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Nkemka, Valentine

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LUND UNIVERSITY

PO Box 117
221 00 Lund
+46 46-222 00 00

Two-Stage Conversion of Land and Marine Biomass for Biogas and Biohydrogen Production

Valentine Nkongndem Nkemka

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Faculty of Engineering, LTH
Lund University
Sweden

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Two-stage conversion of land and marine biomass for biogas and biohydrogen production

Abstract

The replacement of fossil fuels by renewable fuels such as biogas and biohydrogen will require efficient and economically competitive process technologies together with new kinds of biomass. A two-stage system for biogas production has several advantages over the widely used one-stage continuous stirred tank reactor (CSTR). However, it has not yet been widely implemented on a large scale. Biohydrogen can be produced in the anaerobic two-stage system. It is considered to be a useful fuel for the future due to its high energy density and clean combustion with the emission of only water vapour. Anaerobic digestion can be used to treat wastewater and for energy production, leading to a reduction in eutrophication and greenhouse gases. The material remaining after treatment can also be used as a fertiliser as long as certain standards are met. The production of biogas and biohydrogen from a range of land and marine biomasses was studied in this work. The reduction of the heavy metal content of seaweed was also studied in order to improve fertiliser quality.

Two-stage, dry anaerobic digestion of mussels, reeds, seaweed, solid cow manure, and a mixture of seaweed and manure was studied. The system consisted of a leach bed reactor for hydrolysis and an upflow anaerobic sludge blanket (UASB) reactor for methane production. The results showed that mussels with shells, seaweed, and the seaweed and manure mixture were efficiently digested in the two-stage system; 68 to 83% of the methane being produced in the UASB reactor. The manure by itself, and reeds, which are slowly degradable, were efficiently digested in the one-stage dry leach bed process, in which most of the biogas was produced. Seaweed and manure can also be co-digested in the one-stage dry digestion process, since methanogenic conditions prevailed in the leach bed reactor, thus reducing the cost of operating two biogas reactors. Technically, both the new feedstocks and the one- and two-stage dry anaerobic systems have great potential for biogas production. However, economic evaluations are needed to validate practical applicability.

The removal of heavy metals from seaweed hydrolysate was studied in the two-stage system. The heavy metals Cd, Cu, Ni and Zn were adsorbed using iminodiacetic acid Cryogel® carriers. However, removal of the heavy metals resulted in low methane yields, possibly due to the removal of micronutrients needed for anaerobic digestion. It is therefore suggested that the metals be removed after methane production in a UASB reactor. Alkaline and autoclave post-treatment of the seaweed digestate resulted in 86% organic matter solubilisation and the leachate may be treated in a UASB reactor, providing a means of handling digestate with high heavy metal content. Co-digestion of leachates from the leach bed reactor and the post-treatment resulted in a high methane yield, 0.34 l/gVS_{added} in a batch test. Subsequent treatment of the leachate from the leach bed reactor resulted in a high methane productivity at a loading rate of 20.6 g COD/l·day in a UASB reactor. Treatment of the seaweed leachate in the UASB reactor resulted in a stable process without the need for additional nutrients or buffer. As the seaweed leachate was rich in nutrients and buffer capacity, its co-digestion with wheat straw hydrolysate in the UASB reactor resulted in a stable process.

Biohydrogen and biogas were co-produced from wheat straw hydrolysate in a two-stage system consisting of a CSTR and a UASB reactor, employing the thermophile, *Caldicellulosiruptor saccharolyticus* in the first H_2 reactor. Straw hydrolysate was efficiently produced by acid-catalysed steam and enzyme pretreatment, giving a 95% sugar yield of the theoretical yield. High biofuel production rates of 1.8 to 3.5 l H_2/l·day and 2.6 to 4.0 l CH_4/l·day were obtained under stable operational conditions and treatment efficiencies. However, the cost of nutrient supplementation was high, and cheaper nutrient sources will be required to make the production cost economically competitive.

This research has demonstrated the versatility of a two-stage system that allowed the digestion of new kinds of biomass such as seaweed with sand, mussels with shells, reeds, manure and wheat straw. It has also been shown to be possible to remove heavy metal from seaweed to improve fertiliser quality. High hydrogen and methane production rates were also demonstrated, and the two-stage anaerobic system is thus, technically, a promising reactor configuration for the production of biofuels.

Key words

anaerobic digestion; cadmium; *Caldicellulosiruptor saccharolyticus*; biohydrogen; biogas; dry digestion; steam pretreatment

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To the Nkemka family

Abstract

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Popular scientific summary

Peter is a part-time cocoa farmer who produces cocoa beans that are used for chocolate production. However, he has always wondered about the efficient utilisation of the residual cocoa pods, which seem to contain sugar as they attract bees. He discussed this with a student studying a biological process whereby organic material can be converted to biogas and a residue that can be used as a fertiliser.

This student was studying interesting ways of producing renewable sources of energy such as biogas and biohydrogen from organic materials on land and in the sea, and also a new method of improving fertiliser quality. Abundant marine organic materials such as mussels, reeds and seaweed, which do not compete with food grown on agricultural land, were used to produce biogas. Manure, which is an abundant waste resulting from intensive animal farming, was also used for biogas production. The biogas production system consisted of an anaerobic two-tank system. The reason for using a two-tank process is to separate the fast-growing microbes that break down complex organic material from the slow-growing microbes that produce biogas. In this process, both groups of microbes can grow better than if they were mixed in the same tank. The results demonstrated that biogas production in a two-tank process was efficient for mussels including the shells, seaweed, and a mixture of seaweed and manure, as most of the biogas was produced in the second reactor. In the case of the mussels, the shells remained in the first tank and were then easily removed. In addition, the digestion of a mixture of seaweed and manure reduced the effects of toxic substances such as sulphate and ammonia present at high concentrations in each of these materials. On the other hand, biogas production from the reeds or the manure alone was not efficient in the two-tank process since they degrade slowly. Hence, a one-tank process, which is a simple system to operate (even on farm-scale) could be cost effective for reed digestion.

