# STUDYING THE KINETICS OF ANAEROBIC CO-DIGESTION OF COWDUNG AND PAPER WASTE IN A SEMI-BATCH REACTOR

A thesis submitted in the fulfilments of the requirements for the award of the degree of

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# CERTIFICATE

This is to certify that the project entitled **"Studying the kinetics of anaerobic co digestion of cow dung and paper waste in a semi batch reactor"** is hereby approved as a creditable study in the area of **Chemical Engineering** carried out satisfactorily by **Debojyoti Das** (University Exam Roll No: **M4CHE22003** , Registration No: **131139 of 2015-16** , Class Roll No: **002010302003**) to warrant it's acceptance as a partial prerequisite for the award for the degree of **Master of Chemical Engineering** from **Jadavpur University, Kolkata**. It is understood that by this approval the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein, but approve the project only for the purpose for which it is submitted. The contents embodied in this thesis have not been submitted to any other University for the award of any degree or diploma.

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# DECLARATION

This declaration is to certify that the work presented in this thesis was completed by the author, unless specified otherwise, and that no part of it has been submitted in a thesis to any other university or institution.

This thesis was prepared in a compilation style format and is based on the experiments performed in the Energy Lab, Department of Chemical Engineering, Jadavpur University. All the related experiments were conducted and written during the author's candidature enrolled in M.E. Chemical Engineering (2020-2022).

Despite a certain level of repetition between chapters and introductory material and analytic methods, each single chapter was substantially different in focus and content within the scope of the thesis theme.

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# ABSTRACT

A study of the biogas production of paper waste (PW) and mixture of paper waste with cow dung (PW: CD) in the ratio 1:1 was performed. The two variants were charged into two separate 500 ml biodigesters. 25% (i.e. 125 ml) headspace was left and the rest 75% (i.e. 375 ml) was the working space. 6% substrate volume was mixed with 375 ml water. They were subjected to anaerobic digestion for a retention period of 45-day at around 37 $^{0}$C. Results obtained showed that only paper waste system (PW) had a cumulative gas yield of 5.69 $dm^3$/kg of slurry and the biogas production started on the 5$^{th}$ day even though the biogas production went down drastically. Significant biogas production again resumed after around 7 days and continued till the 34$^{th}$ day and finally stopped till the end of retention period. On the other hand, mixing the cow dung with paper waste increased the cumulative gas yield to 10.35 $dm^3$/kg of slurry, which is almost 100% increase. The gas production for this sample (PW:CD) was first observed on the 6$^{th}$day and it sustained throughout the retention period. Biogas production for the (PW:CD) system was much higher throughout the retention period in comparison to the only paper waste (PW) system. The study showed that paper waste which can be found everywhere and is either burnt off or thrown away as both domestic and industrial waste, degrades the environment, would be a very good raw material for biogas production. It also indicates that mixing paper waste with cow dung or any other animal waste will give sustained gas production throughout the digestion period of the waste. If biogas is produced from waste paper, it can be seen with a positive outlook to solutions of environmental issues like this.

# TABLE OF CONTENTS

# CHAPTER 1: Introduction

## 1.1. Anaerobic digestion

Anaerobic digestion (AD) is a relatively mature technique. AD has been widely used over the last century for the management of organic waste such as urban sewage sludge, biomass and agricultural slurry [1]. Despite extensive research and application history, the continued focus on this area has facilitated further application development with the advent of new concepts and technologies. The driving force behind this development in recent years has been a shift in society towards the promotion of sustainable practices. AD offers a particularly attractive way to move towards environmental sustainability through the potential to produce renewable energy in the form of biogas [2]. Therefore, over the last decade, a number of new papers have been published that primarily focus on the production and use of biogas by AD. AD uses a consortium of microorganisms to biodegrade organic matter under anaerobic conditions, stabilizing waste and producing biogas [3]. The diverse community of microorganisms goes through multiple steps, including the decomposition of complex organic compounds and the final production of by-product gases composed of 60% $CH_4$ and 40% $CO_2$ along with other trace gases (such as $H_2S$) promote AD. Decomposition of organic matter ultimately results in a more stable and hygienic biosolid product and more efficient sludge dehydration. Since anaerobic bacteria cannot break down lignin, AD can be used to break down biodegradable organic matter except wood [4]. Early applications of AD relate to the conversion of organic materials such as animal manure for biogas production for streetlight. In addition, this process promotes sludge dehydration, thereby promoting the cost-reducing value of AD in the industry. These economic benefits, coupled with the therapeutic needs for stabilization and pathogen destruction, ensure the continued relevance of AD procedures. Treatment of wastewater with conventional AD includes application to primary or waste activated sludge. The reason for this limited application is related to the organic concentration requirements of AD. Although increasing dilution can be a means of reducing the effects of inhibitors, gastrointestinal tanks require high organic concentrations to operate efficiently and raw wastewater instead of sludge. Diluting can significantly reduce the need to increase the volume of the reactor. Higher organic content promotes an increase in potential decomposition rate and, as a result, biogas production and a decrease in volatile solids (VS) [5]. However, this increase only occurs to concentrations where the inhibitory effect outweighs the benefits of increased availability of organic matter. Both primary activated sludge and waste activated sludge can be efficiently and anaerobically digested by themselves, but biogas production is low. Primary sludge is composed of the precipitated solids that result from the initial separation of the solids that have settled prior to the aerobic treatment. Waste Activated sludge is generated from a secondary aerobic treatment process. The aerobic treatment process also involves the use of microorganisms to break down dissolved organic matter, but in the presence of oxygen (i.e., aerobic conditions). Primary and wastewater activated sludge may differ in their composition and properties.

### 1.1.1. Process requirements

Basically, AD involves exposing organic matter to anaerobic microorganisms in an oxygen-free environment. Maintaining a relatively neutral pH and temperature range is an essential requirement for AD operation [6]. Due to the simplicity of this inherent process, AD does not require a complex infrastructure. To facilitate the AD process when the appropriate organic substrate is introduced, it is sufficient to provide a stable anaerobic environment in the gastrointestinal tract. However, optimizing the process to maximize both therapeutic potential and by-product parameters is complex. This complexity stems from the existence of four different but interconnected biodegradation mechanisms that occur simultaneously in the system. On the other hand, each group of microorganisms has a set of ideal environmental conditions. It has been shown to have inhibitory effects on various types of substances used in AD, including ammonia, sulphides, certain trace elements, and organic compounds [7]. However, the main research gap in AD involves understanding the complex mechanisms by which inhibition occurs. Conditional understanding of AD requirements has significant flaws, and there are few new process evaluation rules that are actually widely adopted [8]. The lack of a framework for AD testing causes problems and leads to widespread discrepancies in results.

## 1.2. Biological degradation during anaerobic digestion

### 1.2.1. Primary degradation mechanisms

The decomposition of organic matter in the AD process can be broadly divided into four stages. Each of these steps is performed by different types of microorganisms and is broadly categorized by the major methods of energy production. The early stages of decomposition, or hydrolysis, involve the solubilisation of complex organic particles. It is carried out by fermentable and hydrolysable bacterial species. The kinetics of hydrolysis are primarily determined by the composition of organic matter, which can be classified as carbohydrates, lipids and proteins, and the level of particulate matter [9]. As a result, hydrolysis is generally considered to be the rate-determining step of AD in many commodities. The longest hydrolysis time is related to lipids and the fastest solubilization involves carbohydrate degradation [10]. As a result, substrates with high lipid concentrations limit the overall rate of degradation due to the symbiotic dependence of the microbial community. Other determinants of hydrolysis kinetics, particle content simply involves limiting the active surface area exposed to microorganism [11]. Higher particlesizes and concentrations inevitably limit the solubilization of org anic matter in this way, which can be an important determinant of the rate of hydrolysis [12].

Decomposition ofcomplex organicmatter involves conversion to sparingly solub -le substances such as amino acids, sugars, fatty acids and glycerol, depending on the composition of the starting material. These intermediates serve as raw materials for the second stage of biodegradation [13].

The kinetics of hydrolysis is primarily determined by the composition of organic matter. Organic matter can be classified into carbohydrates, lipids and proteins, along with the content of particulate matter (canals) [14]. The decomposition of organic matter in the AD process can be broadly divided into four stages. These steps are performed by different types of microorganisms and are mainly categorized by the major methods of energy production. The early stages of decomposition, or hydrolysis, involve the solubilization of complex organic particles. It is carried out by fermentable and hydrolysable bacterial species [15]. As a result, hydrolysis is generally considered to be the rate-determining step of AD in many commodities. The longest hydrolysis time is related to lipids and the fastest solubilization involves carbohydrate degradation. As a result, substrates with high lipid concentrations limit the overall rate of degradation due to the symbiotic dependence of the microbial community. Other determinants of hydrolysis kinetics; B. Particle content simply involves limiting the active surface area exposed to microorganisms. Higher particlesizes and concentrations inevitably limit the solubilization of org anic matter in this way, which can be an important determinant of the rate of hydrolysis. Decomposition of complex organic matter involves conversion to easily soluble substances such as amino acids, sugars, fatty acids and glycerol, depending on the composition of the starting material. These intermediates serve as raw materials for the second stage of biodegradation.

## 1.2.2. Secondary degradation mechanisms

Although the main mechanism involved in most organic matter degradation, the microbial diversity of AD leads to more diverse processes [16]. These secondary processes are rarely related to the calculation of methanogenesis. Instead, biogas production or odour pollution is of particular importance. The most commonly studied secondary processes in the literature include the conversion of sulphates to sulphides [17]. Performed by Sulphate Reducing Bacteria (SRB), H2S is biosynthesized at each stage of acid production, acetic acid production, and methane production [18]. This process is deeply involved in the production of hydrogen sulphide gas. This is because dissolved sulphides can separate from the reactor sludge in the form of H2S. SRB competes with both methanogens and acetogens for intermediate carbon sources while reducing sulphates to sulphides. Other related pathways, including the formation of odorants, especially VOSCs, have also received a great deal of attention in previous studies. Methanethiol is formed in $H_2S$ by biodegradation of sulphur-containing amino acids, and subsequent conversion converts these compounds to dimethyl sulphide and dimethyl disulphide.

## 1.3. Co-digestion

Although AD can generally achieve effective solids reduction, the reuse of biogas for energy purposes in the wastewater and MSW industries is often limited. In Australia, biogas remains an almost undeveloped resource due to many unfavourable economic and political factors. Maintenance costs for Australian cogeneration units are fairly fixed and very high. Therefore, small energy recovery systems are economically impractical. In addition, rebates for renewable energy production in Australia are only available to large producers [20]. As a result, there is a critical size of biogas production where the use of biogas can make economic sense. This important threshold can be overcome by using co-digestion of sewage sludge and concentrated organic waste. The low concentration of inhibitor and high alkalinity make sewage sludge ideally suitable as a base substrate for co-digestion [21].

The practice of co-digestion involves binding multiple substrates in forming a feed solution for AD. The basic principle of its widespread use focuses on optimizing the anaerobic environment in consideration of the desired outcomes of the fermentation process [22] in addition, increasing the load of organic matter increases potential biogas production. In the country, nuclear reactors are usually loaded well below the load capacity of organic matter [23,24]. As a result, the use of concentrated organic co-substrates has the potential to significantly increase biogas production. The benefits of co-digestion go beyond maximizing biogas, especially when it comes to environmental issues. The practice of co-digestion enables the use of organic waste that is land filled for the potential of bio-methane [25]. This extends the benefits of AD to a wider range of applications and facilitates a significant reduction in the total amount of solids entering the landfill. When the produced bio-solids are used in agriculture, the benefits are further realized, enabling the recovery of nutrients as well as the transition from reliance on landfills [26].

## 1.4 Characteristics of cow dung

Cow dung, the excrement of cattle, is a cheap and readily available biological resource on the planet. Many traditional uses of cow dung, such as fuel, mosquito repellent, and burning as a cleaning agent, are already known in India. Cow dung hosts a diverse group of microorganisms that may be beneficial to humans due to their ability to produce a variety of metabolites. In addition to producing new chemicals, many bovine manure microorganisms demonstrate the natural ability to increase soil fertility through phosphate solubilization [27]. Today, there is growing interest in research into developing cow dung microbial applications for biofuel production and environmental pollutant management. This review focuses on the latest findings on cow dung that can be used in a variety of areas such as medicine, agriculture and industry.

