



A perspective on the combination of alkali pre-treatment with bioaugmentation to improve biogas production from lignocellulose biomass

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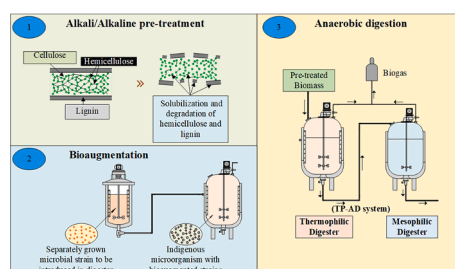
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HIGHLIGHTS

- The benefits of alkali pre-treatment and bioaugmentation are discussed.
- An integrated cascading system is proposed to increase grass biomethane production.
- The modelled integrated system potentially increases biomethane production by 47%.

GRAPHICAL ABSTRACT



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ABSTRACT

Anaerobic digestion (AD) is a bioprocess technology that integrates into circular economy systems, which produce renewable energy and biofertilizer whilst reducing greenhouse gas emissions. However, improvements in biogas production efficiency are needed in dealing with lignocellulosic biomass. The state-of-the-art of AD technology is discussed, with emphasis on feedstock digestibility and operational difficulty. Solutions to these challenges including for pre-treatment and bioaugmentation are reviewed. This article proposes an innovative integrated system combining alkali pre-treatment, temperature-phased AD and bioaugmentation techniques. The integrated system as modelled has a targeted potential to achieve a biodegradability index of 90% while increasing methane production by 47% compared to conventional AD. The methane productivity may also be improved by a target reduction in retention time from 30 to 20 days. This, if realized has the potential to lower energy production cost and the levelized cost of abatement to facilitate an increased resource of sustainable commercially viable biomethane.

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1. Introduction

1.1. Anaerobic digestion of lignocellulosic biomass: State of the art

Anaerobic digestion (AD) is a biological process powered by the metabolism of complex microbial communities primarily consisting of bacteria and archaea with smaller numbers of fungi and protozoa (Coma et al., 2017; Deng et al., 2021, 2020; Donkor et al., 2021; Lin et al., 2021). The microbial community in AD consumes organic matter to produce biogas, a renewable energy source. Aside from biogas production, AD has additional benefits in waste treatment, biofertilizer production, soil carbon sequestration and reducing fugitive methane emissions. A prime example is associated with the agricultural industry, where there is the potential to prevent fugitive methane emissions from open slurry holding tanks, reduce water contamination and produce renewable energy (Stambasky, 2016). In addition, the AD digestate is rich in key elements such as nitrogen and phosphorous and can be used as a biofertilizer to increase crop yield (IEA Bioenergy, 2019). However, the use of digestate as a fertilizer is strictly regulated in some countries and regions such as the European Union to prevent undesirable environmental or health impacts (Saveyn and Eder, 2014). Hence, not all digestate from all feedstocks can be used for fertilizer application. When digestate satisfies regulations, its role as a biofertilizer can enhance and intensify nutrient recycling; it can sequester anthropogenic carbon from the residual organic matter into the soil and in doing so increase soil organic content, enhance photosynthesis and as such sequester further CO₂ from the atmosphere.

AD is an adaptable technology that can make use of a variety of organic feedstocks for biogas production. Feedstock selection is dependent on the feedstock type, availability, accessibility, and ease of collection (Coma et al., 2017). Germany has the largest production of biogas in the EU; this resource is heavily based on silage maize as feedstock for biogas production (Theuerl et al., 2019). However, the use of food crops for biogas is deemed unattractive as per the food fuel debate and as such has led to the use of more sustainable biomass such as agricultural, industrial and municipal wastes and by-products as feedstocks in countries such as the Netherlands, Spain and Italy (European Commission, 2017; Saveyn and Eder, 2014). Denmark does not permit energy crops but does allow grass and clover grass from land that has not been tilled in 5 years (Al Seadi et al., 2018). In Ireland, as 91% of agricultural land is under grass or pasture a strategy is proposed that involves grass as a feedstock for the development of the Irish biogas industry. This involves the use and cultivation of surplus grass (beyond that required for feeding animals) for AD (McEniry et al., 2013).

Ireland is a country known for its characteristic farmlands and grasslands and as such produces sizable amounts of animal waste and surplus grass silage which are both well suited for biogas production (Wall et al., 2013). Studies have demonstrated that anaerobic digestion (AD) can convert grass to renewable biogas (Lehtomäki et al., 2008b; Seppälä et al., 2009; Wall et al., 2013; Xie et al., 2012). However, the feedstock includes for lignocellulosic fibres, which are slow and difficult to hydrolyse and the degradation of these lignocellulosic fibres can be the rate-limiting step in AD (Lehtomäki and Björnsson, 2006; Nizami et al., 2009). The reduction of this barrier to efficient digestion is one important facet in the development of a cost-effective AD technology that can rapidly convert lignocellulose to biogas. Difficulties in bioconversion of lignocellulose are due to the complex cell structure of the lignocellulosic biomass which offer resistance against microbial degradation in AD. The presence of cell wall components such as lignin can provide a rigid and cementing framework for the carbohydrates thereby hindering efficient microbial hydrolysis in the AD process. The lignin and carbohydrate contents of lignocellulosic feedstock such as grass are dependent on external factors such as cultivation practices, climate, location, soil type, age and species (Capstaff and Miller, 2018; Prochnow et al., 2005). The type of grass species such as perennial ryegrass, fescues, timothy and cocksfoot species can significantly affect

biogas production due to differences in matrix structure and chemical composition (Murphy et al., 2013). Ryegrass and fescues are the dominant species of grass in Europe (Rodríguez et al., 2017). Cultivation practices such as harvesting time, fertilizer application and ensiling have been reported to have a significant effect on biogas production due to the varying chemical composition of the grass species (Bedoïć et al., 2019; Krzystek et al., 2020). In a study by Prochnow et al., 2005, biogas production was comparatively low in late-cut grass compared to early-cut grass. This can be attributed to the high crude fiber content and low dry matter digestibility of late-cut grass compared to early-cut grass, hence making it much more resistant to microbial enzymatic attack in AD (Teagasc, 2016).

In the last decade, various research studies have focused on developing thermal, chemical and biological pre-treatment techniques to boost biogas production by overcoming the limitations posed by hydrolysis of fibrous feedstock (Chang et al., 1997; Ecem Öner et al., 2018; Khor et al., 2015). Although some lignocellulose feedstock such as grass has been reported to be more digestible than others, hydrolysis, the rate-limiting step in AD still poses a challenge and limits the productivity of biogas production (Cremonese et al., 2021). This has necessitated the use of pre-treatment techniques to condition the lignocellulosic biomass before AD to improve both hydrolysis and bioenergy extraction. Pre-treatment is targeted at making lignocellulose amenable to enzymatic reaction by combining different types of physical, chemical and thermal processes on a biomass feedstock such as grass (Shah et al., 2015). The pre-treatment process usually decreases lignin content, increases surface area and decreases crystallinity of biomass (Kim et al., 2016). A high degradation of lignocellulose into solubilized components is mostly achieved using severe pre-treatment techniques that involve strong chemicals at high temperatures and pressures. However, such pre-treatment methods are costly and usually generate inhibitory side compounds that can be detrimental to AD.

Bioaugmentation of the AD process is another alternative to improve microbial hydrolysis of lignocellulosic biomass. This is an emerging technology in which separately grown microbes with specific activity for a metabolic pathway are inoculated separately to the digester and added to a digester to improve the efficiency of microbial community in biogas production (Lebiocika et al., 2018; Satoh et al., 2003). Most studies focused on bioaugmentation strategies in which conditions such as temperature and pH are controlled to favor survival and prolonged activity of the specific exogenic microorganism (Nzila, 2017). In most cases, the exogenous microbes are cellulolytic bacteria that target the effective hydrolysis of complex biomass, however, a few studies have also indicated the successful inoculation of acidogenic and methanogenic microorganisms to increase methane production in the AD process (Akyola et al., 2019; Nzila, 2017; Zhang et al., 2015).

Generally, AD processes are temperature dependant as indicated by their kinetic metabolism and degradation reactions (Tassew et al., 2020). AD is therefore operated at mesophilic (30–40 °C) or thermophilic (50–60 °C) temperatures. Although kinetic studies have shown that the hydrolytic rate constant increases between 1.5 and 2 times for thermophilic temperatures in comparison with mesophilic temperatures (Ge et al., 2011; Li et al., 2015c; Siegrist et al., 2002), most studies have focused on operating at mesophilic temperatures. The effects of mesophilic temperatures on hydrolysis, acidogenesis, and methanogenesis within the AD process have been investigated with batch experiments conducted with unacclimatized inoculum (Donoso-Bravo et al., 2009; Membere and Sallis, 2018). A study conducted by Qin et al., 2017 indicated that a sequential hyperthermophilic (above 60 °C) treatment followed by a mesophilic treatment improved the hydrolysis efficiency through a higher (14.5%) organic solids reduction rate. Moreover, a sequential temperature-phased system that combines both thermophilic and mesophilic processes could be a potential solution to obtaining the best output from both processes.

