulose and improved biomethane yield by 120%	Increasing surface area and larger pore size	(Mustafa et al., 2016)
Physical-chemical	Extrusion-alkali	0.45mm sieve extruder with NaOH solution at 35^{0} C, 3-120h	RS	Increased methane production by 54%, energy recovery efficiency from 38.9 to 59.9%, biodegradability	Change in physical and chemical composition	(Zhang et al., 2015)
Physical-biological	Fungal and milling	Pleurotus ostreatus	RS	Improved methane yield by 165%	The incubation time of fungal treatment and removal of lignin	(Mustafa et al., 2017)

2.2.4 Biological pre-treatment

Biological pre-treatment has gained attention throughout the world because of the additional benefits over physical and chemical pre-treatments, such as lesser energy needs, surface and reaction specificity, no initiation of the toxic compounds and huge production of desired products (degradation of inhibitory substances, raise in substrate quality) (Yuan et al., 2012; Yuan et al., 2014; Zhong et al., 2011). Biological treatment is majorly related to the reaction of fungi such as white, brown and soft rot fungi (Cianchetta et al., 2014). In *P. ostreatus* pre-treatment of RS, both incubation time and moisture content had a notable effect on hemicellulose, cellulose and lignin degradation. The temperature and time of biological pre-treatment degradation by domesticated paddy soil microbes are highly important for RS biodegradability (Luo et al., 2022). In contrast, in *T. reesi* pre-treatment, moisture content has no significant change in the degradation of lignin (Mustafa et al., 2016). Degradation of cellulose, hemicellulose, lignin and dry matter has increased in *P. ostreatus* and *T. reesi* pre-treatment. Both treatments degraded hemicellulose and lignin to greater extend compared with cellulose in RS (Mustafa et al., 2016). In *T. reesi* pre-treatment, methane yield improved by 9–78% at 65% and 75% moisture content, whereas at 85%, methane yield decreased by 3–30% compared with raw RS (Mustafa et al., 2016). Selecting the correct combination of incubation time and moisture content results in significant potential of substrate digestion (Mustafa et al., 2016). With cellulolytic microbial consortium pre-treatment on co-digestion of RS and PM, volumetric biomethane production has increased by 62.4% at OLR of 2.5 kg COD/(m^3d), and methane yield has increased by 37.8% (Shen et al., 2018). Even though biological pre-treatment has advantages, it is a long-drawn-out process that needs most control of the growth environment and other complicated needs for the equipment and operator conditions (Chandra et al., 2007). When a high biomass degradability rate is required, biological pre-treatment may be expensive (Sun & Cheng, 2002; Luo et al., 2022).

2.2.5 Anaerobic co-digestion

Co-digestion is a treatment strategy in which several feedstocks are mixed and treated at a time (Kwietniewska and Tys, 2014). AD of the single substrate is ineffective due to raw material inconsistent features, feedstock acidifications, non-availability of trace elements, and improper quality of inorganic substances, which alter the AD performance, and above listed difficulties can be overcome by co-digestion with various biogas anaerobically (Zhang et al., 2018). Co-digestion can minimise the gap between the peak and valley values of methane yield for a stable gas supply (Li et al., 2015). Co-digestion produced more total methane yield than individual mono-digestions (Ye et al., 2013; Jiunn-Jyi et al., 1997) and is considered to be the most economical technique than pretreatment for lignocellulosic biomass (Ye et al., 2013). On comparing with sole substrate, mixing RS at different ratios eventually improved the methane production of the feed (Zhang et al., 2018). Co-digestion can use bacterial diversities and nutrients in several wastes to optimise the digestion process of straw (Babaee et al., 2013). Addition of nitrogen sources such as domestic animal manure like chicken manure (Mei et al., 2016), pig manure (Shetty et al., 2017; Zhang et al. 2015), cow manure (Cai et al., 2017) and pig urine (Meng et al., 2018) has adopted to get the most economical and efficient methane production (Mccarty & Mckinney, 1961). Methane yield of co-digestion of RS and cow manure (CM) is only a little greater than mono-digestion of RS and CM (Li et al., 2015). Co-digestion of RS and MSW produced the best results at a 1:2 ratio and the least in RS alone; on the contrary, digestion of RS produced maximum cumulative methane results with 65 ± 0.93% and least at RS: MSW ratio of 1:1 (Negi et al., 2018). Co-digestion with pig urine produces the highest biomethane yield at lower F/I ratios (Meng et al., 2018). RS at 4% mixed with sewage sludge balanced the C/N ratio and enhanced biogas productivity by around 6 times more than mono sludge, with a methane composition of 60–63% (Dai et al., 2018).

When kitchen waste is used as a co-substrate, from 1:2 concerning RS, methanogenesis was utterly inhibited by VFA accumulation, and digestion did not recover even with pH adjustment with $NaHCO_3$ and NaOH (Ye et al., 2013). It has been stated that low bulk density of RS and high inoculation has enhanced the utilisation efficiency of the digester volume in co-digestion of RS and pig urine (Kargbo et al., 2010). Effective methane production was observed in the co-digestion of high-carbon RS and nitrogen-rich SS in two-stage H_2 and CH_4 fermentation compared with the one-stage CH_4 fermentation process (Albertson, 1961). Co-digestion of UARS and FW reduced the Na^+ concentrations and ionic compounds, which can hinder methanogenesis in AD (Zhang et al., 2018). At a 1:3 ratio of UARS and FW, a combination of Co and Ni showed better performance, and comparatively stable pH indicates merging of Co cooperated with Ni, which is of significant importance in co-digestion, but there is only an 8.83% high methane yield than the sole substrate which is not significant; in contrast, urea-ammoniated RS and raw RS improved biodegradability substantially (Zhang et al., 2018). Co-digestion improves the methane yield of the AD system and balances the C/N ratio of RS when digested along with a low C/N ratio substrate; however, it is important to choose the co-substrate carefully.

Table 2.2 Co-digestion of RS with several substrates for enhancing the performance of the AD process

Feedstock	**Operational conditions**	**Action of co-digestion**	**Influencing factor**	**Reference**
RS+CM	At 37±10^{0}C temperature, 2.5L working volume for batch and 40L for continuous studies	Improve biomethane yield by 5.8% and system stability	High Buffer capacity	(Li et al., 2015a)
RS+MSW	At 37^0 C temperature, 150mL working volume at 100rpm for batch studies	Improved biomethane and biogas yield by 57% and 60%	C/N ratio	(Negi et al., 2018a)
RS+PM	At 55^0 C temperature, 20L digester volume for a semi-batch system with varied TS contents for constant OLR	Achieved stable biogas production at higher TS contents, i.e., 32%	Constant OLR	(Riya et al., 2018b)
RS+SS	At 35±1^0 C mesophilic temperature	enhanced biogas yield by six times	C/N ratio	(Dai et al., 2018)
RS +SS	AT 55 ^{0}C thermophilic temperature in batch studies	High Volatile solids removal efficiency by 60.4% and improved methane yield by 59.6% in the two-stage system to one-stage system	Effective utilisation of VFAs	(Albertson, 1961)
RS+PU	At different F/I ratios, in 500 mL volume at 55±1^{0}C thermophilic temperature in batch scale	Lower F/I ratios are recommended and produce high biogas	Low VFAs produced and permissible pH and ammonia concentration	(Meng et al., 2018a)
RS+PM+KW	At 37±1^0 C mesophilic temperature with a working volume of 2 L	Increased biogas yield than sole substrate by 71.67%	Nutrient balance	(Ye et al.,, 2013)
RS+PM	At 37±1^0 C temperature with a working volume of 2.5 L batch system and 37±2^0 C temperature with a working volume of 40 L	Stable biogas production reduced the gap between valley and peak values	Less ammonia formation	(Li et al., 2015a)
RS+PM	At 35±0.5^0 C mesophilic temperature with working volume 9 L with stirring speed 30r/min	Increased VMPR by 62.4% and methane yield by 37.8%	Balanced total alkalinity	(Shen et al., 2018)
UARS+FW	At 35^0 C temperature, in 1L volume shaken for 60 r/min for 14 days and supplemented with Ni and Co	Increased methane production by 8.83%	Combination of Co with Ni	(Zhang et al., 2018)

MSW – Municipal solid waste, PU – Pig urine, KW – Kitchen waste

2.3 Summary

AD is a reliable technology for digesting RS. RS can be digested productively under mesophilic and thermophilic temperatures but is found to be operated at mesophilic conditions in most cases. Even though VFA is hindering the AD process of RS (more than the threshold value), the VFA accumulation effect is balanced by the buffering capacity of the substrates due to the co-digestion process. As hydrolysis is the rate-limiting step in AD, pre-treatment improves solubility and reduces the complexity and enhances the biogas production by 16–225.6%, whereas milling and alkaline pre-treatment (NaOH) is the most used physical and chemical pre-treatments, respectively, among all and other pre-treatments that can be explored further in research. Co-digestion balances the C/N ratio of the AD system of RS when digested with low C/N ratio substrates like cow manure, chicken manure, food waste in turn, improves the biomethane yield by 5.8–71.67%. However, it is recommended to choose the co-substrate wisely. As RS is high in TS (90–96%) content, SS-AD is suggestible to use L-AD digestate as inoculum. From the literature, it can be concluded that the AD of RS has very good potential to generate biogas by adopting appropriate pre-treatment technology and co-substrates for its effective utilisation. According to the conclusions, co-digestion and pre-treatment technologies were chosen for AD of RS for its effective utilization.

Chapter 3 Materials and Methods

The present chapter is aimed to describe the materials, analytical methods, and experimental procedures carried out in the present study.

3.1 Material collection and preservation

In the current study, the main substrate is RS, and the co-substrates chosen are food waste (FW), sewage sludge (SS), cow manure (CM), and chicken manure (ChM). These organic co-substrates were chosen based on their local availability and suitability for AD of RS. RS was collected from farmlands in Kazipet of Warangal district, Telangana, India (representing locally available RS, the quality may differ with the seed grains). The collected RS was dried in an oven at 50°C for two days to remove the moisture content. The dried RS was cut manually to 1-2 cm and stored in airtight polyethene bags at room temperature for further use. FW is leftovers from the hostel mess in the National Institute of Technology Warangal campus. The FW is mixed and macerated to achieve homogeneity. It consists mostly of cooked rice, vegetables, and legumes dal. Sun-dried sewage sludge is obtained from the wastewater treatment plant at the NIT Warangal campus. It is a conventional wastewater treatment plant that runs using an activated sludge process (ASP) to treat the wastewater generated on the NITW campus. The plant runs daily with a volume of 600 m^3. CM was brought from agricultural lands near Kazipet, Warangal district, Telangana, India. ChM was brought from farms near kazipet, Warangal. The coarse material was removed manually from SS, CM, and ChM, and all the co-substrates were stored at a temperature of 4°C in the refrigerator until further use to maintain freshness and prevent possible degradation. Inoculum is a liquid anaerobic digestate from the anaerobic plant installed at the NIT Warangal campus that processes FW generated in the campus hostels, which has been acclimated for five days at 37°C ± 2°C.

3.2 Measurement methods and instrumentations

The physicochemical characterisation of substrates and co-substrates is carried out by analysing pH, total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) according to the "Standard Methods for the examination of water and wastewater" (APHA, 2017). The biogas volume was manually measured with a gas-tight syringe (140 mL, equipped with a stopcock). Biogas was analysed for its composition using a gas chromatograph (YL Instruments Model 6500) (Plate. 3.3) equipped with a thermal conductivity detector and a stainless-steel column length of 15 feet packed with Porapak Q (80–100 mesh). Hydrogen was used as a carrier gas, and the injection port, detector, and column oven temperature were maintained at 40℃, 100℃, and 50℃, respectively. Biogas samples are analysed on alternative days using a standard biogas mixture having 51.65% carbon dioxide and 48.35% methane by volume. Carbon and nitrogen of substrates were analysed using elemental analysis (Euro EA Elemental Analyzer). Cellulose, hemicellulose, and lignin were measured using the approach outlined by (Li et al., 2004). Volatile fatty acids are computed from the Nordmann method (Purser et al., 2014). Liquid samples were centrifuged at 6000 rpm for 15 minutes at room temperature and filtered with a 0.22 µm membrane filter for VFA analysis.

All feedstock mixtures taken for batch and semi-continuous study were analysed for all the parameters i.e., Total solids (TS), Volatile solids (VS), pH, and Chemical oxygen demand (COD). All the characterisations were duplicated, and the averages were taken for further interpretation. All the chemicals used for the analysis are of analytical grade.

3.3 Experimental setup

3.3.1 Batch scale

AD experiments were carried out in batch reactors (glass bottles) and a pilot-scale reactor. The batch scale reactor has a capacity of 120 mL (Plate. 3.1). Each reactor is fed with appropriate proportions of feedstock and inoculum. Enough space is left for biogas generation and collection in each reactor. The reactor bottlenecks were tightened with a rubber cork and aluminium crimp with an attachment for collecting biogas after flushing with nitrogen in the head space. Reactors were manually shaken to mix the contents two times a day. Duplicate bottles were kept for each mix and were controlled at a mesophilic range of 37˚C ± 2˚C. No external nutrients, external alkalinity, or inocula were added to the bottles. To determine the gas composition, we measured the volume of gas every day with a leak-proof glass syringe and every alternate day after an eventual decrease in gas production.

3.3.2 Pilot scale

The pilot scale reactor has a capacity of 500 L (Plate. 3.2). Biogas was collected with a collection pipe attached to the reactor and measured the biogas every alternate day. The same pilot scale reactor was used for batch pilot scale and semi-continuous pilot scale study. There was set up at the top and bottom with air-tight valves to semi-continuous study for feeding and removal of feed respectively. The top and bottom valves were closed for batch pilot scale study and ensured no leakages. In the homogenisation of RS, four sizes were chosen, i.e., 3-5 cm, 1-2 cm, 5-10 mm, and < 300 µm, where 3-5 cm and 1-2 cm sizes of RS were cut manually, 5-10 mm and < 300 μm were ground and sieved between 9.5 mm and 4.75 mm sieves, and 300 μm sieve respectively.

Plate. 3.1 Batch scale experiments

Plate 3.2 Pilot scale reactor

Plate. 3.3 Gas chromatography system for biogas analysis

3.3.3 Co-digestion with various Total solid contents (TS %)

The solid-state anaerobic co-digestion of RS has been studied to form six ternary mixtures as i) RS+FW+CM, ii) RS+FW+ChM, iii) RS+FW+SS, iv) RS+SS+CM, v) RS+SS+ChM, vi) RS+CM+ChM simultaneously at four different TS contents, i.e., 15%, 20%, 25% and 30%. The co-digestion experiments were carried out for 65 days. RS substrate, high in C/N ratio of around 50, was co-digested with complementary substrates with low C/N ratios, and all combinations were formulated to see a 25 - 27 C/N ratio, as suggested by (Shah et al., 2015).

3.3.4 Pre-treatment with various RS sizes

Various sizes of RS that were opted for were 3-5 cm, 1-2 cm, 5-10 mm, and < 300 µm through literature. 3-5 cm and 1-2 cm RS sizes were cut manually, 5-10 mm RS sizes were ground and sieved remains between 4.75 mm and 10 mm, where < 300 µm RS sizes were ground and sieved through a 300-micron sieve. The samples were stored in air-tight containers. In anaerobic co-digestion of RS, four pre-treatments were adopted, i.e., thermal, H_2O_2, thermal + H_2O_2, and H_2O_2 + thermal pre-treatment. For thermal pre-treatment, RS was set to 100° C temperature for 30 minutes in an autoclave then the sample was sundried and stored for further use. For H_2O_2 pre-treatment, 100 g of RS was soaked in 300 mL of H_2O_2 for five days; then, the sample was sundried and stored for further use. Thermal + H_2O_2 and H_2O_2 + thermal pre-treatments are combined pre-treatments of both thermal and H_2O_2 pre-treatments.

3.4 Modified Gompertz model

The cumulative biogas production and fermentation time are closely related to each other. The modified Gompertz model can simulate the experimental biogas production values obtained for different co-digestion mixtures at various TS % ranges (Nguyen et al., 2016). In the current study, a modified Gompertz model is adopted for the performance evaluation of the co-digestion due to its robustness. The model assumes that the methane yield (mL CH_4/g- VS) from AD is a function of microbial growth (Patil et al., 2012). The model is expressed as (Eq. 3.1).

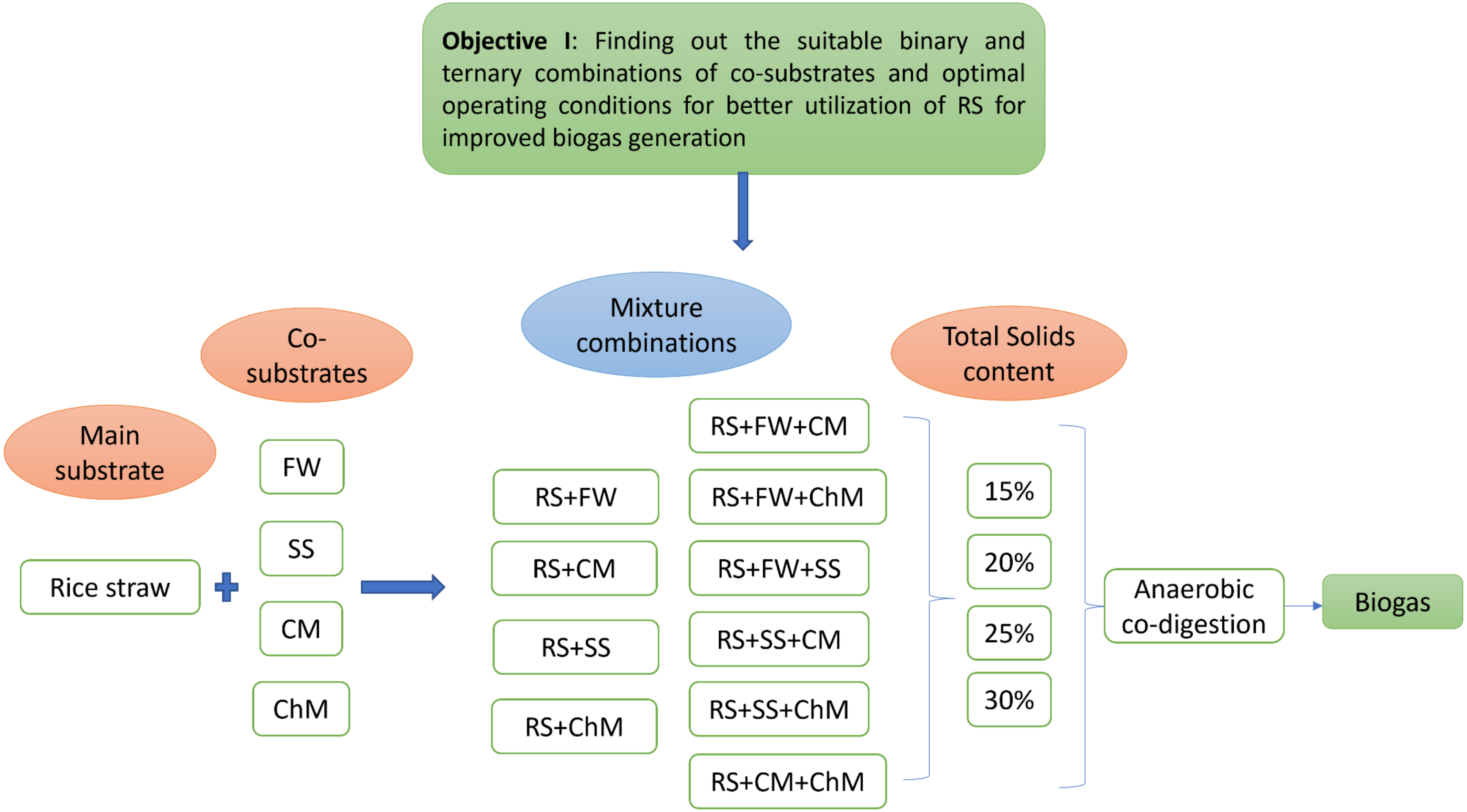
$$Y(t) = P_{max} \times \exp\{-\exp[\frac{R \times e}{P_{max}}(\lambda - t) + 1]\} \qquad \text{Eq. 3.1}$$

where Y(t) (mL/g-VS) is the cumulative biogas production at time t, P_{max} (mL/g-VS) is the maximum methane potential, R (mL/g-VS d) is the biogas production potential, λ (days) is the lag phase time, and e is Euler's constant of value 2.7182. Kinetic parameters P_{max}, R and λ are estimated using a nonlinear least-square regression method using experimentally obtained methane yield. The kinetic parameters are used to predict the methane yield. The predicted methane yield from the model is plotted with the obtained methane yield in the AD experiments. The goodness of fit for the kinetic parameters is diagnosed using a coefficient of determination (R^2). The above equation is fitted with cumulative biogas production curves using OriginPro 2021 software.

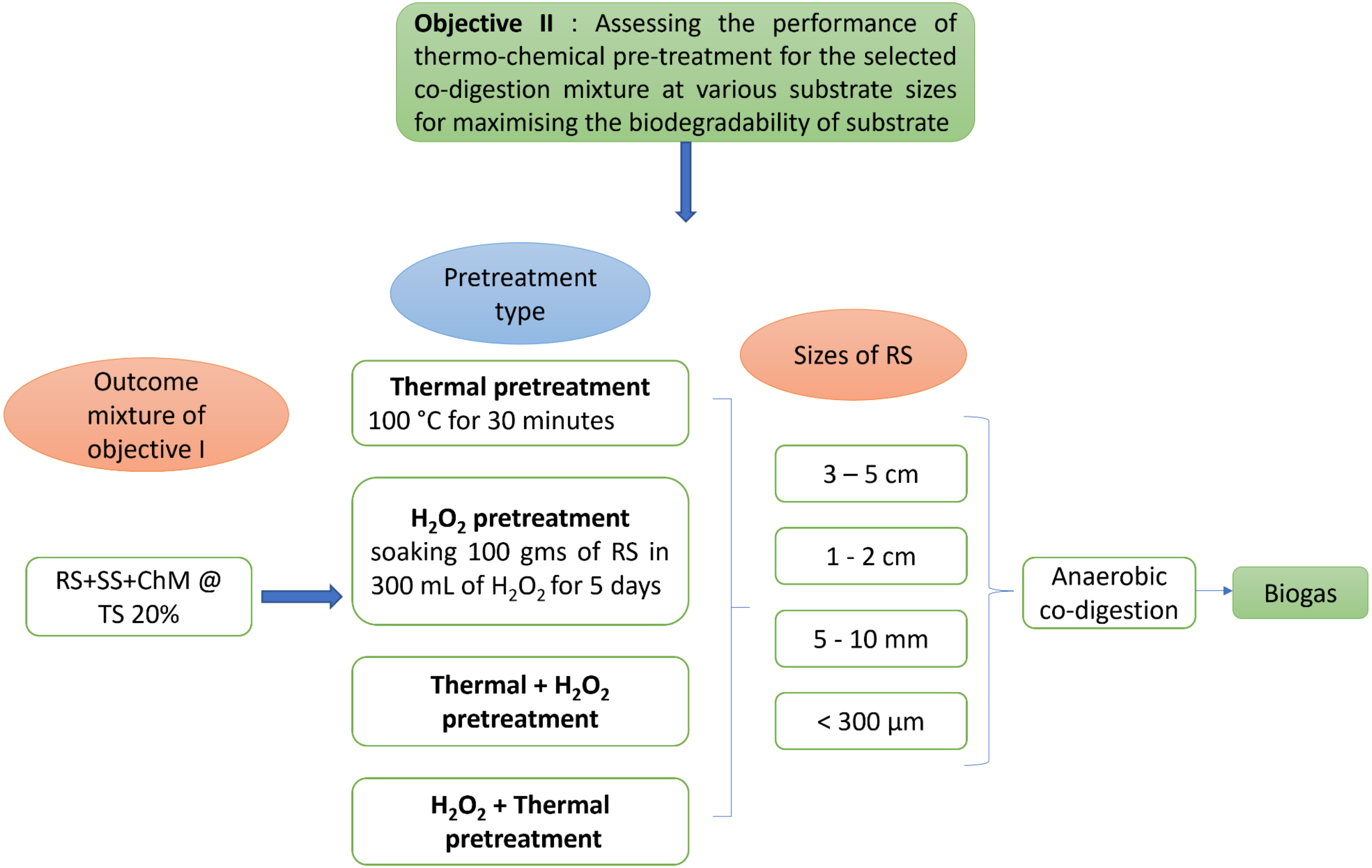
3.5 Computation of GHG emissions

The GHG liberations through the open field combustion of RS are evaluated in different stages with various factors according to (IPCC 1996). There are several kinds of GHG emissions from open burning, harvesting, and, AD of RS which were explained in brief with parameters in this section, and is fully described in Chapter 7. GHG emissions from open burning of RS was computed with parameters: a) residue-to-crop ratio (RCR), b) Dry matter fraction (DMF), c) Fraction burned (FB), d) Carbon fraction (CF), e) Nitrogen carbon fraction (NCR), f) Emission ratio (ER), g) Conversion ratio (CR) and, h) crop or grain Production (P). Emissions of methane (CH_4) and carbon dioxide (CO_2) from AD of RS, the theoretical CH_4, CO_2, and trace gases found with equation by Chen et al. (2015) with elemental analysis representing RS as $C_aH_bO_cN_d$. Emissions of methane from rice farming, Emissions of Nitrous oxide (N_2O) through rice cultivation, and Emissions of carbon dioxide from urea application.

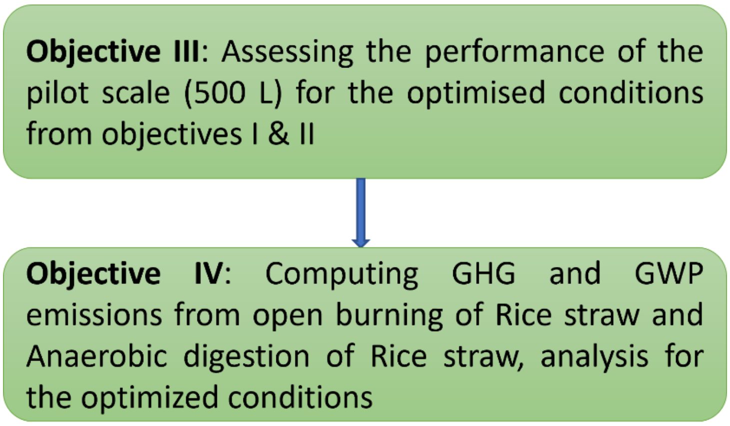
3.6 Workflow chart



Objective I: Finding out the suitable binary and ternary combinations of co-substrates and optimal operating conditions for better utilization of RS for improved biogas generation



Objective II : Assessing the performance of thermo-chemical pre-treatment for the selected co-digestion mixture at various substrate sizes for maximising the biodegradability of substrate



Objective III: Assessing the performance of the pilot scale (500 L) for the optimised conditions from objectives I & II

Objective IV: Computing GHG and GWP emissions from open burning of Rice straw and Anaerobic digestion of Rice straw, analysis for the optimized conditions

Plate 3.4 Work flow chart

Chapter 4 Anaerobic co-digestion of RS with binary and ternary mixtures

This chapter is aimed to describe the characteristics of substrates, co-digestion combinations at various total solids and data analysis. Summery is presented at the end of the chapter.

4.1 Characteristics of substrates

The main substrate in the current study is rice straw (RS), and the co-substrates chosen are food waste (FW), cow manure (CM), sewage sludge (SS), and chicken manure (ChM). These co-substrates were chosen based on their local availability and suitability for AD of RS. All the substrates were characterised in triplicates, and their average value was taken to represent the sample. The obtained proximate and ultimate analysis of all substrates are shown as follows:

4.1.1 Proximate analysis

Proximate analysis indicates the potential suitability of substrates for AD. The proximate analysis gives moisture content, total solids content, volatile content, and ash content. Moisture content represents the water content per unit mass of biomass. It affects the heating value of the substrate. High moisture content indicates a low heating value since heat is required to evaporate the moisture contained. The moisture content of each substrate is presented in Plate 4.1. It can be observed from the plate that FW, CM, SS and ChM have a moisture content ranging between 50 to 65 %, and RS has a low moisture content (10.23 %).

The total solids (TS %) content of each substrate which represents organic and inorganic content in biomass is presented in Plate. 4.2. It can be observed from the plate that RS has high total solids (TS %) content of around 90%, relatively high compared to FW, CM, SS and ChM and a similar range of values is reported by (Ye et at., 2013).

Volatile solids (% of TS) of each substrate which represents the probable biodegradable organic fraction of biomass are presented in Plate. 4.3. It can be observed that the selected co-substrates have quite a good amount of volatile solid content (69 – 93 %), indicating the potential for biological degradation and subsequent biogas production, whereas ChM has low volatile solids content compared to remaining substrates however, AD of ChM reported higher biogas production in earlier studies (Sukesh et al., 2019).

