Chapter 1

Introduction

1.1. Overview

Rice (*Oryzae sativa*) is a crucial dietary element in many countries worldwide, serving as one of the most widely grown food crops and a staple meal for half of the global population. It has an impressive yearly production of 756.74 million tonnes [1]. In recent years, there has been a significant increase in rice production, and it is expected that the demand would grow by 28% by 2050, primarily due to the rising global population [2]. Asian countries make a significant contribution to the cultivation of rice, with more than 90% of global production. China is the biggest producer, closely followed by India [3]. Rice has become a crucial element in the cropping systems of Asian countries due to the widespread availability of high-yielding and short-duration rice cultivars. Rice paddies have a dual impact: they are essential for providing staple food to half of the world's population, but at the same time, they are responsible for almost 48% of greenhouse gas (GHG) emissions from croplands [4]. The growing worldwide need for rice, along with higher levels of production, has resulted in a proportional increase in the production of rice straw. Rice straw, a residual result of the rice harvesting procedure, is either stacked or dispersed in the field, or burned openly (stubble burning) contingent upon whether the sharvest was carried out manually or with combine harvesters [5]. Stubble burning is frequently utilized as a rapid and economical strategy to prepare fields for the subsequent planting season [6]. Although stubble burning has traditionally been a prevalent agricultural practice, it is accompanied by some notable drawbacks. Crop residue combustion emits greenhouse gases, such as carbon dioxide and methane, into the environment [7]. The practice of stubble burning results in the depletion of valuable organic matter present in crop leftovers. Organic matter is crucial for preserving soil structure, retaining moisture, and sustaining nutrient levels. The process of burning intensifies the occurrence of soil erosion and degradation. The act of burning stubble leads to the depletion of these essential nutrients, which then requires the application of extra artificial fertilizers in order to sustain the fertility of the soil [8]. The high temperatures resulting from stubble burning can have detrimental effects on the habitat of numerous microbes, insects, and small creatures that reside in the soil, perhaps causing harm or destruction. This disturbance might have a ripple impact on the ecology and biodiversity [9]. Stubble burning emits toxins that can contribute to the production of smog. This not only impacts the quality of the air but also poses harmful consequences for respiratory health, especially for individuals residing in close proximity to agricultural regions [10]. Stubble burning is now subject to regulation or prohibition in numerous areas, mostly due to its detrimental effects on the environment and human health. Farmers who participate in this activity may have legal repercussions, which might provide difficulties in adhering to and enforcing regulations [11]. In that light, governments all around the world are aiming to utilize crop residues in a sustainable manner to produce products of economic value, thereby reducing GHG emissions as a strategic initiative to achieve Sustainable Development Goals (SDG).

Anaerobic digestion is a sustainable solution for managing rice straw, which helps to address environmental concerns. Anaerobic digestion employs microbial activity in the absence of oxygen to decompose organic substances, transforming them into biogas [12]. This approach not only reduces the negative environmental effects of stubble burning but also has the potential to produce renewable energy from agricultural wastes. By incorporating anaerobic digestion into rice straw management procedures, the ideals of the circular economy are met, as it converts trash into a valuable and useful resource [13].