1.2. Innovation and objectives

The conventional AD technology that is currently applied in the biogas industry may be limited in productivity and efficiency; this can be attributed to low biomass conversion and requirement for long residence times. The opportunity exists to innovate a system that combines the prowess of both pre-treatment, temperature-phased AD (TP-AD) and bioaugmentation to develop an improved and cost-effective digestion process, thereby providing a solution to improving biomass conversion and AD efficiency. Such a study that combines different elements in reactor design, pre-treatment and biological augmentation has not been undertaken for a lignocellulosic biomass such as grass silage. This study: (1) discusses the potential of alkali pre-treatment to improve digestibility in AD of lignocellulosic biomass; (2) assesses the application of bioaugmentation of specific microbes to enhance biomass hydrolysis and biogas production; (3) proposes a novel cascading AD concept consisting of alkali pre-treatment, TP-AD and microbial bioaugmentation strategies that may provide a cost-effective pathway to improve biomethane production from lignocellulosic biomass such as grass silage.

2. Challenges and difficulties in conventional anaerobic digestion of lignocellulosic biomass

2.1. Feedstock associated challenges

The usage of lignocellulosic biomass as a feedstock for AD has a significant impact on biogas yield and productivity. This is mostly due to the quality of biomass which directly affects biomass conversion and hydraulic retention time (HRT) of the AD process. In lignocellulosic biomass such as grass, cell wall contents such as cellulose, hemicellulose and lignin directly impact biomass conversion. Unlike commercial enzymes, cellulases secreted by hydrolytic microbes in the AD process have a relatively slow activity in converting polymeric organic structures into monomers and oligomers (Donkor et al., 2021). This rate-limiting phase of AD is generally responsible for low biomass conversion, especially when there is high crude fiber content such as lignin in the biomass. Lignin and undigestible fiber content, though not inhibitory to microbes, act as blocking agents in hydrolysis of degradable components. Biomass conversion can be severely reduced when using lignin-rich biomass in comparison with one with a lower lignin content (Li et al., 2021; Li et al., 2018b). This limits the rate of hydrolysis and increases the HRT of the AD process, as a longer digestion time is needed to degrade lignocellulose by the slow-acting activity of the hydrolytic bacteria.

The HRT is the average length of time that a substrate spends in a digester and determines the effectiveness of the conversion of the volatile solids (VS) into biogas (Mayer et al., 2014). Due to the resistance to microbial activity exhibited by lignocellulosic biomass, digesters are generally operated at relatively long HRT (30 days and sometimes up to 60 days) (Dong et al., 2019; Liu et al., 2019). Slower biochemical reactions are associated with low biogas productivity and require longer HRT which in turn necessitates larger reactor vessels (Yadvika et al., 2004). To cut down capital cost (and overall cost of energy) and increase process efficiency and productivity, it is desirable to have high biomass conversion with a short HRT. However, care must be taken in selecting a suitable HRT and associated reactor volume that does not result in microbial washout (Muzenda, 2014). Developers need to be informed of the “sweet spot” which minimizes the HRT (and the cost of the energy produced) while not leading to process instability and/or microbial washout. This necessitates innovative approaches to enhance the digestibility of biomass to increase biogas production and reduce HRT in the AD process.

Another operational issue for most digesters treating lignocellulosic substrates such as grass is the high viscosity, and potential for foaming and scum formation when operating at high organic loading rates. Previously reported studies have indicated that high organic loading can

lead to partial degradation of organic matter and can stimulate microbial production of biosurfactants. The lipid and protein content of biomass have been identified as the main compounds responsible for foaming and scum formation in digesters (Kougias et al., 2013). Foaming can result in a 30–50% reduction in biogas yield and was common in about 80% of the industrial biogas plants investigated (Kougias et al., 2013). For example, substrates such as grass, grain waste and manure are known to have high protein content that promotes foaming (Moeller and Görsch, 2015). Foaming was reported as being due to a protein film surrounding a gas bubble (Foegeding et al., 2006); this resulted in a structural bond that kept bubbles locked in place. Lipid foam formation on the other hand is caused by fatty acids in which these acids express properties of surfactants, however, lipid foaming in AD lignocellulosic biomass is quite low due to the low lipid content of these biomass types (Boe et al., 2012). Feeding regimes at high organic loading do not only cause scum and foam formation but can also produce excessive quantities of volatile fatty acids (VFA) that are responsible for biomethanation failures in most digesters. High concentrations of VFAs have been reported to incapacitate methanogenic archaea in the AD process (Xiao et al., 2013). The intolerance of methanogens to high levels of VFAs forms a secondary rate-limiting step in the biomethanation process, as the rate of biogas production is relatively slowed. The difficulties associated with the use of lignocellulosic biomass in the AD process have led to the development of certain remedies to overcome these challenges. In the past decade, a significant amount of research studies have focused on using pre-treatment or biological augmentation (bioaugmentation) techniques to enhance biomass digestibility and improve the overall efficiency of AD. Whereas pre-treatment is used prior to the AD process, bioaugmentation focuses on the supplementation of the digester with specialized and separately grown microbes to enhance biochemical conversion of substrate to biogas while simultaneously preventing scum formation in digesters with high organic loadings.

2.2. Physical pre-treatment to enhance feedstock digestibility

Pre-treatment has been used as one of the means to condition biomass and reduce its resistance to microbial degradation before the AD process. Pre-treatment serves to remove lignin from lignocellulose, thereby reducing cellulose protection, improving biomass porosity and accessibility of surface area (Kim et al., 2016). Furthermore, there is a reduction in cellulose crystallinity and degree of polymerization which promotes efficient AD (Karimi and Taherzadeh, 2016; Mankar et al., 2021). There are several types of pre-treatment primarily including physical, thermal, and chemical pre-treatments. Each type of pre-treatment presents its own merits and demerits (Table 1); it is, therefore, necessary to carefully select the type of pre-treatment based on its effectiveness on digestion performance and the associated cost of the treatment.

Physical pre-treatment is a treatment that does not involve the application of chemicals or microorganisms. This pre-treatment reduces the particle size, compactness, dispersal, degree of polymerization, and cellulose crystallinity while improving the biomass surface area for effective microbial contact in AD. Generally, physical pre-treatment does not produce toxic inhibitors such as furfural and hydroxyl methyl furfural (HMF) (Amin et al., 2017). Although there are some advantages, it has high energy requirements, high capital cost and does not reduce the lignin content which makes it less effective than other pre-treatment techniques (Jain et al., 2015). As a typical physical pre-treatment, thermal pre-treatment involves the use of water, steam, or irradiation within the temperature range (50–250 °C) to enhance the digestibility of feedstock for AD. Pre-treatment techniques such as hydrothermal, steam explosion, microwave and ultrasonication are usually combined with chemical reagents in a thermochemical setting to improve digestibility and bioprocessing of biomass. Thermal pre-treatments present the advantages of removing pathogens from the biomass, reducing the degree

Table 1

Different pre-treatment methods, their advantages and disadvantages (Abraham et al., 2020; Jomnonkhaow et al., 2021; Kumar et al., 2020).

Pre-treatment method	Advantages	Disadvantages
Physical		
Grinding or milling	Easily handling of feedstock, particle size reduction, increased surface area, reduction of cellulose crystallinity, reduction in degree of polymerization, no inhibitor production	High energy demand, high capital requirement, no lignin removal
Chemical		
Alkaline	Lignin removal, improved particle porosity, alteration of cellulose structure, no inhibitor formation	High cost of some alkali reagents such as NaOH and KOH
Acid	Hemicellulose removal, alteration of cellulose structure	High cost of acid reagents, requirement for specialized equipment, inhibitors formation, loss of cellulose, high cost of waste treatment
Oxidation	Lignin and hemicellulose removal	High cost of oxidative reagents, inhibitor formation, loss of cellulose, high cost of waste treatment
Organic solvent	Lignin removal	Cost of organic solvent, extensive solvent removal processes
Thermal		
Hydrothermal	Lignin and hemicellulose removal, alteration of cellulose structure, increased particle porosity	High water usage, high energy demand, high capital requirement, inhibitor formation
Steam explosion	Lignin and hemicellulose removal, alteration of cellulose structure, increased particle porosity	High energy demand, high capital requirement, inhibitors formation
Biological		
Microbial	Low energy demand, lignin and hemicellulose removal, no inhibitor formation, alteration of cellulose structure	Requirement for sterilized environment, extensive treatment time, carbon loss
Enzymes	Low energy demand, lignin and partial hemicellulose removal, no inhibitor formation, alteration of cellulose structure	High cost of enzymes, may require regular enzyme supplementation

of polymerization, and increasing biomass porosity (Jomnonkhaow et al., 2021). However, thermal pre-treatments using water or steam at elevated temperatures (>150 °C) with prolonged treatment time could result in the production of inhibitory products which are disadvantageous to the AD process (Ariunbaatar et al., 2014; Mahmoodi et al., 2018). Although microwave and ultrasonication require short treatment times, they still require a high power input and maximum benefits are usually observed when complemented with alkaline pre-treatment (Janker-Obermeier et al., 2012).