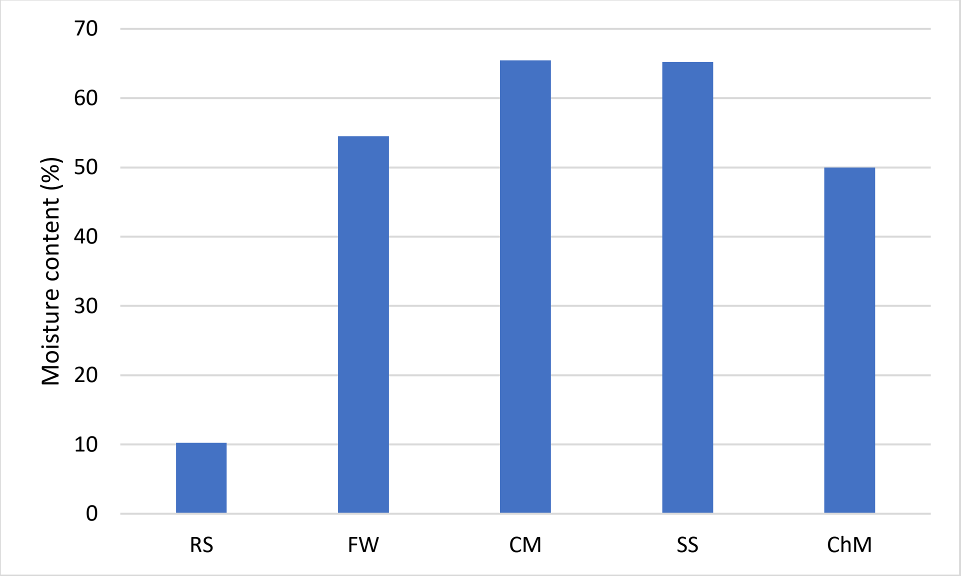


Plate. 4.1 Moisture content of substrates

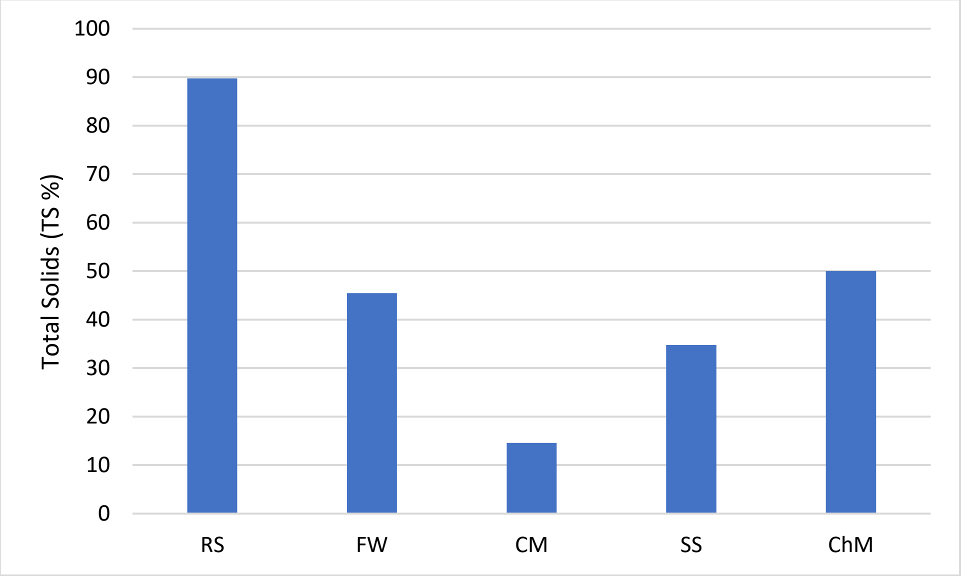


Plate. 4.2 Total solids content of substrates

Ash content is the non-volatile organic matter left after thermal digestion at 550° C. The substrate with lower ash content indicates a better substrate for AD. Ash content for all substrates is presented in Plate. 4.4. It can be observed from Plate. 4.4 that all the substrates have low ash contents (7 to 30 %), indicating the feasibility of organic wastes for AD except for ChM.

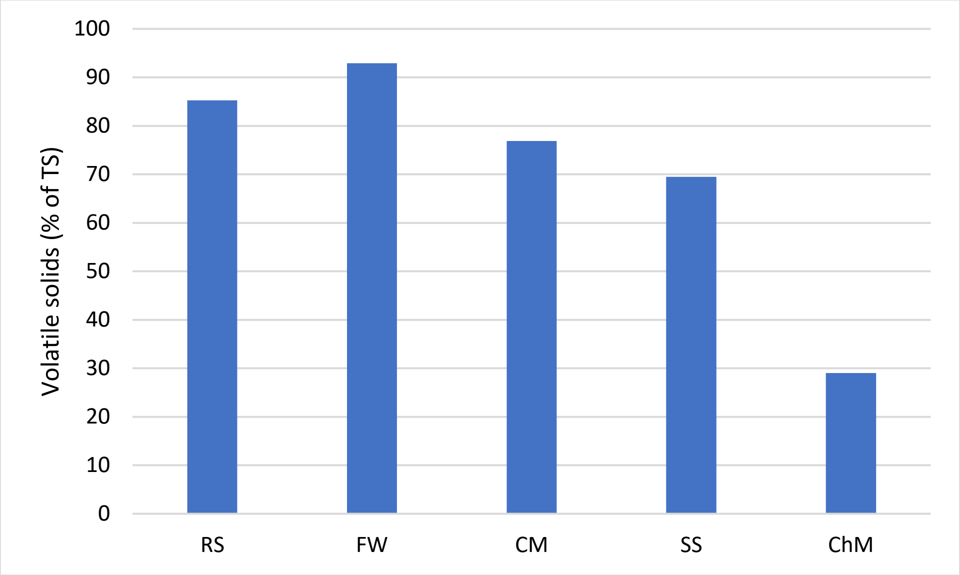


Plate. 4.3 Volatile solids content of substrates

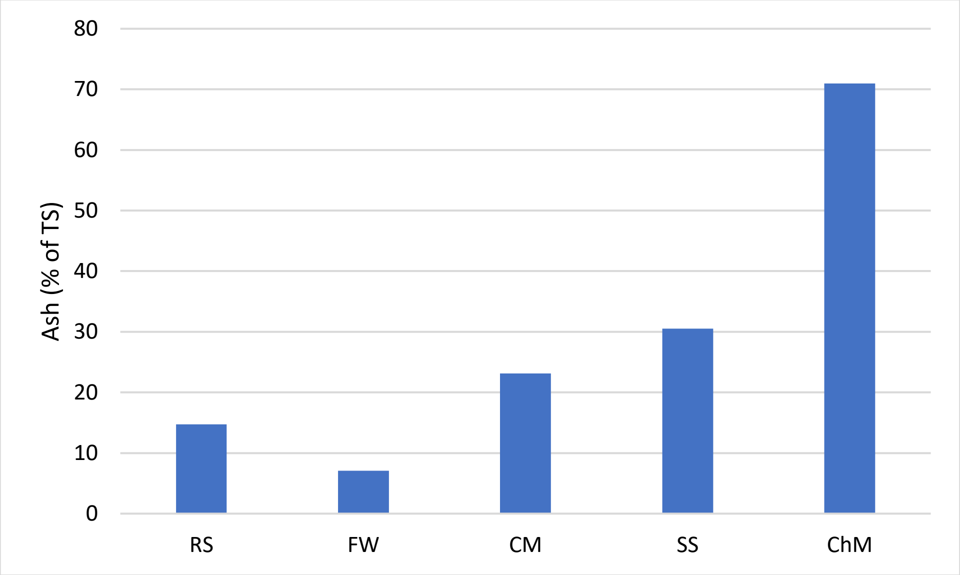


Plate. 4.4 Ash content of substrates

4.1.2 Ultimate analysis

The ultimate analysis is carried out to determine the composition of carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) content of each substrate on a weight basis. The elemental composition of each substrate is represented in Table 4.1. It can be observed that the selected substrates have a reasonably good amount of carbon content (14.42 - 42.36%). The substrates such as FW, CM, SS, and ChM have relatively high nitrogen content (near and more than 2.0 %) compared to RS. The variations in elemental composition are due to the diverse source of organic wastes and constituents present in them. For example, CM has high nitrogen content due to the presence of uric acid and undigested protein (Abouelenien et al., 2014).

Table 4.1 Ultimate analysis of all substrates (weight %)

Substrate	**C (%)**	**H (%)**	**N (%)**	**S (%)**	**C/N ratio**
Rice straw (RS)	37.82 ± 0.0	6.80 ± 0.0	0.74 ± 0.0	0.27 ± 0.0	51.10
Food waste (FW)	42.36 ± 0.0	5.80 ± 0.1	4.07 ± 0.0	0.37 ± 0.0	10.41
Cow manure (CM)	27.23 ± 0.1	4.18 ± 0.0	2.31 ± 0.0	0.25 ± 0.0	11.79
Sewage sludge (SS)	22.23 ± 0.0	3.59 ± 0.1	2.20 ± 0.0	0.86 ± 0.0	10.10
Chicken manure (ChM)	14.42 ± 0.2	1.94 ± 0.0	1.83 ± 0.0	0.54 ± 0.0	7.88

C/N ratios of all the substrates analysed in the present study which is the carbon matter per unit of nitrogen and is a better indicator to represent the nutritional content of feedstock for microorganisms (Kainthola et al., 2019a) are shown in Plate. 4.5. The presence of a high C/N ratio (low nitrogen content) may cause the system to be devoid of nitrogen, which is a structural element for microorganisms. A low C/N ratio (high nitrogen content) may release toxic ammonia nitrogen that could affect the microbial communities in AD (Li et al., 2013). From Plate. 4.5, it is shown that the C/N ratio of RS is higher (51.1), representing more carbonaceous matter, whereas, in FW, CM, SS and ChM, it is less (7.88 to 11.79), representing nitrogen-rich matter. The four co-substrates were chosen accordingly to balance the C/N ratio of the overall digester feed to between 25 – 27 (Shah et al., 2015). The imbalanced nutritional characteristics of the organic matter may not yield biogas at its optimal level, although it has good potential for biogas production (Chen et al., 2008). The balancing of nutritional content is necessary for optimal biogas production.

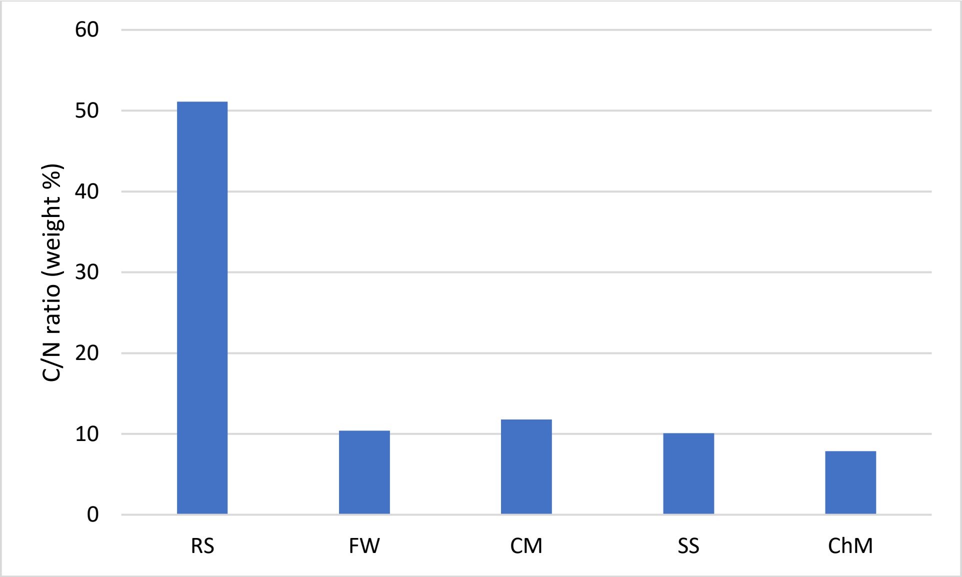


Plate. 4.5 C/N ratio of each substrate (weight %)

4.1.3 Cellulose, Hemicellulose and Lignin analysis

The typical chemical characteristics of RS biomass like lignin, cellulose and hemicellulose of RS are 13.1%, 33.14% and 19.73%, respectively. This falls under the range Japan Institute of Energy (2002) gave, which is 12% lignin, 25% hemicellulose and 38% cellulose.

Table 4.2 Cellulose, Hemicellulose and Lignin analysis of RS (% TS)

Substrate	**Cellulose (%)**	**Hemicellulose (%)**	**Lignin (%)**
Rice straw (RS)	33.14 ± 1.15	19.73 ± 1.28	13.1 ± 0.52

4.2 Anaerobic co-digestion (A-co-D) of RS - Experimental design

In the present study, co-digestion of various mixtures, i.e., binary and ternary co-digestion mixtures at four different TS contents, i.e., 15%, 20%, 25% and 30% at mesophilic temperature (37 ± 2 ºC) were chosen to evaluate their influence on biogas production. The solid-state anaerobic co-digestion of RS has been studied at four binary co-digestion mixtures as i) RS+FW, ii) RS+CM, iii) RS+SS and iv) RS+ChM at four TS contents counts for 16 combinations. In the case of ternary co-digestion, it forms six ternary mixtures as i) RS+FW+CM, ii) RS+FW+ChM, iii) RS+FW+SS, iv) RS+SS+CM, v) RS+SS+ChM, vi) RS+CM+ChM simultaneously at chosen TS contents, i.e., 15%, 20%, 25% and 30%. RS, a high C/N ratio substrate of around 50, was co-digested with complementary substrates with low C/N ratios, and all combinations were formulated to see a 25 C/N ratio (Shah et al., 2015). The co-digestion mixtures are shown in following Table 4.3.

Table 4.3 Experimental design of binary and ternary mixtures for AD of RS

Mixture type	**Combinations – TS%**	**Remarks**
Binary mixtures	1. RS + FW -15% 2. RS + FW - 20% 3. RS + FW - 25% 4. RS + FW - 30% 5. RS + CM - 15% 6. RS + CM - 20% 7. RS + CM - 25% 8. RS + CM - 30% 9. RS + SS - 15% 10. RS + SS - 20% 11. RS + SS - 25% 12. RS + SS - 30% 13. RS + ChM - 15% 14. RS + ChM - 20% 15. RS + ChM - 25% 16. RS + ChM - 30%	High C/N ratio substrate (RS- carbon rich substrate) and Low C/N ratio substrates (FW, CM, ChM, SS- nitrogen rich co-substrates)
Ternary mixtures	17. RS + FW + CM -15% 18. RS + FW + CM - 20% 19. RS + FW + CM - 25% 20. RS + FW + CM - 30% 21. RS + FW + ChM - 15% 22. RS + FW + ChM - 20% 23. RS + FW + ChM - 25% 24. RS + FW + ChM - 30% 25. RS + FW + SS - 15% 26. RS + FW + SS - 20% 27. RS + FW + SS - 25% 28. RS + FW + SS -3 0% 29. RS + SS +CM - 15% 30. RS + SS +CM - 20% 31. RS + SS +CM - 25% 32. RS + SS +CM - 30% 33. RS + SS + ChM - 15% 34. RS + SS + ChM - 20% 35. RS + SS + ChM - 25% 36. RS + SS + ChM - 30%	

	37. RS + CM + ChM - 15% 38. RS + CM + ChM - 20% 39. RS + CM + ChM - 25% 40. RS + CM + ChM - 30%	

4.3 Anaerobic co-digestion of binary combinations of RS

4.3.1 Biogas and methane production

A-Co-D experiments are carried out as per the design, and corresponding biogas production in mL is noted for 70 days; after that, experiments are terminated due to negligible biogas production. Cumulative biogas production for various binary co-digestion mixtures of RS with FW, CM, SS and ChM at four TS contents are illustrated in Plate 4.6, along with the control, which is RS mono digestion. The biogas values are normalised using VS content present in particular mixtures. Plate 4.6 shows the cumulative biogas production in binary combinations at four TS contents. The y-axis (biogas production) is taken the same for all the graphs to show the relative difference in each graph. The purple line is for the control system, which is a mono-digestion of RS adding no co-substrate. From the graph, it is observed that the maximum cumulative biogas production out of all the combinations was observed as 385 mL/g-VS in RS+CM mixture in 52 days at a TS content of 15 % with an average methane content of 51.34 %. For mixture RS+ChM, 376.02 mL/g-VS of cumulative biogas was produced at TS 30%, which is near to the maximum biogas observed in RS+CM and with an average methane content of 54.06%, which is more than the RS+CM combination mentioned above.

Plate 4.7 shows each co-digestion mixtures methane content at selected TS contents. Generally, methane content in biogas is between 40-60 (Panigrahi & Dubey, 2019). Comparing methane content in each mixture, RS+FW has a lesser value than the other three mixtures where RS+CM and RS+ChM have high methane content. Among the four TS contents in RS+ChM, at TS 15%, 25% and 30% produced 352.59, 329.59 and 376.12 mL/g-VS, respectively. Whereas in RS+CM, the highest production is seen in two TS contents, 15% and 20 % with values 385.4 and 384.10 mL/g-VS, with little difference in the two combinations. The least production is observed in RS+FW with 57.94 mL/g-VS at 30% TS, and after 15 days, the biogas production was observed to be diminishing. In the RS+FW combination, while increasing the TS from 15 to 30%, the biogas production decreased gradually, with values of 129.81, 106.77, 78.70, and 57.94 mL/g-VS, respectively. In the RS+SS combination, a lag phase has been observed in the starting digestion, and the digestion has continued for up to 70 days. Overall, the co-digestion has improved the biogas production than the mono-digestion in all combinations at four considered TS contents with average methane content between 40 - 53%.

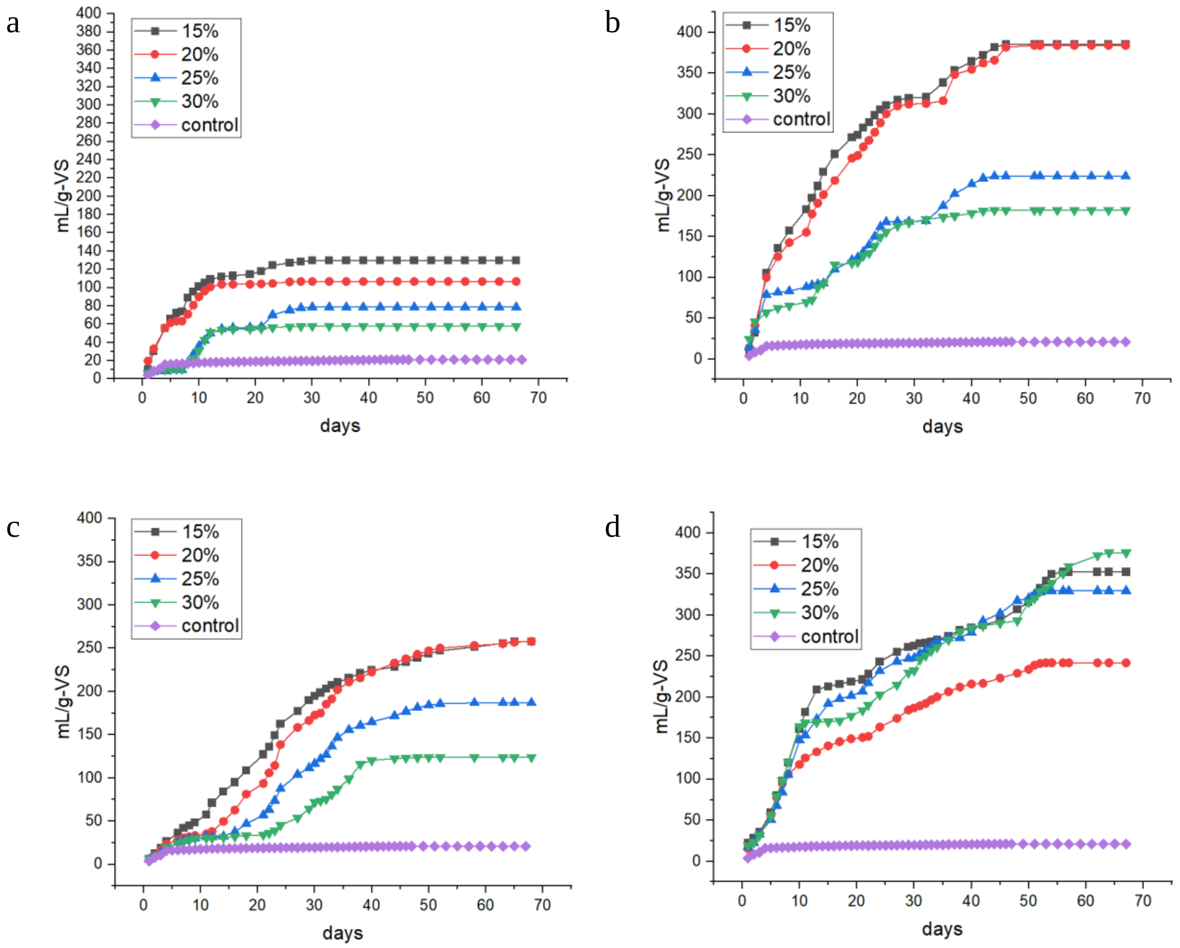


Plate. 4.6 Cumulative biogas yield (mL), a) RS+FW, b) RS+CM, c) RS+SS, d) RS+ChM, control is digestion of RS alone, 15%, 20%, 25%, 30% are TS%

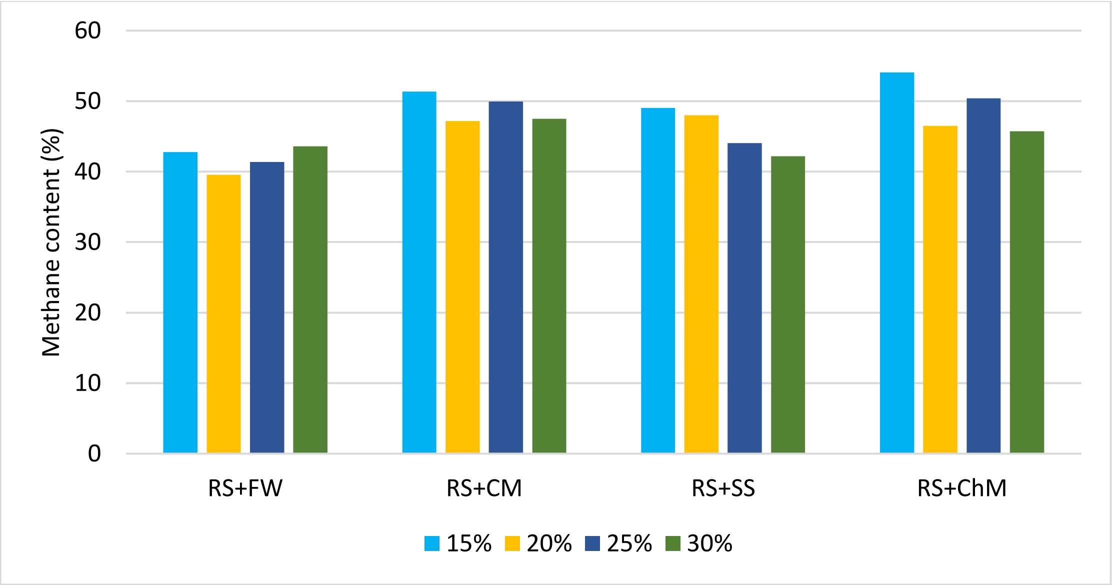


Plate. 4.7 Methane content (%) in each binary mixture at four TS contents

4.3.2 VFA concentration

Lignocellulosic matter degrades anaerobically primarily through hydrolysis (the rate-limiting step in quickly degrading feed), acidogenesis, acetogenesis, and methanogenesis (the rate-limiting step in slowly degrading feed). The efficiency of the hydrolysis and acidogenesis steps can be evaluated by the accumulated concentration of VFAs. The amount of VFAs is the indicator of acids produced from the hydrolysis and acidification process, which methanogenic bacteria cannot consume, on accumulation leads to a reduction of pH and system destabilisation (Cai et al., 2017). Despite this, the indicative VFA level could not be specified with absolute certainty as the composition of the substrates and the operating conditions varied (Murto et al., 2004). The accumulation of VFA restricts methanogenic bacteria, which disturbs AD by significantly lowering the pH levels (Song et al., 2013). VFA concentration of approximately 4000 mg/L inhibits the process (Croce et al., 2016). The accumulation occurs due to the two-stage fermentation of the organic matter during the AD process. The acids generated during the hydrolysis and acidogenesis get converted into methane and carbon dioxide by methanogens in the methanogenesis (Yue et al., 2007).

The VFA variations of all the mixtures at all TS ranges are depicted in Plate 4.8. Among the four mixtures, RS+FW resulted in maximum VFA production of around 7298 mg/L at 15% TS which is far greater than the threshold limit (4000 mg/L). This could be correlated to the low range of biogas produced from the same mix. High VFA accumulation can also be related to the low C/N ratio of the substrates like FW. With a high VFA concentration, improved hydrolysis rates and degradation of RS recalcitrant lignocellulosic structure are observed, thereby enhancing biochemical conditions in the reactor and increasing biodegradability. A similar kind of inhibition was observed in mixtures RS+SS (25% TS) and RS+ChM (30% TS) when the VFA generated crossed 4000 mg/L, whereas there are no traces of inhibition due to VFAs in the RS+CM mixture at four TS contents and also in the other two combinations, i.e., RS+SS (15%, 20%, 30%), RS+ChM (15%, 20%, 25%) it is under the threshold value.

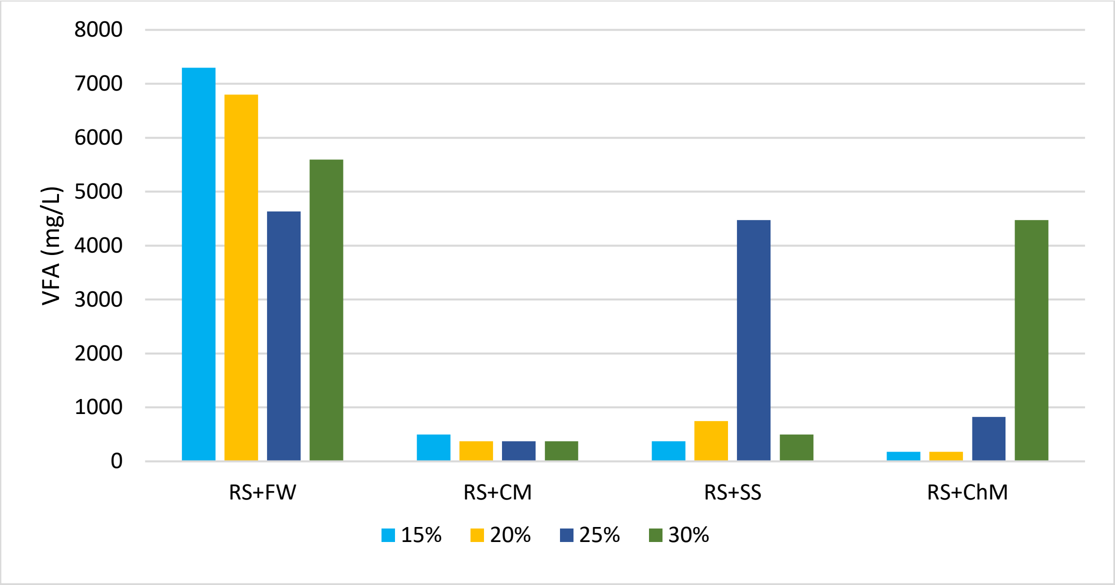


Plate. 4.8 Volatile fatty acids in each binary co-digestion mixture at four TS contents

4.3.3 Total volatile solids reduction

The decrease in volatile solids content is an important parameter in assessing the performance of an anaerobic digester (Kainthola et al., 2019a). The rates of volatile solids removal for all the binary mixes performed in A-co-D is presented in Plate 4.9. The RS+CM combinations showed around 60 % VS removal efficiency at 25% TS, whereas other binary mixtures like RS+SS and RS+ChM attained only 20-30% volatile solids reduction. The biodegradability of substrates improves as VS removal rates increase, and the initial high VS content of substrates allows better degradation. Despite low biogas and methane production values in mixture RS+FW, volatile solids removal efficiency ranges from 35 - 45%. There could be a difference in degradation efficiency due to the organic matter in co-substrates being more easily degradable than in RS. The organic contents present in FW are more easily degradable compared to other substrates. In mixture RS+ChM, however, the biogas productivity is good; comparatively, the VS reduction % is less may be due to the presence of ChM as it has fewer volatile solids (29%).