### 1.4.1 Background

Cow dung can be defined as an undigested residue of ingested food excreted by herbivorous bovine species. It is a 3: 1 mixture of faeces and urine and is mainly composed of lignin, cellulose and hemicellulose. It also contains trace amounts of sulphur, iron, magnesium, copper, cobalt and manganese, in addition to 24 minerals such as nitrogen and potassium [28]. Native Indian cattle contain more calcium, phosphorus, zinc and copper than hybrid cattle. Cow dung hosts a rich microbial diversity, including various types of bacteria and Lactobacillus, Protozoa and yeast (Saccharomyces and Candida), Citrobacter koseri, Enterobacter aerogenes, Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Kluyvera spp., Morgarella morganii, Pasteurella spp., Providencia alcaligenes, Providencia stuartii, Pseudo and many other genera of bacteria have been identified segregated from cow dung [29].

In India, 69.9% of the population lives in rural areas [30], and cattle [31] are the main cattle, producing 9-15 kg of manure per day [32]. Waste is generally destined to be disposed of as it can be a source of pollution. However, it can be considered a by-product if it is in another process, such as when used as a raw material [33]. People in Indian villages bake cow dung directly and use it for cooking. It is also used to plaster the walls and floors of rural homes for winter and summer insulation. It is an ancient practice to use smoke from burnt cow dung as a mosquito repellent and then use ash to clean kitchen utensils [34]. Therefore, the various uses of cow dung by villagers reflect the native knowledge associated with it. It also shows that cattle play an important role in the village economy and have high socio-economic value [35].

Cow dung is also used in India as an agricultural by-product such as fertilizers, biofertilizers, biopesticides and pesticides, and as an energy source. According to Ayurveda, it also acts as a purifier for all waste in nature. Therefore, in India, cows (B. indicus) are not only lactating animals, but also Gomata (mother of all) and Kamdhenu. Detailed research on cow dung has attracted worldwide attention, with few attempts to exploit its potential in the fields of energy production and medicine [36]. This review aims to highlight the potential uses of cow dung in areas ranging from energy, agriculture and the environment to medicine for human well-being [37].

### 1.4.2 Source of Energy

Mankind's reliance on non-renewable energy sources such as coal, oil and gas are increasing around the world. In India, the main source of energy is coal, which accounts for 44% of total energy consumption. Despite being the third largest coal producer in the world, Japan is currently facing a shortage of coal supply. According to the Energy Information Administration (EIA), reliance on imported fossil fuels has increased to 38% [38]. Due to the limited availability of coal, there is a need for readily available, inexpensive and environmentally friendly renewable energy sources. According to the Food and Agriculture Organization of the United Nations (FAO), the excrement of animals on this planet produces about

55-65% of methane, which can affect global warming 21 times more than $CO_2$ when released into the atmosphere [39]. Biogas, a mixture of various gases resulting from the anaerobic fermentation of organic matter by methane-producing bacteria, is mainly composed of methane (50-65%) and $CO_2$ (25-45%). 1 kg of cow dung is 35-40 L of biogas when mixed with the same amount of water and maintained at an ambient temperature of 24-26 °C with a hydraulic residence time (HRT) of 55-60 days can be generated [40]. Green bacteria such as Pseudomonas and Azotobacter and other violet or non-purple sulphur bacteria are known to produce the largest amount of methane gas compared to other photosynthetic bacteria found in cow dung [41].

Cow dung is the main source of biogas or gobar gas production in India. The total population of cows in India is 190.9 million, of which 151 million are native and 39 million are hybrids [42]. Cow dung produced by 3 to 5 cows per day can power a simple 8 to 10 $m^3$ biogas plant that can produce 1.5 to 2 $m^3$ of biogas per day. This is enough for a family of 6-8 people, 2 or 3 people, or 2 lamps can be on for 3 hours, the refrigerator can be run all day, and the 3KW motor generator can be run for 1 hour. A 1 $m^3$ biogas plant produces 28.78 l / kg (0.028 $m^3$) or 32.76 l / kg (0.032 $m^3$) of biogas when supplied daily with 22 kg of fertilizer mixed with the same amount of water. Total solid content 10%. The maximum biogas production of this plant is 39.00 l / kg (0.039 $m^3$) and 40.04 l / kg [43].

For example, cow dung is used only as the main source of biogas production, while research is ongoing on possible sources of other sources, but the addition of pig dung has been shown to be more effective. A mixture of bovine and pig manure (60:40) was taken. As reported by, it showed a 10% increase in methane production. The use of mashed potatoes and cow dung in a 20:80 ratio produced significant amounts of methane compared to pure cow dung [44]. In addition, there is a report of a comparative study of biogas production that reduces the average amount of biogas produced by using various raw materials such as food waste, corn, used tea scraps, and cow dung in a ratio of 1:1. Intestinal disease, hyperacidity, ulcers, wound healing, heart disease, skin infections, tuberculosis, chicken pox, hepatitis, leprosy and some other bacterial and viral infections. Panchgavya also appears to be beneficial for diseases such as cancer, acquired immunodeficiency syndrome (AIDS), and diabetes. The immunostimulatory, immunomodulatory and anti-inflammatory effects of Panchagavya have also been mentioned in Ayurveda. Recently, the effect of Punchgavya on spontaneous movement, muscle tone and pain in the central nervous system of albino rats was determined [45,46].

Cow dung contains antifungal substances that inhibit the growth of coprophilia. Eupenicillium bovifimosum in cow dung produces compounds like patulodin. CK2108A and CK2801B have significant antigenic activity. A large number of enterococci with anti-listeria effect were detected in cow dung. An isolate of Enterococcus faecalis V24 was found to produce mostly hydrophobic antibacterial substances with significant thermal stability against pathogenic Gram-negative bacteria. The potential use of cow dung microorganisms in the pharmaceutical industry was proposed and demonstrated that isolate K4 has antibacterial activity against E. Kori [47,48]. Studies were also conducted on water, ethanol and n-hexane extracts from whole bovine manure against Candida, E. coli, Pseudomonas and Staphylococcus aureus clarify their antibacterial properties [49].

Mycobacterium vaccae, the first non-pathogenic bacterium isolated from cow dung, has antidepressant properties. Inhalation increased the growth of neurons that stimulate the production of serotonin and norepinephrine in the brain. Its effect on anxiety and learning was also tested in mice and showed good results when given live M. vaccae to mice. Immunotherapy with the dead M. vaccae vaccine has also been shown to be effective in the treatment of asthma, cancer, leprosy and psoriasis. These reports suggest that cow dung may serve as a promising and undeveloped source of microorganisms that may be associated with new antibacterial metabolites [50,51].

### 1.4.3 Conclusions

Cow dung hosts a variety of microorganisms with different individual characteristics. The use of cow dung microbiota has the potential to contribute significantly to sustainable agriculture and energy needs [52]. It is one of the world's biological resources that is widely available and not yet fully utilized. Understanding the mechanisms that allow cow dung microorganisms to break down hydrocarbons is useful for bioremediation of environmental pollutants [53]. Recent advances in scientific research and technology in whole-genome sequences have made it possible to identify the genes responsible for bioremediation. Another exciting area of research for future research is the development of microbial enzymes and antibacterial agents [54]. The production of enzymes by microorganisms from this inexpensive biological resource can be found in a wide range of applications in various fields such as agriculture, chemistry and biotechnology [55]. The application of cow dung microflora, which is highly antibacterial, may promote human health. However, it was necessary to investigate a comprehensive screening of these microorganisms for the production of antibacterial, antifungal, and antiviral metabolites [56]. There is still so much room for research and development to achieve industrial scale production of antibiotics and enzymes, so it is certainly clear that more detailed research on cow dung is needed. Thus, cow dung can be seen as a readily available biological resource with great potential for sustainable development in the near future [57].

## 1.5. Paper waste

The results show that a greater part of the 'waste' is recyclable or potentially recyclable and that a well-coordinated recycling programme will not only ensure a huge reduction of waste volume, but can equally lengthen the life of existing dumpsites and possibly, create wealth and reduce poverty [58]. The paper argues that scaling up the project offers the local authority an opportunity to tap into the innovative strengths embedded in the project, particularly its physical and economic synergies, which may bolster community sustainable development as a symptom rather than a victim of poor policies and programmes crowds out its inherent potentials, which can be gleaned to ensure sustainable environmental development [59]. Appreciating the waste value chain means that all stakeholders ought to be made part of the governance structure. Municipal SWM requires planning, foresight and reflexive governance to ensure that sustainable systems are adding value and/or complementary to each other [60]. The current situation, where some urban residents are often prevented from participating and benefiting from the development processes and services because of their perceived lack of knowledge, or due to others' lack of knowledge about their situation, conditions and development needs, must be abated. Traditional waste management protocols and ideas generally assume that waste already exists and should be managed [61]. As a result, most waste management models, especially in developing countries, including Ghana, are merely a response to the existence of waste or something that needs to be disposed of. This paper considers this traditional philosophy of waste management as a potential obstacle to efficient and sustainable management, and adopting an integrated system approach is the process of producing waste (waste treatment) [62]. And insists that it helps control (including use) and allows city managers to minimize waste generation in the first place. This paper explores the potential for turning household waste into resources using a project initiated by a community-based organization in Gamasie. Using multiple survey methods, this survey analyses the properties and composition of waste generated in the Akra community. The results show that a greater part of the 'waste' is recyclable or potentially recyclable and that a well-coordinated recycling programme will not only ensure a huge reduction of waste volume, but can equally lengthen the life of existing dumpsites and possibly, create wealth and reduce poverty [63,64]. The paper argues that scaling up the project offers the local authority an opportunity to tap into the innovative strengths embedded in the project, particularly its physical and economic synergies, which may bolster community sustainable development as a symptom rather than a victim of poor policies and programmes crowds out its inherent potentials, which can be gleaned to ensure sustainable environmental development [65]. Appreciating the waste value chain means that all stakeholders ought to be made part of the governance structure [66]. Municipal SWM requires planning, foresight and reflexive governance to ensure that sustainable systems are adding value and/or complementary to each other. The current situation, where some urban residents are often prevented from participating and benefiting from the development processes and services because of their perceived lack of knowledge, or due to others' lack of knowledge about their situation, conditions and development needs, must be abated [67]. When knowledge and information are developed, accessed and shared at the local level, such trends can put the community on an equal footing. This knowledge can be meaningfully shared between cities and improve the prospects for appropriate investment and development.

Efforts for empirical research need to continue, especially with regard to waste audits. These are to strongly support future decisions made by the city authorities. Yes, it is important to sample other areas to determine the likelihood of replicating a project. In addition, waste separation programs should be accompanied by appropriate education to better understand the separation, reuse and recycling activities of each community [68,69]. Rather, this process is seen as a new approach that encourages residents to consider waste as a potential resource. The result is that the structure of the government improves city planning and management, and the poor in the city.

Consistent with previous studies showing increased urbanization of poverty unless they recognize their right to improve their condition and adapt to poverty [70].

# CHAPTER 2: Literature Review

There is extensive literature on anaerobic digestion techniques and related techniques such as co-digestion. Although certain areas of the literature are mature, research gaps remain, such as the research objectives highlighted in chapter 4. This chapter provides an overview of the current state of the literature in the field of anaerobic digestion, especially co-digestion.

## 2.1. Current trends and issues in anaerobic digestion

In recent years, some trends have emerged in scientific publications related to AD. The development and availability of analytical techniques has expanded the range of techniques applicable in this area. For example, with the advent of more diverse analytical techniques in recent decades, more and more focus has been placed on the elucidation of the complex biological transformations that drive processes. In addition, techniques using spectroscopic and electrochemical principles are increasingly being used to better understand AD. Recent developments in modelling software have flooded with research combining experimental and modelling components. Other trends include increasing application of pre-treatment techniques to both typical AD substrates such as waste activated sludge and less degradable lignocellulosic waste [71].