2.3. Alkali pre-treatment to enhance feedstock digestibility

Chemical pre-treatment involves the disruption of biomass with the assistance of acid, alkaline/alkali, organic solvents, or oxidants. The use of these chemicals results in the solubilization of the cell wall material, thereby improving the enzyme accessibility to the biomass. Chemical pre-treatment (primarily used in conjunction with thermal pre-treatment) is much more beneficial for biomass with high lignin content than biomass with low lignin and high carbohydrate content (Nguyen et al., 2010). Chemical pre-treatment is usually influenced by factors such as the type of chemical used, treatment time, and temperature. Significant issues with chemical treatment agents are the cost associated with specialized equipment and neutralization operations

(Hendriks and Zeeman, 2009).

One chemical pre-treatment is oxidative pre-treatment which involves the introduction of oxygen to biomass to increase the free radical production to dissolve lignin and hemicellulose (Mao et al., 2015). However, oxidative pre-treatments are known to have poor selectivity resulting in degradation of cellulose as well (Hendriks and Zeeman, 2009). The poor selectivity of this process results in chemical reactions with both the carbohydrates and other cell wall components, leading to the production and accumulation of inhibitory products that adversely affect biogas production (Travaini et al., 2016). In addition, oxidative agents such as ozone are known to be costly on an industrial scale due to the large volume of gas required in such a pre-treatment process.

Alkali/alkaline pre-treatment is a treatment method that involves the use of alkali/alkaline solutions such as sodium, calcium, potassium and ammonium hydroxide to enhance the digestibility of biomass feedstock such as rice straw, sorghum silage and grass. In contrast to oxidative and acid pre-treatment, alkaline treatments have a significant effect on the lignin and hemicellulose content, as the process decreases the degree of polymerization for improved enzyme accessibility (Mankar et al., 2021; Xu and Huang, 2014). Additionally, it can cause modification and reduction in crystallinity of biomass feedstock (Yu et al., 2019). Alkaline treatments using chemicals such as Ca(OH)₂ have been found to be inexpensive processes due to low chemical cost and the ability to operate at relatively lower temperatures when compared to other forms of pre-treatment (Chaturvedi and Verma, 2013; Kim et al., 2016). However, an expensive operational and capital cost is unavoidable when moderately expensive reagents such as KOH and NaOH are solely utilized in the pre-treatment of various biomass feedstock (Amin et al., 2017; Song et al., 2014). Alkali/alkaline pre-treatment has shown much effectiveness in lignin and hemicellulose solubilization from biomass (Baruah et al., 2018) (Table 2). This pre-treatment method aims to break down lignocellulosic biomass, reducing lignin content and reducing lignocellulose resistance to microbial degradation. In addition, there is an improvement in fiber porosity, surface area and a reduction to crystallinity which serves to improve microbial degradation of carbohydrates and production of biogas (Kumar et al., 2020; Li et al., 2015a). The hydroxyl group present in the alkaline solutions target and break the lignin-carbohydrate ester bonds while simultaneously weakening the hydrogen bonds between hemicellulose and cellulose (Kumar et al., 2020; Li et al., 2016). This leads to a subsequent release of both lignin and hemicellulose in the solubilized portion with minimal effect on cellulose content (Ismail et al., 2017) (Fig. 1). Alkali reagents such as

Table 2

The effect of different alkali reagents on pre-treatment of lignocellulose biomass (Kim et al., 2016; Kim and Lee, 2007; Li and Kim, 2011; Tajkarimi et al., 2008; Wyman et al., 2005).

Alkali/Alkaline	Conditions	Delignification	Hemicellulose dissolution	Comments
NaOH, Na ₂ CO ₃	Low to high pressure and temperature	60–80%	20–50%	Moderately expensive and difficult to recover NaOH
Ammonium	High pressure and temperature	0–80%	10–50%	Expensive with the requirement of high-pressure equipment
Ca(OH) ₂	Low pressure and temperature	60–80%	20–40%	Less costly with low energy demand and relatively cheap CO ₂ carbonating recovery process

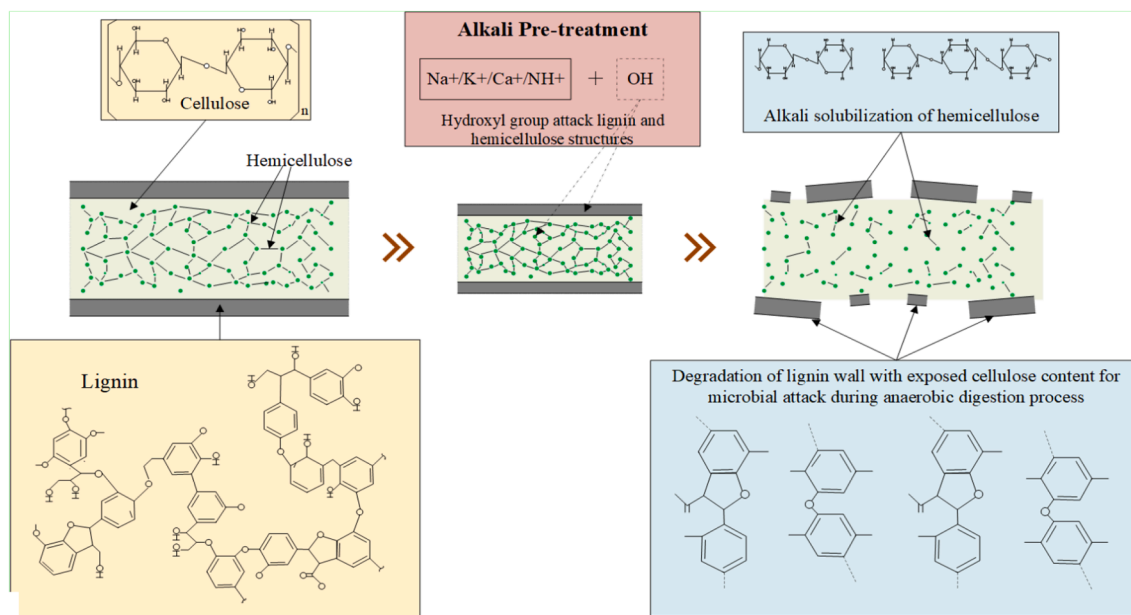


Fig. 1. Effect of alkali pre-treatment on lignocellulose matrix.

NaOH, KOH and Ca(OH)₂ have been reported to result in hemicellulose and lignin dissolution in the range 20–40% and 60–80%, respectively (Sharma et al., 2013) (Kim et al., 2016). In an alkaline (2% NaOH) pre-treatment study by Zheng et al., 2009, digestion time was shortened by 35% while biogas production and biomethane yield of corn stover improved by about 70%. Although NaOH has been reported to be one of the most effective chemical reagents in chemical pre-treatment, Ca(OH)₂ presents a cheaper alternative providing a higher net profit benefit that is more feasible in improving biogas production (Jiang et al., 2017). In the comparison of both acidic (H₂SO₄, HCl, and CH₃COOH) and alkaline/alkali reagents (NaOH, Ca(OH)₂, and NH₃·H₂O) at various concentrations, Ca(OH)₂ pre-treatment at 8% was reported to produce the highest methane yield from corn straw among the seven assessed acid and alkali pre-treatments (Song et al., 2014). The methane yield with Ca(OH)₂ pre-treatment in this study was 105% greater than the untreated corn straw, however, a pre-treatment time of 48 h was needed to achieve this favorable effect (Song et al., 2014). Also, the pre-treatment effect is significantly dependent on the quality of feedstock and other conditions. For example, Khor et al., 2015 reported a significantly less methane increase (37% increase) using 7.5% Ca(OH)₂ to pre-treat grass before the AD process.

Considering the competitiveness of Ca(OH)₂ in relation to other acid and alkaline reagents, Ca(OH)₂ pre-treatment is deemed by the authors to be desirable to enhance biomass digestibility before AD. Some of the reasons for this opinion is that the use of Ca(OH)₂ is less corrosive and less expensive in terms of its recovery process in comparison to an alkaline treatment method such as NaOH; Ca(OH)₂ has also shown promising effectiveness for herbaceous (grass) biomass and in anaerobic digestion. A 37% increase in methane yield have been reported for Ca(OH)₂ pre-treatment of grass (Khor et al., 2015; Kim et al., 2016). A key to further improving Ca(OH)₂ pre-treatment of biomass in converting to biogas production lies in combining with other alkali/alkaline reagents to achieve a low-cost but high-impact effect. Such multi-combination of other alkali agents with Ca(OH)₂ must not only improve biomass digestibility but also have secondary beneficial effects on the subsequent AD process. The presence of alkaline agents such as Ca(OH)₂ could be used as a pH buffer to prevent a drop of pH when rapid acidogenesis occurs, leading to accumulation of VFAs which can be detrimental to methanogenesis in the AD process (McKennedy and Sherlock, 2015; Rodriguez et al., 2017). Apart from the buffering capacity of the alkaline reagents, some alkali earth metals such as potassium and magnesium are

essential macronutrients that have been shown to prevent inhibitory effects by enhancing VFA consumption while also improving biomass solubilization in the AD process (Huang et al., 2016; Li et al., 2018a). Although alkali agents such as KOH and Mg(OH)₂ are relatively expensive, there is a pathway to combine these reagents in optimized low dosages with the majority of the dose from lower cost Ca(OH)₂ to further improve the overall effectiveness of the pre-treatment combined with AD processes without suffering any financial penalties.