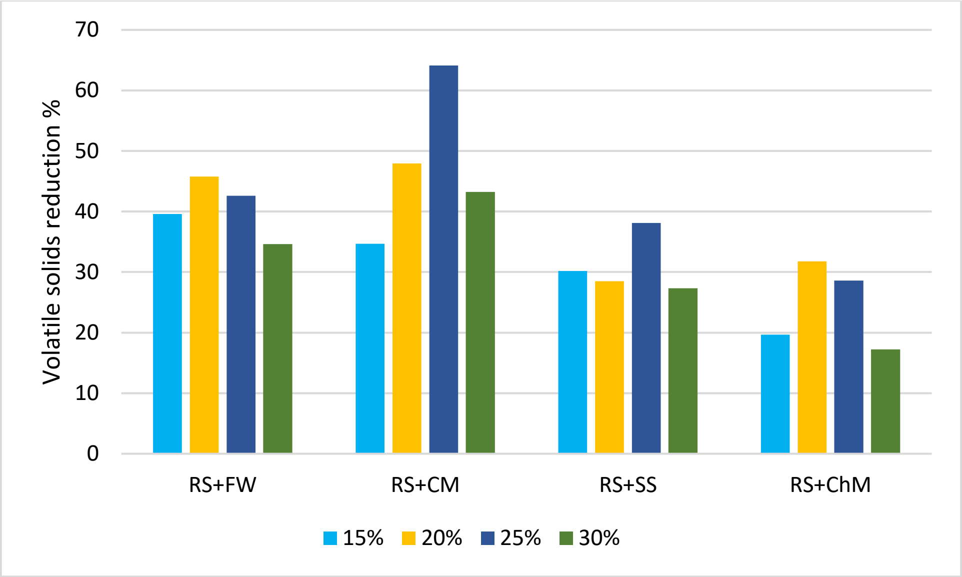


Plate. 4.9 Volatile solids reduction % in each binary mixture at four TS contents

4.4 Anaerobic co-digestion of ternary combinations of RS

4.4.1 Biogas and methane production

The cumulative biogas production for various co-digestion mixtures of RS with dual combinations of FW, CM, ChM, and SS at different TS concentrations is illustrated in Plate 4.10. The biogas production values are normalised using VS content present in the mixtures. The maximum cumulative biogas production was observed as 442 mL/g-VS on the 35th day for the ternary mix RS+CM+ChM at a TS content of 20% with an average methane content of 55.38%. For RS+SS+ChM on the 40th day, a 408 mL/g-VS value was obtained at 20% TS content. Previous studies have shown increased biogas production when RS and nitrogen-rich substrates like chicken manure and cattle manure are co-digested. A study by Wang et al. (2013) showed similar increased biogas production (343 mL/g-VS) values for mixtures comprising chicken manure and cattle manure digested with RS. A desirable C/N ratio may have assisted in increasing biogas production in the current study by mixing RS with CM and ChM. A similar yield of 383.5 mL/g-VS was observed by Li et al. (2015) during AD of RS with CM. The biogas production at TS 25% and 30% are low compared to the other two TS contents (15% and 20%) in all the mixtures.

Next to CM and ChM, FW showed better compatibility with RS. In combinations of SS with RS, a maximum biogas yield of 408 mL/g-VS was obtained at 20% TS content. AD of low C/N ratio feedstock often leads to the release of NH_4-N in the system and causes direct inhibition of microbial activities (Rajagopal et al., 2013). Hence, the RS with SS and CM combination served as an optimum mix for a stable AD process without causing ammonia inhibition. As per some studies by Sasaki et al. (2010; 2011), carbon fibre textiles (CFT) have

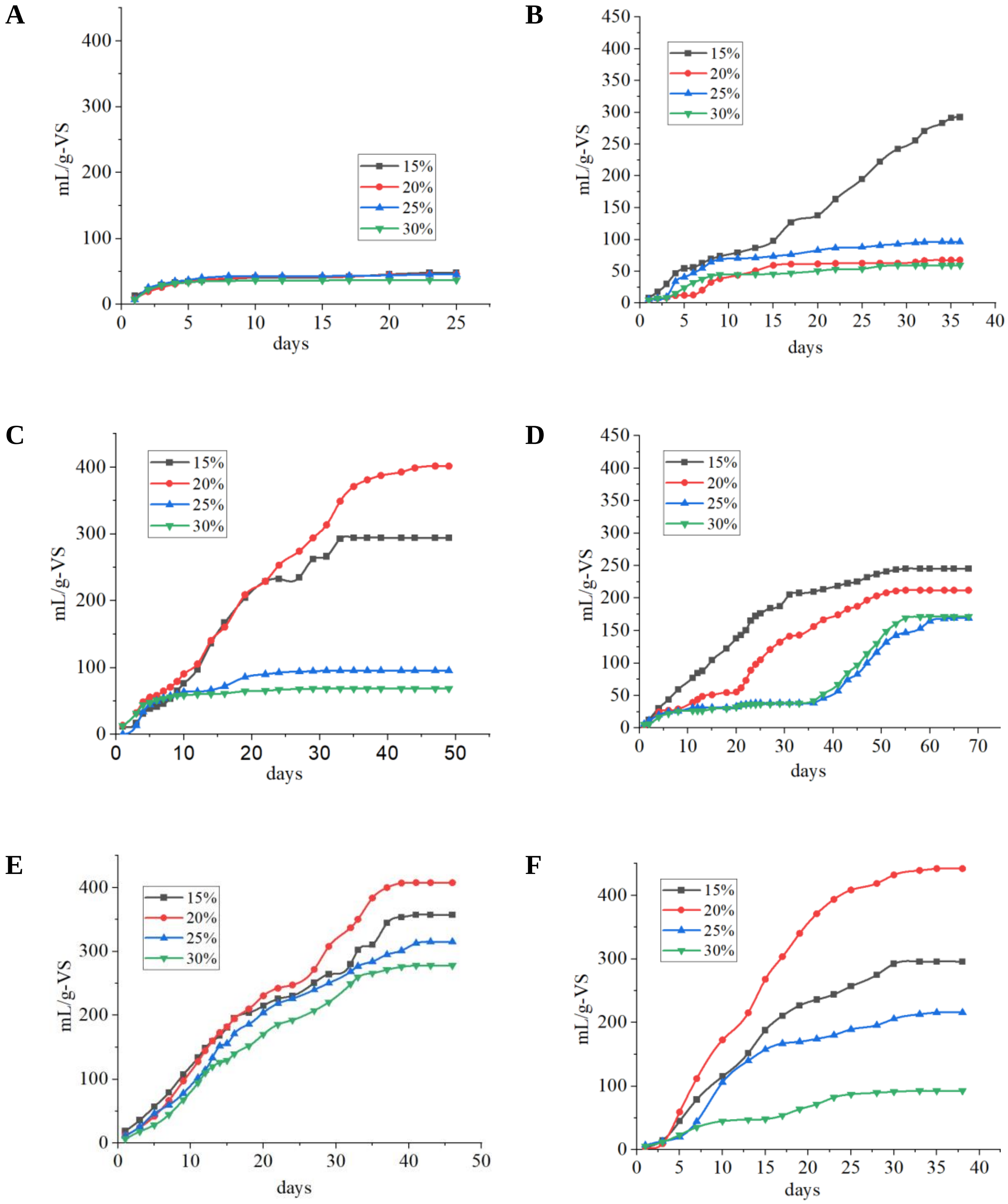


Plate. 4.10 Graphs showing cumulative biogas yield in each mixture, a) RS+FW+CM, b) RS+FW+ChM, c) RS+FW+SS, d) RS+SS+CM, e) RS+SS+ChM, f) RS+ChM+CM

been reported to be effective in treating ammonia toxicity in wet AD systems. In the mixtures comprising SS and ChM, at four TS ranges of 15%, 20%, 25%, and 30%, the biogas production values obtained are 357.3, 408, 315.2, and 278 mL/g-VS, respectively. As for RS+CM+ChM, TS 20% outperformed among four ranges; however, all ranges attained results within 35 days with an average methane content of over 50%.

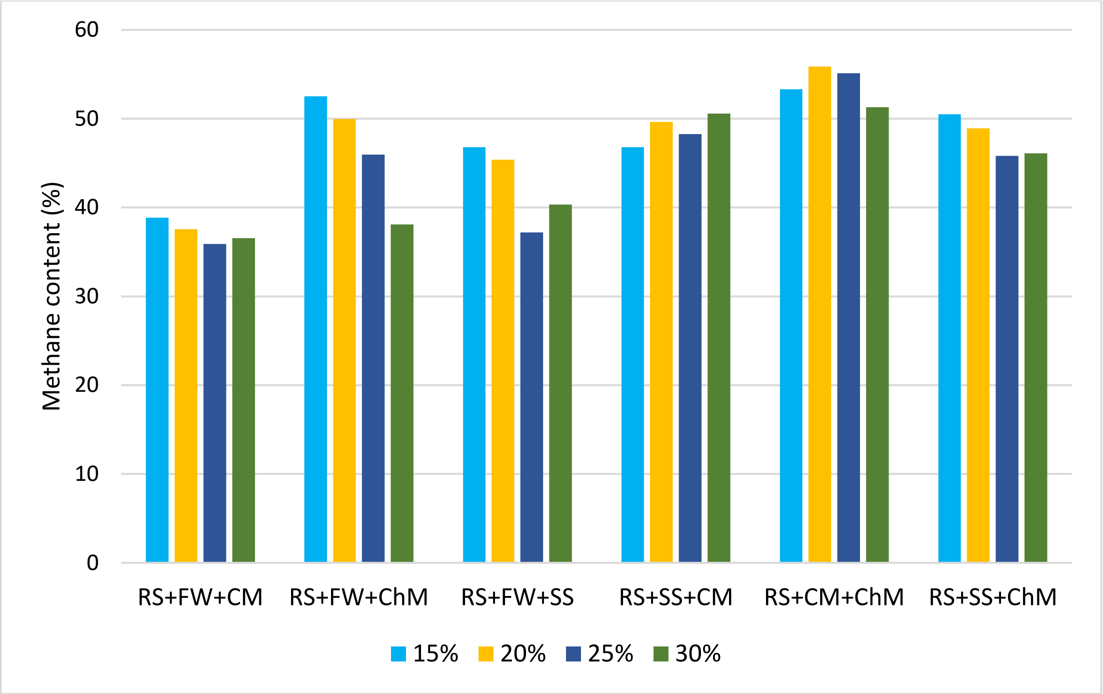


Plate. 4.11 Average Methane content (%) in each ternary mixture at all Total solid contents

The ternary mixture comprising RS with FW and SS showed the lowest biogas production value, i.e., 48.32 mL/g-VS. For other ternary mixtures having FW mixed with CM, and SS, the biogas production values obtained are comparatively more than that with SS, i.e., 245.39 mL/g-VS, respectively. Although the volatile fraction and moisture content in FW are favourable for AD, the low C/N ratio might have caused rapid acidification, resulting in lower efficiency. Li et al. (2015) reported a biogas production value of 478.98 mL/g-VS at a 1:1 VS ratio of RS and PM. Contrary to this, Ye et al. (2013) reported that a lower biogas production of 61.8 mL/g-VS was observed during the co-digestion of RS, PM, and kitchen waste as the mixture comprises a high content of kitchen waste.

The average methane yield per unit mass of volatile solids for all mixtures is presented in Plate 4.11. The ternary mixtures comprising FW and SS are likely to generate less methane than other substrates. This could be correlated with their degradation rates. FW and CM degrade easily, while SS and ChM degrade slowly. Hydrolysis occurs quickly in easily degradable feed, while methanogenesis is the rate-limiting step; on the other hand, among slowly degradable feeds, hydrolysis is slower (Tomei et al., 2009). Ye et al. (2013) reported a lower methane yield ranging from 13.33 - 60.20% for co-digestion mixtures of RS with kitchen waste and PM.

The graphs 4.10, and 4.11 shows that the RS+FW+CM mixture produces the least biogas, the average methane content is below 40%, and digestion ends within 25 days. There is also no substantial difference in biogas value across the ranges of TS. In the case of RS+FW+ChM, at TS 20%, the biogas production is 401.6 mL/g-VS, and from the 12th day, the productivity started increasing. The initial delay in biogas production might be due to the slow rate of degradability of the substrates. In RS+SS+CM, digestion lasted 60 days, and biogas production is approximately 220 ml/g-VS for TS 15% and 20% and about 170 mL/g-VS for TS 25% and 30%. The lower production could be due to high total solids. Among all the combinations, the mixture of slowly degradable feedstocks has given stable results, i.e., RS+SS+ChM. The results confirmed that the anaerobic co-digestion of two or more substrates could improve system stability and increase biogas production (Zhang et al., 2013).

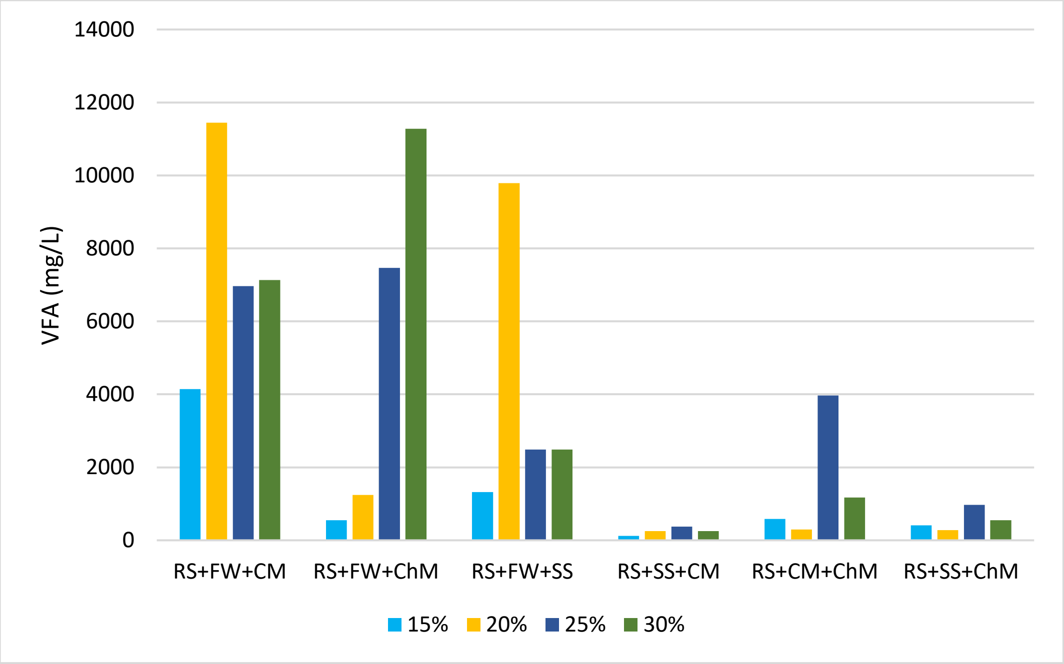


Plate. 4.12 Volatile Fatty Acids (VFA) concentration in each mixture at all Total solid contents.

4.4.3 VFA concentration

The VFA variations of all the mixtures at all TS ranges are depicted in plate 4.12. Among the six ternary mixtures, RS+FW+CM resulted in maximum VFA production of around 11448 mg/L at 20% TS which is far greater than the threshold limit (4000 mg/L). This could be correlated to the low range of biogas produced from the same mix. High VFA accumulation can also be related to the low C/N ratio of the substrates like FW. With a high VFA concentration, improved hydrolysis rates and degradation of RS recalcitrant lignocellulosic structure are observed, thereby enhancing biochemical conditions in the reactor and increasing biodegradability. A similar kind of inhibition was observed in mixtures RS+FW+SS (20% TS) and RS+FW+ChM (25% and 30% TS) when the VFA generated crossed 10000 mg/L. The corresponding biogas generation values obtained at respective TS ranges are also lower (Plate

1), whereas, in the other three combinations, i.e., RS+SS+CM, RS+SS+ChM and RS+CM+ChM, it is under the threshold value.

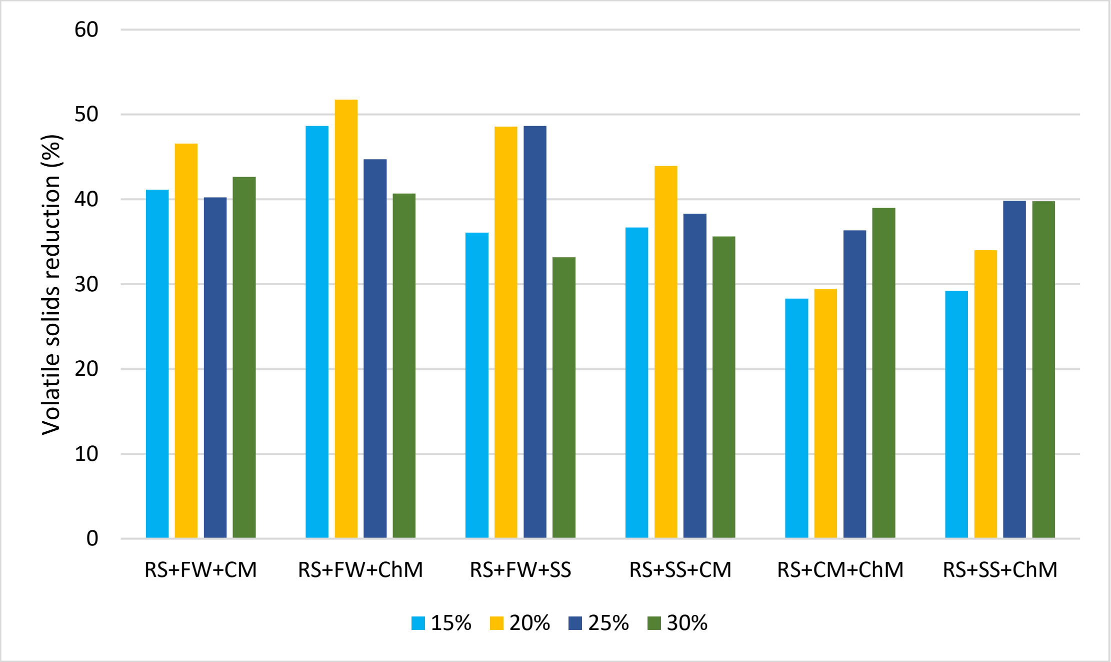


Plate. 4.13 Volatile solids reduction % in each mixture at all Total solids contents

4.4.3 Total Volatile solids reduction

The decrease in volatile solids content is an important parameter in assessing the performance of an anaerobic digester (Kainthola et al., 2019a). The rates of volatile solids removal for all the ternary mixes performed in AD is presented in Plate. 4.13. The RS+FW+CM, RS+FW+SS and RS+FW+ChM showed around 50 % VS removal efficiency, whereas other ternary mixtures like RS+ChM+CM and RS+SS+ChM attained only 20-30% volatile solids reduction. The biodegradability of substrates improves as VS removal rates increase, and the initial high VS content of substrates allows better degradation. Despite low biogas and methane production values in mixtures containing FW, volatile solids removal efficiency ranges from 48.56 to 51.76%. There could be a difference in degradation efficiency due to the organic matter in co-substrates being more easily degradable than in RS. The organic contents present in FW are more easily degradable compared to other substrates. Ye et al. (2013) reported a similar range of VS reduction (51.53-55.76%) during co-digestion of FW. In mixtures RS+SS+CM, RS+SS+ChM, and RS+CM+ChM, however, the biogas productivity is good; comparatively, the VS reduction % is less may be due to the presence of SS and ChM.

4.4.4 Kinetic model results on ternary AD mixtures

A better understanding of fermentation system evolution can be gained by analyzing the kinetic parameters of the AD process. The cumulative biogas production values of each ternary mix at different TS% are simulated using a modified Gompertz model (Eq. 3.1). The kinetic parameters estimated (P_{max}, Q, and ƛ) are summarised in Table 4.4, and the curves of model fitting are shown in Plate 4.14. The correlation coefficient (R^2) values range from 0.90 - 0.99 for all mixtures at four TS contents, showing that experimental values can be well simulated using the chosen model. AD efficiency can generally be determined by the maximum cumulative biogas production potential (P_{max}) and the maximum biogas production rate (Q). The maximum cumulative biogas production value obtained for RS+SS+ChM at 20% TS was 470.01 mL/g-VS, and RS+CM+ChM at 20% TS with 447.47 mL/g-VS. Hence, the BMP values fitted well with the Modified Gompertz model. Wide variations in the lag phase time ($ƛ_{days}$) were observed. The lowest lag phase time was reported for FW mixtures, ranging from 0.03 to 4.46 days. This indicates that FW took a minimum of days to achieve the maximum biogas production. Furthermore, it was found that the anaerobic co-digestion of RS with CM and ChM has an apparent lag phase time ranging from 0.61 to 1.15 days. This value matches the results of Zhong et al. (2021), where a lag phase time of 1.79 and 2.43 days was reported for RS and PM.

The highest lag phase time of 10.04 days was obtained for RS+SS+CM at 30% TS. In RS+FW+CM (all TS ranges), RS+FW+SS (25%, 30% TS), RS+SS+ChM (all TS ranges), and RS+CM+ChM (all TS ranges), comparatively less lag phase times (0.03 to 4.46 days) are reported, which shows that methanogenesis can be accomplished in less time (Table 4.4). Other higher values of lag phase duration may be due to the mixing of easily degradable substrates for which methanogenesis serves as the rate-limiting step. Another reason could be the presence of high solids content. Gompertz's model predicted biogas yields between 0.33% and 15.65% higher than observed yields.

Along with Modified Gompertz model, linear, quadratic, and cubic models were fit to evaluate and compare the significance of applied models. Table 4.15 shows the R^2 values obtained from linear, quadratic, and cubic models for all co-digestion mixtures at four TS% ranges. On comparing R^2 (coefficient of determination) values from Modified Gompertz model to linear, quadratic, and cubic models, it is observed that Modified Gompertz model is more reliable than linear, quadratic, and cubic models. Additionally, running strength and weakness analysis among applied models, linear fit is simple and assumes constant growth but fails in gaining nonlinear or exponential growth. Strength of Quadratic fit is, it is suitable for data of initial rapid growth with eventual downtrend but weakness is depending on the curve of growth path, growth can be underestimated or underestimated. Cubic fit has its strength in capturing more complicated growth patterns with faster and decelerated phases and can provide a closer fit with data exhibiting cubic or S-shaped growth patterns. Whereas, Modified Gompertz model is flexible and capable of capturing both the initial exponential growth and the subsequent saturation of the phenomenon and is widely used in biological studies as a description of growth patterns but, the model presumes a continuous and stable growth, which cannot be used in all scenarios. Summarising the data, Modified Gompertz model has provided reliable fit than the other selected models. In light of these results, it can be concluded that a study of the relationship between kinetic value and operational and process conditions can provide valuable insight into monitoring and controlling anaerobic co-digestion.

Table 4.4 Kinetic parameter values from Gompertz model analysis on co-digestion studies at four TS% ranges

AD mixture Parameter	**Total solids %**			
	15%	20%	25%	30%
RS+FW+CM				
P_{max}	47.49	45.44	43.67	36.29
$P_{experimental}$	48.33	46.10	45.61	36.91
Q	11.48	10.15	10.89	12.60
ƛ	0.52	0.81	0.03	0.32
R^2	0.90	0.92	0.96	0.98
Error (%)	1.73	1.43	4.25	1.67
RS+FW+ChM				
P_{max}	304.26	402.95	99.88	73.31
$P_{experimental}$	294.34	401.61	95.48	68.55
Q	13.16	15.02	3.18	2.93
ƛ	3.73	0.47	1.68	2.15
R^2	0.99	0.99	0.95	0.96
Error (%)	3.37	0.33	4.60	6.94
RS+FW+SS				
P_{max}	300.80	66.11	91.06	56.07
$P_{experimental}$	292.36	67.75	96.35	59.28
Q	9.57	5.44	7.95	5.52
ƛ	4.46	2.46	0.43	0.71
R^2	0.99	0.98	0.95	0.95
Error (%)	2.88	2.42	5.49	5.41
RS+SS+CM				
P_{max}	248.19	235.23	193.56	198.55
$P_{experimental}$	245.40	212.09	169.31	171.67
R^2	0.99	0.98	0.94	0.93

ƛ	1.28	6.05	8.21	10.04
Q	6.67	5.46	11.91	4.41
Error (%)	1.14	10.9	14.32	15.65
RS+SS+ChM				
P_{max}	362.87	470.01	324.79	299.06
$P_{experimental}$	357.31	407.60	315.16	277.91
Q	10.56	12.32	11.12	9.77
ƛ	0.91	1.33	1.45	1.98
R^2	0.91	0.98	0.99	0.99
Error (%)	1.55	15.31	3.05	7.61
RS+CM+ChM				
P_{max}	299.10	447.47	221.75	94.00
$P_{experimental}$	295.95	441.99	216.00	92.79
Q	8.87	11.64	6.12	3.53
ƛ	0.94	0.61	1.15	0.86
R^2	0.98	0.99	0.97	0.97
Error (%)	1.06	1.23	2.66	1.30

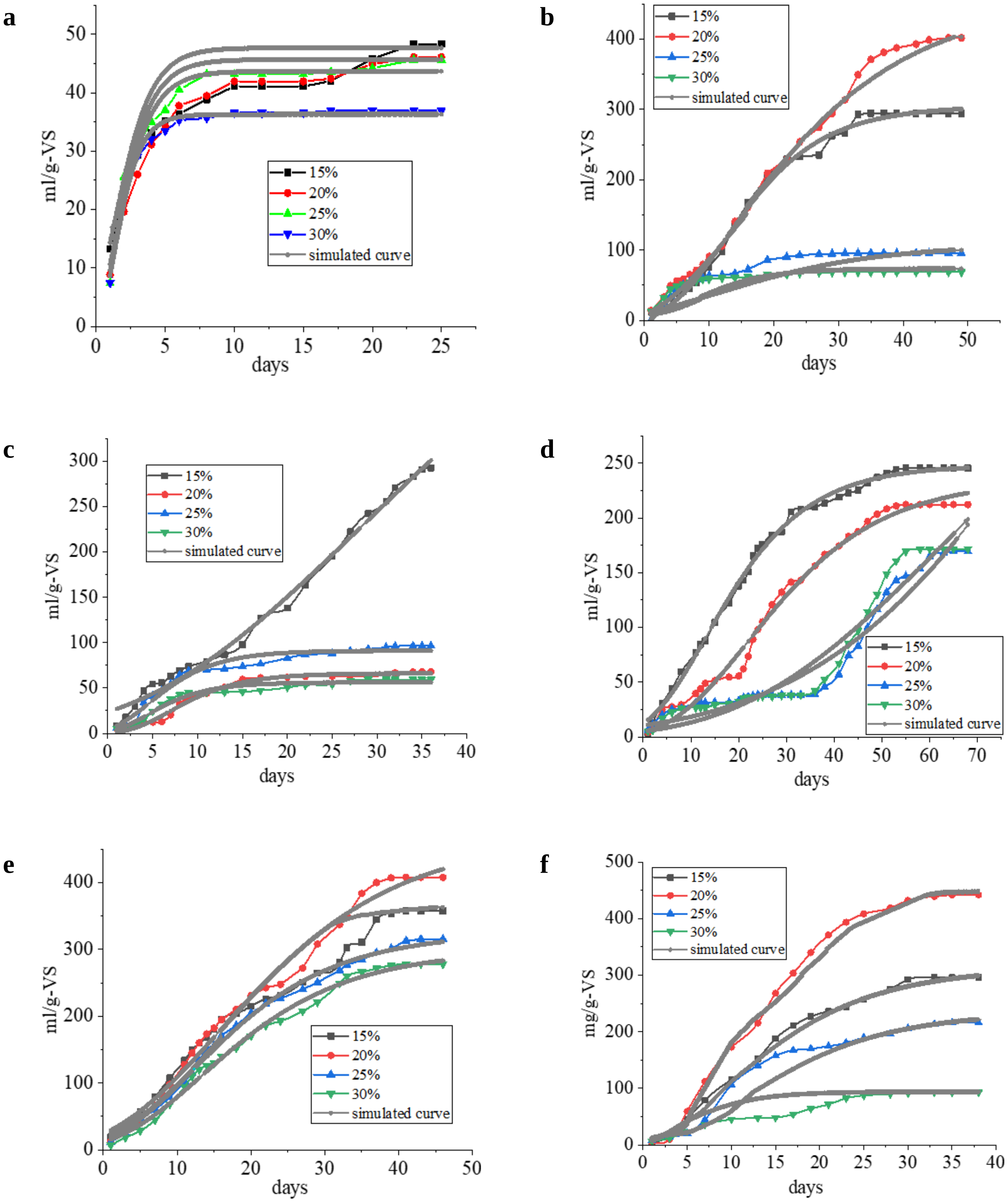


Plate. 4.14 Graphs showing cumulative biogas yield plotted using the Gompertz model in each mixture, a) RS+FW+CM, b) RS+FW+ChM, c) RS+FW+SS, d) RS+SS+CM, e) RS+SS+ChM, f) RS+ChM+CM