Biogas production from seaweed and the reduction of contaminating heavy metals were also studied. As the seaweed contains high levels of heavy metals, the digested residue can not be used as a fertiliser. Biogas production and removal of the heavy metals were performed in the two-tank biogas process. During the breakdown of organic matter, the liquid produced ferments or sours, due to acid production. This process favours the release of metals, which can easily be removed. Removal of the metal was performed with a sponge-like material called Cryogel®, which is highly porous and has special metal-binding sites. The resulting liquid, with low heavy metal content, was used for biogas production. It was found that biogas can be produced from seaweed, and that the seaweed liquid, which was rich in nutrients, can replace the nutrients and buffer that are usually added to biogas processes. The heavy metals could be reduced using the two-tank system, but more research is needed before the residue is used as a fertiliser.

Finally, biogas and biohydrogen were produced from wheat straw, which is an abundant agricultural residue that does not compete with food cultivation. Since straw degrades slowly, and has a structure similar to that of reeds, the material was first treated to release the sugars into a liquid. The liquid was then used for biohydrogen production, and the resultant waste from this process was in turn used for biogas production, thus using most of the sugars contained in the liquid. Biohydrogen is produced in a similar, but incomplete process like biogas. The processes were very efficient, resulting in high production rates of biohydrogen and biogas. The only emission from the combustion of hydrogen is water vapour, and the addition of a small amount of hydrogen during the combustion of methane significantly enhances combustion. Hence, the production of such fuels from cheap renewable resources will be very beneficial for the environment and reduce climate change.

In conclusion, exploring land and marine organic materials and the pretreatment of slowly degrading materials can increase biogas production. In addition, the two-tank biogas process was versatile in handling a wide range of organic materials, and can be optimised for the combined production of biohydrogen and biogas. This system also offers the possibility of heavy metal removal to improve fertiliser quality.

The student's advice to Peter was, thus, to use the cocoa pods for biogas production; providing renewable energy to dry his product especially during the rainy season, avoiding the use of firewood. The residue from the biogas process can also be used to improve vegetable production in Theresia's farm hence, providing enough vegetables for the family.

Preface

The experimental studies presented here were carried out to investigate biogas and biohydrogen production from terrestrial and marine biomass, as well as a novel method for the removal of heavy metals in order to improve the quality of fertiliser obtained from the residue. This thesis gives a summary of my research.

Financial support was received from the Swedish Energy Agency (Energimyndigheten) and the Swedish International Development Cooperation Agency (SIDA), which are both acknowledged.

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The Legion of Mary Praesidium in Lund is acknowledged, especially Anna, Henry, Krystyna, Mary and Rev. Fr. Anders.

Most importantly, I can not thank Almighty God enough for all His marvellous deeds.

List of papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. **Nkemka, V.N.,** Arenales-Rivera J., Murto M.
Co-digestion of seaweed and manure: An evaluation of a dry anaerobic digestion configuration, *Submitted to Journal of Environmental Management*

II. **Nkemka, V.N.,** Murto, M., 2013
Two-stage anaerobic dry digestion of blue mussels and reeds, *Renewable Energy 50, 359-364*

III. **Nkemka, V.N.,** Murto, M., 2012
Exploring strategies for seaweed hydrolysis: Effect on methane potential and heavy metal mobilisation, *Process Biochemistry, doi: 10.1016/j.procbio.2012.06.022*

IV. **Nkemka V.N.,** Murto, M., 2010
Evaluation of biogas production from seaweed in batch tests and in UASB reactors combined with the removal of heavy metals, *Journal of Environmental Management, 91, 1573-1579*

V. **Nkemka V.N.,** Murto M.
Biogas production from wheat straw: The roles of pretreatment and seaweed hydrolysate as a co-substrate, *Accepted for publication in Bioresource Technology*

VI. Willquist, K., **Nkemka, V.N.,** Svensson, H., Pawar, S., Ljunggren, M., Karlsson, H., Murto, M., Hulteberg, C., van Niel, EWJ., Lidén, G., 2012
Design of a novel biohythane process with high H_2 and CH_4 production rates, *International Journal of Hydrogen Energy, doi: 10.1016/j.ijhydene.2012.08.092*

My contributions to the papers

Paper I: I performed parts of the experiments, and Jorge the other parts. I wrote the major part of the manuscript.

Paper II: I performed the experiments and wrote the major part of the manuscript.

Paper III: I performed the experiments and wrote the major part of the manuscript.

Paper IV: I performed the experiments and wrote the major part of the manuscript.

Paper V: I performed the experiments and wrote the major part of the manuscript.

Paper VI: I performed the pretreatment and the methane production experiments, and also read and commented on the manuscript. Karin, assisted in some of the methane production experiments.

Abbreviations

AD: anaerobic digestion
CHP: combined heat and power
COD: chemical oxygen demand
CSTR: continuous stirred tank reactor
DF: dark fermentation
GHG: greenhouse gas
HMF: hydroxymethylfurfural
HRT: hydraulic retention time
IDA: iminodiacetic acid
OLR: organic loading rate
sCOD: soluble chemical oxygen demand
SRB: sulphate reducing bacteria
TS: total solids
UASB: upflow anaerobic sludge blanket
VFA: volatile fatty acids
VS: volatile solids
ww: wet weight

Contents

1. Introduction

Anaerobic digestion (AD) occurs spontaneously in oxygen-free environments such as wetlands, paddy fields and landfills, as well as in the gut of ruminants and some insects such as termites. In past decades, this process has been explored as a method of solid waste and wastewater treatment in municipal wastewater treatment plants [1-2]. The process of AD is now being modified in order to help meet the increasing energy needs of the world today [3-4]. Fossil fuel depletion and the detrimental effects of its utilisation on the environment are the driving forces behind renewable energy production. In addition, EU policies such as the reduction of landfilling of organic waste by 65% by 2016 [5] and the increase in domestic renewable energy consumption by 20% in 2020 [6] have given additional impetus. Consequently, there is a fast-growing market for biogas not only in the EU, but worldwide [3].