2.4. Bioaugmentation

Bioaugmentation is the addition of specific exogenous stress-resistant microorganisms or a mixture of cultures into the AD microbial community to improve biomethane production. Bioaugmentation is mostly applied to enhance the hydrolysis and acidogenesis phases of the biomethanation process, however, a few studies have also demonstrated its effectiveness in the methanogenic phase of the AD process (Table 3). During the microbial hydrolysis step, where the complex biomass structure is broken down into monomeric sugars, bioaugmentation can be performed with substrate-specific microorganisms to improve the breakdown of monomeric sugars and other components. This can be especially effective in a temperature-phased AD system (TP-AD) where there is a clear separation of the methanogenic phase from the hydrolytic phase of the biomethanation process. In the TP-AD system, bioaugmentation of the thermophilic stage can be particularly effective on overall methane production since digester conditions (such as temperature and pH) are particularly conducive to the exogenous hydrolytic and acidogenic microbial species (Martin-Ryals et al., 2015) (Bagi et al., 2007). The thermophilic stage presents favorable conditions for these bioaugmented species to thrive and concentrate on rapidly converting lignocellulose to VFAs which can later be efficiently used to produce biogas in the subsequent methanogenic mesophilic digester. Microorganisms such as *Caldicellulosiruptor lactoaceticus*, *Clostridium cellulolyticum*, and hemicellulolytic bacteria have been used to target cellulose, wheat straw cellobiose, and xylan respectively with reported increases in biomethane production of between 8 and 53% (Peng et al., 2014; Weiß et al., 2010). Other works report various microorganism mixtures used on feedstock such as sweet corn processing residues, *Axonopus compressus* (cowgrass), and brewery spent grain which obtained between a 5 to 70% increase in biomethane production (Cater et al., 2015; Lee et al., 2020; Martin-Ryals et al., 2015).

Table 3
Examples of bioaugmentation studies on AD of various feedstock.

Targeted phase	Feedstock	Bioaugmentation microbes	% increase in CH ₄ yield	References
Hydrolysis	Wheat straw	Cellulolytic anaerobic bacteria <i>Clostridium cellulolyticum</i>	7.6	(Peng et al., 2014)
	Brewery spent grain	<i>Pseudobutyrvibrio xylanivorans</i> Mz5 ^T	17.8	(Cater et al., 2015)
		Coculture (<i>Pseudobutyrvibrio xylanivorans</i> Mz5 ^T & <i>Fibrobacter succinogenes</i> S85)	6.9	
	Sweet corn cellulosic waste	Coculture (<i>Pseudobutyrvibrio xylanivorans</i> Mz5 ^T & <i>Clostridium cellulovorans</i>)	4.9	
		Cellulolytic bioculture mixture, predominantly of the genus <i>Clostridium</i>	56	(Martin-Ryals et al., 2015)
	Cowgrass	Mixed culture of <i>Clostridium cellulovorans</i> (ATCC 35296), <i>Mesotoga infera</i> (DSM 25546), <i>Methanosaeta concilii</i> (DSM 3671)	20.7	(Lee et al., 2020)
	Wheat straw and cow manure	<i>Clostridium thermocellum</i>	39	(Ecem Öner et al., 2018)
	Birchwood xylan	Hemicellulolytic bacteria	53	(Weiß et al., 2010)
	Cereal crops (such as wheat, rye, barley and triticale)	Anaerobic rumen fungus <i>Orpinomyces</i> sp.	15–33	(Akyola et al., 2019)
	Corn straw	<i>Acetobacteroides hydrogenigenes</i>	19–23	(Zhang et al., 2015)
Acidogenesis and Acetogenesis	Maize silage	<i>Enterobacter Cloaca</i>	21	(Ács et al., 2015)
	Dried green biomass and dried tubers of Jerusalem artichoke	<i>Caldicellulosiruptor saccharolyticus</i>	60	(Bagi et al., 2007)
	Wheat straw	A mix of two pure cultures of anaerobic fungi (<i>Neocallimastix</i> sp. and <i>Orpinomyces</i> sp.) with fermenting and hydrogen-producing bacterial pool (F210)	70	(Ferraro et al., 2019)
	Cattle manure and microalgae	Hydrogenotrophic <i>Methanoculleus bourgensis</i>	28	(Tian et al., 2019)
Hydrolysis and methanogenesis	Cattle manure	<i>Clostridium</i> sp. PXYL1 and <i>Methanosarcina</i> sp. PMET1	67	(Akila and Chandra, 2010)

Bioaugmentation has also been used as a means of resolving reactor imbalances such as hydrogen (H₂) deficiency, free ammonia inhibition (NH₃), and VFA accumulation in AD treatments. An adequate presence of H₂ is vital within the methanogenesis phase of the AD process to reduce CO₂ and produce biomethane. H₂-producing microorganisms such as *Acetobacteroides hydrogenigenes*, *Caldicellulosiruptor saccharolyticus*, and *Enterobacter cloacae* have been used on corn straw, dried green biomass, and dried tubers of Jerusalem artichoke and maize silage to obtain a 19–60% increase in biomethane yield (Nzila, 2017; Zhang et al., 2015). A high NH₃ concentration (>1000 mg/L) resulting from protein-rich substrates can cause excessive increases in CO₂ and H₂ production, proton imbalance, and potassium deficiency which collectively affects biomethane yield. To solve such challenges, microorganisms with high NH₃ tolerance and with the ability to utilize H₂ such as hydrogenotrophic *Methanoculleus bourgensis* have been applied in bioaugmentation. Utilization of this microorganism in a bioaugmentation setup was shown to improve biomethane yields by 28% and decrease VFA by 80% in comparison to the control digestion (Tian et al., 2019).

Most of the research conducted using bioaugmentation has focused on the hydrolysis and acidogenesis stages in the AD process. However, Jain et al., 2015 stated that the total biogas produced is typically based on acetate degradation (60%), H₂/CO₂ redox reaction (30%) and methyl compounds (less than 10%). It therefore brings into question why most bioaugmentation treatments have only focused on bacteria in increasing the generation of acetate, H₂ and CO₂ while ignoring the role of methanogens in degradation of acetate and utilization of H₂ to further improve the biogas yield. Methanogens are microbes that obviously in more abundance of select species can improve the methanogenesis stage thereby improving the production of biogas. Methanogens are extremely sensitive to stress and environmental changes which makes their application challenging (Vrieze et al., 2012). However, most of these stresses (low temperature, high ammonium concentration, organic overload, and oxygen stress) can be targeted by microbial bioaugmentation experiments (Nzila, 2017). Methanogens such as *Methanosarcina* sp. which are mixotrophic and coccoid in shape have been reported to be robust, stress-resistant and have a doubling time of 1–2 days leading to an increase in biogas production in comparison with

other methanogens (Vrieze et al., 2012). This therefore presents an opportunity, especially in a TP-AD system, to use a microbial consortium that combines methanogens with hydrolytic or acidogenic/acetogenic bacteria to obtain higher biogas yields. Akila and Chandra, (2010) used a coculture of *Methanosarcina* sp. (PMET1) and *Clostridium* sp. (PXYL1) to stimulate biomethanation of xylose as a feedstock at psychrophilic temperatures and obtained a 67% increase in biogas production as compared to using *Clostridium* sp. (PXYL1) alone. Thus, the addition of *Methanosarcina* sp. to the bioaugmentation mixture could serve as an effective means to deal with the methanogenesis limitations while improving biogas production.

A considerable number of studies have focused on the identification and application of feedstock-specific microbes and enzymes in digesters to improve biogas production, however, research into bioaugmentation strategies such as routine supplementation is key to the success of the bioaugmentation process. Martin-Ryals et al., 2015 went further to determine the effects of a routine and one-time bioaugmentation in the acid phase of a two-phase AD treatment of sweet corn. In a batch system, the routine bioaugmentation led to a significantly improved soluble chemical oxygen demand (sCOD) generation (+25%) and methane production (+15%) compared to one-time bioaugmentation. Furthermore, in a continuous system, the routine bioaugmentation resulted in an increased acid-phase sCOD (29–68%), acetic acid concentrations (31–34%) and methane production (56%) in comparison to a non-bioaugmented system. Routine supplementation with feedstock-specific microbes and enzymes is a promising avenue to implement bioaugmentation in the biogas industry. A typical example has been demonstrated by DuPont which offers short cycle microbial enzyme cocktails to improve biogas production in anaerobic digesters (DuPont Industrial Biosciences, 2017). Furthermore, companies such as Veolia and Dong Energy have pilot applications in bioaugmenting biogas digesters with microbial and enzyme-specific inoculum (Holmes, 2016; Woodcote Media, 2016). These examples indicate the potential of bioaugmentation and establish that this technology is a viable pathway to improving the overall efficiency and productivity of biogas production from biomass.