The most striking and lasting trend in the literature over the last decade is arguably the focus on the by-products produced. Through the formation of biogas and the stabilization of residual sludge, operators have access to two potentially valuable products that can be used for energy or agricultural applications [72].

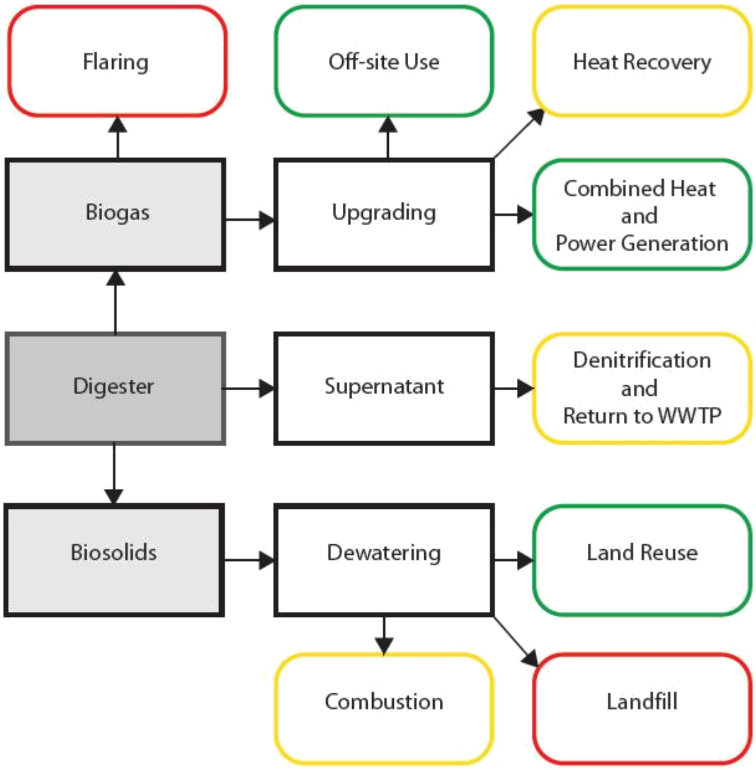


Figure 2.1 shows the potential management options currently available to wastewater treatment companies. The most sustainable practices in terms of environmental and economic factors include the use of both biosolids and biogas resources for land use and power generation.

### 2.1.1. Biosolids reuse

In recent years, there has been increasing interest in potential reuse options for digested solids derived from AD. Focusing on the possibility of using this material as a resource rather than a waste is primarily due to economic factors, while tailoring practices to the needs of social sustainability. Reuse of digests can increase the efficiency of use of existing resources while reducing disposal costs. To date, the most common reuse option for biosolids produced from AD has been related to land use as a soil conditioner. The high nutrient content of the digest makes it an ideal alternative to inorganic fertilizers. With increasing concerns about the lack of phosphorus available for mass use in agriculture, the possibility of offsetting the demand for fertilizers has recently become very important. The suitability of this reuse option is supported by the low viability of pathogens in AD digests, justifying its use as fertilizers and soil conditioners [73].

Despite the great potential of AD biosolid land use options, their use has often been limited by some permanent issues. The main ones are concerns about the presence of odours, pathogens, and harmful pollutants. National standards and agricultural perspectives differ greatly internationally. As a result, the reuse rate also improves. In Australia, about two-thirds of biosolids are reused for agricultural purposes, while New Zealand, a country with a similarly prominent agricultural sector, uses only about 30% of the solids produced. Public interest is primarily related to the stinks emitted by biosolids. The most prominent odour may be primarily due to the release of volatile organosulfur compounds (VOSCs) and inorganic sulphur compounds in biosolids. Common VOSCs present in odour-conscious biosolids include methanethiol and dimethyl sulphide, but the most prominent inorganic sulphur compound is hydrogen sulphide. High concentrations of VOSC in biosolids increase residual biological activity. This reflects the effectiveness of AD treatment in VS removal. To solve the biosolid odour problem, this phenomenon should be utilized in conjunction with other biosolid quality optimization techniques [74].

### 2.1.2. Biogas utilisation

In recent years, biogas production has become increasingly important. Biogas production represents an attractive co-benefits in treating organic waste as a renewable energy source. The potential for anaerobic biogas production for energy recovery is widely recognized throughout the literature. However, current levels of biogas utilization characterize resources as almost unused energy products [75]. In Australia, more than 20% of domestic biogas-producing sewage treatment plants do not generate energy from biogas and do not have a biogas utilization strategy. As a rule, the resulting unused biogas is burned. Methane has a global warming potential that is 23 times higher than carbon dioxide, so biogas flaring is needed to reduce its impact on the Earth's climate. Flare biogas effectively reduces the impact, but energy recovery can provide far greater benefits [76].

Key on-site options include energy recovery in the form of heat, electricity, or a combination of both. Combined heat and power uses a combined heat and power plant to generate energy from wastewater biogas. Such systems are considered optimal because they can balance power generation capacity with the thermal energy demands of wastewater treatment plants (WWTP). However, using acase study, we have shown that fermentation of sewage sludge solely for the purpose of producing energy is economically meaningless. However, this undermines the value of the associated treatment. A study of five different wastewater treatment plants in Catalonia found that 39-76% of total energy demand could be met by AD biogas cogeneration, depending on the plant's configuration and infrastructure quality. UN described two Austrian sewage treatment plants that achieved a net energy balance of 610% by using combined heat and power biogas [77].

The benefits of using biogas may be related to both environmental and economic factors. The ability to reliably produce energy from existing on-site resources is an obvious economic advantage in itself, but an important trend towards the use of biogas is largely related to mitigating the effects of climate change. Co-fermentation of different materials may reduce dependence on other energy sources while reducing the impact of emissions from other sectors. This is especially true for livestock. Methane produced by the natural decomposition of fertilizer is not simply released, but can be captured and used [78].

Despite the ardent optimism about biogas reuse, serious problems still hinder the use of its potential in the world. Limitations on biogas reuse are primarily due to the economics associated with system installation and maintenance. This limitation applies primarily to power recovery due to its higher maintenance requirements than heat recovery systems. Energy recovery is limited, especially in the context of Australian wastewater AD, due to the significant high fixed labour maintenance costs regardless of the size of the system. For an energy recovery system to be economical, it must provide an economic benefit that balances these costs with installation costs and other financial requirements from component replacement. This situation leads to a conceptual break-even point for the minimum biogas production required, as the economic benefits of reuse increase somewhat in proportion to the amount of biogas. Therefore, to exceed this threshold, there is a need for a way to increase biogas production from AD in sewage sludge [79].

A ubiquitous and significant obstacle to biogas utilization is the concentration of hydrogen sulphide ($H_2S$). Depending on the composition of the feed, the $H_2S$ concentration can be between 10 and 2000 ppm. Given the odour and health effects of gas, it is ideal to avoid the production of $H_2S$. This topic also discusses considerations for potential land-use applications for bio-solids that are plagued by concerns related to odour emission. In addition, H2S can cause serious damage to the equipment used to utilize biogas. This causes deterioration of the lubricating oil and corrosion of internal combustion engine components and piping [80]. Coupled with maintenance cost issues, $H_2S$ pollution has significantly hampered the promotion of biogas re-use.

Another immediately recognizable obstacle to the widespread use of biogas is the low methane content in biogas. Observations of methane purity in previous AD studies vary significantly, generally between 48% and 65%, but in some cases as high as 70%. But, the figure of about 60% methane has long been established as a typical average concentration. Most of the remaining 40% is carbon dioxide, along with traces of other compounds. The low methane content in biogas is a major problem, especially for use in offsite plants. To qualify as a fuel grade biomethane resource, the methane concentration must exceed 98%. On the other hand, removal options tend to be expensive. Although the problem of biogas composition can be addressed with post-digestive treatment, researchers are still seeking better solutions in terms of efficacy and efficiency.

An increasingly used technique for improving biomethane production is known as anaerobic co-digestion [81,82].

## 2.2. Anaerobic co-digestion

The practice of co-digestion has been extensively studied in the literature regarding the optimization of biogas production and biosolid removal, accounting for half of the AD publications from 2011 to 2013. Co-digestion involves binding multiple substrates in forming a feed solution for AD. The rationale for its widespread use focuses on optimizing the anaerobic environment. Part of this involves deliberately increasing feed stoichiometry to maximize the degree of resulting degradation. In order to reduce VTS and increase the effectiveness of digestion in terms of biogas production, it is generally necessary to prevent conditions that interfere with the AD process. The benefits of these practices go beyond maximizing biogas, especially when it comes to environmental issues. Co-digestion is likely to prevent inhibition and therefore broadens the range ofsubstrates useful for

the treatment of AD. This extends the benefits of AD to a wider range of applications and keeps organic waste away from landfills. By using agriculturally produced biosolids, the benefits can be further enhanced by enabling the recovery of nutrients [83].

Anaerobic co-digestion studies over the last decade have focused on maximizing biogas production. This is essential for the benefits of energy to exceed the biogas production threshold of cogeneration economics, where the cost of maintaining the system must be exceeded. In the above cogeneration process, the economics of the process are highly dependent on maximizing energy recovery. The potential increase in biogas production provided by co-digestion results from two major mechanisms. The first means of biogas optimization relate to the possibility of manipulating the type and concentration of organic matter present in the feed. The increase in organic matter concentration is usually expressed in VS content, which logically gives the feed a high digestive capacity. This clearly correlates with achievable biogas production. The existing literature is rich in studies aimed at manipulating the organic content of AD feed solutions. A common theme in the literature in this area describes the increased potential for biogas production at higher organic load factors. However, as with digestion of a single substrate diet, inhibition was observed at higher concentrations. Therefore, each of the proposed feed mixtures has an optimal organic content [84].

Optimizing the organic content of the AD feed can go beyond maximizing the VS concentration. It was found that different substrates had opposite ratios of methane potential to total VS concentration. observed that the difference in methanogen potential with respect to VS concentration between sewage sludge and grease trap waste was significant, recording values of 263 and 918L $CH_4$/kg VS added, respectively. Differences in the methane potential of volatile solids complicate the process of optimizing AD biogas production and place great importance on the choice of different substrates in co-digestion. In addition, it can be seen that the factors that influence the dynamics of the biological process differ significantly based on the organic composition of the starting material [85].

The secondary mechanism by which co-digestion practices promote higher biogas production is associated with the potential mitigation of the above inhibition conditions. Logically, the reduced efficiency of AD microbial community function prevents the decomposition of organic matter and thus the formation of biogas. Therefore, avoiding inhibition is necessary in biogas-oriented process optimization. Prevention by co-digestion involves diluting one inhibitor of the substrate or leveraging synergistic effects between different substances leading to mutual reduction of compound / ion toxicity. Higher concentrations of substrates examined in the literature are generally more likely to promote suppression. Therefore, co-digestion of substrates with very different compositions, especially with respect to organic content, often allows for improved microbial activity in AD [86].

## 2.2.1. Substrates for anaerobic co-digestion

Perhaps the broadest segment of existing literature is related to the assessment of potential substrates. Due to the applicability of AD above, a large number of single substrates and organic composites have been evaluated. Screening for new potential substrates remains important for the full-scale uptake and expansion of co-digestion. The focus of substrate screening is on the impact on the degradation process with respect to factors such as total biogas production and solids reduction. In addition to directly identifying useful substrates, the researcher's overall goal is for these studies to characterize the ideal substrate solution and thus provide selection criteria for the initial evaluation of the feed solution [87]. However, numerous variables, method discrepancies, and substrate composition variability hamper the ultimate realization of this goal. Certain new rules have emerged that explain the requirements of AD. The most common raw material requirement is the ratio of carbon to nitrogen, with more focus on the relative concentration of phosphorus and sulphur. Beyond these basic stoichiometric ratios, there is little consensus on challenging concentrations, as the complexity of biological processes exacerbated by the synergistic interactions of substances hinders the separation of ideal concentrations. Therefore, further optimization of the input material is aimed at based on the composition of the starting substrate.