AD is a commercialised process that offers a benign and relatively cheap method of treating organic waste and wastewater. It can also be used to convert energy crops to renewable sources of energy in the form of biogas. Other advantages include nutrient recycling and reductions in eutrophication and greenhouse gas (GHG) emissions [7]. Biogas and the digested residue or liquid, that can be utilised as a fertiliser, are the two main products of AD. Biogas is composed of about 60% CH_4, 40% CO_2 and trace amounts of other gases such as H_2S and water vapour. The gas can be used directly for cooking or combined heat and power (CHP) generation, or upgraded to at least 95% CH_4 (biomethane) and injected into the natural gas grid, as is a current practice in Germany, Sweden and Switzerland [3]. Alternatively, biomethane can be compressed and used more efficiently as a vehicle fuel [8], and this is becoming popular especially in Sweden. Furthermore, biomethane can be liquefied, facilitating its transport. Combustion engines utilising biomethane are less noisy and produce less emissions than those based on fossil fuels [9].

The effluent, or digestate, of a biogas process is rich in nutrients and can be used as a fertiliser. This is true for manure and other wastes with tractable sources, which have low or acceptable levels of contaminants [7]. However, sewage sludge, for example, is heterogeneous and may contain high levels of contaminants such as heavy metals, and its composition must, therefore, be determined before the digestate can be applied as a fertiliser [10]. Hence, reducing the content of pollutants in the digestate is vital for the sustainable recycling of nutrients to agriculture. One aspect of the present work was the improvement of seaweed digestate quality, since seaweed contains high levels of toxic heavy metals, especially Cd.

Biohydrogen is another renewable fuel that can be produced by the AD of carbohydrate-rich materials. It can be produced in a modified anaerobic two-stage process, in which the first hydrolytic stage, or dark fermentation (DF), is optimised for H_2 production. Today, 88% of H_2 is produced from fossil fuels and 4% from the electrolysis of water [11]. Hence, there is growing interest in renewable H_2 production through biological processes such as DF and photobiological processes. In the latter processes, microalgae or photosynthetic bacteria harness solar energy using water or organic compounds, respectively. Photobiological H_2 production is limited by many practical and fundamental factors such as the efficient use of sunlight and complex reactor designs [12]. Biohydrogen production by DF, on the other hand, is rapid and simple, and any carbohydrate-rich organic waste can be utilised [13]. Renewable H_2 can also be produced using a modified fuel cell [14]. Hydrogen is regarded as a fuel for the future as its combustion is very efficient and the only emission is water vapour (i.e. zero emission). The combustion of biohythane (a mixture of H_2 and CH_4) with a low C/H ratio, produces lower CO_2, CO and nitrous oxide emissions than the combustion of methane [15]. There is also a demand for H_2 in the chemical industry for mineral fertiliser production [11].

In the present work, strategies for improving biogas and biohydrogen production were investigated, including the use of new types of biomass, and a two-stage AD process. The use of marine biomass (mussels, reeds and seaweed), which does not compete with agricultural land, was evaluated for biogas production. Biogas production from manure was also studied, as it is an abundant material resulting from intensive animal farming. In addition, wheat straw, which is a cheap and abundant lignocellulosic material and does not compete with food for biofuel production, was used for biogas and biohydrogen production. The two-stage anaerobic configuration used in this research has been reported to offer benefits such as improved process stability, high methane production rates and yields, the possibility of high organic loading rates (OLRs) and a low energy demand [16-18]. Furthermore, leach bed reactors in a two-stage system are useful for the digestion of solid organic materials that seldom require pretreatment.

1.1. Scope of the thesis

The objective of the work described in this thesis was to experimentally explore the benefits of two-stage AD. The research carried out is summarised in Figure 1. In particular, the two-stage AD of marine and terrestrial biomass for biogas and biohythane production was examined. The separate digestion and co-digestion of seaweed and solid cow manure were evaluated using the two-stage AD configuration (Paper I). The two-stage system was also used to evaluate the AD of mussels with shells and reeds (Paper II). Improvement in the quality of seaweed digestate as a fertiliser using iminodiacetic acid (IDA) Cryogel® carriers to remove heavy metals was also assessed. The studies presented in Papers III and IV describe the hydrolysis of seaweed and heavy metal mobilisation, and also the effect of heavy metal removal on methane yield during the treatment of seaweed hydrolysate in a UASB reactor. Biogas and biohythane production from acid-catalysed steam- and enzyme-pretreated wheat straw were also studied (Papers V and VI).

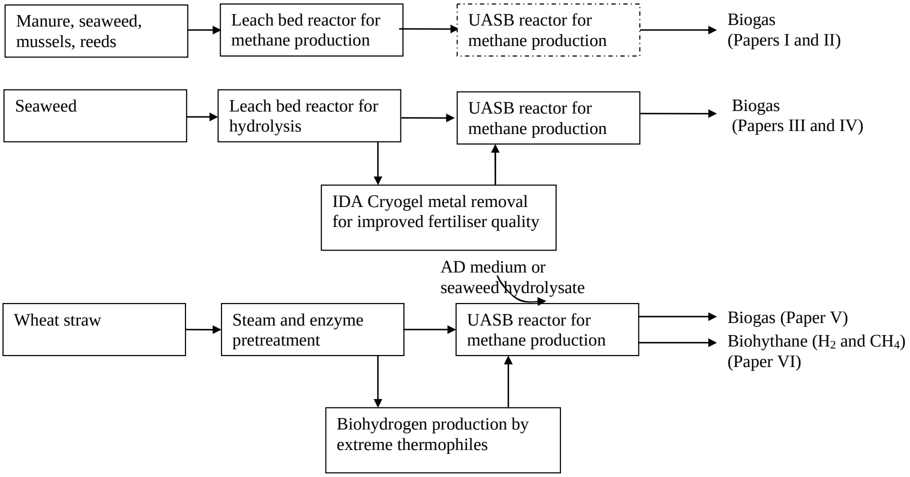

Figure 1. Schematic diagram showing the processes studied. Rectangles with solid lines represent major routes and broken ones alternative routes.

2. Feedstocks

The characteristics of the feedstock are important in the design of a biogas process. These are examined in this chapter.