3. Biomethane production from alkali pre-treated biomass using sequential temperature-phased AD enhanced with microbial bioaugmentation

3.1. Improvement of grass biomethane using Sequential Temperature-phased Enhanced AD using Microbes and/or Enzymes (STEAME)

In the last decade, a significant portion of published studies have focused on improving biogas production from lignocellulose biomass by using the aforementioned pre-treatment and bioaugmentation techniques. However, pre-treatment and bioaugmentation are typically used as an individual means to improve biogas production. From the authors' perspective, a promising avenue to improving biogas production from lignocellulosic biomass would be a process that encompasses a low cost but high impact pre-treatment with an appropriate reactor system bio-augmented with feedstock specific and efficient microbes or enzymes. Therefore, the authors' have proposed a Sequential Temperature-phased Enhanced AD using Microbes and/or Enzymes (STEAME) as illustrated in Fig. 2. The STEAME system targets cost-effective biogas production from recalcitrant lignocellulose biomass.

Although there are a variety of pre-treatment techniques, alkali/alkaline pre-treatment has still been one of the most utilized pre-treatment techniques for lignocellulosic biomass (Mao et al., 2015). This is mainly due to its low cost and remarkable effectiveness in hemicellulose dissolution and lignin removal without compromising the integrity of cellulose in the lignocellulose matrix (Kim, 2013). These attributes of the alkaline pre-treatment method make it attractive to incorporate into AD processes. In most reported studies, alkaline

reagents such as NaOH, Ca(OH)₂ and KOH have been used in standalone applications to enhance the digestibility of lignocellulose biomass (Dussadee et al., 2017; Khor et al., 2015). However, the alkali pre-treatment section of the STEAME technology targets a low-cost but effective process that combines less expensive reagents such Ca(OH)₂ and Mg(OH)₂ with KOH to increase the digestibility and enhance biogas production from biomass. Li et al. (2015b) were able to obtain a similar delignification effect with a 77% increase in methane yield (as compared to untreated corn stover) while combining 0.5% KOH with 2% Ca(OH)₂ as opposed to solely using 2% KOH. This affirms that a combination of multiple types of alkaline reagents in a pre-treatment solution could help lessen the issues with alkali reagent cost while ensuring a satisfactory delignification and increase in biogas yield.

Depending on the feedstock type and severity of the pre-treatment proposed in STEAME, the pH of the reaction mixture drops towards the end of the reaction, as acetyl groups and uronic acids are released together with the release of the hemicellulose fraction into the mixture. This observation was noticed in ongoing alkali pre-treatment experiments of grass, where pH dropped from a high of 11 to a low of 8 at different alkali loading. The pH drop did not reach the optimum pH for AD, however this reduces the chemicals required for neutralization after pre-treatment. This is a desirable effect for a scale-up process and the only adverse effect noticed was a 12 h lag phase from the onset of the AD process which can be overcome with the adaptation of AD microbial consortia. However, the thermophilic digester of the TP-AD system operates at a lower pH (pH 5 to 6), hence neutralization may be essential to reduce the pH to optimum conditions for successful hydrolytic digestion / biological acidification of pretreated biomass. Additionally,

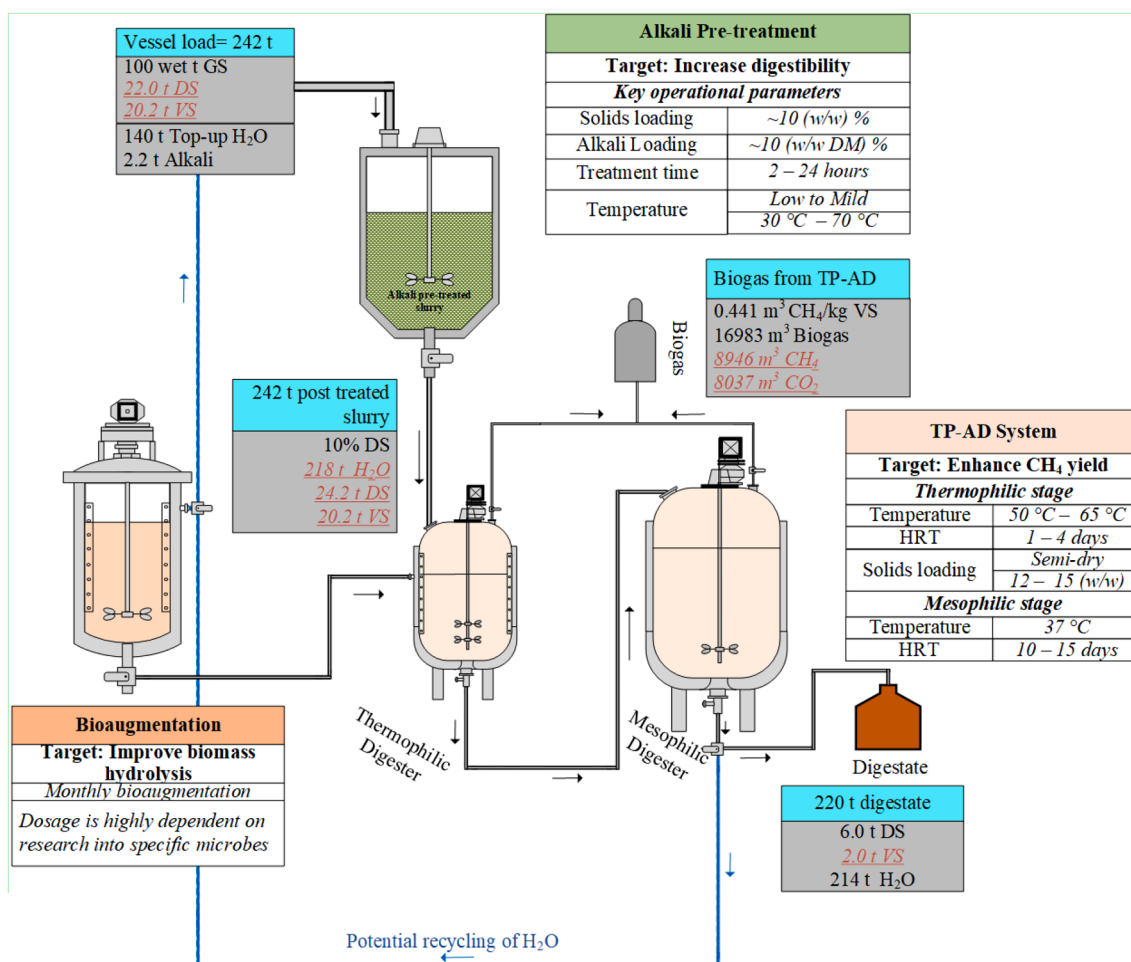


Fig. 2. Authors ambition for daily mass balance for STEAME concept.

in the STEAME system, the CO₂ produced along with the methane can react with calcium present in the solution and form calcium carbonates that can help stabilize the system pH by acting as a system buffer within the mesophilic digester to combat VFA accumulation resulting from the digestion of pretreated biomass at higher organic loading rates (Chen et al., 2015).

Another issue of concern in introducing alkali pretreated biomass into digesters is the potential adverse effect of elements such as calcium on the performance of the microbial community. This can be experienced either through the direct effect of calcium cation (Ca²⁺) species on anaerobes or the impact of precipitates (such as CaCO₃) on the performance of the digester. The effect of Ca²⁺ was recently researched by Chen et al., 2020 and Cristina et al., 2020, and the results indicated significant inhibition of methanogenesis at calcium addition above 2 g/L. The methanogenic inhibition effect was significantly observed with acetotrophic methanogens, however, increasing Ca²⁺ enriched hydrogenotrophic methanogens and could be key to improving methane production in the TP-AD system (Chen et al., 2020). On the other hand, high CaCO₃ precipitate formation affects microbial cell to cell communication and can result in death of microbes in outer layers of the formed anaerobic granules (Cristina et al., 2020). The alkali pre-treatment section of the STEAME concept targets low alkali dosage, however, assuming a maximum dosage of 20 g per 100 g dry biomass at a moderate digester organic loading rate (OLR), the concentration of Ca²⁺ will be significantly less than 1 g/L in the digester and will not lead to the various reported adverse effects. Also, the lower concentration of calcium in the digester will not trigger excessive precipitation and as previously discussed will rather help buffer and stabilize pH in the mesophilic digester.