Substrate compositions from different sources and regions vary to some extent, but certain properties can be expected based on the nature of the organic raw material. When considering a particular substrate, the material is evaluated for a variety of factors such as sulphur concentration, organicity, lignin content, or the presence of toxic compounds. Based on this characterization of the substrates, they can be further classified based on their potential function during co-digestion and their proper mating. Co-digestive substrates can generally be divided into two categories based on their intended function[88]. To increase biogas production or to stabilize the entire feed mixture. Substrate rich in degradable organic matter generally has properties that are not suitable for digestion. Therefore, it is necessary to balance these inhibitory properties through either dilution of contaminants, stoichiometric manipulation, or optimization of factors such as alkalinity. This requirement regularly includes pairing of high methanogenic substrates with robust stabilizers (Figure 2.2).

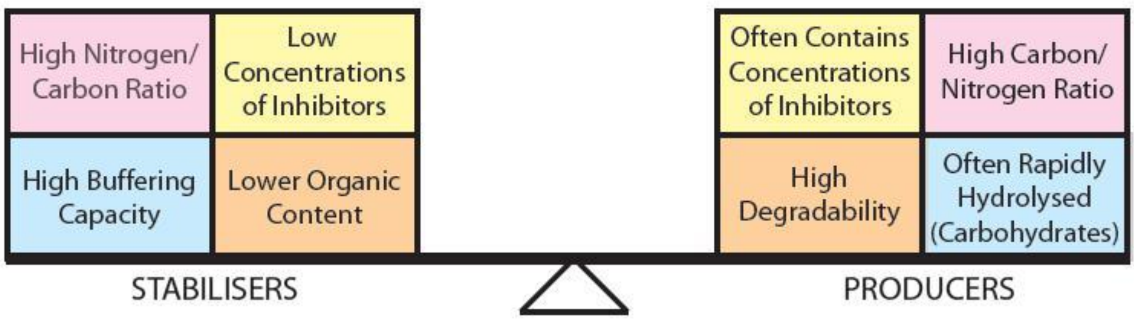


Figure 2.2. Optimisation of co-digestion through the selection of complimentary substrates.

Increasing biogas production on stabilized substrates requires a combination of carbon-rich substrates. A common carbon source for AD can be cellular waste from the paper or textile industry. Studies on the pairing of cellulosic substrates with organically deficient substrates are common in the literature. The optimal biogas yield for the combination of algae sludge and waste paper occurs when 60% VS is derived from the paper substrate. Here, digesting only algae increased methane production by 180% showed a 5994% increase in methane yield using a combination of 67% sisal pulp and 33% fish excrement. This is greater than the yield when either substrate is fermented. The low toxicity of these wastes makes it easier to consider inhibition in the application. Therefore, these applications are managed by optimization of organic stoichiometry, coupled with the problem of the amount of logistic and biosolids. Note that recycled paper with high sulphate concentrations was observed with characteristically high COD values. Internationally, this waste represents the unseparated portion of most of the urban solid waste currently being sent to landfills. As a result, it is very likely that they will be more involved in the practice of co-digestion [89].

A notable carbon-rich substrate used in co-digestion is the organic fraction of MSW. The considerable availability and logistical convenience of MSW's organic fraction has attracted much attention to the use of substrates for C/N balance in co-digestion. The effectiveness of substrate use with nitrogen-rich substrates has been demonstrated in many studies, especially in sewage sludge. This is the most common co-digesting mixture in the literature showed that the stronger dynamics of methanogenesis in the organic fraction of urban solids may be maintained by co-digestion with TS. The applicability of the organic fraction of MSW in co-digestion is facilitated by its ability to prevent acid accumulation in the digestion of high-fat waste [90].

In some cases, separation of fruit and vegetable fractions from MSW was performed for use as a co-substrate. Fruit and vegetable waste is characterized by high concentrations of easily degradable VFA, which inevitably results in rapid hydrolysis and accumulation of VFA at higher organic load factors. As expected, the fruit and vegetable fractions were observed as successful co-substrates for strongly buffering base substrates such as sewage sludge and animal manure. However, general concerns about the use of fruit and vegetable waste in AD are related to the potential for H2S outbreaks. It has been observed that feeds containing food waste have high sulphate concentrations and ultimately high H2S concentrations in the biogas produced [91].

Food waste also leads to higher phosphorus concentrations, which accumulate in concentrates. When this happens in a processing plant, struvite precipitation is often observed at inconvenient points in the processing cycle.

Energy crops are perhaps another common basis for code digestion, which is most optimistic. Such substrates that are commonly considered can be corn, sunflower, and rapeseed residues. A decisive parameter for plants is the extraordinary net energy yield per hectare, which has a positive impact on the outlook for biogas utilization [92]. Energy crops also have the high C / N ratio required for co-digestion with protein-rich substrates. However, a significant obstacle to use in AD is the concentration of lignin in energy crops. In nature, lignin degradation occurs by enzymatic or microbial-based chemical reactions that require aerobic conditions. Therefore, significant pre-treatment is required to increase the bioavailability of organic matter and achieve optimal AD results. Nevertheless, the promotion of this substrate in co-digestion studies persists due to its good availability and logistics, as well as synergistic factors associated with biosolids. The ideal reuse option for AD biosolids is to grow energy crops to circumvent legal land use restrictions in certain countries [93].

This potential ofthe nutrient cycle further facilitates the incorporation of energy crops into co-digestion practices.

In terms of maximum theoretical gas yield, lipid-rich substrates are predominantly superior to substrates containing other organic species. These substrates are classified as fats, oils and fatty wastes and can be generated from slaughter houses, food processing and municipal solid wastes. However, despite the high potential for methane, fat, oil and fat digestion suffers from slowing production as well as lipid-based inhibition. We found that inhibition originated from a VS concentration of 65% lipid [94].

## 2.3. Implications of Substrate Characteristics on Reactor Performance

Choosing the right co-substrate is the most effective and cost-effective way to deal with downstream conditions. Reactor management and post-treatment processes are usually ineffective or costly.

Inhibitor concentration, stoichiometry, and organic content are all factors that have a significant impact on the end result of the digestive process. Once the substrate has been selected, decisions regarding reactor conditions and post-treatment requirements are also required to counteract the adverse effects of different substrate compositions.

### 2.3.1. The role of carbon/nitrogen stoichiometry in co-digestion

The ratio of carbon to nitrogen in the reactor feed can be considered a major factor in the choice of co-substrate. The widespread use of C/N stoichiometry in AD considerations reflects the impact of ammonia concentration on the process. High nitrogen levels combined with low relative carbon concentrations result in high levels of ammonia formation in the reactor. Ammonia is required as a nutrient for methanogenic activity and is a by-product of early protein and amino acid degradation. In addition, ammonia provides the buffer capacity needed to reduce the effect of VFA accumulation on pH. Ammonia (NH3) is produced by the decomposition of nitrogen-rich proteins and increases bicarbonate by forming bicarbonate and ammonium salts.

Keep in mind that nitrogen is an important nutrient utilized by the microorganisms involved in digestion. Many studies on this subject have identified various optimal values. However, it is generally within a fairly consistent range. For a mixture of milk, chicken and straw. A stable C/N range from 25: 1 to 30: 1 is observed, with optimal mixing occurring at 27.2: 1 (Wang, 2012). For a mixture of algae sludge and paper mill waste. According to one study, the ideal range is also within this range of C/N ratios from 25: 1 to 30: 1. It is generally accepted that the C/N ratio should be between 15: 1 and 30: 1, but a 600: 15: 5: 1 carbon-nitrogen-phosphorus-sulphur mixture is considered sufficient.

Insufficient or excessive levels of ammonia can result in pH levels that interfere with the biological process of AD. pH-based inhibition is particularly complex due to the interaction of various pH-dependent toxicities of the substance. Depending on the inhibition pathway associated with ammonia concentration, the extent of inhibition can range from reduced methanogenesis to complete lack of methanogenic activity. We have observed that non-ammonia-based pH inhibition is classified as "direct" pH inhibition. Associated with the interaction of substances such as ionized and non-ionized VFA with hydroxide or hydrogen ions. Under conditions associated with inadequate ammonia concentration, the expected decrease in pH shifts the composition of total ammonia nitrogen to a decrease in free ammonia concentration, a non-ammonia-based suppression. Will occur. Ammonia pathway (Fig. 2.3).

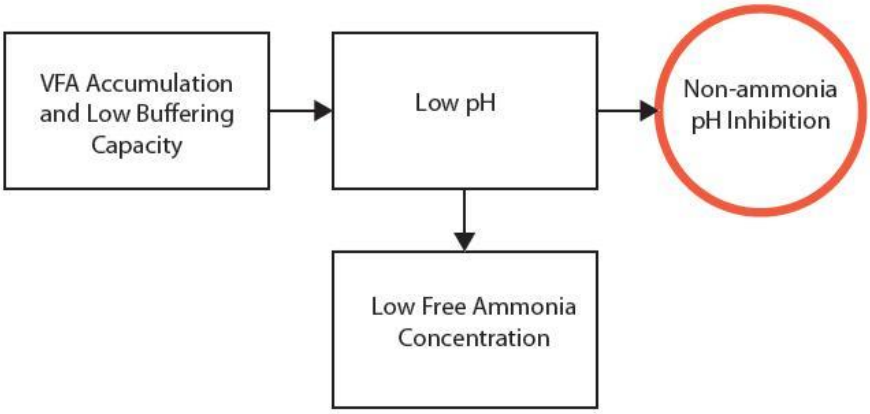


Figure 2.3. Inhibition due to low ammonia concentrations.

Ammonia nitrogen exists in two basic forms in AD reactors. Unionized free ammonia or ammonium ($NH_4^+$). Free ammonia is generally considered to be the more toxic of the two compounds [95]. However, the mechanism by which ammonia inhibition occurs is not yet fully understood, and theories have been proposed to cover changes in intracellular pH, specific enzyme inhibition, proton imbalances, and other possibilities. It was noted that bacterial ammonia suppression requires its presence within the cell, following the observation that suppression affected bacteria in a less permeable cell wall structure. It was later argued that the increased permeability of free ammonia compared to ionized ammonium was responsible for the higher toxicity of the compound. Maintaining a low pH in the AD reactor can reduce the concentration of free ammonia [96].

Ammonia level-based inhibition includes the interaction between VFA concentration and pH, as well as the concentration of ammonia. Therefore, the total ammonia nitrogen concentration of the important is primarily context-sensitive. Ammonia inhibition was observed at concentrations of about 1400 to 1700mg/L of ammoniacal nitrogen on certain substrate with a sharp decrease in methane production activity at about 2000 mg/L N in the pH range of 7 to 7.5. However, other publications show considerable variability in the initiation of ammonia inhibition. Inhibition was observed at about 1500-2000 mg/L at pH 7.8, whereas inhibition occurred at concentrations above 1000 mg/L with another substrate at pH 8.4. This study is consistent with the scientific consensus that ammonia sensitivity increases with pH. This phenomenon stems from the effect of pH above on the total composition of ammonia-nitrogen. Higher pH values result in an increase in the free ammonia to ammonium ratio. As a result, raw materials with high total ammoniacal nitrogen levels exceed the critical level of free ammonia (Fig. 2). Four). These cases of pH values being too high or too low can be caused by an imbalance in the biochemical processes of methanogens and acid-producing bacteria. It should also be noted that the toxicity of ammonia does not depend solely on pH and free ammonia concentration. The presence of other ions can potentially create synergistic scenarios and reduce toxicity. This is the case for $Na^+$, $Ca2^+$, and $Mg2^+$ concentrations [97].