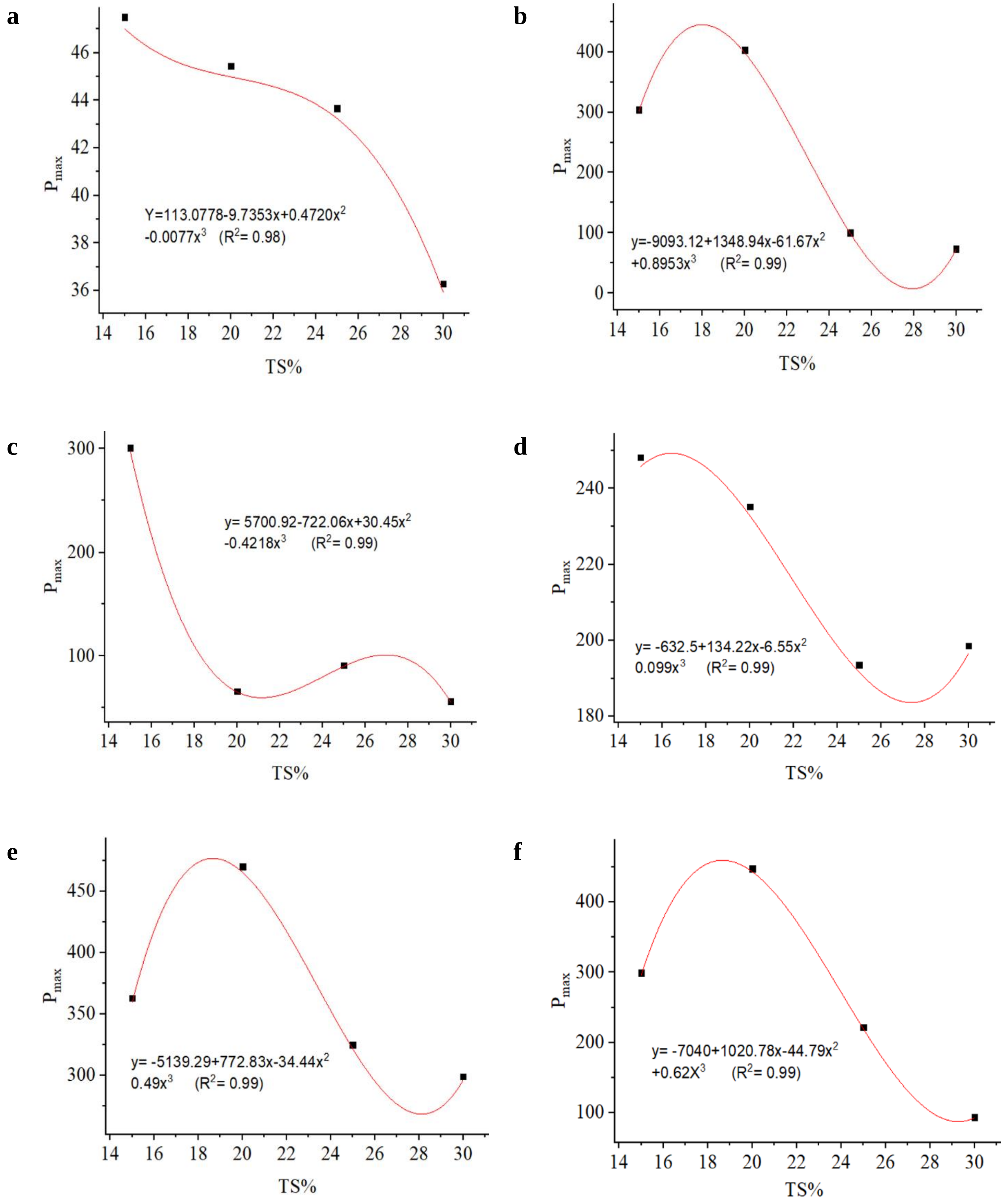


Plate. 4.15 Graphs showing maximum cumulative biogas yield plotted against TS% using the Gompertz model in each mixture showing the fit equation with R^2 value, a) RS+FW+CM, b) RS+FW+ChM, c) RS+FW+SS, d) RS+SS+CM, e) RS+SS+ChM, f) RS+ChM+CM

Table 4.5 Kinetic parameter (R^2) values from Linear, Quadratic, and Cubic fit model analysis on co-digestion studies at four TS% ranges

AD mixture Parameter	**Total solids %**			
	15%	20%	25%	30%
RS+FW+CM				
Linear fit	0.71776	0.62254	0.45883	0.3398
Quadratic fit	0.83174	0.83664	0.70845	0.62571
Cubic fit	0.94583	0.962	0.89194	0.82754
RS+FW+ChM				
Linear fit	0.90669	0.97403	0.71792	0.53582
Quadratic fit	0.98411	0.98833	0.93039	0.7773
Cubic fit	0.98824	0.99728	0.96388	0.89046
RS+FW+SS				
Linear fit	0.98575	0.81536	0.78306	0.75991
Quadratic fit	0.99218	0.95996	0.92731	0.91102
Cubic fit	0.99178	0.96188	0.96878	0.95491
RS+SS+CM				
Linear fit	0.8662	0.94554	0.85531	0.86917
Quadratic fit	0.99409	0.96717	0.94618	0.93254
Cubic fit	0.99474	0.98907	0.94461	0.9374
RS+SS+ChM				
Linear fit	0.96712	0.97522	0.95211	0.95684
Quadratic fit	0.98806	0.98962	0.9942	0.99306
Cubic fit	0.9888	0.99007	0.994	0.99459
RS+CM+ChM				
Linear fit	0.9166	0.90524	0.85643	0.92987
Quadratic fit	0.99448	0.99194	0.97029	0.97337
Cubic fit	0.99465	0.99469	0.96975	0.97595

4.5 Conclusion

The binary and ternary mix combinations employed in this study using RS as the main substrate and FW, SS, CM, and ChM as co-substrates showed promising results in biogas production and degradability. In all binary and ternary mixtures, the biogas produced exceeded the control (RS alone digestion). In binary co-digestion, maximum biogas production was observed in RS+CM at TS 15% with 385.4 mL/g-VS and RS+ChM at 30% TS with 376. mL/g-VS. In ternary digestion, at 20% TS, RS co-digested with ChM and CM, ChM and SS, and CM and SS produced maximum biogas production of 442, 407 and 245 ml/g-VS, respectively. Hence it can be concluded that ternary co-digestion of RS with a mixture of SS, CM, and ChM is a competent approach for improving the biogas. Among the ternary mixes tested, maximum biogas production and methane content was obtained for the RS+CM+ChM mixture at 20% TS. A decrease in biogas productivity was observed for all the mixtures as TS% increased. In addition, biogas production for RS+SS+ChM at all TS contents was stable with no VFA accumulation, with a maximum production of 408 ml/g-VS at 20% TS. The maximum VS reduction was observed for the RS+FW+ChM mixture at 20% TS. VFA accumulation is much higher in RS+FW+CM at all TS contents, possibly due to easily degradable substrates. The order of adaptability for choosing a co-substrate for RS can be listed as ChM>CM>SS>FW. The study has concluded that co-digestion with ternary mixtures is a systematic approach for enhancing biogas production.

Chapter 5 Pre-treatment on anaerobic co-digestion of rice straw

This chapter describes the effect of various pre-treatment techniques, i.e., thermal, hydrogen peroxide and their combinations on different sizes of Rice straw, i.e., 3-5 cm, 1-2 cm, 5-10 mm and < 300 µm with the co-digestion combination selected from objective I (co-digestion-chapter 4) which is RS+SS+ChM (rice straw, sewage sludge, and chicken manure) at 20% TS.

5.1 Pre-treatment

The primary aim of pre-treatment technology on RS is to change or alleviate the structural and compositional impediments to hydrolysis (Kaur & Phutela, 2016). There are three types of pre-treatments: (1) physical pre-treatment (milling, grinding and chipping), (2) chemical pre-treatment (acids, alkalis and oxidants) and (3) biological pre-treatment. The pre-treatment technology results in chemical and physical changes in the lignocellulosic biomass (Mosier et al., 2005). Most chemical pre-treatment methods use a huge quantity of chemicals and liquids to infuse solid substrate; this process generates a large quantity of toxic effluents, which cause high investment in the facility, huge treatment price and impending environmental contamination.

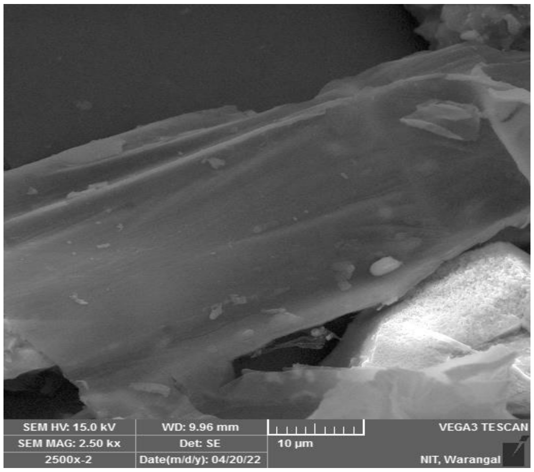
Thermal pre-treatment of lignocellulosic biomass has recently gained huge importance due to no requirement for additional chemical and corrosion-resistant tools. Moisture under high temperature and pressure can infiltrate substrate, hydrate cellulose, and detach the hemicellulose and partial lignin concentration in the process. Hydrothermal pre-treatment involves lignocellulosic feedstock and water and has been widely accepted as a green technology without potential chemical consumption and potential pollution (Saha et al., 2013). Typically, it can remove most of the hemicellulose and part of lignin in biomass by degrading them into soluble fractions and loosening the recalcitrant structure. Therefore, hydrothermal pre-treatment has been widely applied to facilitate biofuel production from lignocellulosic feedstocks (Cybulska et al., 2013). Also, by increasing the surface area of lignocellulosic biomass by particle size reduction, increasing the contact with microorganisms may improve the methane yield and thus increase biodegradability (Mshandete et al., 2006).

However, the studies that examined the effects of particle size reduction of RS on the digestibility for biogas production are limited in literature and the current study explored whether the RS properties and microbial community could explain the changes in the AD performance following the RS comminution (Dai et al., 2019). The objective of the current study is to study the effect of different pre-treatment techniques (thermal, hydroxy, thermal + hydroxy and hydroxy + thermal) on anaerobic co-digestion of RS at various sizes of RS (3-5 cm, 1-2 cm, 5-10 mm and < 300 µm). This study is to understand how far the pre-treatment could enhance the digestibility and enhances the accessible available surface area of RS, and makes it more feasible for hydrolytic bacteria. This study also focused on the correlation between the compositional changes and the physical alternations of RS in various sizes at respective pre-treatment methods.

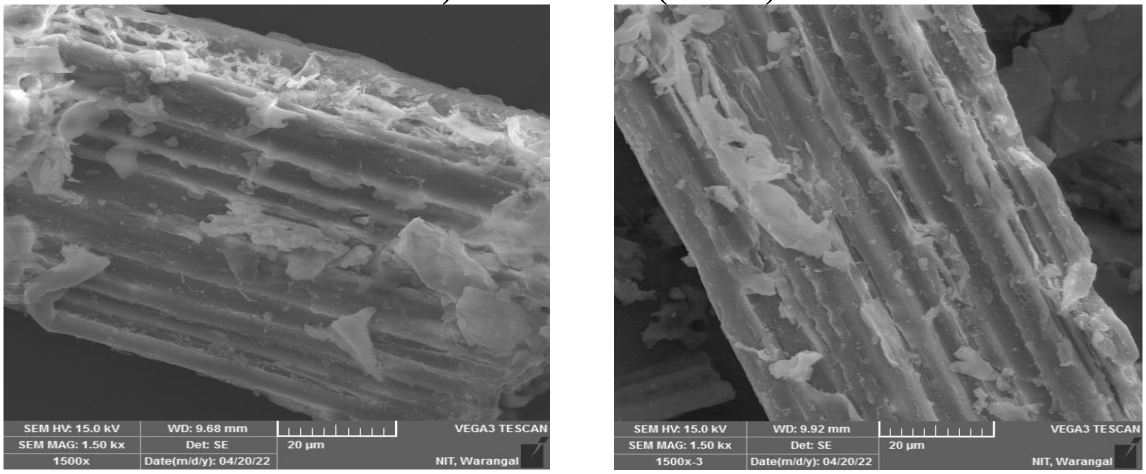
5.2 Field emission scanning electron microscopy (FESEM)

FESEM micrographs of untreated and pre-treated RS samples under thermal, H_2O_2, thermal + H_2O_2, and H_2O_2 + thermal pre-treatments at chosen four sizes of RS are presented in Plate. 5.1, 5.2, 5.3 and 5.4 respectively. The structural morphology of the untreated RS sample is clearly defined in Plate. 5.1 a), which depicts that is regular and complex, crystalline and rigid structure of the untreated sample of RS. Whereas Plate.5.1 b) shows a 3-5 cm size rupture which increased the surface area of RS due to thermal pre-treatment. In Plate. 5.1 c), 1-2 cm size depicted the ruptured and amorphous structure, which has increased the more accessible area of pre-treated RS to microbes attach. In Plate. 5.1 d), 5-10 mm size indicated collapsed and broke down the structure of pre-treated RS. In Plate. 5.1 e) < 300 µm size represented the severe effect of thermal pre-treatment, which completely collapsed and high structural breakdown has observed. So, we can easily distinguish the structural morphology of untreated and pre-treated samples of RS and select the best pre-treatment method and size of RS with the help of these images taken at the magnification more than 2000 times.

In Plate. 5.2 b), 3-5 cm size showed a more chiselled structure than untreated RS after H_2O_2 pre-treatment, which increased the surface area of RS. Plate. 5.2 c), 1-2 cm size depicted ruptured and amorphous structure after pre-treatment in RS. Plate. 5.2 d), 5-10 mm size indicated collapsed and broke down the structure at lesser sizes of RS. Plate. 5.2 e), < 300 µm size represented the severe effect of H_2O_2 pre-treatment, which completely collapsed, and high structural breakdown has been observed in lesser sizes of RS. So, we can easily distinguish the structural morphology of untreated and pre-treated samples of RS and select the best pre-treatment method and size of RS with the help of these images taken at the magnification more than 2000 times.

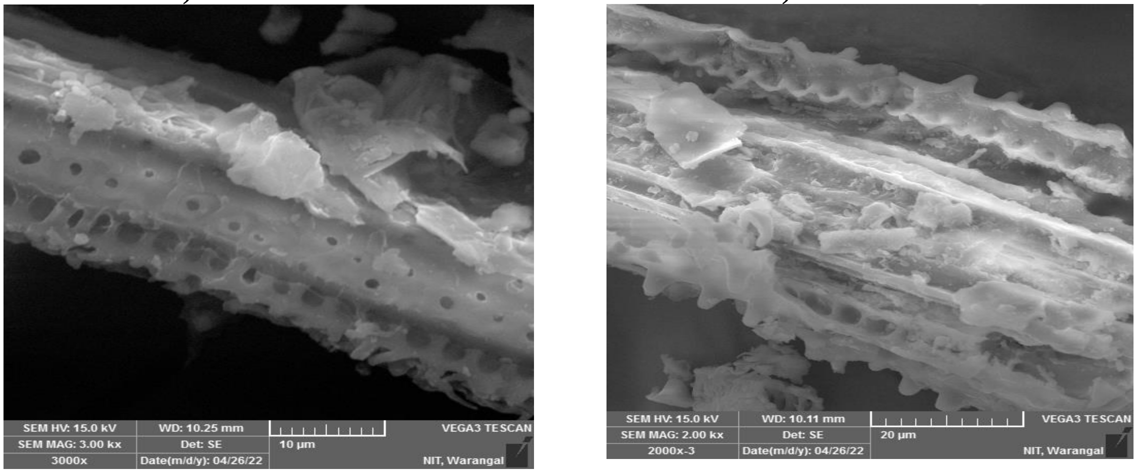


a) Untreated RS (Control)



b) Thermal 3-5 cm

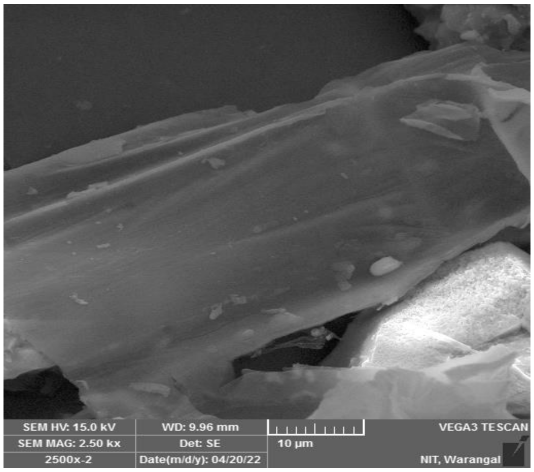
c) Thermal 1-2 cm



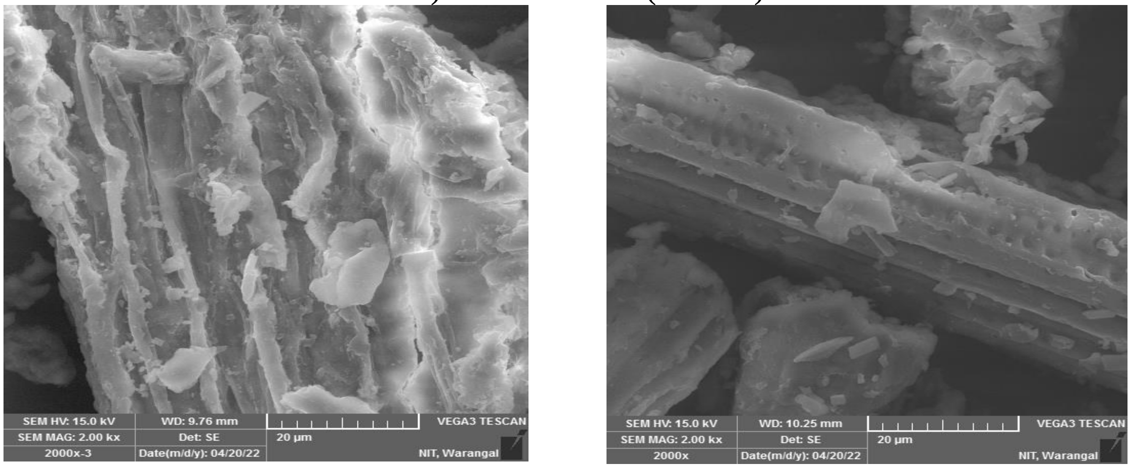
d) Thermal 5-10 mm

e) Thermal < 300 µm

Plate. 5.1 FESEM micrographs of thermal pre-treated RS at a) Control b) 3-5 cm, c) 1-2 cm, d) 5-10 mm, e) < 300 µm

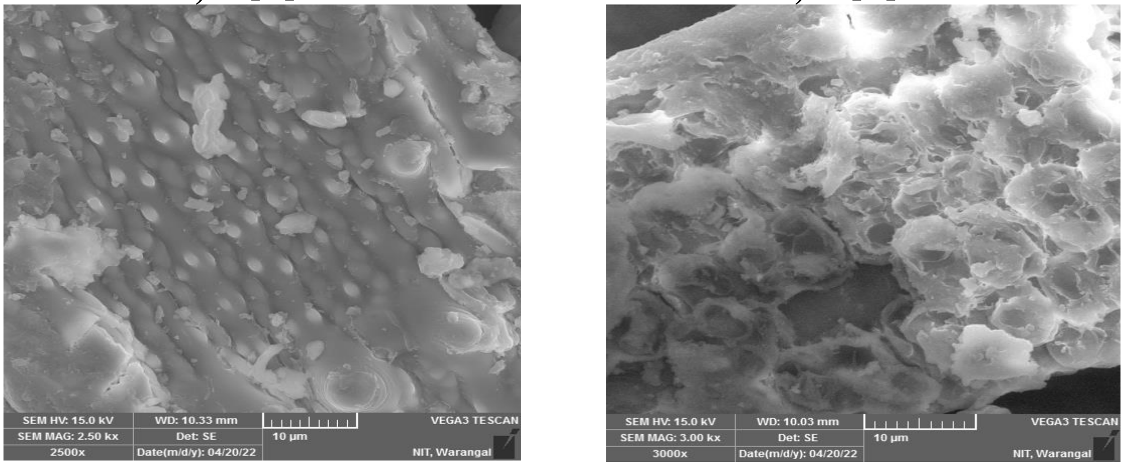


a) Untreated RS (Control)



b) H_2O_2 3-5 cm

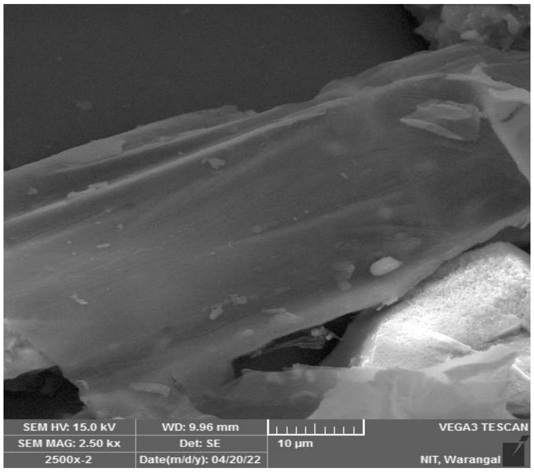
c) H_2O_2 1-2 cm



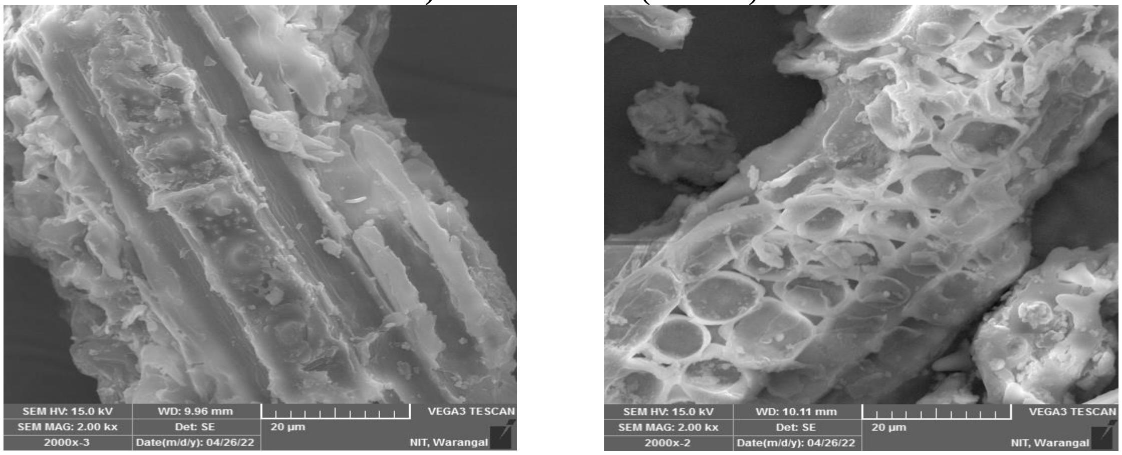
d) H_2O_2 5-10 mm

e) H_2O_2 < 300 µm

Plate. 5.2 FESEM micrographs of H_2O_2 pre-treated RS at a) Control, b) 3-5 cm, c) 1-2 cm, d) 5-10 mm, e) < 300 µm

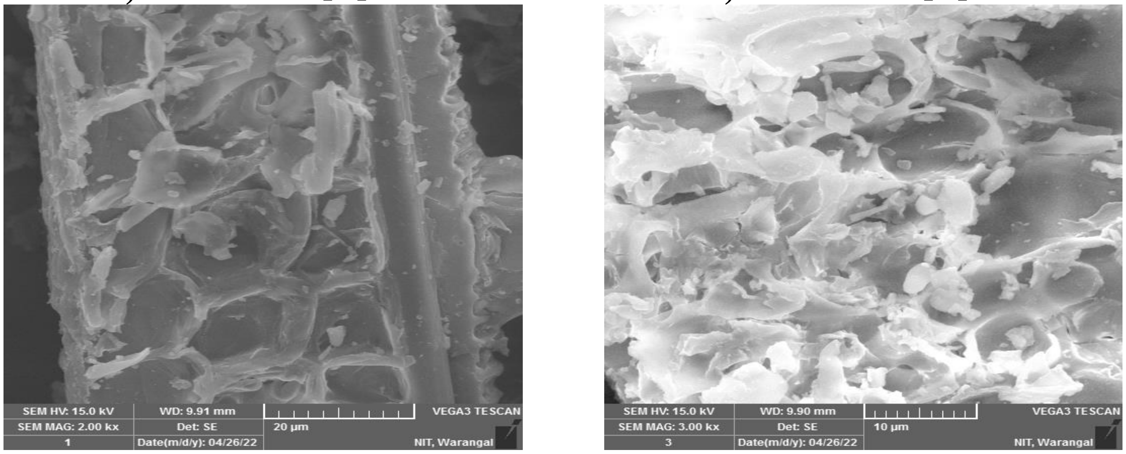


a) Untreated RS (Control)



b) Thermal + H_2O_2 3-5 cm

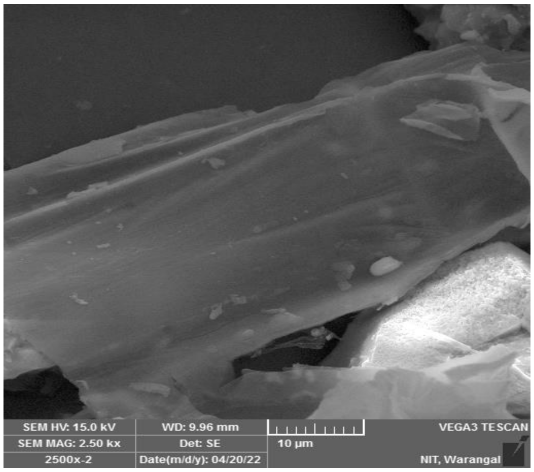
c) Thermal + H_2O_2 1-2 cm



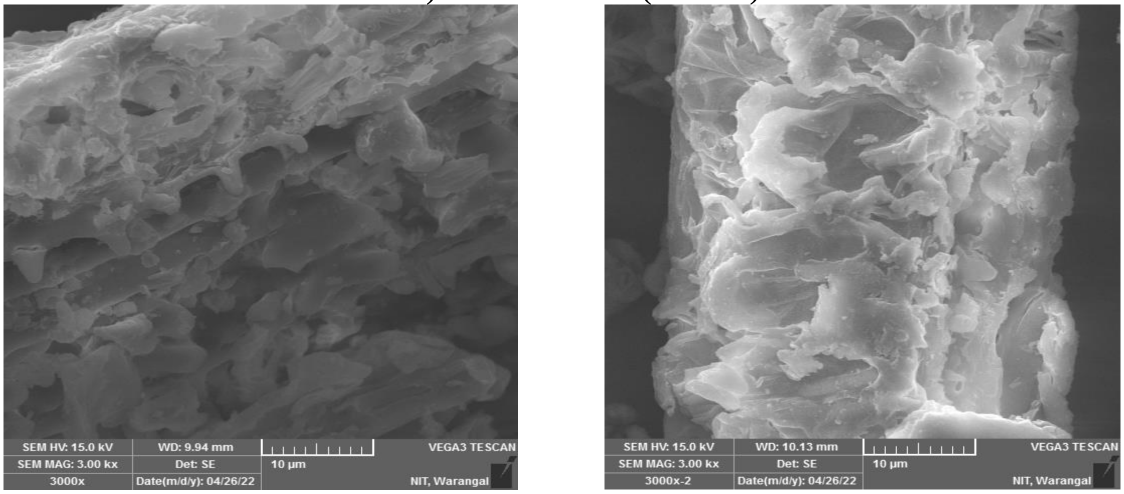
d) Thermal + H_2O_2 5-10 mm

e) Thermal + H_2O_2 < 300 µm

Plate. 5.3 FESEM micrographs of Thermal + H_2O_2 pre-treated RS at a) Control, b) 3-5 cm, c) 1-2 cm, d) 5-10 mm, e) < 300 µm

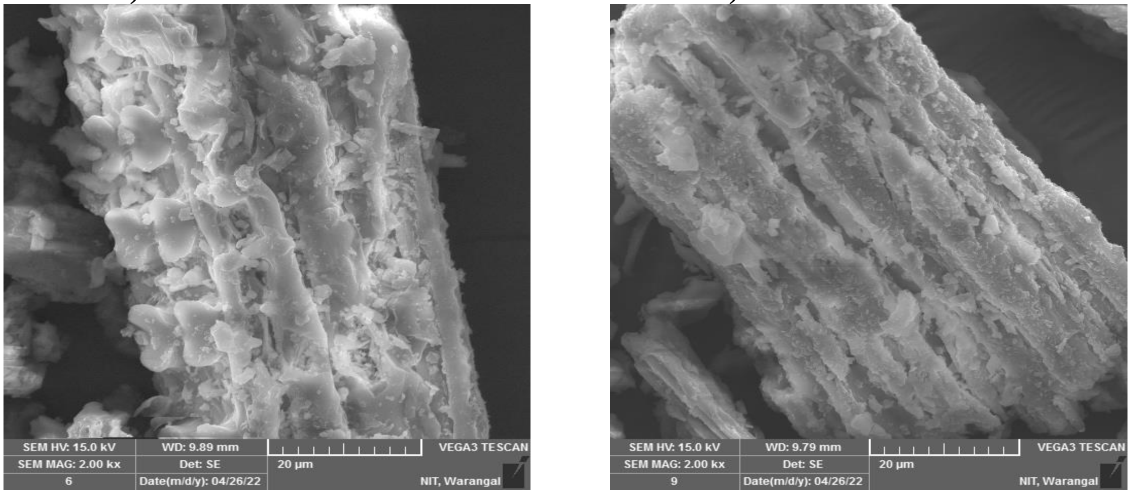


a) Untreated RS (Control)



b) H_2O_2 + Thermal 3-5 cm

c) H_2O_2 + Thermal 1-2 cm



d) H_2O_2 + Thermal 5-10 mm

e) H_2O_2 + Thermal < 300 µm

Plate. 5.4 FESEM micrographs of H_2O_2 + Thermal pre-treated RS at a) Control, b) 3-5 cm, c) 1-2 cm, d) 5-10 mm, e) < 300 µm

In Plate. 5.3 b), 3-5 cm size showed more chiselled and ruptured structure than untreated RS after thermal + H_2O_2 pre-treatment, which increased the surface area of RS. Plate. 5.3 c), 1-2 cm size, Plate. 5.3 d), 5-10 mm size and Plate. 5.3 e), < 300 µm size represented the severe effect of H_2O_2 pre-treatment, which completely collapsed, and highly structural breakdown has observed in lesser sizes of RS other than 3-5 cm due to both the pre-treatments. So, we can easily distinguish the structural morphology of untreated and pre-treated samples of RS and select the best pre-treatment method and size of RS with the help of these images taken at the magnification more than 2000 times.