A principal component of the proposed STEAME process is the TP-AD system. This follows the pre-treatment section and is aimed at accelerating the conversion of pretreated biomass to biogas (Borowski, 2015; Doooms et al., 2018; Ge et al., 2010; Lv et al., 2016). Anaerobic digesters are mostly operated at either mesophilic or thermophilic conditions (Akgul et al., 2017; Demirbas and Ozturk, 2005). The operation of AD digesters at either of these two conditions presents particular limitations due to the actions of microbes at each stage of the AD process. In mesophilic AD, there is a limitation of operating at high organic loading rates due to relatively slow hydrolysis and low volatile solids conversion, as mesophilic conditions favor methanogens in the conversion of acetate to biogas (Han et al., 1997). Contrarily, operating at thermophilic conditions kinetically enhances the rate of microbial hydrolysis of lignocellulose structure in the hydrolytic phase of the biomethanation process. However, it also presents the limitation of accumulation of short-chain fatty acids that can be detrimental to methanogenic activity and can lead to methane reduction (Iranpour, 2006; Speece et al., 2006); there is also the issue of reduced CO₂ solubility at higher temperatures reducing levels of bicarbonate alkalinity and ability to buffer high levels of VFAs. A TP-AD digester system sequentially operates a thermophilic digester (optimised for hydrolysis and acidification) and a mesophilic digester (optimized for methanogenesis). This AD system is designed to bring the best out of the two individual digester configurations. The thermophilic digester makes use of elevated temperatures (45–65 °C) to increase the rate of hydrolysis in the first stage while also improving the acidogenesis/fermentation process through operation at very high organic loading rates and optimal pH levels for hydrolysis and acidogenesis (Ge et al., 2010; Yu et al., 2013); this is sometimes referred to as biological acidification. This requires a low solids retention time (2–6 days), a high organic loading rate, and high pH associated with elevated production of VFAs (Montañés Alonso et al., 2016; Wu et al., 2015; Yu et al., 2013). The mesophilic stage makes use of milder temperatures (35–37 °C) to facilitate a significant improvement in the acetogenesis and methanogenesis phases at an optimal pH range to enhance the production of biogas from the hydrolysate rich in VFAs from the thermophilic biological acidification digester (De Bok et al., 2004; Lafitte-Trouqué and Forster, 2000). The primary benefits of using a TP-AD

system in comparison to a single-stage digester include lower total HRT allowing for a higher organic loading rate (up to 15 kg VS/m³/day in thermophilic first phase biological acidification reactor) with higher VS removal and increased methane production (Ge et al., 2010; Lv et al., 2010; Nizami and Murphy, 2010).

The STEAME process is also based on the notion of further improving the TP-AD system by using bioaugmentation to target the specific phases of the biomethanation process. A bioaugmented TP-AD system in the STEAME process presents additional benefits such as further improving the rate of hydrolysis and shortening the HRT when highly hydrolytic bacteria are introduced in the thermophilic biological acidification stage. A similar effect can be attained in the mesophilic stage by improving acetogenesis and methanogenesis phases with methanogenic archaea. Methanogens such as *Methanosarcina* sp are capable of resisting the stress of organic loading shocks and robustness in biogas conversion (Vrieze et al., 2012).

Bioaugmentation of microbes has much more long-term effectiveness as compared to the application of enzymes. The STEAME concept serves to demonstrate the potential of the TP-AD system for lignocellulose biomass such as grass silage, especially when the thermophilic section is bioaugmented with thermotolerant cellulolytic and xylanolytic microorganisms to enhance biomass degradation and further improve the biogas production. Dosage and frequency of dosage are typically the two key factors of any bioaugmentation process. An optimum bioaugmentation dosage needs to be realized by testing different dosage frequencies. Reported studies such as Jiang et al., 2020 and Lebioccka et al., 2018 investigated dosage ranging from 10% to 20% (v/v) of digester inoculum with dosage frequencies of 3, 5 and 7 days; these are really not feasible for commercial operations. In commercial operations that use bioaugmentation for various purposes (one example is to reduce the ammonia concentration), highly concentrated microbial biomass is dosed once a month. Furthermore, such bioaugmentation strategies will lead to the survival and evolution of the most competent elements of the microbial population; enzymes do not have such a possibility. The study of such a bioaugmented system in a lab and pilot continuous STEAME concept is necessary to yield an insight into identification and adaptive evolution of effective microorganisms to optimize the conversion of lignocellulosic biomass to biogas. Molecular techniques and whole-genome sequencing play a big role in understanding the role and efficiency of bioaugmentation in AD. Unlike the pre-treatment and the TP-AD technology, bioaugmentation is an underutilized technology in the biogas industry, hence the STEAME process seeks to apply the promising capabilities to further enhance the overall efficiency of biogas production from lignocellulosic feedstock.

The potential of the modelled STEAME concept to significantly improve AD is illustrated with biogas production from grass silage (Fig. 2). The chemical composition and attributes of grass silage used for this initial mass balance are referenced from Smyth et al. (Smyth et al., 2009). This refers to a conservative assessment of Irish grass silage with a moisture content of 22% and VS content of 92% of dry solids content. A pilot AD plant operating at mesophilic conditions was assessed to be able to produce 300 L CH₄/kg VS from this grass silage at an HRT of 60 days (Nizami and Murphy, 2010). This is similar to reported studies where the methane yield for various grass varieties (silage, first and late-cut) varied between 200 and 370 L CH₄/kg VS in conventional 30 to 60-day mesophilic digestion process (Lehtomäki et al., 2008a; Mähnert et al., 2002; Nizami and Murphy, 2010). This normally corresponds to a biodegradability index (BI) of about 50 to 60%. However a more recent study by Wall et al. (2014b) indicated BI of 90% for first-cut grass silage from a model farm in Ireland. This was achieved with substantial recirculation of separated liquor effluent which kept the ratio of fatty acids to buffering capacity as defined by the FOS/TAC titration to values below 0.30 and indicated that maximum effective degradation of such lignocellulosic substrate requires long residence time (>20 days). For a significant improvement in methane production and productivity (such as shorter HRT), Smyth et al., 2009 indicated necessary improvements

are essential for the AD process. The STEAME concept as modelled in Fig. 2 has the essential tools to significantly improve the AD process. The probable improvements stem from the summed effect of the alkali pre-treatment, bioaugmentation and TP-AD aspects of the STEAME process. The calculation as described in e-Supplementary material highlights the improvement potential of the STEAME concept. The assumptions used in developing the STEAME concept in Fig. 2 and e-Supplementary material are highlighted in Table 4.

In the STEAME concept, a late-cut grass was utilized, and a biodegradability index of 90% (Wall et al., 2014a,b) was assumed from the combinational application of pre-treatment, bioaugmentation and TP-AD system for digestion of the specified grass silage. Different types of grass (such as ryegrass, cocksfoot, meadow foxtail, and switchgrass) have distinct chemical compositions that can affect biogas yield. The more recalcitrant biomass such as switchgrass and Napier grass usually have lower biogas yield because of the high lignin content and hence require harsh pre-treatment to significantly improve biogas yields (Murphy et al., 2013; Wang et al., 2020). Perennial ryegrass is the dominant grass species in Ireland; hence it was selected as the feedstock for the STEAME concept. However, it is worth noting that various types of grass in relation to harvesting time (such as early-cut, first-cut, second-cut and late-cut grass) have different compositions and characteristics that can also affect pre-treatment efficiency and bioconversion of grass into biogas. The compositional and characteristic effect of different grass types on biogas production was reviewed by (Murphy et al., 2013) and indicated higher biogas production from early-cut grass and lower biogas production from late-cut grass. Late-cut grass silage is assumed for the STEAME concept because of its high recalcitrance and lower digestibility as compared to first-cut grass which is predominantly preferred as feed for livestock farming. The use of the stoichiometric formula from grass as stated in e-Supplementary material to estimate the biomethane yield is bespoke and though it highlights a process calculation, the result does not apply to all grass species. However, the assumed biodegradability index of 90% for the late-cut grass also considered the impact of bioaugmentation and pre-treatment on late-cut grass in relation to its high lignin content and low biodegradability. The biomethane yield for the STEAME concept is determined from the assumed biodegradability. This may be considered as a model based on reported literature on the effect of pre-treatment and bioaugmentation on late-cut grass and other different species of grass (Akila and Chandra, 2010; Deng et al., 2019; Lee et al., 2020; Nizami et al., 2012; Wall et al., 2014a,b; Xie et al., 2011).

The assumed biodegradability of the late-cut grass corresponded to about a 90% destruction of VS and conversion to biogas. In contrast to the grass biomethane study by (Wall et al., 2014b), biomethane yield

markers can potentially be accomplished with an HRT of less than 20 days in a TP-AD system, indicating high methane productivity as compared to most reported studies. The STEAME model presents a subset of a range of possible cascading systems including for a range of pre-treatments (for example, white-rot fungi ligninolytic enzymes treatment, ionic liquids, organosolv process and CO₂ explosion pre-treatments), a specific configuration of multi-phase digestion systems, and bioaugmentation which when employed for specific feedstocks may be able to produce more biogas from smaller cheaper digestion systems leading to a more cost-effective biogas industry. However, experimental, modelling and techno-economic studies are needed to assess the individual and collective benefits on the STEAME concept on AD and will act as a segue to further papers.