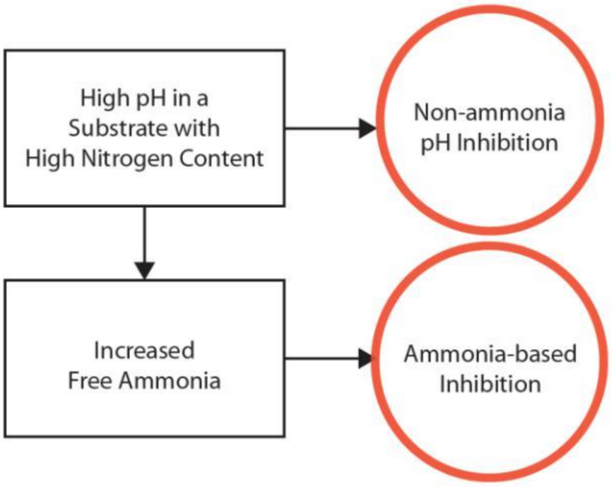


Figure 2.4. Inhibition due to high ammonia concentration

There are various ways to prevent the suppression of ammonia and pH. As already mentioned, considerable attention has been paid to the production of C/N balanced feeds for co-digestion. A good C/N balance is usually a reasonable indicator to prevent pH and ammonia suppression. In addition, artificial maintenance of pH levels can be used to prevent inhibition levels and the accumulation of free ammonia in AD. Several combinations of treatment options were evaluated for the potential to reduce ammonia concentration, including acidic air stripping, struvite precipitation, dilution, and activated sludge processes. Each option has shown the potential for raw material pre-treatment, depending on the situation [98].

### 2.3.2. Impacts of readily degradable organic concentrations in co-digestion

High concentrations of organic carbon in AD raw materials are expected to increase methane yields. However, over-concentration of degradable components can lead to rapid hydrolysis of these organics, which can interfere with biological processes. This suppression mechanism is necessarily associated with the C/N ratio described above, which may lead to the rapid degradation of such organic matter leading to the accumulation of VFA.

This happens when the VFA formation rate exceeds the consumption rate. As mentioned earlier, the associated lowering of pH, coupled with a high C/N ratio, can result in inhibition of microorganisms, especially methanogens. Nitrogen concentration requirements depend not only on the load factor of the organic matter, but also on the composition of the organic matter. This has the potential drawback of relying on C/N values for feed composition management, as the rate of hydrolysis and subsequent VFA formation are determinants of buffer requirements as well as carbon concentration is showing [99].

### 2.3.3. Implications of sulphur content in co-digestion

The presence of sulphur in AD raw materials has several important implications for both the reactor process and downstream product quality. Reactor problems are related to the suppression of methanogens indirectly caused by the presence of sulphur-containing compounds. The simplest form of suppression is associated with additional competition with methanogens. Alternative pre-treatment techniques provide similar improvements in methane yield, but the additional benefit of FA pre-treatment is its favourable economic outlook. In fact, a design to recycle ammonia in a wastewater treatment plant to achieve nitrogen removal during anaerobic digestion and enhanced biomethane production [100].

Despite optimistic and promising results in existing studies, FA pre-treatment is still in its infancy. There is still a lack ofunderstanding of the mechanism behind the improved biodegradability of substrates. This technique was applied only during batch testing and has not yet been demonstrated in continuous systems. In addition, FA pre-treatment was applied only during sludge digestion, not during co-digestion [101].

## 2.4. Summary of Literature Review

Anaerobic co-digestion in municipal wastewater treatment is a promising practice to improve sustainability and utilisation of resources. Co-digestion allows for the treatment of substrates that are unsuitable for conventional mono-digestion. Anaerobic co-digestion utilises the spare organic loading capacity of existing digesters at wastewater treatment plants without requiring significant modification of existing infrastructure. As sewage sludge possesses significant buffering capacity yet low organic content, it may be suitable for co-digestion with countless potential co-substrates. Furthermore, the potential for synergistic effects between subjects offers greater methane yields and degradation kinetics.

Despite the fervent optimism surround anaerobic co-digestion, persistent knowledge gaps restrict uptake of the practice. Lack of available co-substrates can hinder full scale co-digestion in rural areas, thus the identification of further potential co-substrates is essential. Indeed, several high-volume waste streams have yet to be tested in anaerobic co-digestion. Additional shortcomings relate to uncertainty in the nature of the synergistic effect. Conflicting views arise in whether synergistic effects solely influence biodegradability, process kinetics or both. Meanwhile it has not been differentiated if separate mechanisms are behind the two forms of synergisms. Furthermore, there is a persistent deficit of pilot scale research, whilst laboratory experiments tend to provide overly favourable results through the elimination of variables. Laboratory-scale papers also tend to focus overly on the volume of biogas produced during co-digestion, without sufficiently considering its impacts on downstream processes. Factors such as biosolids quality and odour are insufficiently covered in the literature, as is the composition of biogas, particularly in terms of the hydrogen sulphide content. Further research is also required to elucidate the impact of different pre-treatments when used in conjunction with co-digestion. In the literature most pre-treatments applied during co-digestion involve thermal based processes. Free ammonia pre-treatment has demonstrated promising results and favourable economics, yet has only been applied during mono-digestion of sewage sludge and has not been demonstrated in a continuous system or in conjunction with co-digestion.

The identified gaps within the literature form the basis on which this study was developed. These objectives of the research were addressed by methodologies derived from prior research, whilst also introducing techniques that were previously absent from the field anaerobic co-digestion.

# CHAPTER 3: Studying the kinetics of anaerobic co-digestion of cow-dung and paper waste in a semi-batch reactor

## 3.1 Introduction

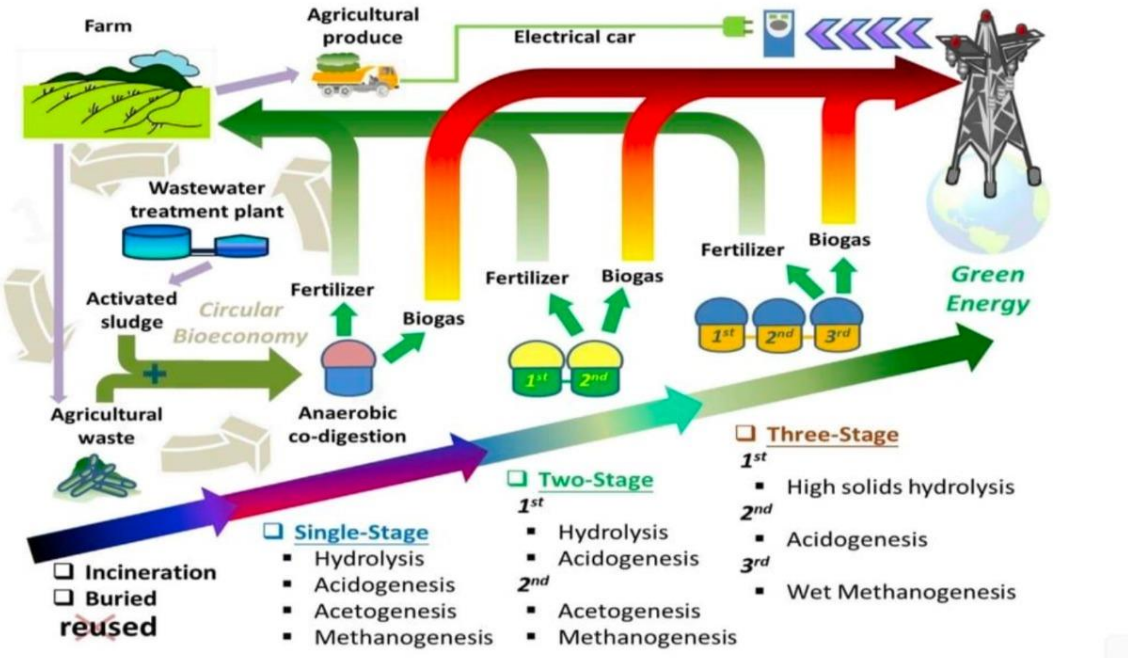


Fig 3.1: How waste materials are a great propellent for a greener future

The anaerobic digestion (AD) technology [102] has rapidly developed because of serious concerns regarding the disposal of organic wastes from several sources like industrial, agricultural and domestic sources [103]. The waste water treatment plants widely use anaerobic digestion for stabilising the sewage sludge and it produces biogas at the same time which is a renewable fuel [104] Elements like nitrogen and phosphorus in the form of ammonia and phosphate are released during anaerobic treatment and hence anaerobic digestion is a very good way to recover the nutritional contents from waste products.

One of the recent innovations of the anaerobic digestion technology involves co-digesting two or more substrates together. This can be beneficial in several ways where there are problems associated with digestion of single substrate [105]. Some of the problems include imbalanced C/N ratio, lack of micronutrients and many more. Co-digestion is a brilliant idea in this regard as financially it can be helpful to lower the capital investments that is required in additional waste management facilities. Although there are many substrates which can be co-digested beneficially several fundamental aspects of anaerobic co-digestion process remain poorly understood.

It has been observed that co-digestion can improve the pace of anaerobic digestion of each individual substrate. That can either lead to an increase in methane yield or the individual substrate or can boost the kinetics of biogas production. There is a popular hypothesis that co-digestion can improve the process performance because of a more balanced C/N ratio. For e.g., paper waste when co-digested with cow dung of low C/N ratio, the optimum C/N ratio is about 20-25 [106].

Anaerobic digestion of sorted organic wastes from municipal solid waste especially paper waste is a cost-effective technology. Methane fermentation is a complex process. The general process of anaerobic digestion is a series of processes like enzymatic hydrolysis, acidogenesis, acetogenesis and methanogenesis and each metabolic stage is assisted by a series of microorganisms. Amongst the four stages, hydrolysis is the rate limiting stage for the process. The acid forming microbes convert macromolecules like carbohydrates, proteins, starches, cellulose etc. to organic acids (step 1 and 2). In the 3rd step, organic acids are converted to acetate & finally in the 4th step the acetate is converted to $CH_4$ and $CO_2$ by the methanogens. The time needed for step 1 and 2 is more and this consumes most of the time [107]. However, experiments conducted in batch processes are usually lengthy in terms of more retention or detention time. Carbohydrate rich substrates like paper wastes are quicker producers of volatile fatty acids and leads to excess acid accumulation leading to acidity, low pH and process inhibition. So, higher concentration of substrates for paper wastes like cow dung leads to lowering of pH and thereby produces more biogas. Nevertheless, an important factor affecting AD process is temperature. Generally, AD process is operated under mesophilic or thermophilic condition in which thermophilic digestion is reported as more efficient method. Compared with wet AD process, dry AD process is much beneficial to compact digester with high organic loading rate and its energetically effective performance. Literature review shows that India stands second in the production of paper wastes in the world. It contributes about 8 and 10 % of paper production in the world. Paper wastes are created by marketing, processing, transportation etc. that takes a considerable amount of space in factories and domestic households. Many researchers studied anaerobic digestion of paper waste in one stage systems in laboratory scale reactors [108].

This study aims to sequentially fit models and explain the nature of outcomes that relates to the kinetics of biogas production. We also would be doing a comparative study between the biogas production trend in paper waste sludge and the same when mixed with cow dung proportionately.