In Plate. 5.4 b), 3-5 cm size, Plate. 5.4 c), 1-2 cm size, Plate. 5.4 d), 5-10 mm size and Plate. 5.4 e), < 300 µm size represented the severe effect of H_2O_2 + thermal pre-treatment which completely collapsed, and highly structural breakdown has observed in all sizes of RS due to both the pre-treatments and the effect of pre-treatments were increasingly drastic in collapse from 3-5 cm to < 300 µm RS size was observed. So, we can easily distinguish the structural morphology of untreated and pre-treated samples of RS and select the best pre-treatment method and size of RS with the help of these images taken at the magnification more than 2000 times.

5.3 Fourier Transform Infrared Spectroscopy (FTIR)

FTIR spectra are used to analyse the bond transformation in lignin carbohydrate complexes. Lignin is associated with cellulose and hemicellulose (polysaccharides) by phenyl glycosidic, ester and α bongs (Li et al., 2014). The FTIR spectra of untreated and pre-treated RS samples under thermal, H_2O_2, thermal + H_2O_2, and H_2O_2 + thermal pre-treatments at chosen four sizes of RS are presented in Plate. 5.5, 5.6, 5.7, and 5.8, respectively. Pre-treatment can dissociate the lignin-carbohydrate matrix by hydrolysing bonds, thus improving RS's degradability. The declining absorbance pattern of untreated and pre-treated RS samples depicts the intermolecular and intramolecular changes. The wavelength at 3338 cm^{-1} represents a hydrogen bond in the hydroxyl group of cellulose, and the compressed band suggests the breaking of hydrogen bonds, which shows the formation of soluble monomers. The wavelength at 1718 cm^{-1} depicts a carbonyl (C=O) bond; the reduction in the band indicates that lignin was broken. At wavelength 1374 cm^{-1} represents a C-H (covalent) bond; its stretch implies that pre-treatment eliminated the linkage between lignin and carbohydrate. At a wavelength of 691 cm^{-1}, it shows the β-D-glycosidic linkages, and the band's disappearance

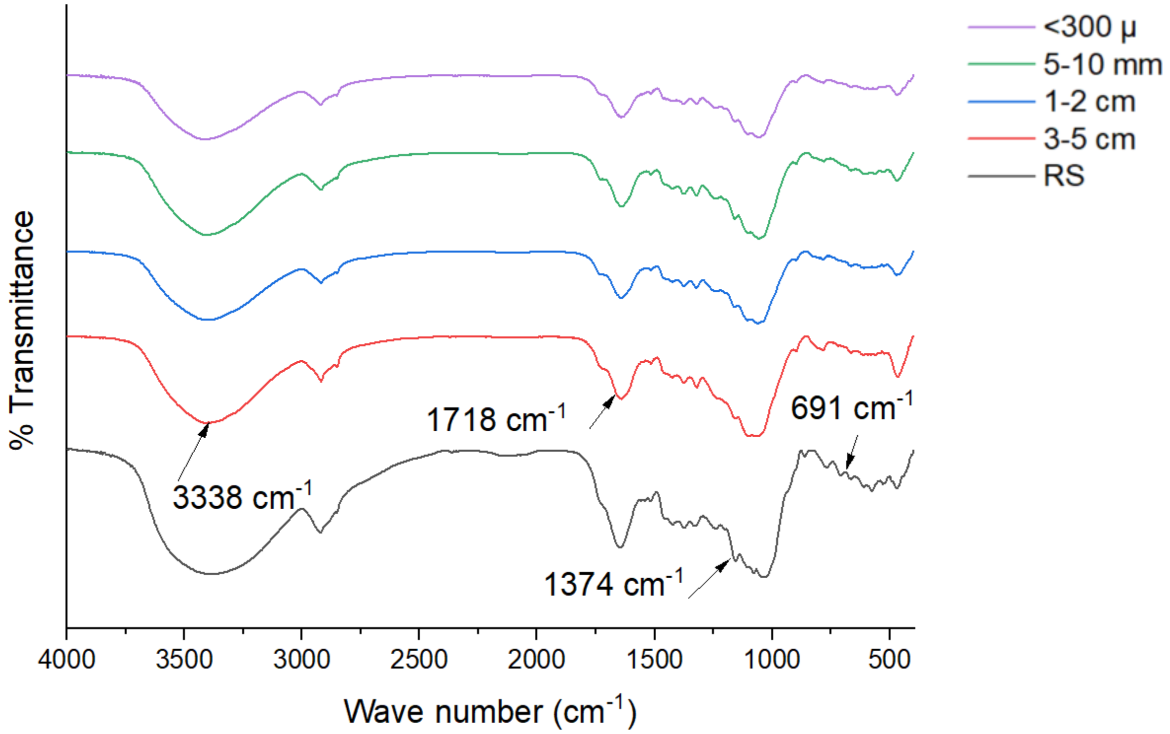


Plate. 5.5 FTIR spectra of untreated and thermal pre-treated RS

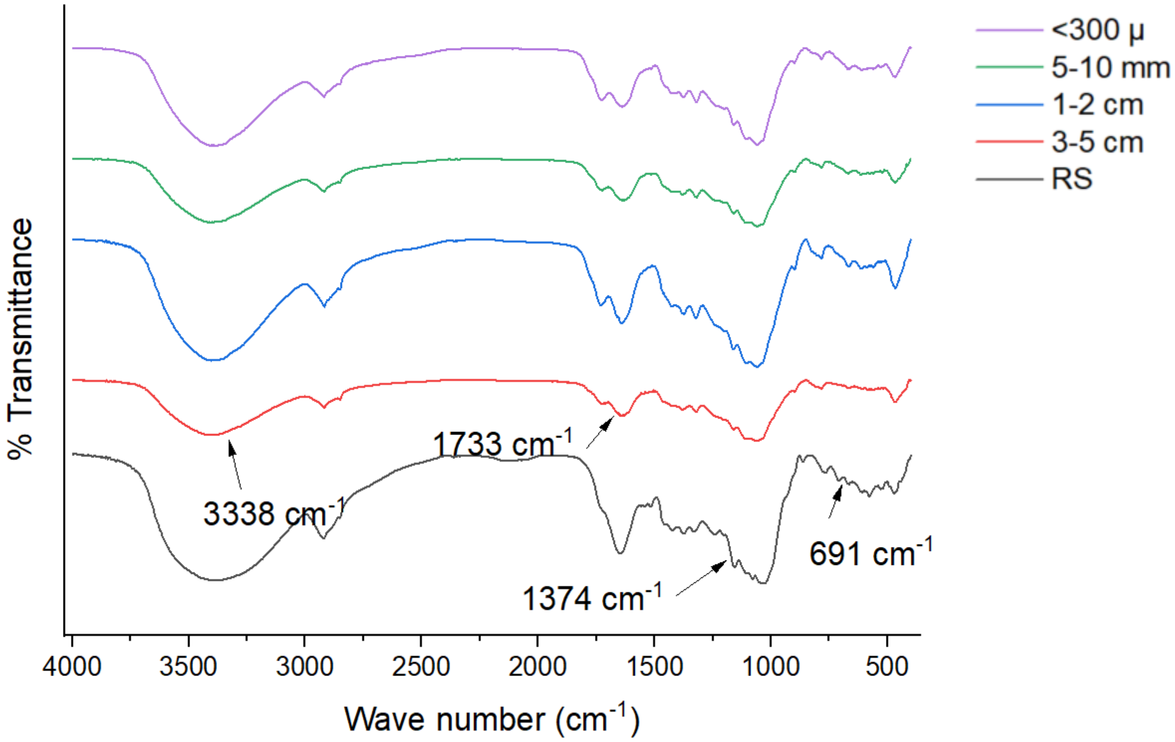


Plate. 5.6 FTIR spectra of untreated and H_2O_2 pre-treated RS

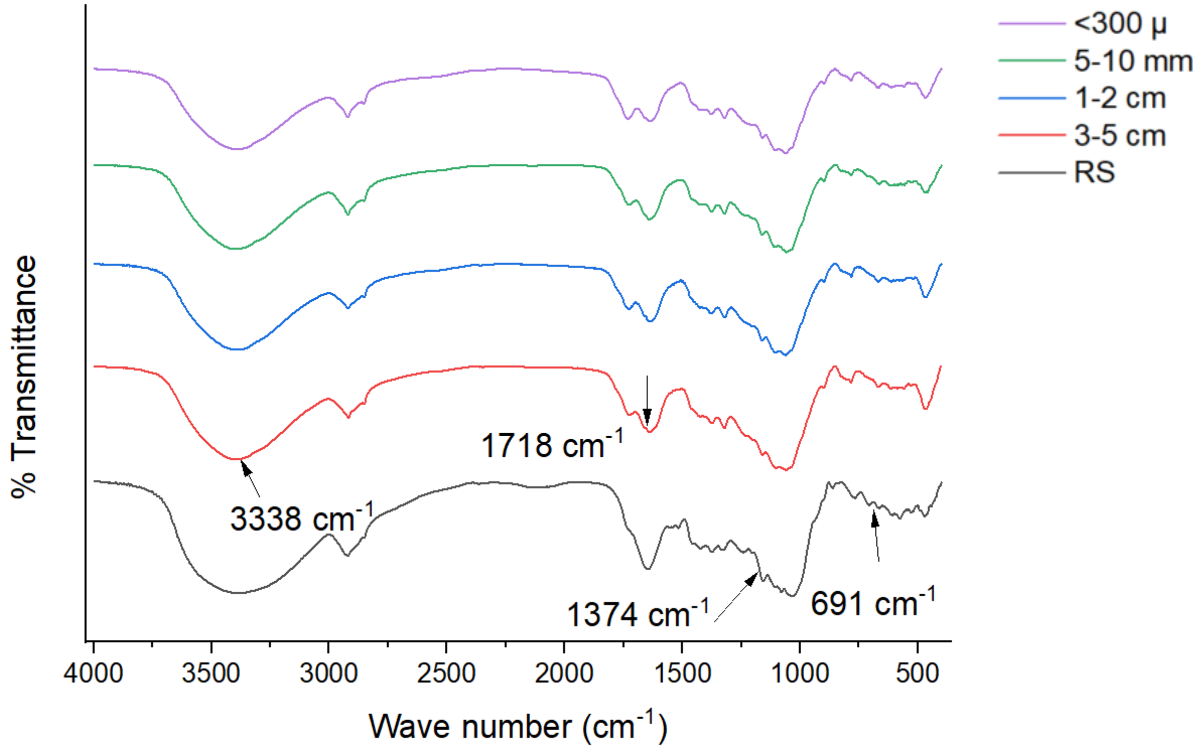


Plate. 5.7 FTIR spectra of untreated and Thermal + H_2O_2 pre-treated RS

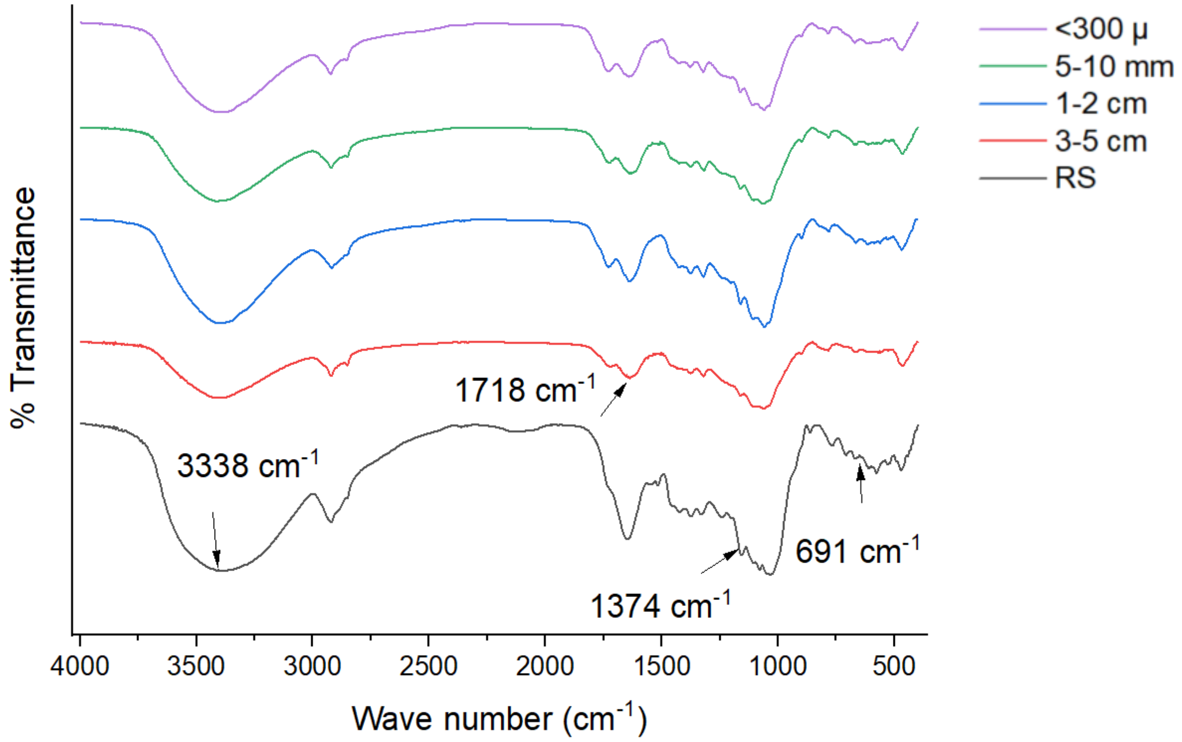


Plate. 5.8 FTIR spectra of untreated and H_2O_2 + Thermal pre-treated RS

shows the conversion of crystalline cellulose to amorphous compounds. In Plate. 5.5, gradually from 3-5 cm to < 300 µm, the band got softened and enlarged more towards < 300 µm comparatively to 3-5 cm on thermal pre-treatment. Plate. 5.6 depicts the FTIR of H_2O_2 pre-treatment on four sizes of RS. Compared to untreated RS, pre-treated RS bands were altered. On comparing with thermal pre-treatment, H_2O_2 and other two combined pre-treatment bands showed more changes, meaning more bond transformations were seen in H_2O_2 and the other two combined pre-treatment.

5.4 Compositional analysis

RS is complex, as it is recalcitrant to enzymic or microbial degradation, because of its composition and structure (Hendriks & Zeeman, 2009). Some parameters that alter the lignocellulosic biomass biodegradability are the grade of polymerisation, crystallinity, solubility, surface area and lignin content (Monlau et al., 2013). Pre-treatment of lignocellulosic biomass is an important step in the conversion process and improves the accessibility of the cellulose during the hydrolysis of the lignocellulosic structure to the enzymes (Song et al., 2013). The aim of the Pre-treatment is to alter the complex structure of RS to make it feasible for microbes to digest. Different pre-treatment techniques may affect the parameters to different degrees; all methods chosen have a major effect on cellulose, hemicellulose and lignin. Untreated RS is consisting 33.14 % cellulose, 19.2 % hemicellulose and 13.1 % lignin. Decreasing the lignin content of the pre-treated RS sample promotes the delignification, and the reduction in hemicellulose percent ensured the breaking of hemicellulose crosslinking across cellulose. Removal of lignin and hemicellulose made cellulose easily accessible to hydrolytic bacteria responsible for the destruction of b-1, 4 glycosidic linkages to soluble D-glucose subunits, which could be readily available food for microbial activity in AD (Kainthola et al., 2019b). An increase in hemicellulose may hinder digestion as it acts as a physical barrier limiting the accessibility of enzymes to cellulose.

Plate. 5.9, 5.10, 5.11, 5.12 shows the cellulose, hemicellulose and lignin contents before and after thermal, H_2O_2, thermal + H_2O_2 and H_2O_2 + thermal pre-treatment on RS. In Plate. 5.9, thermal pre-treatment of RS has decreased cellulose and lignin content in all four sizes, i.e., 3-5 cm, 1-2 cm, 5-10 mm, and < 300 µm whereas hemicellulose was random, increased in 3-5 cm and 5-10 mm, decreased in 1-2 cm and < 300 µm. Such a drastic reduction in these parameters confirmed that thermal pre-treatment not only gave rise to maximum solubilisation in the form of soluble

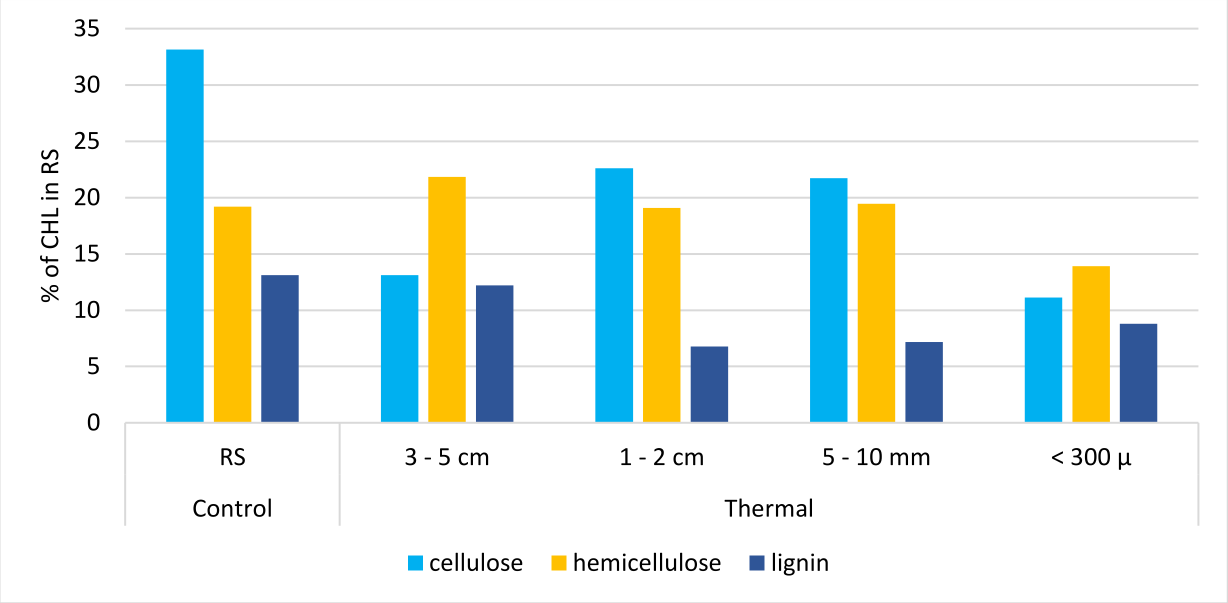


Plate. 5.9 Cellulose, hemicellulose and lignin (CHL) degradation before and after thermal pre-treatment

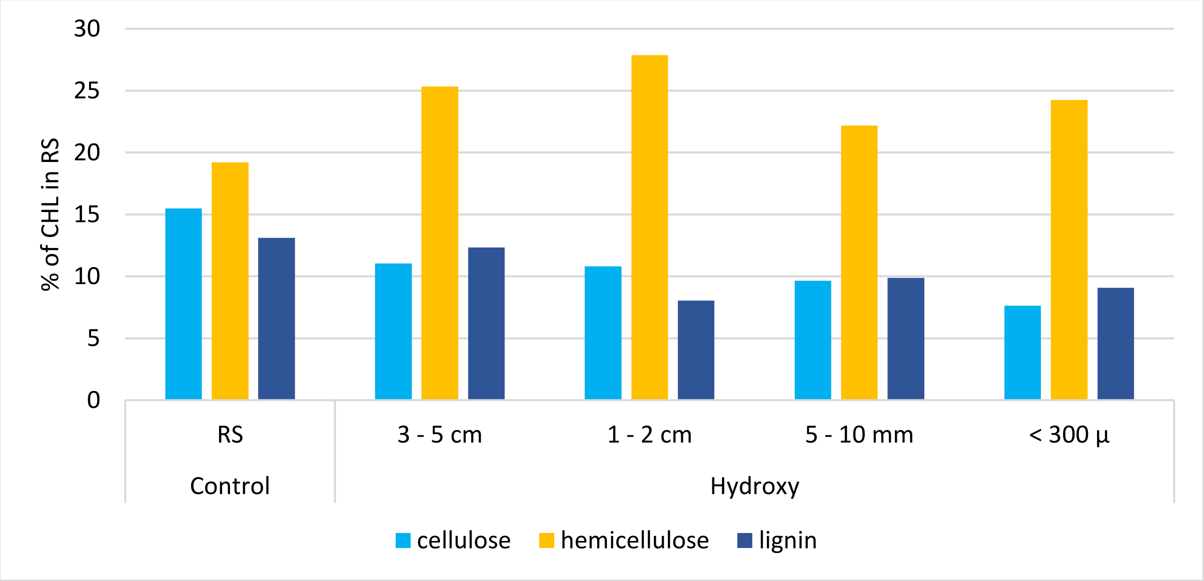


Plate. 5.10 Cellulose, hemicellulose and lignin (CHL) degradation before and after hydroxy pre-treatment

sugars in the fraction of RS but also removed lignin and made cellulose easily available to microbes for bacterial hydrolysis (Demirbas & Ozturk., 2015). In general, decreased lignin content also increases biogas production and biodegradability. Plate. 5.10, 5.11 and 5.12 showed a decrease in cellulose and lignin and a drastic increase in hemicellulose. Even after reducing the lignin, which may improve the biogas production, hemicellulose increment may not access the enzymes to cellulose for degradation by being a physical barrier. The same kind of scenario is seen in H_2O_2, thermal + H_2O_2 and H_2O_2 + thermal pre-treatment on RS other than thermal pre-treatment.

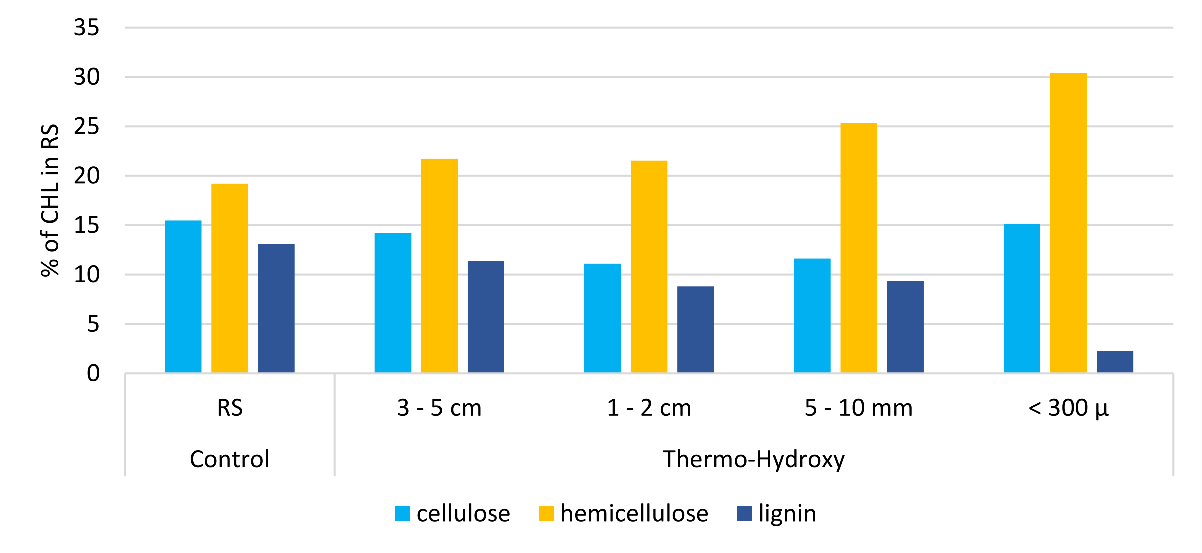


Plate. 5.11 Cellulose, hemicellulose and lignin (CHL) degradation before and after thermal + H_2O_2 pre-treatment

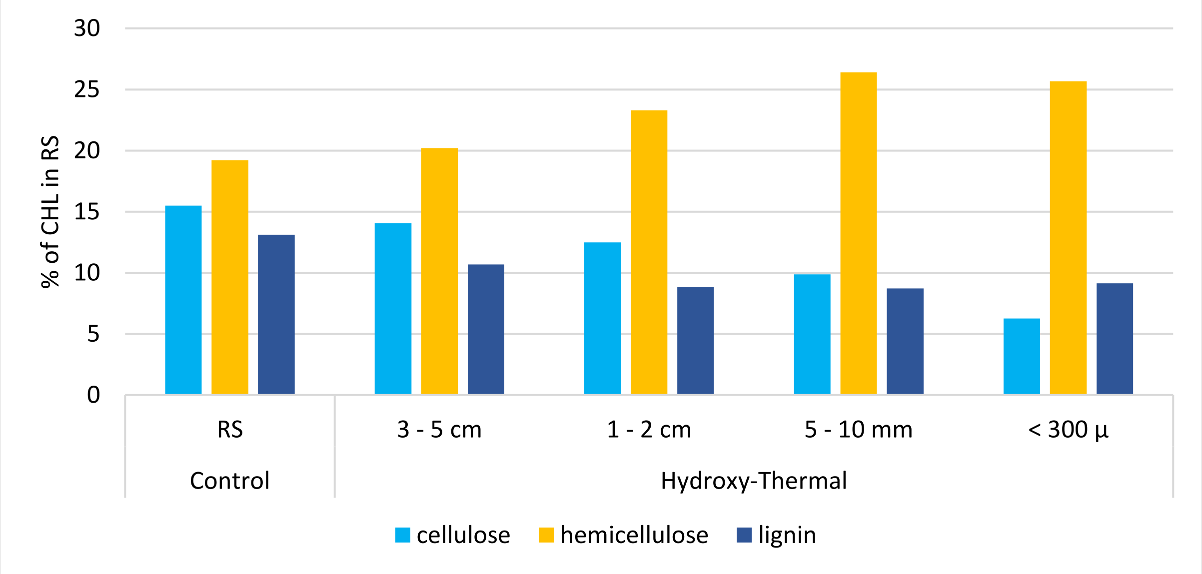


Plate. 5.12 Cellulose, hemicellulose and lignin (CHL) degradation before and after H_2O_2 + thermal pre-treatment

5.5 Biogas and methane production in pre-treated A-co-D of RS

Plate. 5.13 depicts the VFA concentration, Plate. 5.14 shows the cumulative biogas yield and Plate. 5.15 represents the average methane content in thermal, H_2O_2, thermal + H_2O_2 and H_2O_2 + thermal pre-treatment on RS at 3-5 cm, 1-2 cm, 5-10 mm and < 300 µm sizes of RS including the control which is RS+SS+ChM at 20% TS selected from chapter 4. VFAs form when the acids produced

from acidogenesis and acetogenesis are not able to consume by methanogenic bacteria (Song et al., 2013). The threshold concentration for VFAs is 4000 mg/L; more than this value may hinder the process by reducing the pH and destabilising the methanogenic bacteria (Croce et al., 2016). From Plate. 5.13, among the four pre-treatments, maximum VFA production of more than 4000 mg/L is observed in H_2O_2, thermal + H_2O_2, and H_2O_2 + thermal pre-treatment may be due to fast hydrolysis of RS because of its ruptured and collapsed structure after pre-treatment whereas in thermal pre-treatment the VFA concentration is in limit.