3.2. A review of different AD processes and the advantages of the STEAME concept

Different types of processes that enhance AD of biomass are presented in Fig. 3 and Table 5. The AD configuration commonly used in the biogas industry is the single or multiple CSTR (Continuous Stirred Tank Reactor) digesters (single-phase) system (Nizami and Murphy, 2010). These single-phase digester systems are generally operated at mesophilic conditions with a few operated at thermophilic temperatures to facilitate higher OLRs. Commercially, there is minimal adoption of mono-digestion of grass silage for biomethane production with only one reported case noted by the authors, a biogas plant located in Eugendorf, Austria (Smyth et al., 2009). This as reported is a two-digester CSTR (single-phase) system that mono digests grass silage to yield 300 L/kg VS at an HRT of 60 days. The methane yield was achieved at a considerably longer residence time of 60 days and is 64% lower than the theoretical maximum methane potential of grass silage (in an Irish context); this indicates the need for better processes such as the STEAME concept. The majority of grass silage AD systems have been performed in laboratory scale CSTR systems with studies by Nizami et al., 2012, Wall et al., 2014a, Wall et al., 2014b and Voelklein et al., 2016 indicating high biomethane yields with 90–95% VS destruction of grass silage. However, these studies reported lower productivities (longer HRT) than was modelled for the STEAME system and were performed on less recalcitrant early-cut silage under either thermophilic conditions or employed recirculation of digestate.

The two-phase AD concept is another AD process that separates the hydrolytic and acidogenic phases of the biomethanation process from acetogenic and methanogenic phases. This is generally applied in TP-AD and SLBR-UASB (Sequential Leach Bed Reactors followed by Upflow Anaerobic Sludge Blanket) systems to shorten retention time while

Table 4
Assumptions made for the calculations of grass biomethane in the STEAME concept.

STEAME Concept	Component/Description	Assumptions/Basis	References
Feedstock	Grass silage (GS)	Quantity of grass silage = 100 wet tonnes/day	(Smyth et al., 2009)
	Dry (DS) and volatile solids (VS) content	DS @ 22% & VS @ 92%	
Alkaline Pre-treatment	Carbon, Hydrogen, Oxygen and Nitrogen	CHNO = C _{28.4} H _{44.5} O _{17.7} N	(Li et al., 2018b) (Huang et al., 2016; Khor et al., 2015; Li et al., 2015a; Sharma et al., 2013) (Chang et al., 1997; Khor et al., 2015; Kim et al., 2016)
	Maximum/Theoretical CH ₄ yield	From Buswell equation = 491 m ³ CH ₄ /tonne VS	
	Considered alkaline reagents	Ca(OH) ₂ , Mg(OH) ₂ & KOH	
	Pre-treatment reactor load	10% solids loading	
TP-AD system with bioaugmentation	Alkaline loading	10% (w/w) of DS (0.1 g alkaline per g dry Grass Silage (GS))	(Wall et al., 2014b)
	Targeted temperature range	Target from a low 10 °C to a high of 100 °C	
	Biodegradability index of GS	Assumed BI of 90%	
	Methane production	442 m ³ /tonne VS	(Qin et al., 2017)
	Thermophilic digester temperature	50 °C to 65 °C	
	HRT for thermophilic digester	1 to 4 days	
	Mesophilic digester temperature	37 °C	(Dooms et al., 2018; Orozco et al., 2013)
	HRT for mesophilic digester	10 to 15 days	

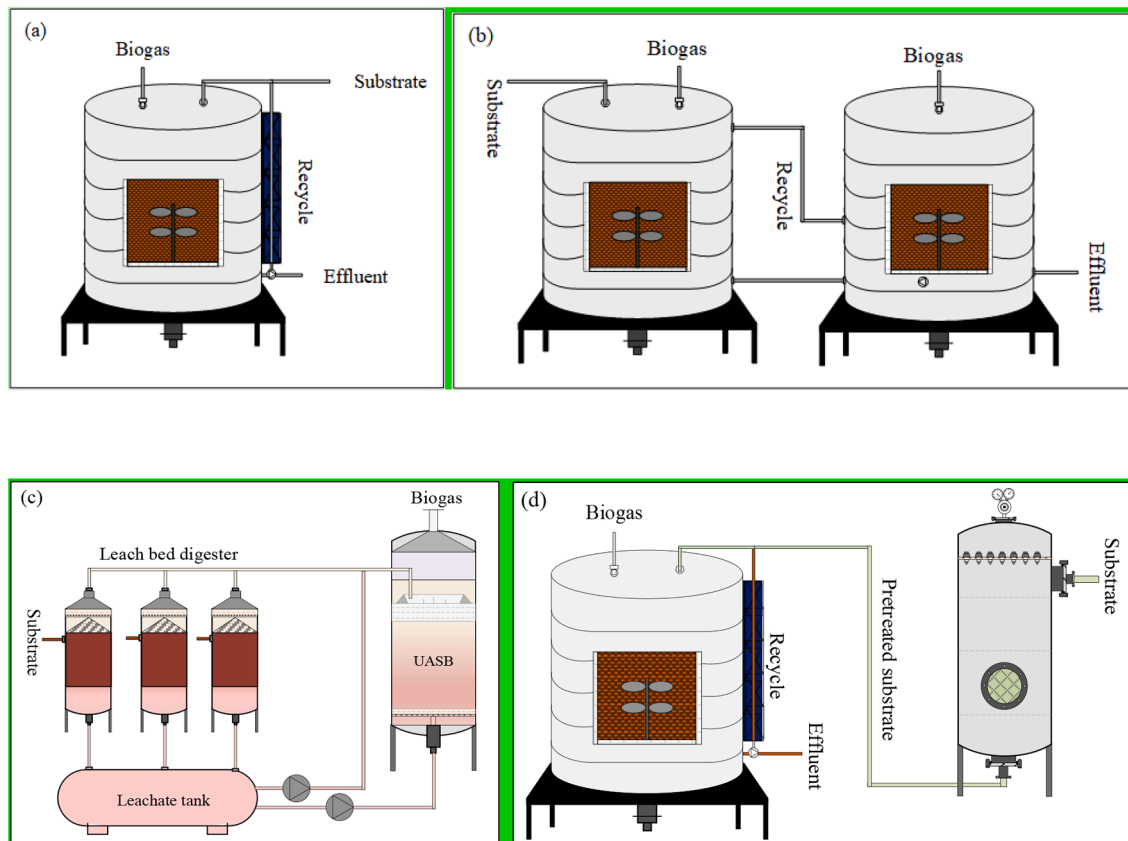


Fig. 3. (a) One-stage/single phase AD; (b) Two-stage AD; (c) Sequential Leach Bed Reactor coupled with UASB; (d) Pre-treatment coupled with AD.

aiming for high methane yields. The successfulness of the two-phase AD system is highly feedstock specific (Janesch et al., 2021). The utilization of grass silage in the two-phase AD concept usually shortened the HRT and increased OLR, however, the increase in OLR moderately reduced methane yields and VS destruction (Table 5). This is in contrast to two-phase AD of food waste (Ding et al., 2021), which yielded higher VS destruction (>90%) and methane yield. This indicates that the additional constituents of the STEAME concept such as pre-treatment and bioaugmentation are potentially critical to efficient conversion of grass to biomethane. Pre-treatment techniques such as acid, alkali, hydrothermal and ionic liquids have individually been applied in research studies to enhance biomethane production from biomass (Deng et al., 2019; Khor et al., 2015; Li and Xu, 2017; Xie et al., 2011). Regarding pre-treatment of grass silage, harsher treatment conditions (such as high temperature and reagent dosage) usually lead to higher biodegradability with improved biomethane yield (Table 5). However, severe pre-treatment processes are regarded as costly enterprises that are difficult to implement in the biogas industry. Hence, there is the need to maximize yield at less demanding conditions to develop a cost-effective pre-treatment process that enhances biomethane production from grass silage. This is the target of the STEAME concept; to improve current AD technology.

3.3. The potential economic competitiveness of the STEAME concept

The alkali pre-treatment, TP-AD system and bioaugmentation sections of the STEAME concept have been utilized separately to improve biomethane production from grass and other lignocellulosic biomass in various reported studies (Table 5). Most of the research on grass AD has focused on first-cut grass with only a few studies on late-cut grass, however these studies serve as a good barometer to assess the potential economic feasibility of the STEAME concept. High operational costs

(19–22% of operating expense) have been reported for various pre-treatment methods except for $\text{Ca}(\text{OH})_2$ alkali pre-treatment (Banu J et al., 2021; Baral and Shah, 2017). This is especially true for alkali pre-treatment at mild to low temperature conditions (10–50 °C) and low alkali loading (>0.15 g/g dry biomass fed), as the financial liability generally is attributed to the heating cost and chemical reagent expenditure. Antonopoulou et al., 2020 and Reilly et al., 2015 indicated the potential economic sustainability of pre-treatment of grass and wheat straw at lower alkali loading (0.02–0.10 g/g dry biomass fed), and determined that extra net income can be gained after considering the cost of the alkali pre-treatment. The STEAME concept targets low alkali loading (~0.10 g/g dry biomass fed) coupled with low to mild temperature conditions for pre-treatment of late-cut grass. This will have a favorable effect on the overall process economics of the STEAME concept as compared to using expensive methods such as acid, steam explosion and ionic liquid pre-treatments.