2.1. Seaweed

Beach cast seaweed in Trelleborg, southern Sweden, is as a result of eutrophication of the Baltic Sea (Figure 2). Not only does it create a significant waste handling problem for the municipality, but prevents the beaches from being used for recreational purposes due to the smell and reduced access. Incineration could be used to deal with this seaweed, but that would be expensive due to the high water and ash content [19]. At the moment, the seaweed is collected by the municipality during the spring and summer, stored under controlled conditions, and then returned to the beach in the autumn and winter.

Figure 2. Cast seaweed on the beach at Trelleborg on the coast of Scania, southern Sweden, September 2007 (Photo: M. Murto)

The municipality has conducted pilot-scale trials for the production of biogas from this material as a means of waste treatment, while producing renewable energy [20]. Some sand is collected together with the seaweed, and the accumulation of sand in the wet digestion process using a CSTR can be problematic. An attractive alternative is, thus, to digest the seaweed in a two-stage dry digestion system, where the sand can be easily removed from the first stage leach bed reactor. A major drawback of using seaweed for biogas production is its high heavy metal content, which can limit the use of the residue as a fertiliser. Another disadvantage is the high sulphate content (see Section 2.6).

The seaweed studied in this work had a low P content compared to manure (Paper I), implying that more seaweed would have to be added to farmland in order to reach the Swedish standard for fertilisers, which is based on guideline values of 100 mg Cd/kg P or 2 mg Cd/kg total solids (TS) [21-23]. Hence, there is a risk of the application of high concentrations of heavy metals such as Cd. Jogbratt (2011) reported values of 1.84 mg Cd/kg TS (245 mg Cd/kg P) in mixed seaweed collected from the west coast of Sweden, rendering it unsuitable as fertiliser [24]. Furthermore, the concentrations of heavy metals would be even higher per unit TS after AD due to the reduction in the amount of organic matter. The challenge therefore lies in reducing the concentration of heavy metals so that the nutrient-rich digestate can be recycled on farmland.

2.2. Mussels

Mussels are filter feeders and can accumulate nutrients. The cultivation of blue mussels (*Mytilus edulis*) has been reported to be an effective method of nutrient removal from the Baltic Sea [25]. Blue mussels have been cultivated in the Kalmar Strait in an attempt to reduce eutrophication. Due to the very small size of these mussels, they are not suitable as food, but can be used for biogas production. Therefore, the AD of these mussels could provide both renewable energy and a biofertiliser, however, the high costs of cultivation, harvesting and transport are the main hurdles [25]. AD of mussels with shells would cause problems in wet digestion systems, and two-stage dry digestion could, therefore, be an option.

2.3. Reeds

Eutrophication also leads to the establishment and growth of reeds (e.g. *Phragmites australis*) around the coastlines, hindering beach access. Harvesting these reeds could improve recreation along the beaches, and provide renewable energy through biogas production. The common reed has been reported to be a suitable candidate for renewable energy production using dry anaerobic digestion due to its high energy density, i.e. TS content [26]. The high TS content can also reduce the cost of transport. However, reeds are not found to be efficient for nutrient removal from eutrophied waters [25]. Another problem associated with using reeds for biogas production is the high cost of harvesting due to the need for special equipment and the difficult terrain. Moreover, reeds are lignocellulosic in nature and degrade slowly under anaerobic conditions [27].

2.4. Manure

Large amounts of manure are generated by intensive animal farming. This manure poses a waste handling problem due to the smell, its potential to cause GHG emissions, eutrophication and the spread of pathogens. For these reasons, the Nordic EU countries have legislation that ensures effective manure management, such as stabilisation in storage facilities for six months before use as a fertiliser [7]. Manure stabilisation requires a large amount of space and can thus be costly. The use of manure for biogas production has been reported to be a good method of manure management, as it reduces eutrophication effect and GHG emissions, kills pathogens and also provides a nutrient-rich fertiliser and renewable energy [7].

A major concern associated with the use of manure as a feedstock in the biogas process is the high transport cost, which is due to the high water content. In the region of Scania in southern Sweden, where the present research was performed, pipeline transport of manure has been evaluated in a preliminary study [28]. This could allow the transport of manure and digestate between farms, and a centralised biogas plant might reduce the production cost. Outdoor grazing during summer can be another hindrance, which reduces the volume of manure available for biogas production. In the current work, the suitability of dry anaerobic digestion of solid cow manure was studied (Paper I).

2.5. Wheat straw

Wheat straw is an abundant and cheap lignocellulosic agricultural material which does not compete with food for biofuel production. However, this material is difficult to degrade to soluble sugars. Lignocellulosic materials have a complicated structure of interwoven complexes of cellulose, hemicellulose and lignin. The crystalline nature of lignocellulose also prevents access by hydrolytic enzymes [29]. The structure of wheat straw is similar to that of reeds. Pretreatment is therefore needed to hydrolyse the sugars bound in the straw, which can result in a liquid suitable for biohydrogen and biogas production (Papers V and VI).

2.6. Nutrient composition of the feedstocks

The nutrient contents of seaweed, solid cow manure, reeds, mussels and wheat straw are presented in Papers I, II and V. Biogas processes have special requirements regarding macro- and micronutrients, as well as the alkalinity or buffering capacity, as discussed in Section 3.2.1. The C/N ratios of wheat straw and reed were found to be high (47.7 and 32.5, respectively), while those of seaweed and mussels were low (9.2 and 7.4, respectively). The C/N ratio of solid cow manure was about 16.9, close to the optimum ratio of 25 recommended for AD processes [30]. Thus, co-digestion using these materials could provide a means of balancing the C/N ratio. The micronutrients Co, Fe, Ni, Mo, W and Se were comparable in wheat straw, solid cow manure and seaweed, and were around the levels recommended for AD processes [31-32]. Pretreatment of the wheat straw, however, resulted in the loss and dilution of these nutrients, which could be costly for the entire AD process due to the need to add nutrients. The concentration of Ca in seaweed (5.62 g/kg wet weight ((ww)) was found to be high, and is a component of calcium carbonate. This compound dissolves in water forming bicarbonate ion, which can reduced the need to add buffering compounds to biogas reactors.

The seaweed, however, contained high concentrations of heavy metals, which might prevent its use as a fertiliser. The concentration of toxic heavy metals such as Cd was higher in the seaweed than in the manure and straw (Paper I).