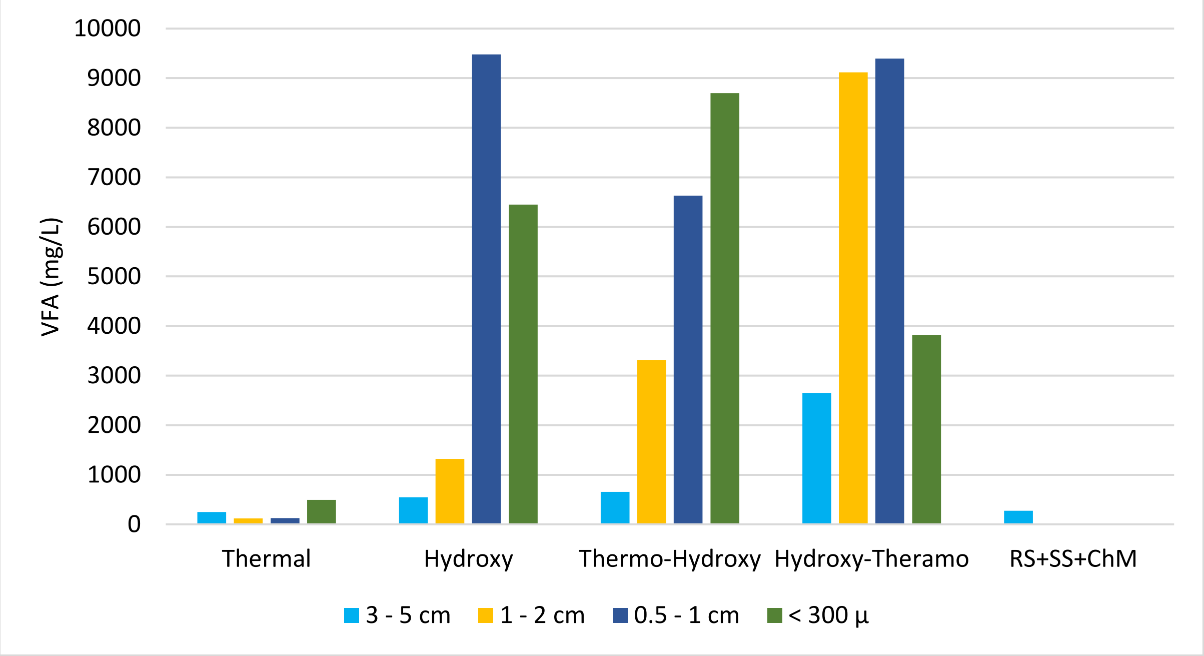
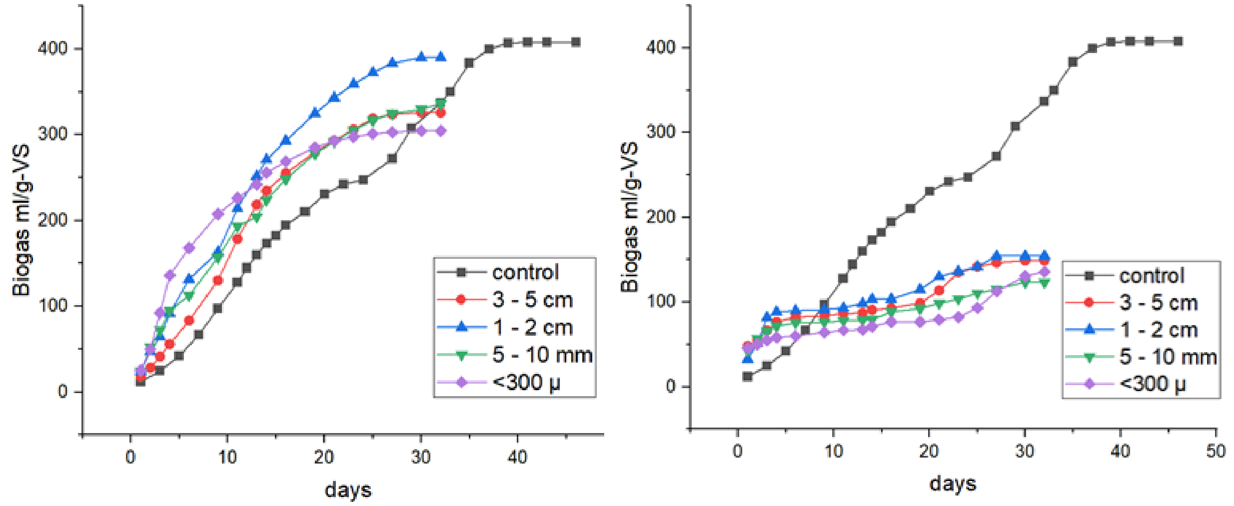
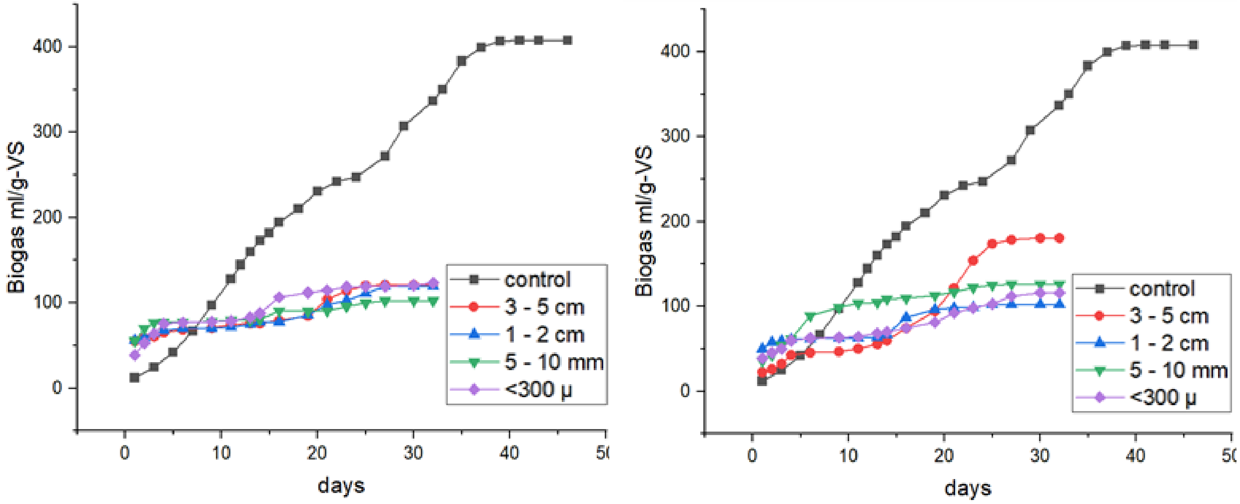


Plate. 5.13 VFA concentration in each pre-treatment at all RS sizes

From the Plate. 5.14, the cumulative biogas yield in all pre-treatment methods and at chosen RS sizes, the maximum biogas yield was observed in thermal pre-treatment at 3-5 cm, 1-2 cm, 5-10 mm and < 300 µm RS size with 325.8 mL/g-VS, 389.9 mL/g-VS, 336.3 mL/g-VS and 304.6 mL/g-VS respectively. A similar result was seen in the thermal pre-treatment of RS with 325.76 mL/g-VS (Kainthola et al., 2021). The reason for enhanced biogas production was due to accelerated hydrolysis of the substrate, enhanced cell membrane fragility, desirable cellular disruption and efficient release of soluble compounds (Kainthola et al., 2021). In the AD process, lignocellulosic biomass needs an intensive pre-treatment to accelerate the hydrolysis step, which is a rate-limiting step among the four steps. Thermal pre-treatment de-structure the lignin, the RS's main conferring structural support unit. As a result, the accessibility of cellulolytic enzymes improves. Due to decreased cellulose crystallinity and a rise in the available area for hydrolysis, the specific



a) Thermal pre-treatment b) H_2O_2 pre-treatment



c) Thermal + H_2O_2 pre-treatment d) H_2O_2 + Thermal pre-treatment

Plate. 5.14 Cumulative biogas yield of pre-treated RS in A-co-D, a) Thermal pre-treatment, b) H_2O_2 pre-treatment, c) Thermal + H_2O_2 pre-treatment d) H_2O_2 + Thermal pre-treatment

methanogenic activity is also increased. Whereas in H_2O_2 pre-treatment, it is observed to be only around 154 mL/g-VS, in thermal + H_2O_2 pre-treatment, nearly 123 mL/g-VS and in H_2O_2 + thermal pre-treatment of RS could produce only 180 mL/g-VS which are significantly less than the thermal pre-treatment. The reduced biogas production was due to rapid hydrolysis of RS (collapse and break down of structure) and increase in hemicellulose, which hinders the accessibility of microbes to cellulose, and also due to the formation of VFA accumulation. Plate. 5.15 shows the average methane content, it is observed to be more than 50% in thermal pre-treatment, and with nearby methane content ranging from 38-55 in H_2O_2 pre-treatment, Thermal + H_2O_2 pre-treatment and H_2O_2 + Thermal pre-treatment where the general methane content in biogas is between 40-60, all the methane contents in pre-treatment methods are in range. However, the average methane yield in thermal pre-treatment is more than the control (RS+SS+ChM at 20% TS). On the contrary, among the four pre-treatment methods, thermal pre-treatment outperformed but not more than the control biogas production, which is 407 mL/g-VS more than the thermal pre-treated A-co-D production (389 mL/g-VS), that suggests the co-digestion over pre-treatment for AD of RS.

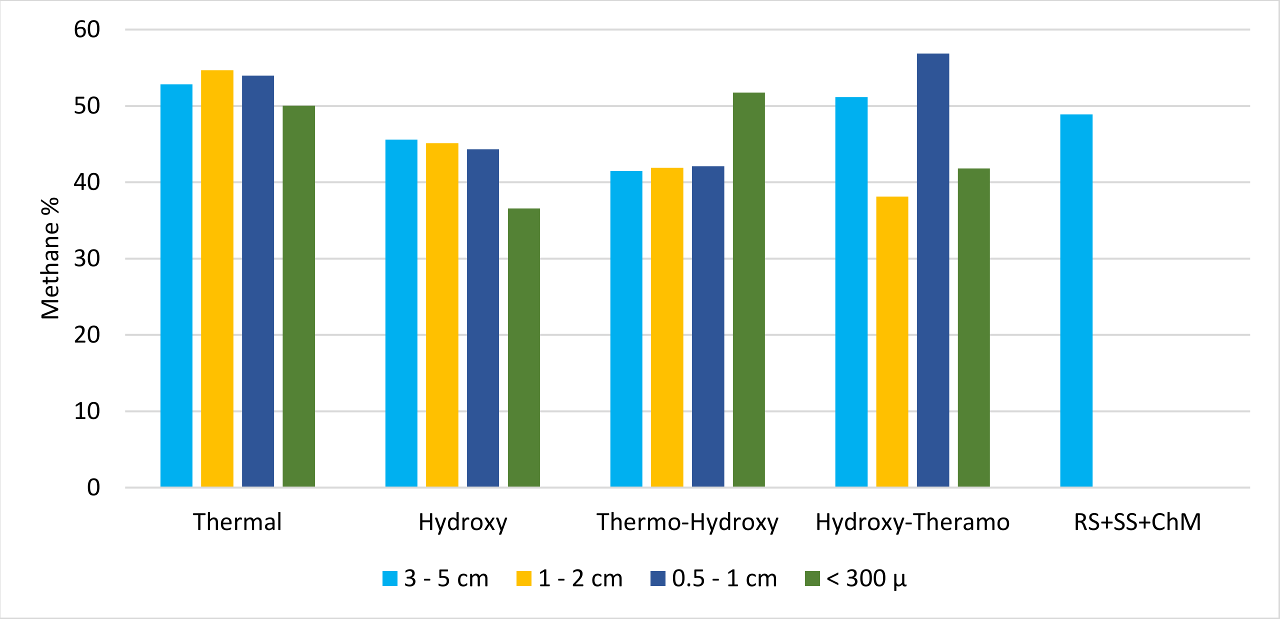


Plate. 5.15 Average methane content in each pre-treatment at all RS sizes

5.6 Effect of size reduction on AD of RS

Four sizes of RS have been chosen to increase the surface area of lignocellulosic biomass, thereby increasing the contact with microorganisms and further enhancing the degradation of the biomass. Particle size reduction releases cell compounds and directly creates new surfaces available to the microorganisms, thus increasing biodegradability (Mshandete et al., 2006). Studies have found that maximum substrate utilisation has increased twice with particle size reduction from 2 mm to 1 mm (Kim et al., 2000). In the current study, the chosen RS sizes are 3-5 cm, 1-2 cm, 5-10 mm and < 300 µm; the maximum biogas production was observed in thermal pre-treatment at 1-2 cm.

In thermal pre-treatment, the order of adaptability for choosing an RS size can be listed as 1-2 cm > 5-10 mm > 3-5 cm > 300 µm. The size reduction provided limited help improve the biogas production in AD of RS. The contradicting results were observed in the study by Dai et al. (2019), who adopted four sizes of RS, i.e., 2 cm, 1 mm, 0.15 mm and 75 µm the maximum biogas production was observed in 75 µm size of RS with adaptability order 75 µm > 0.15 mm > 1 mm > 2 cm and also high degradation of lignin and increased cellulose content were seen similar to the above order. On contrary, a study on AD of wheat straw with three different sizes of 1.45 mm, 0.67 mm, and 0.11 mm, increasing the particle size has induced better results (Motte et al., 2013). Supporting the above statement another study on AD of sewage sludge carried out by reducing the size of substrate from 2 cm to 8 mm, where there is no significant improvement in biogas production (Silvestre et al., 2015), the results in the current study are similar to this literature, and further study is needed to understand better the effect of size reduction on AD of RS.

5.7 Conclusion

Among the four pre-treatment methods adopted, thermal pre-treatment was obtained as the best pre-treatment method for RS at 1-2 cm RS size with 389 mL/g-VS. Microstructure observations (FESEM and FTIR) also proposed thermal pre-treatment as the best method to destruct the recalcitrant structure of RS. VFA accumulation is also not seen in thermal pre-treatment. In the other three pre-treatments, reduced biogas production was seen due to collapse and breakdown structure, which led to rapid hydrolysis and high VFA accumulation, which is also an inhibition for methanogenic bacteria and an increase in hemicellulose. Size reduction has improved bacterial activity on basic morphology and dissolution abilities. However, pre-treatment methods could not produce more biogas than control (RS+SS+ChM at 20% TS). As the pre-treatment methods require energy, it is suggested to opt for co-digestion than pre-treatment in AD of RS.

Chapter 6 Assessing the performance of the AD using pilot scale reactor

This chapter consists of feasible studies on pilot scale (500 L) batch and semi continuous anaerobic digestion (AD) of RS with the optimised results from objective I (co-digestion) & II (pre-treatment) which is RS+SS+ChM (rice straw, sewage sludge, and chicken manure) at 20% TS.

6.1 Batch scale pilot study

The AD of RS has been studied for nearly a century, but the implementation of full-scale biogas plants using RS as the primary substrate has not yet been demonstrated. Rice is the most important staple food for over half the world's population, and RS is one of the most abundant and renewable energy sources in the world (Zhao et al., 2010). Asia accounts for the 2/3 production of RS produced globally which amounts to about 500 Mt (Zealand et al. 2017). India accounts for about 12% of RS produced in Asia amounting to 60.8 Mt annually (Sarnklong et al. 2010). Common solutions for dealing with RS are open-field burning or tilling the straw back into the field, both contributing to increased greenhouse gas emissions (Gadde et al., 2009). Methane (CH_4) emissions from anoxic soils amended with RS are much higher than those without straw (Koga & Tajima, 2011), and one mitigation strategy is to collect the biomass and convert it into a clean-burning fuel through AD. The problem is a lack of realistic operational parameters on how to efficiently convert RS into energy as a sustainable practice (i.e., net energy producer versus a net energy consumer).

One of the major challenges associated with using RS as a substrate in the AD process is the complex, lignocellulosic structure which makes it difficult to decompose (Kadam et al., 2000). Several biological and chemical pre-treatment strategies have proven successful in lab-scale experiments to break down the lignin and accelerate decomposition (Zhao et al., 2010). However, most of these approaches may be inappropriate for a farm-scale application because of large chemical quantities, high energy inputs, excess water required and waste disposal issues associated with the residues, or digestate. The co-digestion of RS with sewage sludge (SS) and chicken manure (ChM) can help to overcome this challenge because it provides not only the necessary microorganisms but also the appropriate balance of nutrients to create favourable conditions for the methanogens to thrive. Biogas production increased approximately 35% and the methane yield increased from approximately 270 - 340 L-CH_4 /kg-VS when inoculated RS was co-digested with pig manure (2:1 ratio dry basis) compared to the digestion of the inoculated RS alone (Sun et al., 1987).

Numerous bench-scale experiments have been published on RS digestion, which define optimal parameters such as temperature, nutrient balances, inocula ratios and pre-treatment strategies, various co-substrates, however, only a few studies involving RS have been conducted in dry digestion conditions (i.e., total solids (TS) concentration - 20%) (Zheng et al., 2022; Liu et al., 2019; Du et al., 2019; Meng et al., 2018; Mustafa et al., 2017). Data from a larger pilot-scale system is limited. Though principles can be better understood through bench-scale studies, it is very difficult to operate a pilot scale plant. A pilot-scale batch reactors (500 L) filled with untreated RS and co-digested with SS and ChM were constructed and parameters such as TS concentration, digestion temperature and digestion time were evaluated. Specific objectives of this study were to compare gas production in lab and pilot scale study varying in temperature conditions (mesophilic for lab scale and ambient for pilot scale) in order to minimize management and disposal issues, and to avoid pre-treatment or additional inocula in an attempt to simplify the loading strategy for a pilot-scale plant.

For pilot batch scale study 500 L volume reactor is opted for current study to assess the performance comparing to lab scale study. Co-digestion was chosen over pre-treatment in chapter 5 which is also being studied in pilot study. The ternary combination of RS+SS+ChM at 20% TS was adopted at ambient temperature conditions. Inoculum is the liquid digestate from AD plant in National Institute of Technology warangal and biogas measured every alternative day up to 70 days.

6.1.1 Biogas production from pilot batch scale study

The biogas and methane yields were calculated as the volume of biogas or methane produced per unit weight of feed VS added. Plate. 6.1 showed the cumulative biogas in both lab scale and pilot scale studies in A-co-D of RS. The total cumulative biogas production from pilot scale study in digestion time is 167.90 mL/g-VS and 407.6 mL/g-VS in lab scale A-co-D. the duration of pilot scale study is 75 days whereas in lab scale, it is 46 days. The digestion of pilot scale continued up to the negligible biogas observed from the reactor. A pilot scale study of 1m^3 volume with white rot fungi pre-treated RS co-digested with piggery wastewater consisting of reactor TS of 19.5% which is near to the current study (20%), and with the digestion time of 89 days at ambient temperature has produced 570 mL/g-VS (Lianhua et al., 2010). The RS has pre-treated with 5% white rot fungi and an additional 2.5% of sodium bicarbonate ($NaHCO_3$) to balance C/N ratio of the reactor feed (Lianhua et al., 2010). Another study of A-co-D of RS with pig wastewater with 1 m^3 reactor volume and initial TS of 20% at mesophilic temperature with no pre-treatment has produced 28 mL/g-VS (Mussoline et al., 2012). In the current study, the pilot scale biogas production is nearly 42% in lab scale study, this reduction in biogas may be due to various reasons. The probable reasons could be, there is no mixing in the pilot scale reactor because of its heavy weight and volume whereas in lab scale, it is easy to mix the reactor because of its smaller volume. Another reason could be because of variable ambient temperature conditions, the pilot scale study is in ambient temperature which drastically varies throughout the day (maximum temperature in the day and minimum temperature in the night) whereas in lab scale it's in constant mesophilic temperature. From literature the reactor with mesophilic temperatures involves wide range of microorganisms and more stable temperature systems (Appels et al. 2008).

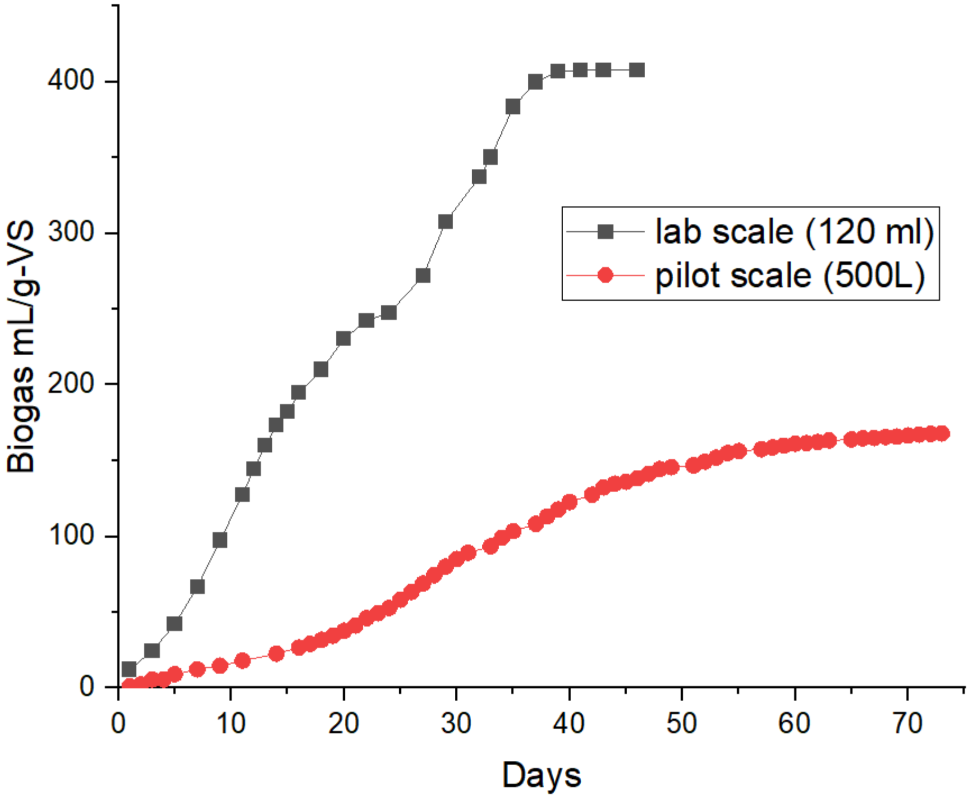


Plate. 6.1 Cumulative biogas production in pilot batch scale and lab scale study

Temperature plays a critical role in the AD process. The ideal temperature range for the AD of RS is between 35 and 40 °C (Lianhua et al., 2010). Plate. 6.2 showed the cumulative biogas production in pilot scale study with ambient maximum and minimum temperature with time. The lab scale experiments were kept in mesophilic temperature range for maximum time of the digestion period whereas in pilot scale its ambient for the digestion. The daily biogas production from batch pilot scale study had a lag phase at the beginning until day 15, followed by a growth phase from day 16 to day 42, then a stationary phase from day 43 to day 49, and eventually a decline phase from day 50 to day 73. The maximum temperature steadily increased from 36 to 40 °C whereas the minimum temperature increased from 23 to 29 °C in the digestion period. A pilot scale study by Lianhua et al. (2010) observed that there is no obvious relationship between temperature and biogas production. Whereas in pilot scale study by Mussoline et al. (2012), when the temperature dropped from 37 to 34 °C the biogas has also dropped significantly. In the current study, there is notable variation among maximum and minimum temperatures, which could be one of the reasons for less biogas production when compared to lab scale yield.

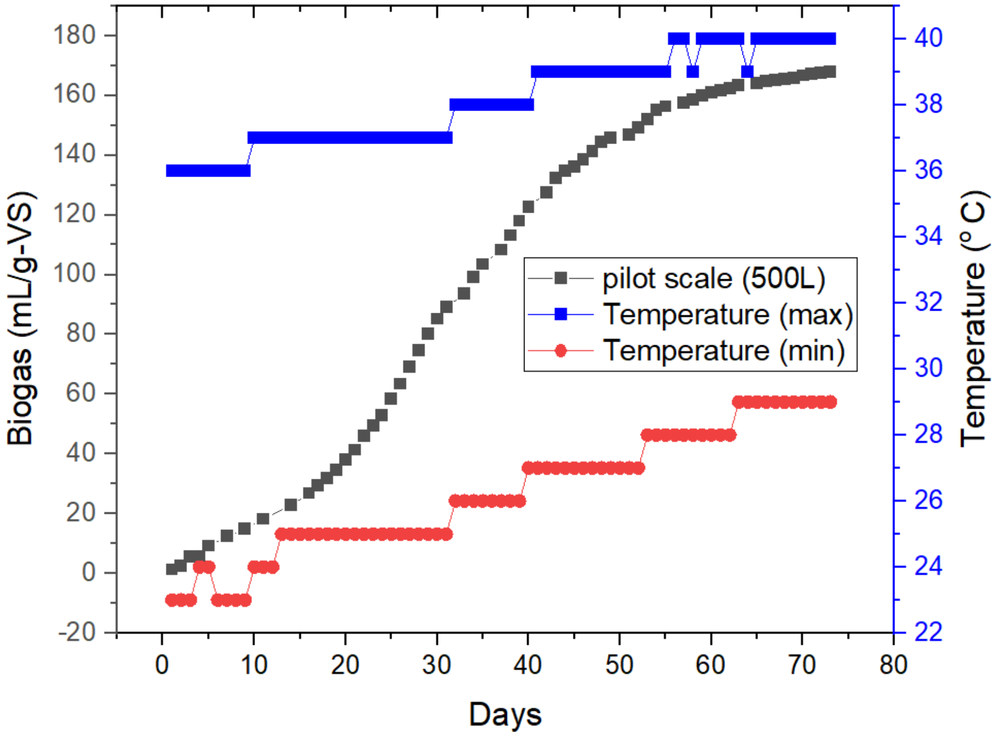


Plate. 6.2 Cumulative biogas production in pilot batch scale study with ambient maximum and minimum temperature versus time

6.1.2 VFA and VS reduction in pilot batch scale study

VFAs are intermediate compounds formed in acidogenesis and acetogenesis of organic substrate and can be accumulated when they are not consumed by methanogenic bacteria. The threshold limit of VFA accumulation that inhibit the methanogenesis and thereby reduce the pH of anaerobic system is 4000 mg/L. Plate. 3 showed the VFAs in pilot scale and lab scale studies. It is observed to be less than 4000 mg/L in both cases which depicts that there is no VFA accumulation and no inhibition due to VFAs.

Reduction in volatile solids (VS) is useful for estimating the performance of AD in SS-AD. Plate. 6.4 shows the VS reduction percentage in both pilot scale and lab scale study. In the pilot scale VS reduction percentage is 28.7%, whereas in lab scale its 34% unlike biogas production, there is no big difference in VS reduction in the pilot and lab scales. Overall, VS reduction is depicting the organic conversion of substrate into biogas.

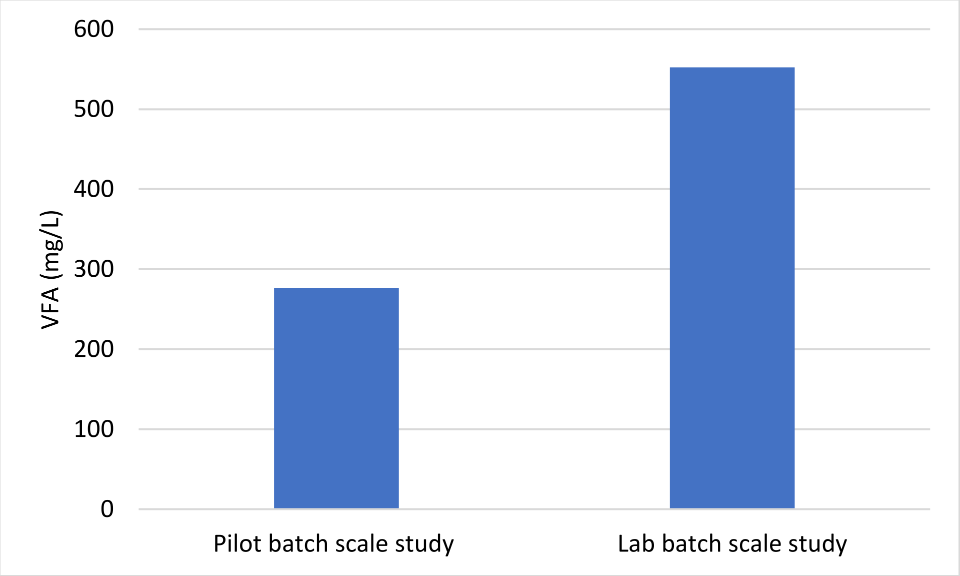


Plate. 6.3 VFA concentration in pilot batch scale and lab scale study

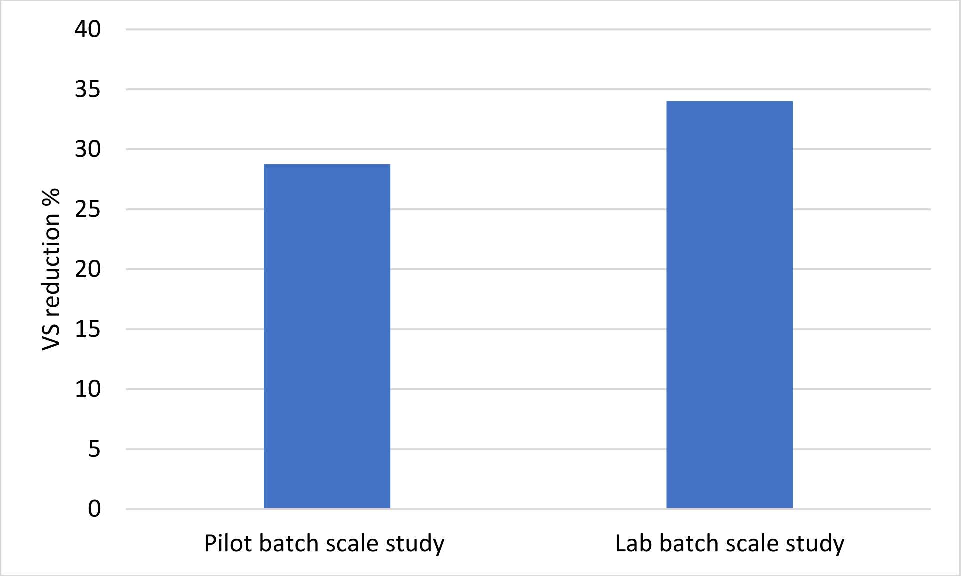


Plate. 6.4 VS reduction percentage in pilot batch scale and lab scale study

6.2 Semi-continuous pilot scale study

6.2.1 Biogas and methane production from semi-continuous study

The pilot scale semi-continuous reactor of capacity (500 L) was operated with 10% feed removed and fed every alternative day for 90 days. The feed for pilot semi-continuous study is opted from objective I i.e., ternary combination RS+SS+ChM (rice straw + sewage sludge + chicken manure) at 20% TS. Plate. 6.5 showed the daily biogas yield for 90 days, while Plate. 6.6 showed the daily biogas yield with maximum and minimum ambient temperature versus time. Plate. 6.7 showed the cumulative biogas and methane in pilot semi-continuous study with A-co-D of RS. The biogas and methane yields were calculated as the volume of biogas or methane produced per unit weight of feed VS added. The maximum daily biogas yield observed at 8th day with 7.56 mL/g-VS-day and on 58th day with 7.89 mL/g-VS-day. The total cumulative biogas production is around 271.67 mL/g-VS and average methane content is 48.55% which gives 131.90 mL/g-VS of cumulative methane yield. Initially biogas production increased up to day 8 of digestion may be because of higher initial activity of acidogenic and methanogenic bacteria in A-co-D.