The TP-AD system is a broadly studied digester design that enhances biomethane production from biomass. The two main components that account for the extra expenditure in a TP-AD system are the capital cost associated with the thermophilic digester and the operational cost for operating the digester at thermophilic conditions (50–65 °C). In recent years, most AD plants in the biogas industry generally have multi-stage digesters to extend retention time and allow for recirculation of digester liquor for better biomass conversion and to further reduce the usage of fresh water (BayWa r.e., 2015). Hence, the capital cost for the TP-AD system in the STEAME concept will probably be similar to that of multi-stage digester system employed in most biogas plants.

The operational cost associated with working at thermophilic temperatures is the distinguishing feature in TP-AD system when compared to conventional digesters, however in the STEAME concept, the thermophilic digester is preceded by the alkali pre-treatment stage which targets mild temperature conditions at a retention time of 1 day. The

Table 5
Comparison of the STEAME concept to different processes to enhance biomethane production from grass.

	Process configuration	SC (% DS)	OLR (kg VS m ⁻³ d ⁻¹)	HRT (days)	Temp (°C)	Methane yield and VS destruction	Economic competitiveness	References
Single phase AD [Fig. 3a & 3b]	Two stage mesophilic CSTR ^(*)	10	1.4	60	38	300 L/kg VS 60 % VS destruction	Not profitable under current sale of net energy output and would require capital grants to break even (Smyth et al., 2012)	(Smyth et al., 2009)
	One stage mesophilic CSTR ^{(*) (1)}	10	4.0	–	37	404 L/kg VS		(Wall et al., 2014a, Wall et al., 2014b)
	Two stage mesophilic CSTR	2 to 14	2.0	50	37	451 L/kg VS 90 % VS destruction	Improved energy output, however, the longer HRT makes profitability difficult without further capital grants	(Nizami et al., 2012)
	One stage thermophilic CSTR ^(*)	12	3 to 4	46–63	55	405 L/kg VS 95 % VS destruction	Lin et al., 2019 indicated economic unfeasibility due to longer HRT and higher capital cost arising for cost of digester	(Lin et al., 2019; Voelklein et al., 2016)
Two-phase AD [Fig. 3c]	TP-AD ^(*)	2	–	20	TM-55 MS-38	368 L/kg VS 74 % VS destruction	Janesch et al., 2021 reviewed the non-competitiveness of multi-phase AD due to higher investment and operational costs (6% more costly than single phase AD). This is highly dependent on feedstock type and there is the potential to overcome the costly gap if the biomethane yield and productivity can be boosted. Recent trends suggest the utilization of more multi-phase processes in biogas industry and indicate the potential for feasibility when done right	(Orozco et al., 2013)
	TP-AD ⁽²⁾	12	–	20		235 L/kg VS		(Dooms et al., 2018)
	SLBR-UASB ^(*)	14	–	36	37	330 L/kg VS		(Singh et al., 2011)
	SLBR-UASB ^(*)	11	–	30	37	230 L/kg VS 61 % VS destruction		(Wall et al., 2016)
	SLBR-UASB ^(*)	11	–	30	37	341 L/kg VS 75 % VS destruction		(Nizami et al., 2012)
Pre-treatment for AD [Fig. 3d]	NaOH Alkali-thermal at 100 °C ^(*)	5	–	15–20	35	359–452 L/kg VS 77–97 % VS destruction	Baral and Shah, 2017 indicated the cost of pre-treatment embodies about 19–22% of the operating expenses. This forms a significant portion of process cost and renders most pre-treatments unfeasible	(Xie et al., 2011)
	H ₂ SO ₄ Acid -thermal at 135 °C (Late-cut silage)	2	–	30	37	304 L/kg VS 61 % VS destruction		(Deng et al., 2019)
	Hydrothermal pretreat at 200 °C (Napier grass)	–	4.0	–	35	248 L/kg VS 48 % VS destruction	without effective bioenergy production. However, alkali pre-treatment at mild conditions has been found to be cost-effective	(Phuttaro et al., 2019b)
	Ionic liquid pretreat at 120 °C (Fresh grass)	–	–	35	35	221 L/kg VS 60 % VS destruction	at the pilot scale level (Banu J et al., 2021)	(Li and Xu, 2017)
	Ca(OH) ₂ Alkali pretreat at 10 °C (Landscape grass)	–	–	30	37			(Khor et al., 2015)
STEAME Concept [Fig. 2]	Joint Alkali Pretreat Bioaugmented TP-AD ^(*) (late-cut silage)	10	1 to 4	less than 20	TM-55 MS-38	442 L/kg VS 90 % VS destruction		As modelled in this study

(*) First-cut grass silage utilized for AD; ⁽¹⁾ Recirculation of digestate liquor; ⁽²⁾ Grass silage and cattle slurry mixture; SC (solid contents); SLBR-UASB (Sequential Leach Bed Reactor and Upflow Anaerobic Sludge Blanket); TM (Thermophilic phase); MS (Mesophilic phase).

combination of the alkali pre-treatment and TP-AD system reduces the HRT for the biomethanation process to below 20 days as compared to 30–60 days for conventional AD plants. This significantly increases productivity and income, hence compensating for any additional operational cost from operating the TP-AD system. Furthermore, heat energy can be recovered in the TP-AD system since the mesophilic phase operates about 15–20 °C less than the thermophilic phase. The recoverable heat can be used to further reduce the heat energy demand of the thermophilic phase and could be achieved with recirculation of digestate liquor mixed with pretreated biomass.

The economic cost of bioaugmentation has been considered a barrier to its implementation in the biogas industry. However, the recent application of bioaugmentation using low-cost enzymes in pilot biogas plants at companies such as DuPont, Veolia and Dong Energy suggest its feasibility and an economic advantage. A recent study by Jiang et al., 2020 indicated the possibility of low-cost bioaugmentation that significantly increased OLR and led to greatly improved biomethane production from food waste. The economic analysis of the bioaugmented food waste AD process indicated a 95% increase in net income as compared to the non-bioaugmented AD (Jiang et al., 2020). The main cost of the bioaugmentation process was due to the operational cost of the cultivating media. This accounted for about 94% of the total expenses of the bioaugmented process and could potentially be reduced by utilizing cheaper media or industrial by-product streams to cultivate the microbes.

Another important parameter in AD process operations is feedstock preparation such as particle size reduction of the feedstock prior to feeding to the AD process. This is a mechanical pre-process operation that is required for any potential grass biomethane plant. The type of chopper coupled with the resulting energy consumption and cost depends on the type of grass species and the required particle size of grass prior to the AD process. A study by Wall et al., 2015 indicated an optimum particle size of 1 cm or below is needed for effective biomethane production from grass silage while Phuttaro et al., 2019a suggested a particle size of 0.6–2.0 mm for optimal biomethane production from Napier grass. The smaller the particle size requirements, the higher the energy demand and cost, hence, it's always desired to operate the largest possible particle size without significantly adversely affecting methane production efficiency. Large scale macerators are generally used for size reduction in grass species to prevent floating and/or wrapping around mixers; the energy for this was stated to equate to 1.8% of parasitic energy in biogas production from grass (Smyth et al., 2009).

Thus, an assessment of the STEAME concept suggests the main investment cost will be attributed to the operational cost of alkali reagents and cultivating media for pre-treatment and bioaugmentation sections. This is advantageous because these are factors that can be optimized through low loading dosages to improve the overall profitability and feasibility of the STEAME concept. Assuming 10 kWh per 1 m³ of the produced biomethane (Wu et al., 2021), and from e-Supplementary material, 220 kWh t⁻¹ VS d⁻¹ can be obtained from methane productivity in the STEAME concept, this represents a significant increase (120% increase) in bioenergy recovery as compared to conventional AD system (calculation in e-Supplementary material). This is promising from an economic standpoint, however ongoing experimental studies and subsequent techno-economic analysis are essential to fully underscore the economic competitiveness of the STEAME concept.

4. Conclusion

Optimization of the biomethanation process can improve biogas yields, reduce required retention times and generate more energy for less cost. Bioaugmentation, pre-treatment and innovative reactor configuration are methods of individually improving biogas production, however, the authors' proposed STEAME system employs a cascading circular economy system including for alkaline pre-treatment prior to a TP-AD configuration to significantly increase feedstock biodegradability

while keeping HRT below 20 days. In grass biomethane, the STEAME concept as proposed by the authors has the potential to increase the methane yield by a target of 47% while doubling methane productivity as compared to conventional AD system.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.biortech.2022.126950>.

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