The sulphur content of seaweed was also high, 1.84 g/kg ww. High S content is detrimental in AD, as it favours the domination of sulphate reducing bacteria (SRB) and the production of H_2S, which inhibits the biogas process. Co-digestion of seaweed with other organic materials could, therefore, offer a means of avoiding the problems associated with a high S content. The concentration of Na was high in both the manure (1.2 g/kg) and the seaweed (1.9 g/kg), but low in the straw (0.2 g/kg). A Na ion concentration of 3.0 to 16.0 g/l has been reported to cause 50% inhibition in AD in the absence of other nutrients or salts [33-34].

In conclusion, seaweed and manure may constitute cheap sources of nutrients for the biogas process, but seaweed must be handled with care as it may contain high concentrations of heavy metals. Co-digestion of these materials may afford an efficient and stable AD process.

3. Process concept

The concept of AD, including the two-stage system, pretreatment, heavy metal removal and co-production of biogas and biohydrogen, is discussed in this chapter.

3.1. Anaerobic digestion

A series of metabolic reactions occurs during the anaerobic conversion of organic matter to CH_4 and CO_2, which can be summarised in four main steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Figure 3).

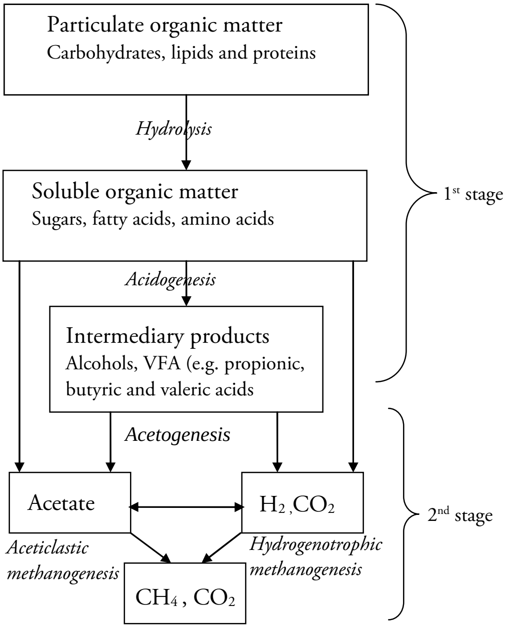

Figure 3. Schematic illustration showing the two-stage anaerobic digestion process.

During hydrolysis, facultative anaerobes secrete extracellular hydrolytic enzymes that break down carbohydrates, fats and proteins into their respective monomers. These monomers are in turn converted during acidogenesis into alcohols and short-chain volatile fatty acids (VFAs) such as acetic, propionic and butyric acids. Fermentation is carried out by a spectrum of obligate and facultative anaerobic bacteria. This is the fastest step in the degradation of easily hydrolysable organics, and the main pathway is via acetate, CO_2 and H_2 [2, 35]. Reduced fermentation intermediates, called electron sinks, can also be formed in another pathway. The accumulation of electron sinks (lactate, ethanol, propionate, butyrate and higher VFAs) is a bacterial response to high hydrogen concentration in the system [36]. The concentration and relative amounts of VFAs produced during this phase are essential indicators of the overall performance of the AD process.

Obligate hydrogen-producing bacteria convert the electron sinks produced during acidogenesis into additional acetate, CO_2 and H_2. This step is vital in degradation since the methanogens can not utilise the electron sinks directly. Acetogens thrive only in an environment with a low partial H_2 pressure. This is possible when there is a well-functioning syntrophic relationship with the H_2-consuming methanogens [36-38].

In the final step, methanogens (*Archaea*) utilise the acetate, CO_2 and H_2, and sometimes other products such as methanol, methylamine and formate, to produce CH_4 and CO_2. About 70% of the CH_4 is produced via the aceticlastic pathway by only a small group of methanogens [39]. However, not all microbes are capable of producing CH_4 through the hydrogenotrophic pathway. The latter pathway is beneficial in reducing the partial H_2 pressure, which is favourable for the acetogens. Gas sparging has been reported to maintain low H_2 pressures during DF, resulting in improved H_2 production [40]. Alternatively, some species of H_2 producers, such as the extreme thermophilic *Caldicellulosiruptor saccharolyticus* can tolerate high H_2 partial pressures [41]. The hydrogen-utilising methanogens are amongst the fastest-growing microbes with a generation time of 6 h, while some slow-growing acetate-consuming methanogens have a generation time of 1 to 12 days [16, 35]. In addition, aceticlastic methanogens are more sensitive to environmental changes than the hydrogen-consuming methanogens [42].

3.1.1. Process parameters

Operating parameters that influence AD include temperature, nutrients, pH and alkalinity, toxins, hydraulic retention time (HRT) and organic loading rate (OLR). These are described below.

Full-scale biogas plants are usually operated under mesophilic (25 to 40 °C) or thermophilic (45 to 55 °C) conditions. Mesophilic processes require lower amounts of nutrients and are less sensitive to toxic compounds. Thermophilic processes, in contrast, can lead to more pathogen destruction and methane production, but require more nutrients and are more sensitive to toxic substances [43].

Both micro- and macronutrients are required for efficient AD. In addition, methanogens need key micronutrients such as Co, Fe, Ni, Mo, W and Se [31, 44]. Nutrient deficiency can be solved by the simultaneous AD of several organic materials (co-digestion) [1, 22]. The optimum pH for acidogens and methanogens is about 6 and 7, respectively [45]. Bicarbonate is often the main buffering species [37] and the range of alkalinity recommended to maintain a suitable neutral methanogenic pH in a biogas digester is 2 to 4 g $CaCO_3$/l [46]. Some raw materials, such as seaweed, manure and mussels, have inherent alkalinity and their co-digestion with carbon-rich organic material can provide a feasible alternative to improve the buffering capacity.

Methanogens are sensitive to toxic substances, but this situation can be reversed under favourable conditions. Indicators of toxicity in AD include the production of H_2, low methane yield, low pH and alkalinity, as well as the accumulation of VFAs. Several substances can exert toxic effects on the microbes in AD, for instance, NH_3, HCN, H_2S, long-chain fatty acids, heavy metals and other aliphatic and aromatic compounds [47].