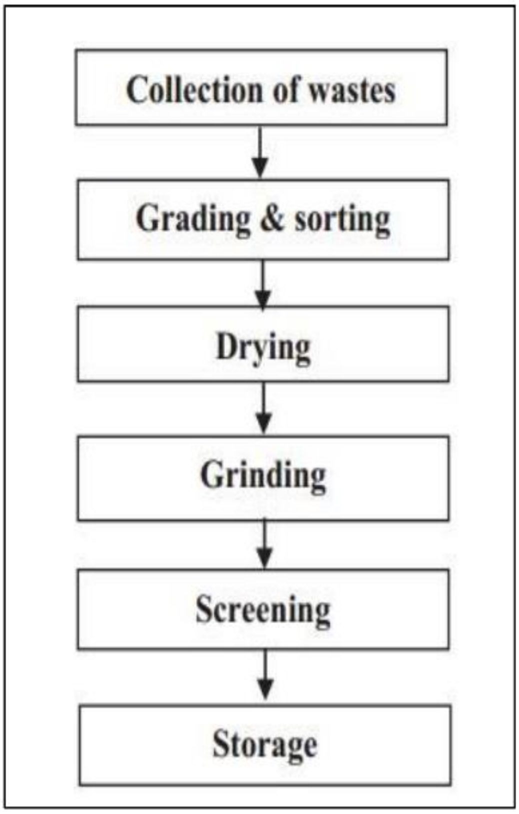


Fig 3.2: Process flow of preparation of paper waste and cow dung

## 3.2 Reactions

Reactions involved in the anaerobic digestion process are as follows:

3.2.1. **Hydrolysis** :

$C_6H_{10}O_5 + H_2O \rightarrow C_6H_{12}O_6$

$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2$

3.2.2. **Acidogenesis :**

$C_{12}H_{22}O_{11} + 9H_2O \rightarrow 4CH_3COO^- + 4HCO_3^- + 8H^+ + 8H_2$

$C_{12}H_{22}O_{11} + 5H_2O \rightarrow 2CH_3CH_2CH_2COO^- + 4HCO_3^- + 6H^+ + 4H_2$

3.2.3. **Acetogenesis :**

$CH_3CH_2COO^- + H^+ + 2H_2O \rightarrow CH_3COO^- + H^+ + CO_2 + 3H_2$

3.2.4. **Methanogenesis:**

$CH_3COO^- + H^+ \rightarrow CH_4 + CO_2$

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$

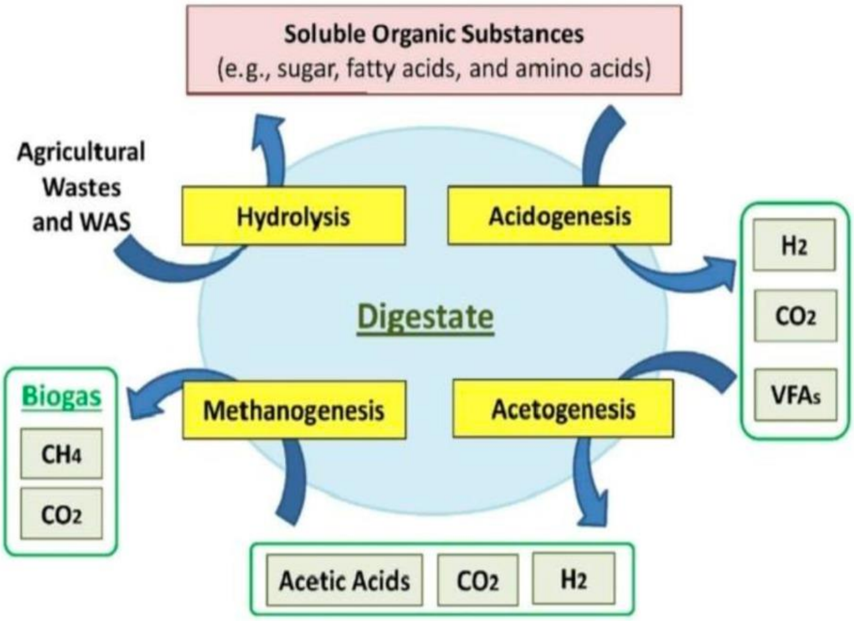


Fig 3.3: The process involved in digestion of bio-waste

## 3.3 Materials and Methods

The waste paper used for this study was collected from soft cardboard boxes that was readily available, while the cow dung was collected from the house of a milkman in Raiganj, West Bengal. The study was done between December 2021 to May 2022 at Jadavpur University. Jadavpur University is located at (22.4988 0 N, 88.3714 0 E) and 11m above sea level. Other materials which were used for this study are:

(i) Measuring cylinder
(ii) Beaker (1Litre)
(iii) Semi-Batch Digester (500 ml)

(iv) Magnetic stirrer

(v) Incubator

(vi) Digital pH meter

(vii) Apparatus to calculate biogas produced by downward displacement of water

(viii) Balance

(ix) Petri dish with cover

(x) Hot air oven

(xi) Muffle furnace

(xii) Crucible with lids

(xiii) Tongs

(xiv) Stop watch

(xv) Plaster of Paris

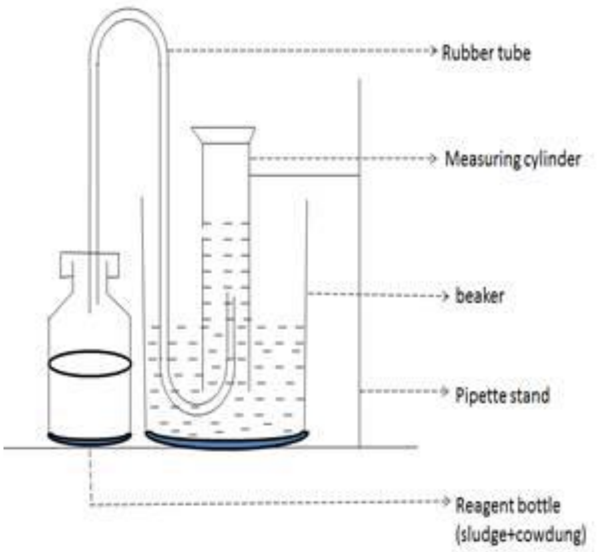


Fig 3.4: Schematic of a semi batch digestor used in the experiment

## 3.4 Digestion Studies

### 3.4.1 Preparing the wastes

The Paper Waste (PW) was soaked in a 1 Litre beaker overnight to allow the aerobic microbes to partially decompose the paste and the pH reading was taken. For the experiment involving only paper waste sample A (PW) 22.5g of paper waste was mixed with 375g of water.

Again, a slurry was prepared by mixing paper waste with cow dung (PW: CD) in the ratio of 1:1. The quantity of PW: CD was 22.5g in the slur, water content being 375g. Hence it implies that out of 22.5g, 11.25g was paper waste and 11.25g was cow dung.

### 3.4.2 Experimental Protocol

The experiment has been performed in a 500ml batch digester essentially made of glass. Depending on the number of solutions a set of digesters maybe used at a time. Pre-digested waste has been used as a source of mixed bacterial community for anaerobic digestion process. After all necessary additions the digesters have been rubber corked, sealed with plaster of Paris (to ensure complete air tight situation) and allowed to stand still inside the incubator at $37^0$C and the gas volume have been checked at an interval of 24 hours by method of downward displacement of water for the next 45 days.

## 3.5 Analysis of Waste

### 3.5.1 Physicochemical Analysis

Ash, moisture and volatile matter were determined using the Proximate Analysis method.

The Proximate Analysis of a sample determines the percentages of moisture, ash, volatile matter in the sample.

### 3.5.1.1. Moisture:

**Apparatus required:** Hot air Oven, petri dish with cover, Balance

A weight of sample is weighed in a petri dish provided with a well-fitted cover. The uncovered sample is heated for about an hour at a temperature of about 105 0C. Then the sample is taken out from the oven and allowed to cool. The moisture content is then determined by calculating the loss in weight as a percentage.

Weight of the petri dish with cover = W1

Weight of the petri dish with cover + the sample = W2

Weight of the sample taken = W3 = W2-W1

(Weight of the petri dish with cover + the sample) after heating = W4

Hence, loss in weight = W2-W4

Percent moisture in the sample = $\frac{W2-W4}{W3}\ x\ 100\ \%$

### 3.5.1.2. Ash content:

Ash refers to the inorganic residue left after combustion of a sample under specified conditions.

Apparatus required: Muffle furnace, crucible with lids, tongs, balance.

A weight of sample is weighed in a silica crucible and placed in a muffle furnace at room temperature without lid. The sample is initially heated to a temperature of around $450^0$C for 30 minutes and subsequently heated at $800^0$C for 1 hour.

After heating is completed the crucible with residue is cooled and weighed. The residue remaining after heating is taken as a percentage of coal sample taken initially to obtain the percentage of ash.

Weight of the crucible = W1

Weight of the crucible + sample = W2

Weight of the sample taken = W3

(Weight of the crucible + sample) after heating = W4

Hence, weight of the residue = W4-W1

Thus, percent ash in the sample = $\frac{W4-W1}{W3} x100\%$

### 3.5.1.3. Volatile matter:

Apparatus required: Muffle Furnace, crucible with lid, tongs, stop watch, balance.

A weight of sample is weighed in a silica crucible and transferred to a muffle furnace and maintained at a temperature of $880^0$C for 8 minutes and the door of the furnace is properly closed. After 8 minutes the crucible is removed and cooled. When it has cooled down it is weighed. The loss in weight as a percentage of the sample taken is calculated and the percentage of moisture is deducted from it to calculate the percentage of volatile matter.

Weight of the (crucible + lid) = W1

Weight of the (crucible + Lid + sample) = W2

Weight of the sample taken = W3

(Weight of the crucible + sample) after heating for 8 minutes = W4

Hence, loss in weight = W2-W4

Thus, total percentage loss in weight = $\frac{W2-W4}{W5} x100\%$

Hence, percent volatile matter content in the sample = W5- % Moisture content

The values hence found by this method is given in the table.

The carbon content was found using Walkey and Black method and the nitrogen content was found using SD-Kjeldahl method [108].

Fat, energy content, etc were determined by carefully studying many research papers. [109]

### 3.5.2 Biochemical Analysis

pH of the paper soaked in water was taken before charging of the waste while the ambient and influent temperatures of both the samples (PW and PW:CD) were monitored on a daily basis throughout the retention period.

## 3.6. Kinetic Model

Study of kinetic behaviour of the experimental samples helps us to understand and draw several conclusions about the behaviour of the experiment. In building the kinetic model we try to fit our experimental values to the pre-existing models. In this study we do comparative kinetic evaluation of biogas production by the method of anaerobic digestion of PW and PW: CD. We fit the experimental data of daily biogas production and cumulative biogas production into two simple kinetic models respectively, namely (1) Gaussian equation, and (2) Gompertz relation. Simple kinetic models like (3) Logistic growth equation, (4) Exponential, and (5) Linear could also have been used to build the models.

However, here the first two models, namely Gaussian equation and Gompertz relations have been mainly used.

The daily biogas production was measured with respect to retention period and the graph showing the comparative biogas production of the two samples was shown in the fig 3.7

The Gaussian equation represented by (1) was used for fitting the experimental outcomes of biogas production rate for both the ascending and descending limbs (ascending limb refers to the part of the curve before it reaches a maximum and descending limb refers to the part of the curve that is post the maximum value). The equation is as follows:

$$y = ae^{-0.5[\frac{T-T_0}{b}]^2} \text{ ------------------------------- (1)}$$

Where 'y' is the biogas production rate ($dm^3$/kg.sl), T is the hydraulic retention time (in days) and T0 is the time at which the maximum biogas production occurred and 'a' ($dm^3$/kg.sl) and 'b' (in days) are constants which are found by fitting the model.

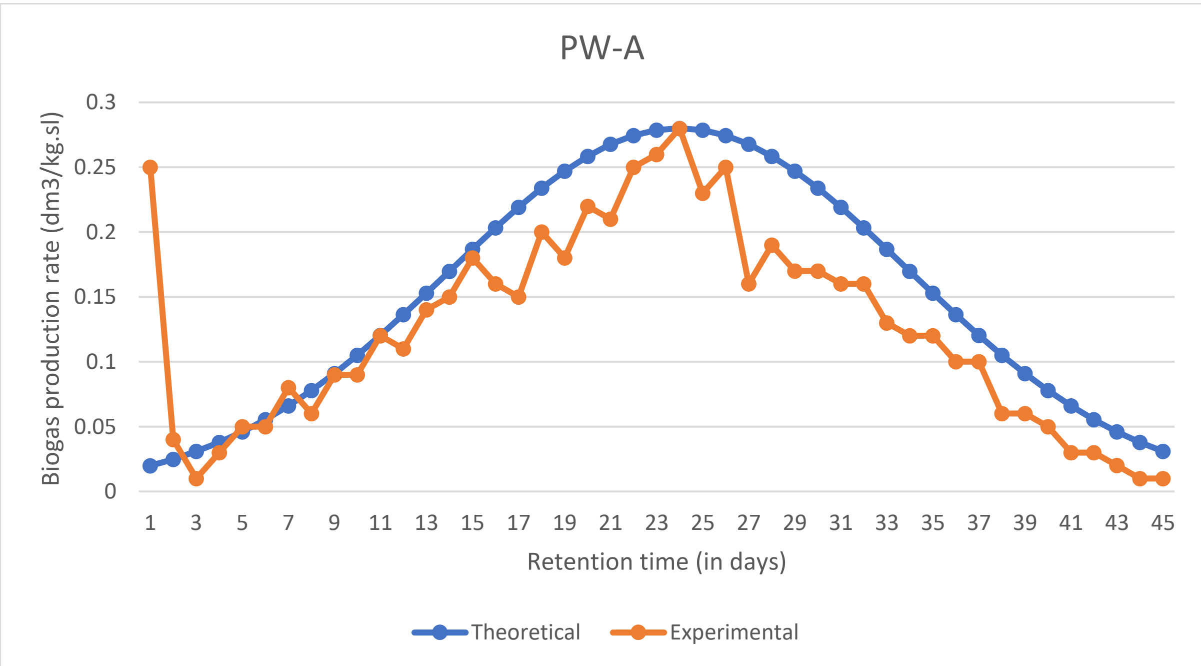


Fig. 3.5(a): Fitting the biogas production rate values into gaussian curve (paper waste slur)

By fitting the model, we obtain the values of a=0.28, b=10, T0=24.