Temperature being a critical parameter in AD, influence the efficiency of the reactor stability and optimal temperature is the basic need for reducing the vulnerability of the anaerobic system and its sustainability (Shetty et al. 2017). From Plate. 6.6, it is observed that the maximum temperature ranged from 33 to 29 °C in declining steadily and minimum temperature steadily decreased from

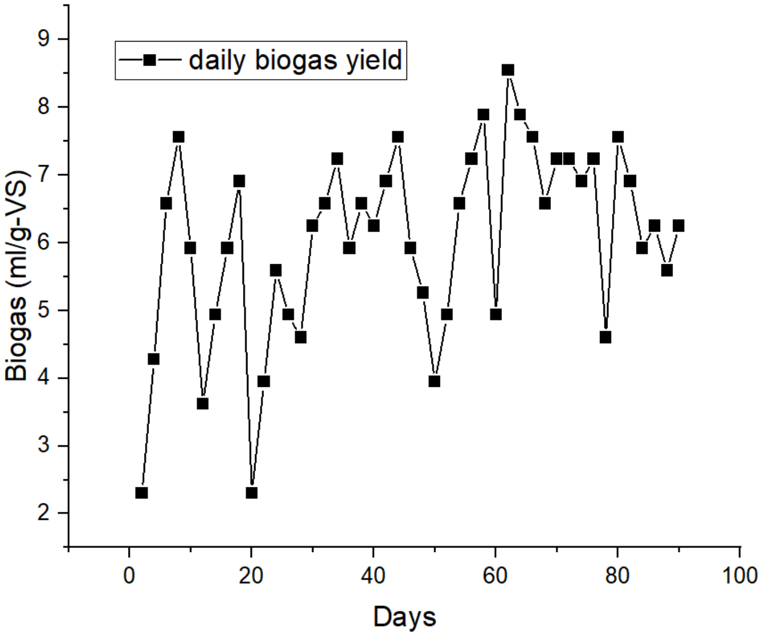


Plate. 6.5 Daily biogas yield in pilot semi-continuous study of A-co-D of RS

26 to 24 °C. The adulations of biogas production can relate with the temperature fluctuations as in day 11, 20, 22, 43, 60, 64, 79 and, 84 the biogas productions were reduced accordingly with reduced maximum ambient temperatures. There are mixed results in literature with the relationship between temperature and biogas production, in a study of AD of sludge, temperature played significant role in biogas production and suggested that increasing temperature shortening solid retention time could be an effective strategy for improving biogas production (Mortezaei et al., 2023), whereas there is no relation between them in A-co-D of RS (Lianhua et al., 2010) on contratry, in Mussoline et al. (2012) pilot study, the temperature reduced with reduction of biogas, and the same scenario is seen in current study as well.

6.2.2 VFA and pH variation in semi-continuous study

Digestate samples were collected every alternate day through out the experiment, and VFA and pH were analysed to monitor the A-co-D of pilot scale semi-continuous study. VFAs are intermediate products in acidogenesis and acetogenesis in AD process and accumulation of more than 4000 mg/L inhibit the methanogenesis whereas the pH less than 6.6 inhibit the methanogenic bacteria. Plate. 6.8 shows the VFA and pH variation in pilot semi-continuous study of A-co-D of RS. With the presence and routine recirculation of the feed every alternative day provided sufficient buffer for the AD system so that the pH never dropped below 7 and VFAs were less than 3000 mg/L shows there in no inhibition due to VFA accumulation in the reactor.

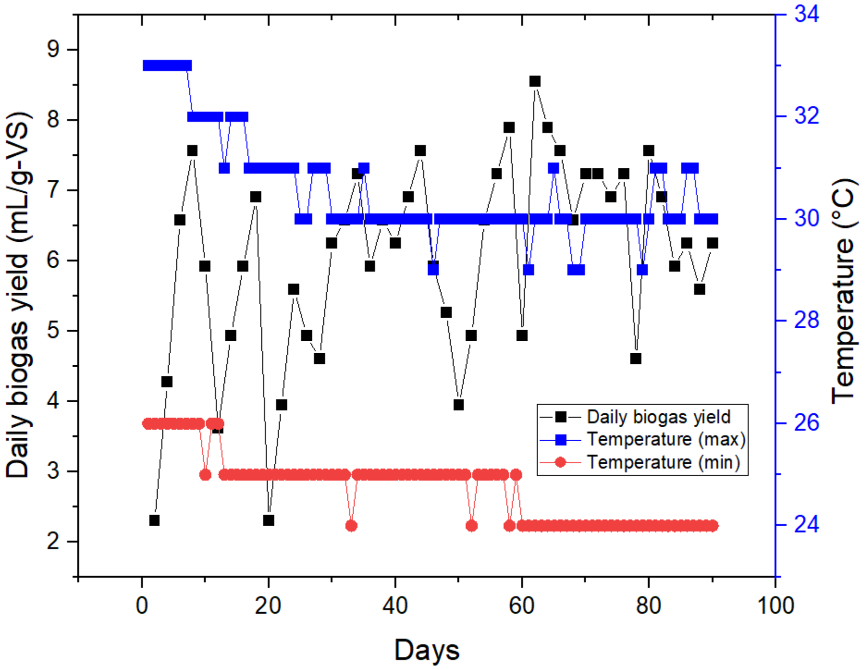


Plate. 6.6 Daily biogas yield with maximum and minimum temperature versus time

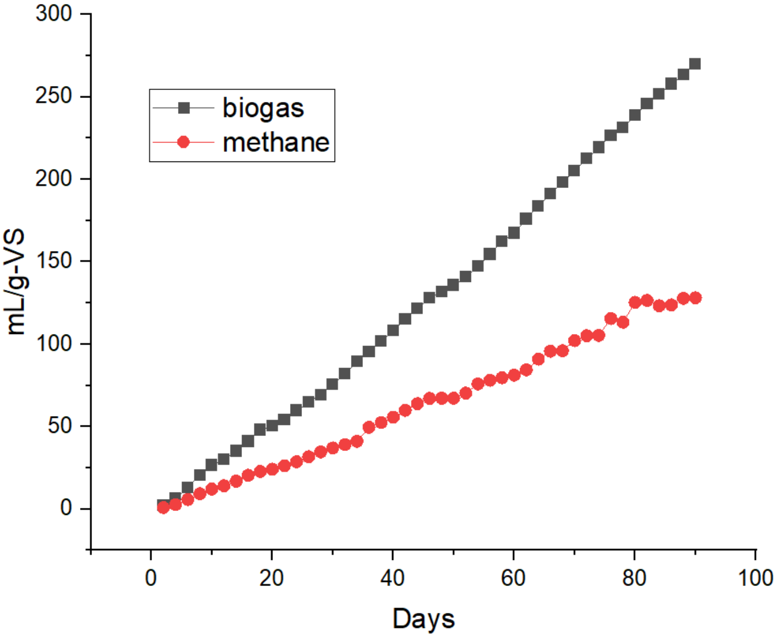


Plate. 6.7 Cumulative biogas and methane yield in pilot semi-continuous study of A-co-D of RS

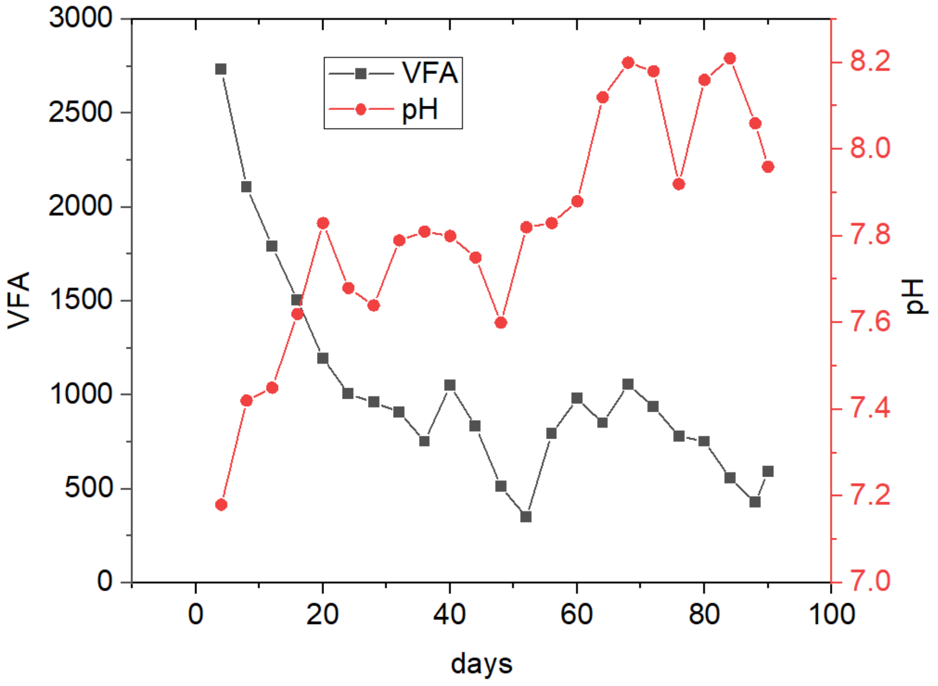


Plate. 6.8 VFA and pH variation throughout the digestion time in pilot semi-continuous study

6.3 Conclusion

The batch pilot scale study of A-co-D of RS has shown significant performance with 42% of lab scale study. There is no VFA inhibition in the reactor and comparable VS reduction % of 28.75% in the pilot scale whereas, in the lab scale, it is 34.02%. The reduced biogas production compared to lab scale is may be due to random ambient temperature conditions, and low mass transfer (no mixing patterns due to high total solids). The pilot scale semi-continuous A-co-D of RS has shown stable performance with cumulative biogas production of 271.67 mL/g-VS with 48.55% of average methane content. It is observed that inhibition due to VFA accumulation is not seen in both batch and semi-continuous studies of A-co-D of RS.

Chapter 7 Comparison of GHG emissions of RS

This chapter aims to describe the greenhouse gas emissions from open burning of RS, AD and other sources of emissions from the cultivation of rice and global warming potential from the sources mentioned above was computed and compared. A summary is presented at the end of the chapter.

7.1 Introduction

The majority of India's land is used for agriculture, and rice is the second most important crop. Rice cultivation has spread over many countries, with a total harvested area of close to 160 million hectares and an average yield of 760 Mt (metric tons) per year (FAO 2018). During rice crop processing, two residues are produced, i.e., rice straw (RS) and rice husk, with RS being the primary fraction of the rice field. It is approximated that 60.8 Mt of RS residues were produced per year in India (Sarnklong et al. 2010). Mainly, RS is used as animal food and roof thatching in India (Meshram, 2002), in addition to other methods for its utilisation (e.g., mechanical collection, composting, mulching, power production, biogas production, ruminant feed, and composite materials). Nevertheless, RS is susceptible to open field burning, a practice common in north India and many places around the world (Meshram, 2002). Being rice a major contributor to greenhouse gas emissions accounting for 10% of global emissions from agriculture (FAO 2015). This number is even higher for Southeast Asia, where 90% of the world’s rice is produced, making up 10–20% of the region’s total anthropogenic emissions and 40–60% of its agricultural emissions (UNFCC 2019). RS combustion has positive impacts on farm activities but has negative impacts on the environment (Romasanta et al. 2017). Despite the negative repercussions of the open burning of RS regarding the environment, health, and soil quality, farmers still decide to burn it due to its cheaper price, ease of tillage handling, and reduced weed growth. In addition, farmers get a short period amid two rice crops (Sahai et al., 2011).

However, RS burning is a leading cause of air pollution in many cities and deficiencies in soil fertility result from the loss of organic matter (Athira et al., 2019; Kumar et al., 2013). The open burning of RS generates carbon dioxide (CO_2), methane (CH_4), carbon monoxide (CO), nitrous oxide (N_2O), oxides of nitrogen (NO_x), hydrocarbons, etc. The release of these gases and particulates (when their concentration exceeds the environmental threshold limit) adversely affects the ecosystem, ecology, and human well-being. Also, it contributes to tropospheric ozone and the formation of Atmospheric Brown Cloud (ABC) - a cause of severe human health concerns (Cheng et al., 2000). Among the above gases, CO_2, CH_4, and N_2O are the principal greenhouse gases (GHGs) (Levine, 2003). Nevertheless, extensive work on GHG emissions by the open burning of RS in India has yet to be made available (Sahai et al., 2011). However, biomass combustion is not considered a source of CO_2 emissions (Intergovernmental Panel on Climate Change (IPCC) 1995) because the next growing season, plants reabsorb the CO_2 released (Levine, 2003; Houghton, 1991). The volume of residues resulting in open burning is significant and has the potential to be utilised as a resource. Furthermore, efforts are required to consider the application of the residue as a resource, in turn implementing agroecosystem sustainability (Sahai et al., 2011).

The anaerobic digestion (AD) of RS is among the appropriate techniques for converting wastes (e.g., RS, crop straw, or manure) into a potential energy source (Forster et al., 2008; Kaur et al., 2016). Co-digestion and pre-treatment are major technologies to improve the AD process and stability (Mothe et al., 2020). Anaerobic co-digestion of RS with several co-substrates, such as cow manure, sewage sludge, food waste, municipal solid waste, pig manure, chicken manure etc., was conducted to enhance the biogas production. Along with co-digestion, pre-treatment technologies were also evaluated, resulting in improved digestibility and stability. The various pre-treatment technologies include physical (milling, shredding, grinding), chemical (acids, alkalis, heavy metals, oxidants), biological, and combined pre-treatment. To reduce the field burning of RS, the Indian government has called for bio-compressed natural gas (bio-CNG) produced from RS for 46 rupees per kilogram, with subsidies of 700 rupees for projects using 70 tons of paddy per day (Krar et al., 2018). Support from the government in terms of policy and financing will encourage the industries to set up more AD plants to use RS produced by the agricultural sector. Even though the Indian government offers farmers several incentives in the form of subsidies for technology that makes straw management simple, the amount of burning has barely decreased. The government should endeavor to start a self-running system instead of making isolated efforts, and should also empower the stake holders by ensuring them of complete investment safety, according to a three-step mitigation approach that has been advocated in order to handle the pressing issue (Bhuvaneshwari and Hettiarachchi 2019). Additionally, it has been advised to encourage nexus thinking rather than sectorial thinking because this issue affects several sectors, including the environment, education, agriculture, economics, energy, and social issues. (Bhuvaneshwari and Hettiarachchi 2019).

Therefore, the country can expect a higher demand for AD of RS and justifiable compensation for peasants in the upcoming years. Despite these efforts, there are insufficient data on the emissions from the open burning and the use of RS as a resource for converting to bioenergy via AD processes.

The present article focuses on RS generation, utilisation, surplus RS subjected to open burning and energy production from AD of RS in India. The motivation and novelty of this work are to forecast and correlate GHG emissions from open burning and reduction of GHG emissions through AD of RS. To our knowledge, there is no literature available in this area. In this study, the methodology proposed by the IPCC was used to compute the GHG emissions from the open field burning and AD of RS, and the results were compared for Global Warming Potential (GWP) per day.

7.2 Methodology

7.2.1 Yielding and area of rice straw in India

Asia is forecasted for the two third generation of RS yielded in the overall world, which sums up to nearly 500.0 Mt (Zeeland et al. 2017), where it is 840 Mt worldwide (Mussoline et al., 2013). India counts to almost 12% of RS generated in Asia, tallying 60.8 Mt per annum (Sarnklong et al. 2010). In India, rice is cultivated in 43.86 million ha with a generation level of 104.80 Mt, and the yield is approximately 2390 kg per hectare (Government of India (GOI), 2013).

Table. 7.1. Typical properties of RS based on different sources

Parameter	**(Kaur et al. 2016)**	**(Mustafa et al. 2016)**	**(Gao et al. 2013)**	**(Mustafa et al. 2017)**
TS (%)	96.7 ± 0.19	89.9 ± 0.2	90.2 ± 0.8	90.6 ± 0.2
VS (%)	89.2 ± 0.21	80.6 ± 0.2	75.5 ± 1.0	81.5 ± .0.2
C/N	NA	31.8 ± 0.4	25.93	32.8 ± 0.5
Cellulose (%)	43.6 ± 0.09	37.8 ± 0.2	39.6 ± 0.7	36.7 ± 0.2
Hemicellulose (%)	23.8 ± 0.11	29.6 ± 0.7	18.5 ± 0.3	28.3 ± 0.7
Lignin (%)	6.0 ± 0.07	14.8 ± 0.4	22.7 ± 0.5	13.1 ± 0.4
Ash content (%)	10.8 ± 0.26	10.3 ± 0.5	24.1 ± 0.4	NA

Annually, RS is the major sector of feasible lignocellulosic residue globally, which is 7.31 × 10^4 tons of RS (Kim et al. 2004). 104.80 Mt produces 30,390 kg of RS. In the following section, GHG emissions were computed based on rice production levels in India annually. About 60.8 Mt of rice was produced in 43.86 million ha of land, resulting in 104.80 Mt of GHG emissions. The typical RS properties are presented in Table 7.1.

7.2.2 Estimation of GHG emission through the burning of RS

The GHG liberations through the open field combustion of RS are evaluated in different stages with various factors. The residue-to-crop ratio (RCR) is a factor that represents the ratio of crop residue produced to primary production (Sahai et al. 2011). RCR for RS is 1.29 ± 0.29 (Sahai et al. 2011). Dry matter fraction (DMF) is the water proportion of the RS in the course of cropping. For RS, it is around 0.83 (0.78-0.88) (IPCC 1996). By IPCC 1996, fraction burned (FB) is the ratio of collectively dried residue (RS) to its further use options after burning. Fraction actually oxidized (FAO) is the proportion of residue left on the field after burning, which is not oxidized; FAO for RS is 0.9 (IPCC 1996). Carbon fraction (CF) is the percentage of carbon in residue and is 0.4144 for RS (IPCC 1996). Nitrogen carbon fraction (NCR) is the fraction of nitrogen to carbon contents present in residue, which is opted to estimate the overall emissions of nitrogen. NCR for RS is 0.014 (IPCC 1996). The emission ratio (ER) of C (carbon) is the ratio of carbon liberated from compounds of carbon to overall carbon liberated from open burning of the residue for methane and carbon monoxide. Likewise, the emission ratio of N (nitrogen) is nitrogen liberated from compounds of nitrogen to overall nitrogen liberated from fuel. ER for CH_4, CO, N_2O, and Oxides of nitrogen (NO_x) are around 0.005 (0.003-0.007), 0.06 (0.04-0.08), 0.007 (0.005-0.009), and 0.121 (0.094-0.148) respectively (IPCC 1996). Conversion ratio (CR) accounts for the ratio of atomic weight to modify the carbon and nitrogen contents to the total atomic weights of the corresponding liberating

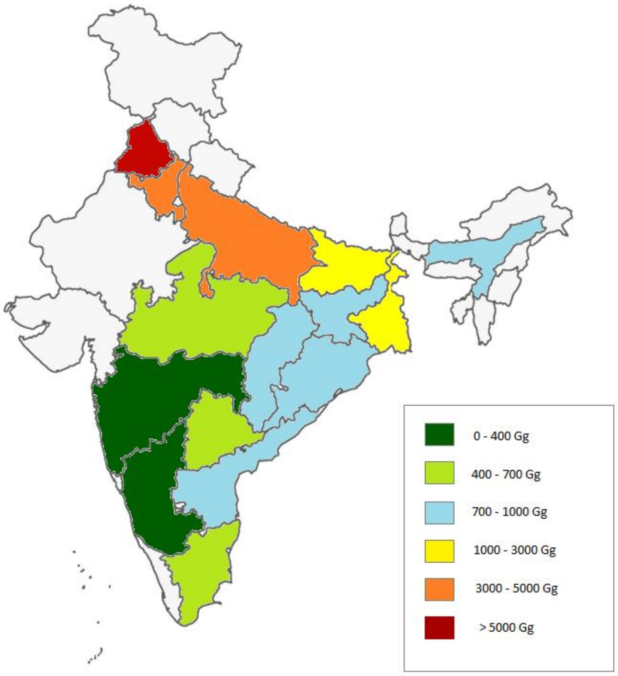


Plate. 7.1. Surplus RS subjected to open burning in various states of India

compounds like CH_4, CO, CO_2, N_2O, and oxides of nitrogen; CR values for the gases as mentioned above are 16/12, 28/12, 44/28, and 46/14, respectively (Sahai et al. 2011). From the factors mentioned above, Equation 7.1 gives the overall emissions:

$$E\ (Gg) = \sum(P \times RCR \times DMF \times FB \times FAO \times CF \times ER \times CR) \qquad \text{Eqn. 7.1}$$

Where E (Gg) is the total emissions of each gas, and P is crop or grain production data in Gg and other factors mentioned in the above section. Based on the given equation, emissions of various gases can be computed from any crop with grain production, and the parameters for the particular country are based on IPCC.

7.2.3 Estimation of methane and carbon dioxide from AD of RS

Based on the elemental analysis, the organic fraction of RS can be represented with the formulation of $C_aH_bO_cN_d$ (Chen et al. 2015). One can find the theoretical methane, carbon dioxide, and trace gases from AD of RS with Eq. 2:

$$C_aH_bO_cN_dS_e + \left(a - \frac{b}{4} - \frac{c}{2} + \frac{3d}{4} + \frac{e}{2}\right) H_2O \rightarrow \left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right) CH_4 + \left(\frac{a}{2} - \frac{b}{8} + \frac{c}{4} + \frac{3d}{8} + \frac{e}{4}\right)$$

$$CO_2 + dNH_3 + eH_2S \qquad \text{Eqn. 7.2}$$

Then, one can compare those with the experimental results of AD of RS and several co-digestion and pre-treatment techniques. However, the literature has not found co-digestion and pre-treatment effects on the above equation.

7.2.4 Boundaries of GHG accounting

The current study has considered the GHG emissions from the open burning of RS and AD of RS. However, there are other ways of GHG emissions in the cultivation of RS, which are CH_4 emissions from rice farming, N_2O emissions from rice cultivation and CO_2 emissions from urea application on rice farming lands. The above three ways are also computed for their emissions to the environment. But only emissions from the combustion of RS and AD of RS are considered in conclusions to compare the GWP according to the objectives (Streets et al., 2013; Sahai et al., 2011). Following Plate 3 shows the system boundary and possible ways of RS GHG emissions.

7.3 Results and Discussions

7.3.1 GHG production rates through the combustion of RS

In Cambodia, rice cultivated during 2005 - 2006 in the 1st and 2nd crop seasons is 2,048,360 and 298,529 ha, respectively (Vibol et al., 2010). Both paddy crops in Cambodia liberate 342.65 Gg of CH_4 and 2.33 Gg of N_2O, respectively, while total GHG emissions due to the combustion of RS is 11723 Gg CO_2 eq (Vibol et al., 2010). Gadde et al. (2009) calculated the releases of CH_4, N_2O, CO, and NO_x from India, Thailand, and the Philippines and found that a total of 97,192 Gg of RS is produced in India annually, out of which 23% is surplus. In India, 13915 Gg of RS is subjected to open burning, releasing 13.36 Gg of CH_4, 0.78 Gg of N_2O, 386.29 Gg of CO, and 34.51 Gg of NO_x, respectively. A total of 10451 grams and 10146 grams of RS were subjected to open burning in Thailand and the Philippines, producing 10.03 grams and 9.74 grams of CH_4, 0.58 grams and 0.57 grams of N_2O, 290.12 grams and 281.64 grams of CO, and 25.92 grams and 25.16 grams of NO_x, respectively (Gadde et al., 2009). Emissions of open burning of rice, Cassava, Corn and Sugar cane contributed 12–14 % of global GWP (Andini et al., 2018).

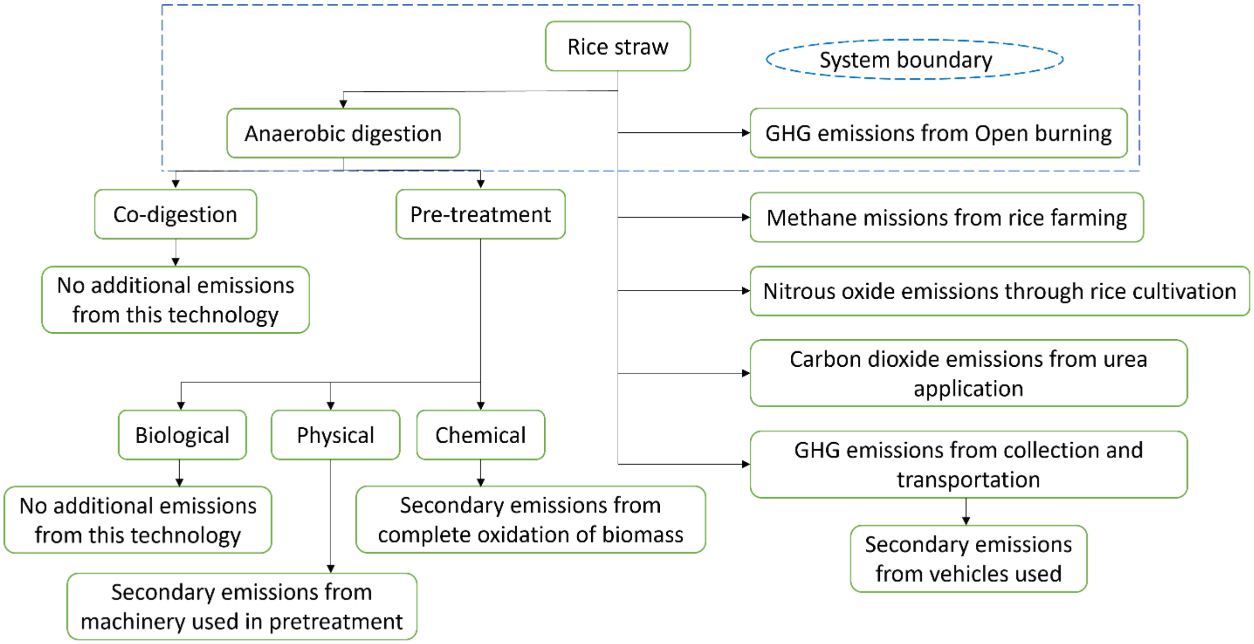


Plate. 7.2. Possible ways of RS GHG emissions with system boundary shown in blue dashed line

It has become a major global concern because of the increased emissions of GHGs, where regulating and mitigation steps have become crucial for sustainability and climate change. In the red river delta in Vietnam, PM2.5 and PM10 emissions are 27 and 29.5 Gg; along with them, CH_4, CO, and NO_x emissions are 31, 301 and 7.4 Gg due to open burning of 32400 Gg of RS (Le et al., 2020). It is observed that the main portion of RS combustion is happening in India, Indonesia, China, and Vietnam. The highest concentration of the emission zones is in the eastern and south-eastern parts of Asia, which also happens to be the major rice-growing areas of the world (Carlson et al., 2016). With the development and increment in agricultural production globally in upcoming years, the challenge is that it may require more nitrogenous fertilisers, which in turn increases the CH_4 and N_2O emissions into the environment (Kim et al., 2014).

The overall dry biomasses produced in India are predicted as 217, 239 and 253 tetra grams (Tg); out of the above values, 20.73%, 4.18%, and 1.19% of dry residue are prone to field burning, that is, 45, 10 and 3 Tg in 1994, 2005 and 2010 years, respectively (Sahai et al. 2011). Andhra Pradesh, Uttar Pradesh, Haryana, west Bengal, Bihar, Punjab and Orissa all together produced nearly 87 Tg (67%) and 95 Tg (69%) out of overall RS in 1994 and 2005, respectively (Sahai et al. 2011). In 1994, 2000, 2005 and 2010, the CH_4 produced from open burning of RS in India were 54.0 ± 21.5, 57.09 ± 22.7, 59.48 ± 23.7, 63.65 ± 25.4 Gg; CO emissions were 946 ± 428.3, 999.13 ± 452.4, 1040.98 ± 471.4, 1113.84 ± 504.4 Gg; N_2O emissions were 1.25 ± 0.37, 1.32 ± 0.48, 1.37 ± 0.50, 1.47 ± 0.54 Gg, and NO_x emissions were 45 ± 15.8, 47.67 ± 16.7, 49.66 ± 17.3, 53.14 ± 18.6 Gg, respectively (Sahai et al. 2011). Athira et al. (2019) computed emissions of open burning of RS in various states of India with surplus RS subjected to open burning, being shown in Plate 2. Plate 3 shows a GWP comparison among the various states and open burning among the different states with a total GWP of 1078.75 (Athira et al., 2019). According to official data, Punjab, Haryana, and Uttar Pradesh produce the bulk of RS with 12439, 4486, and 13628 Gg, respectively; and the surplus RS involved in open field burning in these states is 9951, 3589 and 3407 Gg, respectively, which play an important role in Delhi's air pollution (Athira et al., 2019). Table 2 has featured information about the RS produced, RS subjected to open burning, and CH_4, N_2O, CO, and NO_x emissions.