The HRT and OLR are operational parameters that determine the feeding rate of a biogas digester, and these have to be controlled in order to avoid hydraulic and organic overload, which may lead to process failure due to wash-out of microbes or VFA accumulation. The HRT is the time the liquid feed is retained in the reactor, while OLR is the amount of organic matter added per unit reactor volume per unit time. Biogas processes are usually operated below the optimum loading rates, although they can accommodate higher loads under stable conditions [37]. The reason for this is to ensure a safety margin, since process recovery can be time consuming and costly. The application of higher OLRs requires online process monitoring and control for safe operation [48].

3.1.2. The two-stage process using a leach bed and UASB reactor

The techniques used for biogas production can be classified according to whether they are dry or wet, single-stage or two-stage, batch or continuous, or combinations thereof [17, 35]. Biogas processes that are operated with a TS content of less than 15% are classified as wet processes, while those with a TS content ranging between 15 and 35% TS are categorised as dry digestion processes [17-18]. The advantages of dry over wet processes are the low costs of handling and heating, and higher loading rates. In a one-stage process, all the microbial processes: hydrolysis, acidogenesis, acetogenesis and methanogenesis, take place in a single reactor. In the two-stage process, hydrolysis and acidogenesis take place mainly in the first reactor, while acetogenesis and methanogenesis take place in the second, methane reactor.

Acidogens and methanogens differ significantly in their physiology, nutrient requirements, growth kinetics and sensitivity to environmental conditions [45], and separating these microbes in hydrolytic and methane reactors has been reported to be a good optimisation strategy [16, 45, 49]. Both one- and two-stage processes can be operated as a wet or dry process, or a combination of the two. In batch processes, the organic material is loaded and allowed to digest for a long period of time until the biogas production reaches a preset minimum. Batch processes are common in one-stage dry digestion. Continuous dry digestion processes operated in plug flow mode also exist [50]. Continuous processes are either fed continuously or less frequently (semi-continuous), as in the case of the one-stage conventional CSTR.

In a two-stage process, organic matter is broken down into soluble organics, mainly VFAs. These soluble organics are subsequently treated in a methane reactor [4]. In this set-up, hydrolysis is efficient and methane production can be performed at high OLRs, hence, utilising the maximum reactor capacity. In practice, the two-stage system is not completely separated as some methane production occurs in the leach bed reactor. This is particularly so in the case of slowly degradable organic materials, and materials with a high buffering capacity [51]. Thus, the use of a leach bed reactor only can be an alternative operation unit for biogas production (Figure 4a).

Methane can be produced in the second stage using a CSTR, a methane filter, a UASB reactor, an expanded granular sludge bed reactor or another sequential leach bed reactor [17, 35]. In this research, a UASB reactor was used (Figure 4b). The formation and retention of granular anaerobic sludge is a unique feature of UASB reactors and, hence, they can accommodate high OLRs, methane production rates and organic matter degradation under stable operational conditions [4]. The granules offer protection to the more sensitive methanogens in the interior, while the less sensitive microbes are located around the periphery [52]. UASB reactors are used in the treatment of both dilute and high-strength wastewater that may contain toxic substances. Granular sludge retention over time, enables adaptation to toxic compounds and this technology is available on an industrial scale [53-54].

Benefits of the two-stage process include process stability, high methane production rates and yields, high OLR and low energy demand: as in the case of the dry AD leach bed coupled to a high-rate methane reactor [16-18, 35]. The one-stage dry digestion system also has its own intrinsic advantages such as the direct digestion of organic materials, requiring little pretreatment. One-stage dry digestion is also simple to operate, since the material is loaded less frequently, and the labour cost is thus lower [17].

The main disadvantages of the two-stage process compared to the widely used one-stage CSTR wet digestion process are the loss of inter species and the higher capital and operational costs associated with two reactors [17, 37]. These disadvantages have limited the implementation of the two-stage process in many full-scale processes. For these reasons, the one-stage dry digestion system has become popular as farm-scale biogas digesters in Germany [35], and full-scale dry digestion equipment such as that marketed by Dranco, Valorga, Linde and Kompogas are now available [50, 55-56]. The main drawbacks of the one-stage process are the long digestion time, especially for slowly degrading materials, and the fact that the process is preferred for the digestion of structured materials that facilitate liquid percolation. Co-digestion of municipal solid waste with compost, the latter acting as an inert material, has been reported to improve liquid recirculation and the general performance of the process [57]. Floating of materials such as energy crops in the leach bed reactor is another problem that has been reported in association with the one-stage process [50]. Moreover, uneven distribution of heat has also been reported in this one-stage system. A remedy to this problem is to aerate temporarily, leading to increase temperatures due to aerobic metabolism. Aeration technique also prevents the rapid acidification of the leach bed reactor when digesting easily degradable organic materials [50].

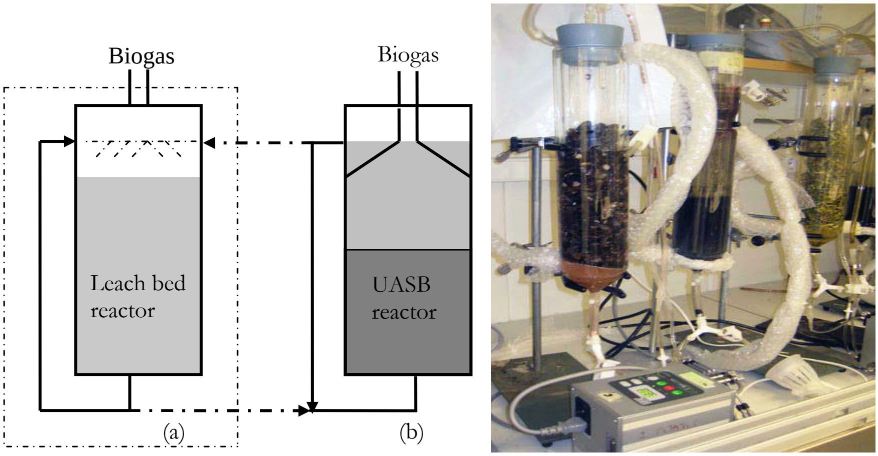

Figure 4. (a) A single-stage leach bed reactor for methane production, and (b) the UASB reactor used in the two-stage system. The photograph shows two-stage anaerobic digestion of mussels (left) and reeds (right).