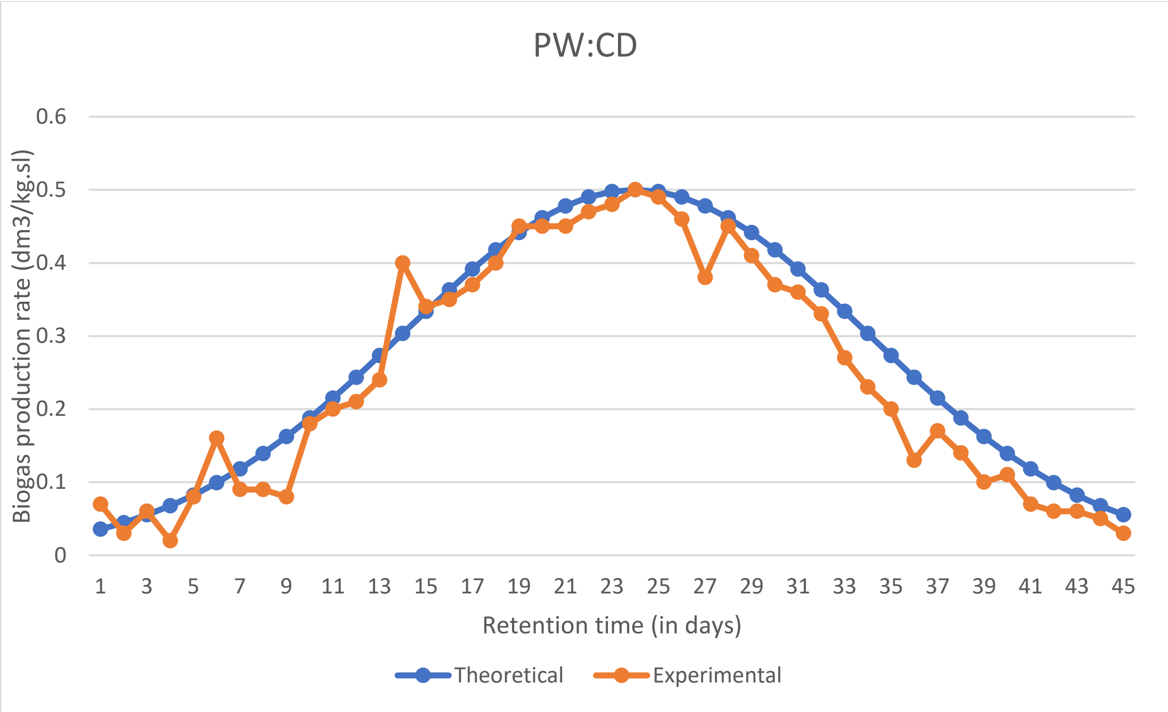


Fig 3.5(b): Fitting the biogas production rate values into gaussian curve (paper waste mixed with cow dung slur)

By fitting the model, we obtain the values of a=0.5, b=10, T0=24.

Similarly, cumulative biogas production can be modelled using the Gompertz equation (2) as follows:

$$y = ae^{-be^{-cT}} \text{ ---------------------------------------- } (2)$$

Where ‘y’ is the production rate of biogas ($dm^3$/kg.sl) , ‘a’ ($dm^3$/kg.sl), ‘b’ and ‘c’ are constants, T is the retention time (in days).

The cumulative curves that were fit into the model are given below:

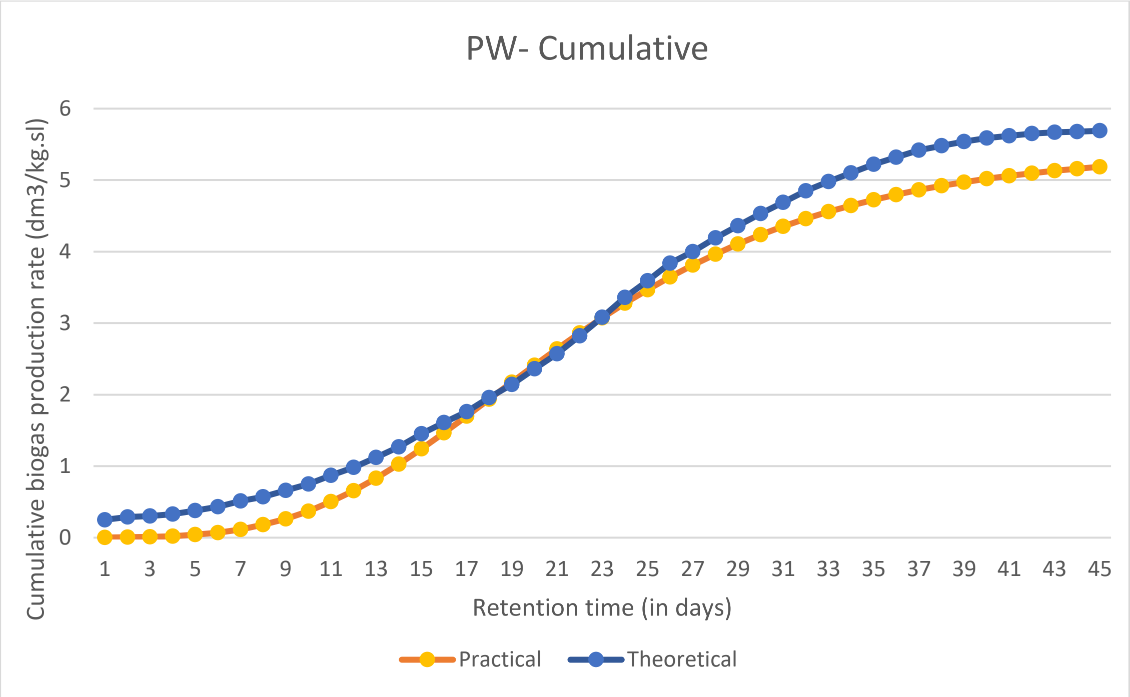


Fig 3.6(a): Distribution of cumulative biogas production rate for paper waste

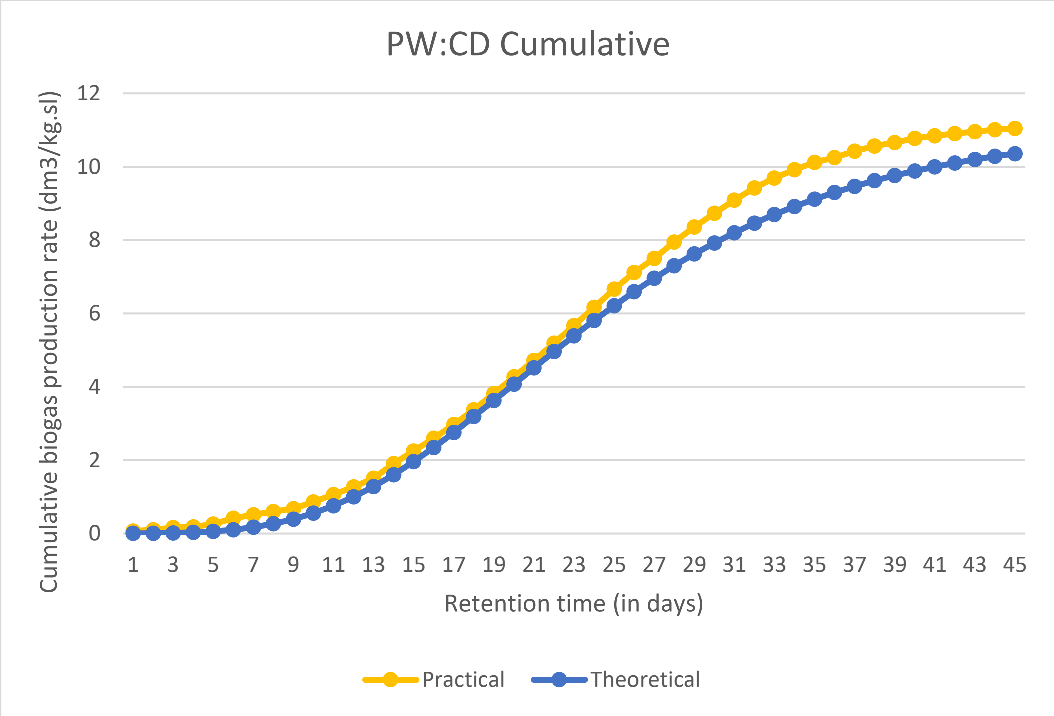


Fig 3.6(b): Distribution of cumulative biogas production rate for the mixture of paper waste and cow dung slur.

The ascending and descending limb of biogas production rate can also be simulated using the exponential (4) model. The ascending limb of cumulative production rate can be modelled using the exponential model and the descending limb of cumulative biogas production rate can be modelled with linear model (5) respectively. We assume that the production rate of biogas increases exponentially with time and after reaching a peak value, decreases exponentially.

The equations (4) & (5) are described below:

$$y = ae^{bT} \text{ ----------------------------------------(4)}$$

Where 'y' is the biogas production rate ($dm^3$/kg.sl), "T" is the hydraulic retention time (in days), 'a' ($dm^3$/kg.sl), 'b' ($day^{-1}$) being constants.

$$y = a + bT \text{ ----------------------------------------(5)}$$

Where 'y' is the biogas production rate ($dm^3$/kg.sl), 'T' is the hydraulic retention time (in days), 'a' ($dm^3$/kg.sl) and 'b' ($dm^3$/(kg.days).sl) being constants.

## 3.7. Results and Discussion

Co-digestion study was done by waste paper and cow dung where waste paper was used as a substrate for this digestion process. The temperature of this study was maintained around 37°C since it is the optimal temperature for co-digestion process [109]. The daily biogas production rate for the retention period of 45 days is graphically shown below (Fig 3.7). The biogas production from only paper waste (PW) was initiated after 5 days of charging the digester whereas biogas generation for the co-digested sample (PW:CD) was started from the 6th day of the charging period.