Figure 1.1 briefly describes the use of rice straw for anaerobic digestion to produce value-added products. Rice straw, which makes up from 40 to 60% of the total weight of the rice plant, is a type of biomass called lignocellulosic biomass [14]. The rice straw contains a significant amount of carbon, primarily in the form of cellulose, which serves as a valuable organic resource for microbial populations. The microbes participating in anaerobic digestion flourish in the presence of carbon-rich substrates, deriving energy from the complex polysaccharides found in rice straw [15]. The outcome of this process is the generation of biogas that is rich in methane, which may be utilized for diverse energy applications. LCBs, despite their evident benefits of being abundant, eco-friendly, and cost-effective, are significantly underutilized as raw materials for generating renewable energy. The main reason for this is the chemical makeup of LCB, which consist of three main polymers namely cellulose, hemicellulose, and lignin forming an intricate and resilient structure [16]. LCB is highly resistant to microbial breakdown because of its recalcitrant nature. Methanogenesis is considered as the rate-limiting step in anaerobic digestion. However, the complex and resistant nature of rice straw makes hydrolysis as the rate-limiting step [17]. Therefore, a delignification step is necessary for improved hydrolysis and efficient utilization of rice straw as a feedstock for anaerobic digestion. The implementation of effective pretreatment methods can yield substantial improvements in the overall efficiency of anaerobic digestion systems. Pretreatment ensures expedited breakdown of rice straw and the subsequent enhancement of biogas generation [18]. The methods used for pretreatment of lignocellulosic biomass can be classified into four main categories: physical, chemical, biological, and combination procedures. Every category includes a variety of specialized approaches, each possessing unique processes and exerting various effects on the lignocellulosic matrix [19]. Physical pretreatment procedures encompass several mechanical operations, including milling, grinding, and steam explosion, which induce physical modifications to the structure of rice straw [20]. Chemical pretreatment techniques involve the utilization of acids, bases, or solvents to facilitate the dissolution or degradation of lignin, hence enhancing the accessibility of cellulose and hemicellulose. In contrast, biological pretreatment employs microorganisms to facilitate the natural degradation of lignin and improve the rice straw's susceptibility to later anaerobic digestion [21]. This study has explored the possibility of using fungal treatment as an additional method to standard pretreatment techniques. The utilization of combined pretreatment methods involves the integration of two or more procedures to attain improved results through the synergistic resolution of different aspects of biomass recalcitrance [20].

The microbial consortium plays a crucial role in the success of anaerobic digestion by decomposing complex organic molecules. The dynamics and functioning of this consortium are impacted by multiple factors, with the inoculum playing a crucial role [22]. The inoculum, obtained from established anaerobic digesters, consists of a wide range of microorganisms that can effectively transform complex organic compounds into biogas [23]. Comprehending the importance of inoculum in anaerobic digestion is vital for maximizing the process and guaranteeing consistent and effective biogas generation [24]. Efforts have been made in recent years to monitor complex microbial communities and identify the key characteristics of an anaerobic microbiome that promotes optimal performance. The ideal performance entails achieving both efficient methane production at high rates and ensuring process stability.

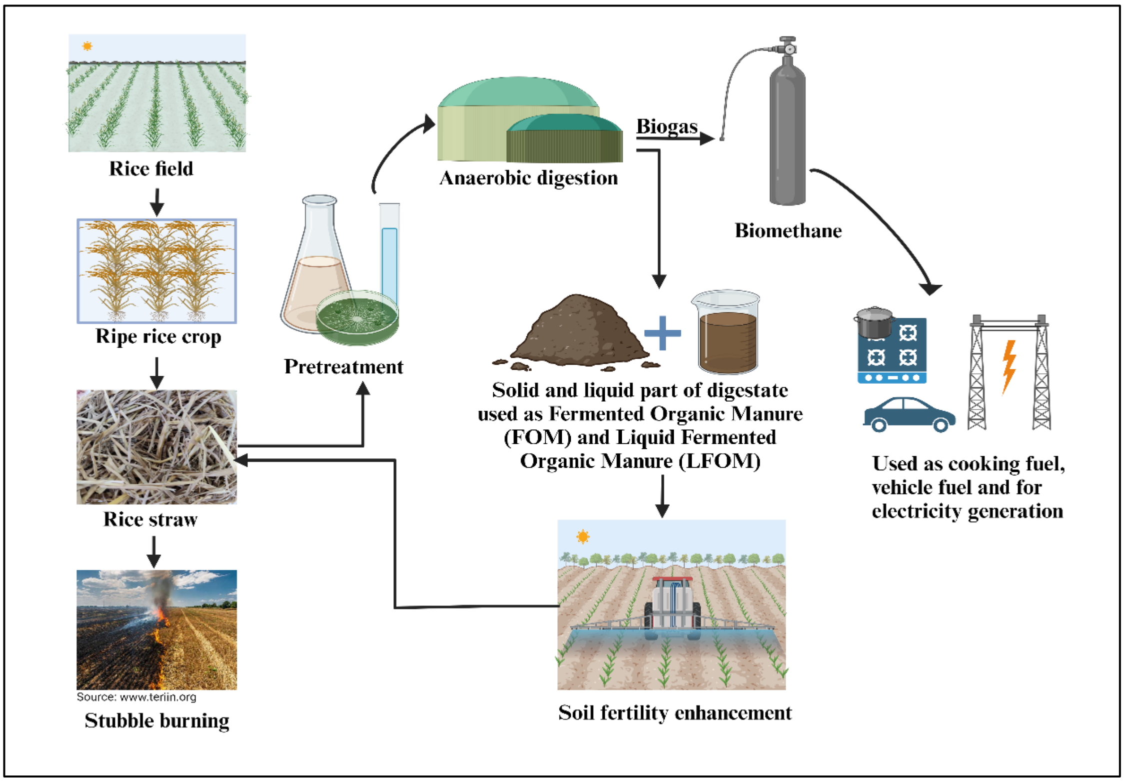

Figure 1.1. Sustainable utilization of rice straw for the production of value-added products (created with www.biorender.com)