3.1.3. Pretreatment for improved biogas production

Some form of pretreatment of the feedstock is usually required prior to digestion in a biogas reactor. This may involve the removal of unwanted inert materials such as plastics, sanitation (pathogen destruction), size reduction, dewatering in the case of very dilute waste streams, or dilution with water or other waste streams to facilitate pumping of materials with high TS content [35]. Another type of pretreatment is intended to improve the substrate biodegradability, and was studied in this research.

Lignocellulosic materials such as wheat straw have a complex interconnected structure of cellulose, hemicellulose and lignin. The degradation of lignocellulosic material is limited by cellulose crystallinity, the degree of polymerisation, the exposed surface area and the lignin and moisture content [58]. Lignocellulose can be degraded under anaerobic conditions, but requires a long digestion time, which can be expensive in a full-scale process [59]. Pretreatment is therefore performed to shorten the digestion time and speed up the conversion of the sugars to biofuels [58]. Several efficient methods of lignocellulose pretreatment have been reported, an example being steam pretreatment at high temperatures (160-240 °C) using a dilute acid catalyst followed by enzymatic hydrolysis [58, 60]. This treatment results in the dissolution of hemicellulose and then lignin, releasing the cellulose fibrils for further enzymatic hydrolysis. The goal of pretreatment is to obtain high sugar yields, preserve the sugars, and limit the formation of inhibitory compounds; it should also have a low energy demand and cost [61]. Although acid-catalysed steam pretreatment is efficient for the release of sugars, it results in the formation of inhibitory compounds such as furfurals, hydroxymethylfurfural (HMF), and levulinic and formic acid, which inhibit fermentative microorganisms [47, 58, 60, 62]. The operation of a steam pretreatment unit can be energy demanding, however, the use of heat exchangers and process integration with other processing steps in bioethanol production have been reported to reduce the energy demand [63]. Furthermore, system integration with other CHP industries that produce excess heat can provide a cheap energy source for steam pretreatment. Demonstration plants that use steam pretreatment techniques in bioethanol production from lignocellulose materials are operational or under construction, and their implementation will increase in the near future due to the need to increase biofuel production [60, 64].

Seaweed degrades easily, but its hydrolysis is incomplete. Brown seaweed, for instance, has a tendency to form insoluble calcium alginate gels, which limit the accessibility of hydrolytic enzymes [65]. Solubilisation of calcium alginate gels occurs at high sodium to calcium ion ratios and at temperatures above 100 °C [66]. Post-treatment of seaweed hydrolysis residue is therefore necessary for total solubilisation and conversion to biogas. The hydrolysis of seaweed in leach bed reactors and the post-treatment of the recalcitrant residue were studied in the present work (Paper III).

3.2. Heavy metal removal using IDA Cryogel

Heavy metals are defined as those with a density > 4.5 kg/dm^3 and atomic number 63.5 to 200.6 [67]. Some heavy metals are required at low concentrations for the metabolism of living organisms, although higher levels have detrimental effects on health and the environment. Some heavy metals are toxic; the threshold for toxicity varying among the metals [68]. Techniques for heavy metal removal include chemical precipitation, ion exchange, adsorption, membrane filtration, coagulation-flocculation, flotation and electrochemical methods [69]. The choice of method is dependent on the economy and efficiency, and combining two or more methods can reduce the heavy metal concentration to acceptable levels [69]. IDA Cryogel® carriers can be used to treat particulate wastewaters due to their porous structure, high mechanical stability and high binding capacity [70-72].

Hydrolysis and acidogenesis of organic matter result in low pH and the mobilisation of heavy metals [73]. The mobilised metals can then be removed from the hydrolysate before methane production (Figure 5). In the present research (Paper IV), IDA Cryogel prepared in Kaldnes carriers was used to bind and remove heavy metals from seaweed hydrolysate.

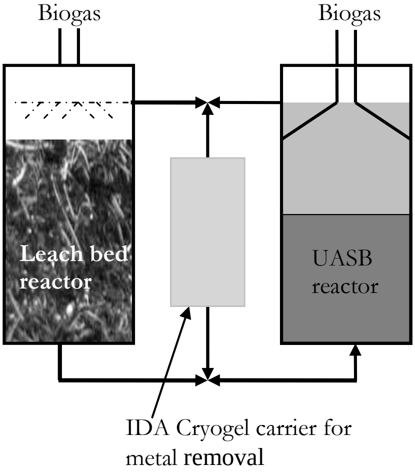

Figure 5. Heavy metal removal from seaweed in the anaerobic two-stage system.

IDA Cryogel carriers are produced by cryogelation of a mixture of monomers and an initiator. The mixture is poured into a glass cylinder filled with stacked Kaldnes carriers (mould) and then frozen rapidly. Most of the water freezes, but the rest of the substances are collected in non-frozen regions. It is in this region that gelation occurs. Upon thawing, the ice crystals that occupy most of the gel melt, leaving behind large interconnected pores with pore sizes between 1 and 100 µm (Figure 6) [70-71, 74]. The functional ligand, IDA, which is a typical ligand for immobilised metal affinity chromatography, is introduced prior to gelation to bind divalent metal ions. This gel can be regenerated and reused [71].

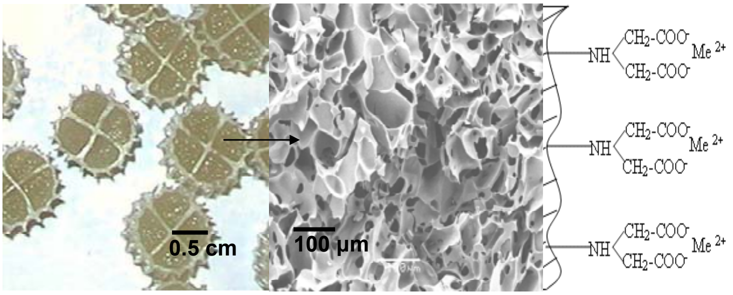

Figure 6. IDA Cryogel in Kaldnes carriers showing the principle of metal binding.