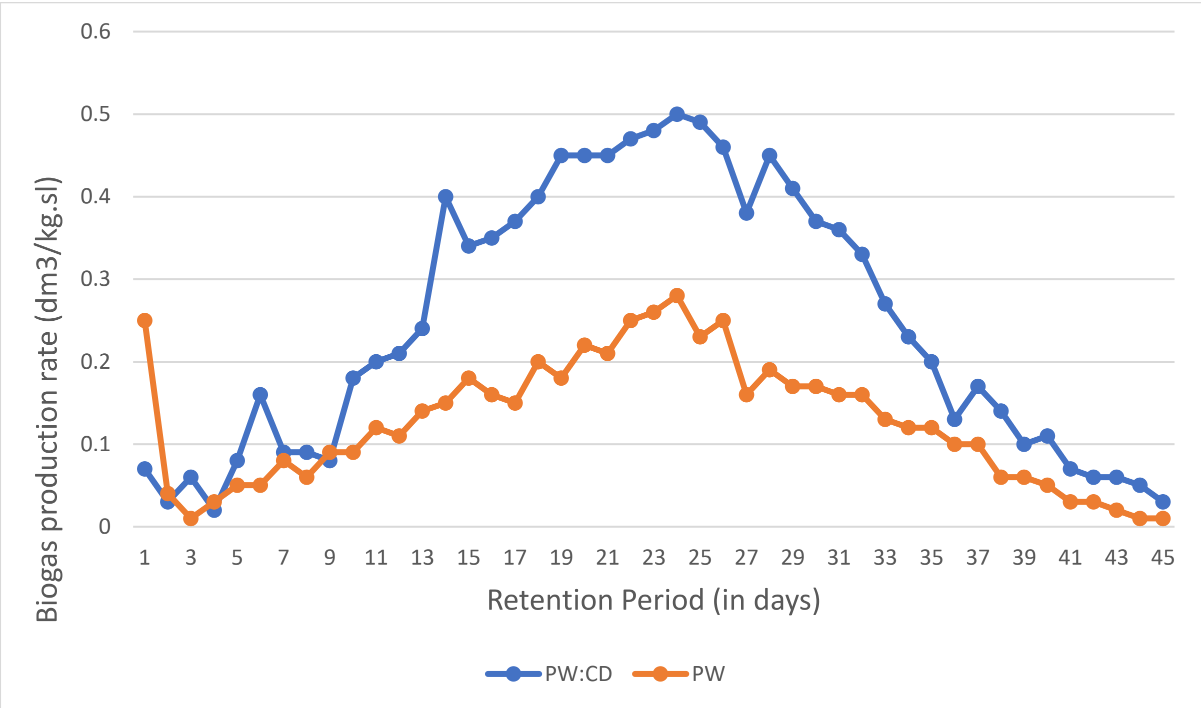


Fig 3.7: Comparison of biogas production rate in PW and PW:CD

The sample with only paper waste (PW) started biogas production within 5th day of digester charging, although the biogas production was almost negligible for the next one week. Significant amount of biogas production for the (PW) system started increasing from the 13th day and was highest on the 24th day (0.28 $dm^3$/kg sl.). From the 25th day it again started to drop. The biogas production almost ceased from the 34th day and no biogas production was observed henceforth till the end of the retention period. The cumulative gas yield for this sample was 5.69 $dm^3$/kg sl. It can be said that the hydrolysis step can be a rate determining step in the anaerobic di gestion process. The production of the gas that took place initially, within 24 hours of charging the sample into the heating chamber (as is seen in the fig. 3.7), may be as a result of the microbes present in the charged digester initially. With the onset of hydrolysis and acidogenesis, there may be a high release of free fatty acids. These fatty acids make the environment more susceptible to the bacteria that convert waste into biogas. These bacteria are known to be more sensitive to pH and usually survive at a pH range of 6.5 to 8.0 [110]. The pH measured for the (PW) sample was 8.5 which is higher than the optimum range. This is likely to cause a sudden decline in biogas production. However, when the significant production of biogas resumed after the 13th day, the gas production thereafter was somewhat high and continued for long, and then the sample had almost stopped biogas production from around the 34th day. The physicochemical properties like the volatile solids, nutrients, energy content and C/N ratio for this sample also had less favourable values that affect the production of biogas. The C/N ratio is optimum within the range of 20–30 [111]. But the C/N ratio for this sample was measured to be around 50. This is because bacteria that convert waste into biogas take up carbon almost 30 times faster than nitrogen [112]. The C/N ratio of paper waste was much higher than the appropriate range needed for the efficient production of biogas. Adequate physicochemical properties are also known to affect biogas production. For example, volatile solids (VS) must be high enough to make reasonable biogas production [113]. In this sample it was measured to be 65% which is not high enough for significant biogas production.

On the other hand, paper and cow dung (PW: CD) sample showed gas production within the 6th day. The gas production went on increasing day by day and it hit the maximum volume on the 24th day (which was around 0.5 $dm^3$/kg sl.). It started dipping down from 25th day but the production was still significantly high compared to the other sample. The cumulative gas yield for this sample was 10.35 $dm^3$/kg sl. which is almost double of the first sample. The biogas production for this sample continued during the entire retention period (i.e. 45 days). Also, blending of cow dung with paper waste resulted in enhancement of physicochemical properties of waste and the microbial load of the mixture.

Cow dung, which is a waste from rumen animal, is known to contain the qualities that result in the immediate production of biogas [114]. The energy content, volatile solids and nutrients of the waste were increased. They have been showed in the table 3.1 below. Volatile matter was around 73.6% which is significant enough for biogas production. C/N ratio was also reduced to an optimum range, which in this sample was measured to be 22.2. The pH was measured to be 7.4 which is well within the optimum range. Co-digestion is known to be the most famous optimisation method used to enhance the production of biogas. All the above-mentioned factors were responsible for better performance of PW: CD sample in comparison to the alone PW sample.

## Table 3.1: Physicochemical properties of the wastes

| Parameter | PW | PW:CD |
|---|---|---|
| Moisture (%) | 2.6 | 6.5 |
| Ash (%) | 18 | 20.2 |
| Volatile matter (%) | 65 | 73.6 |
| Crude fat (%) | Trace | 0.7 |
| Crude fibre (%) | 72 | 52 |
| Crude protein (%) | 1.4 | 8.6 |
| Crude nitrogen (%) | 0.22 | 1.5 |
| Carbon content (%) | 11 | 33.3 |
| Energy (Kcal/g) | 2.44 | 4.18 |
| C/N ratio | 50 | 22.2 |
| pH at charging | 8.5 | 7.4 |

PW = paper waste alone, PW:CD = paper waste mixed with cow dung in the ratio 1:1

## Table 3.2: Lag period, cumulative and mean volume of gas production for the wastes

| Parameter | PW | PW:CD |
|---|---|---|
| Lag period (days) | 5 days | 6 days |
| Cumulative gas yield ($dm^3$/kg.sl) | 5.69 | 10.35 |
| Mean volume of gas production ($dm^3$/kg.sl) | 0.126±0.05 | 0.245±0.03 |

PW = paper waste alone, PW:CD = paper waste mixed with cow dung in the ratio 1:1

# CHAPTER 4: Conclusions & recommendations for future work

Studies have shown that abundant waste paper everywhere is a very good raw material for biogas production and the production is even better when mixed with cow dung. This waste can be used to generate energy instead of being incinerated or left unattended to cause environmental pollution. This study also provides sustained gas production when mixing waste paper with cow dung or other animal waste throughout the waste digestion period, as animal waste is a good substance to mix with, for better biogas production rate.

Fossil fuels are increasingly getting scarce. Environment is getting more polluted day by day. Renewable energy seems to be the only way out. The world today requires long-term potential actions for sustainable development. Biogas has been a very dependable renewable energy source. Being an excellent source of natural gas, it can be definitely considered as an alternative for fossil fuels. Also, it has been adopted as one of the best alternatives for fossil fuels after the 1970 world energy crisis.

## **Research gap**:

There remains a tremendous amount of research potential in this subject. Apart from biodegradable or other cellulosic wastes that have been effectively worked upon to produce biogas, non-biodegradable wastes are also a major concern. The scope of future work remains in finding out the substrates that can easily digest the non-biodegradable wastes to produce energy, which, when kept untreated, pollutes the environment heavily and takes millions of years to decompose at a natural pace.

Furthermore, there is persistent deficit of pilot-scale research, whilst laboratory experiments tend to provide overly favourable results through the elimination of variables. Laboratory-scale papers also tend to focus overly on the volume of biogas produced during co-digestion, without sufficiently considering its impacts on downstream processes. Factors such as biosolids quality and odour are insufficiently covered, as is the composition of biogas, particularly in terms of the hydrogen sulphide content. Further research is also required to elucidate the impacts of different pre-treatments when used in conjunction with co-digestion. In laboratory experiments most pre-treatments applied during codigestion involve thermal based processes. Free ammonia pre-treatment has demonstrated promising results and favourable economics, yet has only been applied during mono-digestion of sewage sludge and has not been demonstrated in a continuous system or in conjunction with co-digestion.

# APPENDICES

## Resources used

Fig 4.1: Apparatus to calculate the volume of biogas produced by downward displacement of water


Fig 4.2: 500 ml digester containing only paper waste and water (PW)

Fig 4.3: 500 ml digester containing paper waste and cowdung in 1:1 ratio (PW:CD)

Fig 4.4: Digesters kept inside the incubator at 37 $^{0}$C

# Data used

| PW:CD-A Th | PW:CD-A Pr |
|---|---|
| 0.035502677 | 0.07 |
| 0.044460809 | 0.03 |
| 0.055125263 | 0.06 |
| 0.067667642 | 0.02 |
| 0.082237228 | 0.08 |
| 0.09894935 | 0.16 |
| 0.117873038 | 0.09 |
| 0.13901865 | 0.09 |
| 0.162326234 | 0.08 |
| 0.187655549 | 0.18 |
| 0.214778679 | 0.2 |
| 0.243376128 | 0.21 |
| 0.273037213 | 0.24 |
| 0.30326533 | 0.4 |
| 0.333488405 | 0.34 |
| 0.363074519 | 0.35 |
| 0.391352269 | 0.37 |
| 0.417635106 | 0.4 |
| 0.441248451 | 0.45 |
| 0.461558173 | 0.45 |
| 0.477998741 | 0.45 |
| 0.490099337 | 0.47 |
| 0.49750624 | 0.48 |
| 0.5 | 0.5 |
| 0.49750624 | 0.49 |
| 0.490099337 | 0.46 |
| 0.477998741 | 0.38 |
| 0.461558173 | 0.45 |
| 0.441248451 | 0.41 |
| 0.417635106 | 0.37 |
| 0.391352269 | 0.36 |
| 0.363074519 | 0.33 |
| 0.333488405 | 0.27 |
| 0.30326533 | 0.23 |
| 0.273037213 | 0.2 |
| 0.243376128 | 0.13 |
| 0.214778679 | 0.17 |
| 0.187655549 | 0.14 |
| 0.162326234 | 0.1 |
| 0.13901865 | 0.11 |
| 0.117873038 | 0.07 |
| 0.09894935 | 0.06 |
| 0.082237228 | 0.06 |
| 0.067667642 | 0.05 |
| 0.055125263 | 0.03 |

| PW Th | PW Pr |
|---|---|
| 0.019881 | 0.25 |
| 0.024898 | 0.04 |
| 0.03087 | 0.01 |
| 0.037894 | 0.03 |
| 0.046053 | 0.05 |
| 0.055412 | 0.05 |
| 0.066009 | 0.08 |
| 0.07785 | 0.06 |
| 0.090903 | 0.09 |
| 0.105087 | 0.09 |
| 0.120276 | 0.12 |
| 0.136291 | 0.11 |
| 0.152901 | 0.14 |
| 0.169829 | 0.15 |
| 0.186754 | 0.18 |
| 0.203322 | 0.16 |
| 0.219157 | 0.15 |
| 0.233876 | 0.2 |
| 0.247099 | 0.18 |
| 0.258473 | 0.22 |
| 0.267679 | 0.21 |
| 0.274456 | 0.25 |
| 0.278603 | 0.26 |
| 0.28 | 0.28 |
| 0.278603 | 0.23 |
| 0.274456 | 0.25 |
| 0.267679 | 0.16 |
| 0.258473 | 0.19 |
| 0.247099 | 0.17 |
| 0.233876 | 0.17 |
| 0.219157 | 0.16 |
| 0.203322 | 0.16 |
| 0.186754 | 0.13 |
| 0.169829 | 0.12 |
| 0.152901 | 0.12 |
| 0.136291 | 0.1 |
| 0.120276 | 0.1 |
| 0.105087 | 0.06 |
| 0.090903 | 0.06 |
| 0.07785 | 0.05 |
| 0.066009 | 0.03 |
| 0.055412 | 0.03 |
| 0.046053 | 0.02 |
| 0.037894 | 0.01 |
| 0.03087 | 0.01 |

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