1.2. Significance and scope of the study

This work aims to tackle the environmental concerns related to the mishandling of rice straw. The traditional approaches of rice straw disposal, such as combustion, not only contribute to atmospheric pollution and climate change but also result in the wastage of rich biomass resources and soil fertility. This study has explored anaerobic digestion as a sustainable way of mitigating the environmental impact of rice straw. The significance of an enriched microbial community of the inoculum in AD performance has been established in terms of microbial composition and dynamics. The recalcitrant structure of rice straw poses difficulty in its breakdown by microbial community, therefore thermal, alkali and fungal treatment by *Pycnoporous sanguineus* and *Trichoderma* *longibrachiatum* have been explored and their efficacy in biogas production has been compared. The research has also aimed to develop predictive models by incorporating machine learning algorithms to study biogas generation based on several operational parameters. Data from the pretreatment experiment has been used to train and validate the models. The objective is to harmonize with worldwide efforts towards creating a more eco-friendly agriculture industry. The study's interdisciplinary nature boosts its relevance and potential influence in both academic and practical domains.

1.3. Research objectives

The present study encompasses a comprehensive investigation to study the potential of rice straw to be used as a valuable feedstock for energy production. This study has evaluated and compared alkali, thermal and fungal pre-treatment to address the recalcitrance of rice straw. The study has also investigated the microbial diversity and dynamics to understand the underlying mechanisms in AD. The research has also sought to create predictive models by integrating machine learning algorithms to analyse biogas production using various operational indicators. The main objectives of this study are as follows:

1. Evaluating the effect of digested versus raw manure on the anaerobic digestion of rice straw and assessing adaptability and diversity of core microbiome through 16S rRNA metagenomics.
2. Study the effect of alkali, thermal, and fungal pre-treatment by *Pycnoporus sanguineus* and *Trichoderma longibrachiatum* on the anaerobic digestion of rice straw.

3. Testing the efficiency of fungal treatment by *Pycnoporus sanguineus* and *Trichoderma longibrachiatum* at high substrate loading and assessing the microbial community dynamics
4. Utilize machine learning algorithms to create prediction models that analyse the relationship between various operational characteristics and the generation of biogas.

1.4. Organisation of the thesis

This thesis contains eight chapters divided into sections and subsections. The chapters are summarised briefly as follows:

Chapter 1: The problem of rice straw mismanagement, harmful effects of its burning, the potential of rice straw as a valuable feedstock for anaerobic digestion, challenges en-route, pretreatment techniques, significance and scope of the study, and the objectives have been discussed briefly.

Chapter 2: Detailed literature review covering the biochemistry of anaerobic digestion, the structure of lignocellulosic biomass, the importance of inoculum performance, pretreatment technologies to enhance biogas production from rice straw and important operational parameters have been discussed elaborately.

Chapter 3: A detailed description of the materials used and the methods employed have been documented.

Chapter 4: The efficacy of raw and digested manure as inoculum sources for the anaerobic digestion of untreated rice straw has been tested on the basis of biogas production potential and microbial community composition.

Chapter 5: Comparison of the effect of thermal (autoclaving), alkali (NaOH), and fungal pretreatment (*Pycnoporus sanguineus* and *Trichoderma longibrachiatum*) methods on biogas generation has been studied.

Chapter 6: The AD potential of fungal treatment by *Pycnoporus sanguineus* and *Trichoderma longibrachiatum* at an increased substrate loading has been studied for the improvement of biogas yield.

Chapter 7: Different machine learning algorithms have been compared to predict the performance of AD based on different operational parameters.

Chapter 8: Important findings and conclusions obtained from the execution of different objectives and directions for future work have been covered in this chapter.