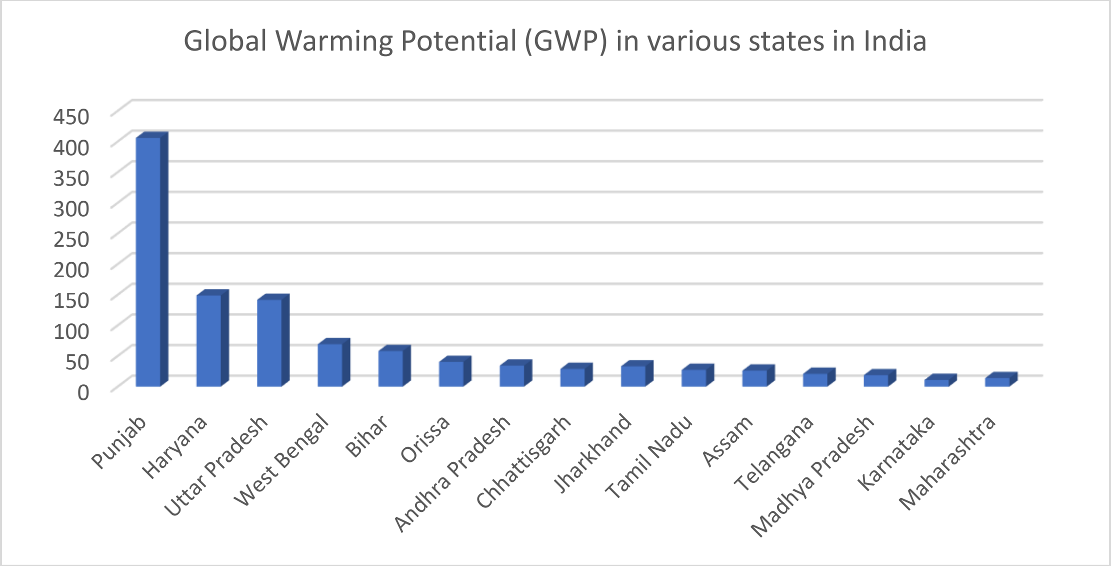


Plate. 7.3. Global Warming Potential (GWP) in Gigagram caused in various states of India due to open burning of RS (Athira et al., 2019)

Equation 3 can be used to convert CH_4 and N_2O (as these two are GHGs) into CO_2 eq:

$$GWP = (CH_4 \text{ emission} \times 25 + N_2O \text{ emission} \times 298) \text{ kg } CO_2 \text{ equivalents} \qquad \text{Eqn. 7.3}$$

Parameters used to evaluate the emissions from RS are presented in Table 7.2. In India, RS was estimated (using Equation 7.1) to liberate 69.75 Gg of CH_4, 1164.73 Gg of CO, 115.08 Gg of N_2O, and 4159.55 Gg of NO_x by open combustion, which would result in around 745.72 Gg and 34253.84 Gg CO_2 eq. The summarised GHGs from RS burning in India is nearly 35995.56 Gg

CO_2 eq per year. As the RS produced is 60.8 Mt annually, GWP is 592032.52 kg CO_2 eq/kg of RS produced and 1663.01 kg CO_2 eq/kg of RS per day.

Table. 7.2. Parameters for RS in computing the emissions

Crop	**P (Mt)**	**DMF**	**FB**	**FAO**	**CF**	**NCR**	**ER**	**CR**
Rice	104.80	0.83	0.25	0.9	0.4144	0.014	CH_4 – 0.005 CO – 0.06 N_2O – 0.007 NO_x – 0.121	CH_4 – 16/12 CO – 28/12 N_2O – 44/28 NO_x – 46/14

P = grain production; DMF = dry matter fraction; FB = fraction burnt; FAO = fraction actually oxidised; CF = carbon fraction; NCR = nitrogen carbon fraction; ER = emission ratio; CR = conversion ratio.

7.3.2 Emissions of methane (CH_4) from rice farming

The source of methane and nitrous oxide liberations is through flooded paddy cultivation. Anaerobic conditions of submerged soils of rice fields produce methane that escapes into the environment. Methane emissions were estimated using the IPCC (1996) methodology with the following equations,

$$E_{methane(rice)} = f_e \times A_h \times t \qquad \text{Eqn. 7.4}$$

$$f_e = f_{ec} \times SF_w \times SF_p \times SF_o \qquad \text{Eqn. 7.5}$$

where $E_{methane(rice)}$ is methane emissions from rice harvesting per year (kg per year); f_e is the liberation fraction per day (kg per hectare per day); A_h is cropping stretch per year (ha per year); and t farming duration of paddy (days); f_{ec} is baseline liberation component of overflow crop which are continuous with no organic acts is 1.3 kg CH_4 per hectare day; SF_w is the adjusting element to represent variations in the water system at the time of the farming cycle is 0.78; SF_p is the adjusting element to detail the variations in the water system in the prior season before the farming cycle is 0.68. The above values are code numbers advised by the IPCC considering humid subtropical and continuously flooded areas. SF_o is estimated at 1.90 for the baseline and 1.32 for the alternative systems. The total area allocated is 43.86 million ha, as mentioned. Upon the calculations, the methane production from rice cultivation is 6,320.65 Gg CH_4, which is 31,613.25 Gg CO_2 eq. Using Equation 3 to convert the methane emissions into carbon dioxide emissions. As RS produced is 60.8 Mt per year, GWP is 1,424.08 kg CO_2 eq/kg of RS per day.

Table. 7.3. Rice straw produced, open burned and the associated emissions of GHGs from various places (Gg = Giga gram; 1 Gg = 1000 ton)

Place	**RS production (per year) (Gg)**	**RS subjected to burning (Gg)**	**% RS burned**	**Methane emissions (Gg)**	**Nitrous Oxide emissions (Gg)**	**CO emissions (Gg)**	**NO_x emissions (Gg)**	**Reference**
India	97192	13915	14.32	13.359	0.779	386.287	34.51	(Gadde et al., 2009)
India	110918	25874	23.33	24.91	1.53	718.36	64.25	(Athira et al., 2019)
India	-	84000	-	277	5.88	7728	322	(Streets et al.,2003)
India	102400	-	-	63.65 ± 25.4	1.47 ± 0.54	1113.84±504.4	53.1 ± 18.6	(Sahai et al., 2011)
Punjab	12439	9952	80.01	9.56	0.56	276.27	24.69	(Athira et al., 2019)
Haryana	4486	3589	80.00	3.45	0.21	99.65	8.91	(Athira et al., 2019)
Uttar Pradesh	13628	3407	25	3.28	0.2	94.58	8.45	(Athira et al., 2019)
West Bengal	16438	1644	12.06	1.58	0.1	45.64	4.08	(Athira et al., 2019)
Bihar	7119	1424	20.00	1.37	0.08	39.53	3.54	(Athira et al., 2019)
Orissa	9293	929	9.99	0.9	0.06	25.81	2.31	(Athira et al., 2019)
Andhra Pradesh	8101	810	9.99	0.78	0.05	22.5	2.01	(Athira et al., 2019)
Chhattisgarh	7080	708	10	0.68	0.04	19.66	1.76	(Athira et al., 2019)
Jharkhand	3765	753	20	0.73	0.05	20.91	1.87	(Athira et al., 2019)
Tamil Nadu	6415	642	10	0.62	0.04	17.81	1.6	(Athira et al., 2019)
Assam	5849	585	10	0.57	0.04	16.24	1.46	(Athira et al., 2019)
Telangana	4973	497	10	0.48	0.03	13.81	1.24	(Athira et al., 2019)
Madhya Pradesh	4060	406	10	0.39	0.03	11.28	1.01	(Athira et al., 2019)
Karnataka	3965	198	4.99	0.2	0.02	5.51	0.5	(Athira et al., 2019)
Maharashtra	3299	330	10	0.32	0.02	9.16	0.82	(Athira et al., 2019)
Cambodia	-	-	-	342.65	2.32885	-	-	(Vibol et al., 2010)
Thailand	21859	10451	47.81	10.033	0.585	290.116	25.918	(Gadde et al., 2009)
Thailand	-	7700	-	21	0.54	708	29	(Streets et al.,2003)
Philippines	10680	10146	95	9.74	0.568	281.64	25.161	(Gadde et al., 2009)
Philippines	-	7100	-	19	0.50	653	27	(Streets et al.,2003)
Indonasia	70850	19300	27.24	16000	100	2800	5100 g	(Andini et al., 2018)

7.3.3 Nitrous oxide liberations through rice cultivation

Normally, nitrogen oxide is created through a two-step process called nitrification (where nitrate is formed by ammonium oxidation) and denitrification (where nitrogen gas is produced by nitrate reduction), respectively. Once produced, N_2O seeps through microbial units to the soil, then to the environment, and the intermediate gas is formed by denitrification (da Silva et al., 2021). Direct N_2O emissions are products of crop residues returned to the soil, synthetic fertilisers applied, organic soil cultivation emissions, and indirect N_2O emissions come from environmental deposition of nitrogen vaporised from managed soils (E_{ATD}) and liberations from runoff and leaching (E_L). Both are calculated using the following equation according to IPCC:

$$E_{Direct} = E_{inputs} + E_{MOS} \qquad \text{Eqn. 7.6}$$

$$E_{Direct} = \{[(F_{SN} + F_{CR}) \times EF_{1FR}] + (F_{MOS} \times EF_2)\} \times \frac{44}{28} \qquad \text{Eqn. 7.7}$$

$$E_{Indirect} = E_{ATD} + E_L \qquad \text{Eqn. 7.8}$$

$$E_{Indirect} = \{[(F_{SN} \times Frac_{GASF} \times EF_4)] + [(F_{SN} + F_{CR}) \times Frac_{LEACH} \times EF_5]\} \times \frac{44}{28} \qquad \text{Eqn. 7.9}$$

where E_{Direct} is direct N_2O emissions generated from operated soils per year (kg N_2O/yr); E_{Inputs} is direct N_2O emissions out of nitrogen (N) inputs to operated soils per year (kg N_2O per year); E_{MOS} is direct N_2O liberations from opted organic soils per year (kg N_2O/yr); F_{SN} is supply of synthetic fertilizer utilized to soils per year and is 7.44×10^7 (kg N/yr), where the straw is collected and used; F_{CR} is volume of N in crop biomass restored to operated soils per year is 2.41×10^6 (kg N per year); F_{MOS} is stretch of operated organic soils per year (ha); EF_{1FR} is the liberation fraction for N_2O liberations out of N inputs to overflown paddy, i.e. 0.003 (kg N_2O-N per kg of N input); EF_2 is emission fraction for N_2O liberations out of operated organic soils per year, i.e, 8 (kg N_2O-N/ha/yr); $E_{Indirect}$ is direct N_2O liberations out of operated soils per year (kg N_2O per year); E_{ATD} is volume of N_2O generated out of environmental settlement of N volatilized through operated soils per year (kg N_2O per year); $Frac_{gasf}$ is the ratio of synthetic fertilizer N that volatilizes as oxides of nitrogen and ammonia and is 0.1 (kg N volatilized per kg of N utilized); EF_4 is emission element for N_2O liberations out of environmental settlement of N on ground and water cover and is 0.01 (kg N-N_2O/ (kg NH_3-N + NO_x-N volatilized)); E_L is volume of N_2O contributed from runoff and seepage of N supplements to operated soils in areas where runoff/seepage happens annually (kg

N_2O per year); $Frac_{leach}$ is the ratio of all N added/mineralized in operated soils in areas where runoff/seepage arise, which is lost by runoff, and seepage is 0.3 (kg N per kg of N additions); and EF_5 is liberation element for N_2O liberations out of N runoff/seepage is 0.0075 (kg N_2O/(kg N runoff/seepage). The estimations were presented with the default numbers advised by the IPCC. Using equations 7 and 3, the N_2O emissions from the direct pathway is 551.74 Gg N_2O/y. When converted into CO_2 equivalents, this is 164,419.99 Gg CO_2 eq. As RS produced is 60.8 Mt per year, GWP is 7,408.97 kg CO_2 eq/kg of RS per day. According to equation 9, indirect N_2O emissions are 0.388 Gg N_2O/y, converting it to GWP is 5.21 kg CO_2 eq/kg of RS. Total N_2O emissions from rice cultivation are 7,414.18 kg CO_2 eq/kg of RS per day.

7.3.4 Carbon dioxide emissions from urea

Calculated urea-employed emissions are given in the following equation (IPCC, 2006; De Klein et al., 2006).

$$CO_2^{urea} = UR_{urea} \times EF_{urea} \times \frac{44}{12} \qquad \text{Eqn. 7.10}$$

CO_{2urea} is carbon dioxide emission in kg CO_2/kg; UR_{urea} is the volume of urea utilised (in kg); and EF_{urea} is an emission fraction of 0.2. The amount of urea applied on rice fields is around 81.7 kg/ha. Calculating the CO_2 emissions from urea application is 2,627.79 Gg CO_2. The GWP is 118.41 kg CO_2 eq/kg of RS per day.

7.3.5 Methane and CO_2 production rates from AD of RS

RS is digested with several substrates to improve the biogas yield. Anaerobic co-digestion (Aco-D) of RS with kitchen waste and pig manure produces higher biomethane yields than using only RS as a substrate, resulting in 71.67% substrate digestibility with nutrient balance (Ye et al., 2013). With food waste as a co-substrate, Aco-D of RS produced the highest digestibility of 95% due to butyric acid-type fermentation (Chen et al., 2015). Co-digestion of ammoniated RS with food waste has enhanced biomethane productivity by 8.83% with the supplementation of cobalt (Co) and nickel (Ni) (Zhang et al., 2018). In Aco-D of RS and sewage sludge, a high reduction of volatile solids (VS) was observed, along with an improved methane yield by 59.6% in a two-stage process compared to a one-stage process with effective utilisation of volatile fatty acids (VFA) (Kim et al., 2013). Aco-D with RS and municipal solid waste (MSW) has improved biogas production by 60 % with balancing in the carbon to nitrogen ratio (C/N), which is crucial in AD stability (Negi et al., 2018). In a continuously-stirred tank reactor for co-digestion of RS and chicken manure, the positive synergistic effect in the digester leads to a 28.2 % improvement in CH_4 production (Mei et al., 2016).

Biological pre-treatment with *Pleurous ostreatus* on AD of RS has improved biomethane yield by 120%; reduction of LCH (lignin, cellulose, hemicellulose) has been observed in both *Pleurous ostreatus* and *Trichoderma reesei* because of the enhancement in surface area and larger pore size of the substrate (Mustafa et al., 2016). With NaOH + microwave pre-treatment on AD of RS, biogas production has improved because of lignin reduction and improved biodegradability (Kaur et al., 2016). NaOH pre-treatment on RS in AD has enhanced methane yield by 34% with the removal of lignin, de-crystallization of cellulose and eventual swelling of the substrate (Shetty et al., 2017). Trace element supplementation and NaOH pre-treatment have improved CH_4 yield by 24% due to increased hydrolysis in the reactor (Mancini et al., 2018). With sodium carbonate (Na_2CO_3) treatment on RS, the productivity of biogas has enhanced by 54.4 % with a reduction in lignin, which in turn improved the digestibility of RS (Kaur et al., 2016). Acidic pre-treatment with acetic-propionic acid on RS has reduced lignin by 34.19% and enhanced RS's biodegradability with a 35.85% improvement in CH_4 production (Zhao et al., 2010). In combined pre-treatment of fungal (*Pleurous ostreatus*) and milling on AD of RS, CH_4 yield has improved by 165% due to the fungal pre-treatment incubation period and lignin reduction in the substrate (Musthafa et al., 2017). Physicochemical combined pre-treatment on AD of RS has improved the CH_4 yield by 54% and biodegradability.

Table 7.3 presents the detailed production rates of CH_4 for various ADs with and without co-digestion and various pre-treatment techniques. In biogas, the amount of CH_4 varies between 50 and 60 percent, depending on digestion efficiency and stability (Panigrahi et al., 2019). GWP is calculated as the maximum biogas yield from the AD mixtures in kg of CO_2 equivalents per day; the particular AD mixtures are highlighted in Table 3. Based on the maximum methane production rate over the reactor-operated period and the amount of volatile solids (VS) fed into the digester, GWP is calculated. Overall, the GWP for methane emissions from AD of RS is 0.19 and 0.34 Kg CO_2 eq/kg-day.

Table. 7.4. Biogas, methane production rates from AD of RS

Technique	**Temperature**	**Raw materials**	**Additional Treatment**	**Biogas produced (L/kg VS)**	**Methane produced (L/kg VS)**	**% of CH_4 improved with Aco-D or Pre**	**Reference**
Aco-D	37±2◦C	**RS+SS+ChM**	**-**	**408**	**199.55**		**Current study**
Aco-D	37±2◦C	**RS+ChM+CM**		**442**	**246.9**		**Current study**
Aco-D	37±1◦C	RS+PM+KW^{a}	-	674.4	383.9	71.6	(Ye et al., 2013)
Aco-D	37◦C	RS+MSW^{a}		766.9	468.44	57	(Negi et al., 2018)
Aco-D	35±1◦C	RS+FW	NaOH	535	321*	105.36	(Chen et al., 2015)
Aco-D	55◦C	RS +SS	-	341.02	266	70.12	(Kim et al., 2013)
Aco-D	35◦C	UARS+FW	Trace element supplementation	-	293.44	8.83	(Zhang et al., 2018)
Fungal Pretreatment	37±1◦C	Pleurous ostreatus	-	-	263	120	(Mustafa et al., 2016)
Chemical Pre	37±2◦C	NaOH-microwave	-	297	178.2*	54.7	(Kaur et al., 2016)
Physical-biological	37±1◦C	Pleurotus ostreatus	milling	-	258	165	(Musthafa et al., 2017)
Physical-chemical	35◦C	Extrusion	alkali	-	-	54	(Zhang et al., 2015)
Aco-D	37±2◦C	RS+ChM	-	440	264*	28.2	(Mei et al., 2016)
Chemical Pre	37◦C	NaOH	-	514	308.4*	34	(Shetty et al., 2017)
Chemical Pre	37±2◦C	NaOH	Trace element supplementation	-	342	21.4	(Mancini et al., 2018)
Chemical Pre	35◦C	Acetic-propionic acid	-	1056 ml	604.5ml	35.85	(Zhao et al., 2010)

Note: From biogas, 60% of it has taken as methane for which the data has not given in the particular article (methane in biogas is in the range of (50-70%)) (Panigrahi et al., 2019).

Chemical Pre – Chemical pre-treatment. Aco-D – Anaerobic co-digestion. * Considered AD mixture for computation of methane emissions.

7.4 Comparison of GHG emissions out of combustion and AD of RS

From the above sections GWP from open burning of RS, CH_4 and NO_x emissions out of paddy farming, emissions due to urea application are 1663.01, 1424.08, 7414.18 and 118.41 kg CO_2 eq /kg-day, respectively and for AD of RS produces around 0.34 kg CO_2 eq /kg-day. Table 5 shows that open burning of 1 ton of RS will contribute GWP of 1663010 kg CO_2 eq per day into the atmosphere, whereas AD of 1 ton RS utilisation will produce around 336.66 kg CO_2 eq per day. From 2016–2019, a single rice crop in China with continuous flooding produced an annual CO_2 and NO_x emissions of 426.4 ± 17.7kg C per hectare and 0.38 ± 0.04 kg N per hectare, and the GWP was 19.51 ± 0.42 (Song et al., 2021). A 2-year (2012−2013) experiment on the rice fields in china showed a GWP of 1257.17 ± 187.45 kg CO_2 /ha annually and 7240.48 ± 385.07 kg CO_2 /ha annually with urea and manure treatment in rice fields (Zhao et al., 2015). In Thailand, a GWP of 3883.10 kg CO_2 eq/ha has been produced in rice crop fields with RS burning included (Maneepitak et al., 2019). In India, rice cultivation was examined in the Tamil Nadu state for its GHG emissions; in the monsoon season, the GWP generated was 5419 ± 518 kg CO_2 /ha (Oo et al., 2018). Thus, AD of RS will reduce the GHG emissions released into the atmosphere. Additionally, biomethane generated from AD of RS can also be used as an energy replacement, reducing the need for fossil fuels and GHG emissions.

Table. 7.5. Comparison of GWP between sources of RS emissions and AD of RS

Category	**GWP (kg CO_2 eq/kg-d)**	**1-ton RS GWP (kg CO_2 eq/d)**
Open burning of RS	1663.01	1663010
Methane emissions from rice cultivation	1424.08	1424080
Nitrous oxide emissions from rice cultivation	7414.18	7414180
Carbon dioxide emissions from urea application	118.41	118410
AD of RS	0.3366	336

7.5 Discussion and Implications

Results of this study indicate that RS open burning would result in GWP 493873% higher than that of AD of RS. Opting AD over open burning of RS can reduce the gases CH_4, N_2O, CO_2, and NO_x, which are produced from the combustion of RS, out of which CH_4, CO_2 and N_2O are the GHG gases. Still, they might not reduce emissions from other sources mentioned above. Additionally, AD can reduce CO and NO_x along with GHG, as both are major environmental pollutants. Nevertheless, AD produces CH_4, which can be a substitute for natural gas that depicts AD as a circular economy that may also alleviate the air pollution in Delhi caused due to combustion of RS in Punjab and Haryana. Further studies are needed on the open burning of RS in India and other places to reduce the uncertainties in the emission factors. As the open burning of RS is found to be an environmentally deteriorating process in terms of GHG emissions, it is important to educate the farmers about the adverse effects and abstain from doing so. Additionally, governments need to provide incentives and suggest management options to utilise RS residue sustainably. Furthermore, using RS for biogas/energy production should be endorsed by governments to produce sustainable bioenergy. For AD of RS, more pilot-scale studies have to be conducted, particularly in major RS producing areas. Studies are needed on adopting appropriate RS co-digestion with other locally available substrates. Further studies on pre-treatment technologies like hydrodynamic cavitation, nano-bubble, etc., on RS, are required to improve the process in terms of biogas yield and process stability. A detailed techno-economic analysis is required to ensure the processes are energy- and cost-effective.

7.6 Conclusion

The amount of GHGs released by the open burning of RS in India is several orders of magnitude greater than that released by the AD of RS. In India, open burning of RS results in CO_2 emissions of 592032.52 kg CO_2 eq/kg-year, whereas AD results in about 122.86 kg CO_2 eq/kg-year, which is 0.02% of open burning. The AD of RS is a promising sustainable method, which is recommended to implement to reduce emissions significantly. There is great potential for the waste RS to be used in off- and on-field applications. In terms of off-field utilisation, bio-energy production is the most sustainable technique.

Chapter 8 Conclusions and Recommendations

8.1 Conclusions

RS is an abundantly generating lignocellulosic biomass and has been subjected to open burning causing huge emissions of greenhouse gases whereas RS has a high potential for biogas production. However, a high C/N ratio (about 51) of RS and recalcitrant nature (hard to digest) cause poor system stability, reduced digestibility, and low biogas yield. The current study is to utilize the RS and locally available substrates (which are compatible with the co-digestion of RS) in Warangal as well as various parts of India and to adopt pre-treatment techniques to reduce the recalcitrance of RS. The experiments were conducted with a pilot batch and semi-continuous scale on a better-performed combination of co-digestion and pre-treatment. Following are the significant conclusions based on the current study.

RS is one of the major crop residues generated in India and has huge potential for AD. The chosen locally available substrates according to literature which are compatible for AD of RS are food waste (FW), cow manure (CM), sewage sludge (SS), and chicken manure (ChM). The basic parameters that influence the AD system are temperature, VFA and pH, C/N ratio, metal elements, organic loading rate, and total solids content along with major techniques that affect the performance of AD of RS are co-digestion and pre-treatment. The combinations chosen for co-digestion of RS are RS+FW, RS+CM, RS+SS, and RS+ChM (binary mixtures) and RS+FW+CM, RS+FW+ChM, RS+FW+SS, RS+SS+CM, RS+SS+ChM, and RS+CM+ChM (ternary mixtures) at TS content 15%, 20%, 25%, and 30%. At 20% TS, RS+CM+ChM has given highest biogas production whereas RS+SS+ChM mixture has shown consistent biogas yield at all four TS contents with the maximum yield at TS 20%. The modified Gompertz model accounted well for understanding the kinetic behaviour in the co-digestion of RS. Four pre-treatments opted for A-co-D of RS (RS+SS+ChM at TS 20%) are thermal, H_2O_2, thermal + H_2O_2, and H_2O_2 + thermal at four sizes of RS i.e., 3-5 cm, 1-2 cm, 5-10 mm, and < 300 µm. At 1-2 cm RS with thermal pre-treatment has shown maximum biogas yield however it did not exceed the co-digestion results (RS+SS+ChM at TS 20%). Depending on the results of co-digestion and pre-treatments, co-digestion has been chosen over pre-treatment for pilot scale studies. Pilot scale batch and semi-continuous study were operated with RS+SS+ChM at TS 20% and performed significantly. Upon the methane and carbon dioxide yield from AD of RS, the GHG emissions were computed and compared with the emissions from open burning of RS and other possible emissions from RS harvest. It is concluded that the amount of GHGs released by the open burning of RS in India is several orders of magnitude greater than that released by the AD of RS. The AD of RS is a promising sustainable method, which is recommended to implement to reduce emissions significantly.

The key findings of the research are as follows.

i. The AD of surplus crop residues like RS has significant bio-energy potential and needs to be seriously considered as an alternate source of renewable energy.
ii. Co-substrates CM, ChM, and SS have shown better digestibility in AD with RS, out of which RS+SS+ChM at 20% TS outperformed with consistent biogas production.
iii. Ternary mixtures have better performance than binary mixtures
iv. The order of adaptability for choosing a co-substrate for RS can be listed as ChM>CM>SS>FW.
v. Co-digestion outperformed thermochemical pre-treatment in AD of RS.
vi. Thermal pre-treatment is more suitable for RS than hydroxy and their combinations.
vii. Among the four RS sizes chosen in the current study, 1-2 cm is suggestible to opt for AD. The order of adaptability for choosing an RS size can be listed as 1-2 cm > 5-10 mm > 3-5 cm > 300 µm
viii. Pilot scale studies were feasible to conduct on AD of RS with suitable co-substrates (ChM and SS).
ix. GHG emissions from open burning of RS is high compared to emissions from AD of RS.
x. The bio-energy potential from the AD of RS with suitable co-substrates could significantly avoid GHGs from open burning being released into the atmosphere.

8.2 Scope for further study

The following future work is recommended for further study. Other locally available co-substrates other than chosen ones can be experimented for feasibility of co-digestion with RS. Other mixtures of RS in the present study can also be tested at a large scale to assess the performance and effectiveness of co-digestion with each mixture. Energy economics would be suggested for a pilot scale study to conclude on positive energy balance for better understanding of AD in economical perspective. It would be also interesting if life cycle analysis (LCA) is carried out for biogas production of the mix proportions with binary and ternary mixtures along with pre-treatment.

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Appendix

FESEM (Field-emission Scanning Electron Microscope):

FESEM is microscope that works with electrons (particles with a negative charge) instead of light. These electrons are liberated by a field emission source. The object is scanned by electrons according to a zig-zag pattern. A FESEM is used to visualize very small topographic details on the surface or entire or fractioned objects. Researchers in biology, chemistry and physics apply this technique to observe structures that may be as small as 1 nanometer (= billion of a millimeter). Electrons are liberated from a field emission source and accelerated in a high electrical field gradient. Within the high vacuum column these so-called primary electrons are focussed and deflected by electronic lenses to produce a narrow scan beam that bombards the object. As a result, secondary electrons are emitted from ech spot on the object. The angle and velocity of these secondary electrons relates to the surface structure of the object. A detector catches the secondary electrons and produces an electronic signal. This signal is amplified and transformed to a video scan-image that can be seen on a monitor or to a digital image that can be saved and processed further.

FTIR (Fourier Transform InfraRed):

In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum. This makes infrared spectroscopy useful for several types of analysis. It can identify unknown materials, can determine the quality or consistency of a sample, and can determine the amount of components in a mixture.

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Pilli, S., Kunnoth, B., **Mothe, S**., Rao, P. V. (2022). Life cycle assessment of various anaerobic digestate management technologies. Anaerobic Digestate Management, 385. doi: 10.2166/9781789062755_0385.

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M. Sagarika, and Dr. P. Venkateswara Rao, (2019). Review on Pretreatment of Rice straw in anaerobic digestion, International International Conference on Innovative Trends in Civil Engineering for Sustainable Development (ITCSD), India September 13-15, 2019, National Institute of Technology Warangal, Telangana.

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