3.3. Biohythane production

Two-stage AD for hydrogen and methane (biohythane) production is similar to two-stage AD for biogas production. However, the first hydrolytic reactor is optimised for H_2 production (Figure 7). The theoretical yield for the conversion of hexoses to H_2 when growth is neglected is 33% [75]. This implies that 66% of the energy present in hexoses is trapped in acetic acid or other degradation metabolites and, hence, a second methane reactor is required to recover the remaining energy [76]. A high H_2 yield of 4 mol/mol of hexose is achieved when the degradation end product is acetic acid. But in practice, there can be the production of a mixture of butyric acid, with a yield of 2 mol/mol of glucose, and propionic acid, with a yield of 1 mol/mol of glucose. Other degradation end products such as lactic acid and ethanol do not result in H_2 production, however, these compounds contain energy that can be recovered in the biogas process.

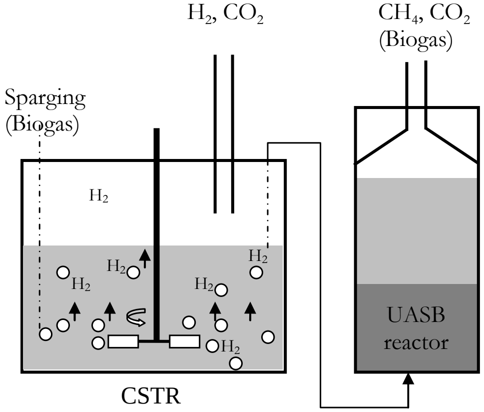

Figure 7. Co-production of biohydrogen and biogas.

The production of H_2 can be improved by heating or chemical inactivation of methanogens, pH control and use of specialised microorganisms [77-78]. In the present work (Paper VI), the extreme thermophile *C. saccharolyticus* was used for H_2 production, which produces a high H_2 yield as the end metabolite is mainly acetic acid. It can grow under high osmotic and partial H_2 pressure, and can also utilise complex carbohydrates, thus making it an interesting candidate for industrial applications [41]. However, gas sparging is needed to maintain a low H_2 partial pressure in order to achieve high yields and productivities. On the laboratory scale, N_2 is often used as a sparging gas due to its low cost, however, its separation from H_2 is difficult and costly on industrial scale. CO_2, on the other hand, can be easily separated from H_2, but has a detrimental effect on the growth of *C. saccharolyticus* [79]. The biohythane process was modelled by combining kinetic models of the fermentation steps (DF and AD) and the gas upgrading unit model. Gas upgrading by CO_2 removal was performed with an amine solution (40% methyldiethanolamine, 10% piperazine and 50% water, by weight). A techno-economic analysis of the biohythane process was performed on small scale process treating wheat straw at 2 tonne/h.

4. Two-stage dry anaerobic digestion of solid substrates

The AD of different kinds of substrate, seaweed, manure, mussels and reeds, were studied in a two-stage system and in a one-stage leach bed reactor.

4.1. Effect of substrate type

Seaweed, seaweed/solid cow manure, mussels with shells and reeds were digested in a dry anaerobic two-stage process (Papers I and II). The two-stage system consisted of a leach bed reactor and a UASB reactor (as described above). Solid manure was also digested in a dry one-stage leach bed process. The results showed that the two-stage system was efficient in the digestion of seaweed and mussels, since 68 to 80% of the methane was produced in the UASB reactor, while only 20 to 32% of the methane was produced in the leach bed reactor (Table 1). It was therefore beneficial to include a second UASB reactor, allowing for faster methane production. Similar results, of high methane production (56%) from a UASB reactor in comparison to a leach bed reactor, have been reported for maize, which is a widely used crop for biogas production [51]. The one-stage leach bed system was, however, efficient for the digestion of manure, seaweed/solid manure and reeds, since most of the methane was produced in this reactor. Operation of a single reactor can be beneficial, as the capital and operating costs are lower [17, 80]. Reeds have a higher energy density than the other materials investigated, and the methane yield based on ww was 5 to 16 times higher than those from seaweed, manure and mussels. The low methane yields based on ww of seaweed and manure were due to the high water content, while for the mussels it was due to the presence of shells. It therefore suggests that in the design of a biogas plant of these materials, transport cost has to be minimised as it may lead to a feasible process. High water content may also lead to the design of large reactors, hence, leading to higher capital cost.

Table 1. Two-stage anaerobic digestion of seaweed, solid cow manure, mussels and reeds.

	Seaweed	Solid cow manure	Seaweed/manure (1:1 g VS basis)	Mussels	Reeds	Maize [51]
Duration (days)	24	77	30	44	107	28
Methane produced in leach bed (%)	20	100	17	32	80	46
Methane produced in UASB reactor (%)	80	-	83	68	20	54
Total methane yield (l CH_4/g VS_{added})	0.16 ± 0.02	0.14 ± 0.01	0.11 ± 0.01	0.33 ± 0.03	0.22 ± 0.02	0.44
Total methane yield (l CH_4/g ww)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.16 ± 0.01	0.05

The two-stage system was very stable for the digestion of seaweed and mussels, both of which exhibited a fast rate of hydrolysis in the leach bed reactor, efficient solubilisation of solids, and efficient conversion of soluble organics to biogas, as evidenced by the low chemical oxygen demand (COD) concentration of < 1 g/l in the UASB reactor. Process stability of the two-stage system has previously been reported in the literature as a particular feature of this reactor configuration [17, 49, 81].

Ammonia inhibition can be a serious problem in the digestion of manure and mussels. Initial ammonia inhibition (139 mg/l) was experienced in the digestion of manure in the leach bed reactor which, however, was overcome by the lowering of pH as hydrolysis proceeded. Long-term treatment of these substrates may result in subsequent inhibition due to the accumulation of ammonia. Co-digestion of manure and seaweed resulted in a favourable process as the ammonia concentration was low, about 23 mg/l, and hence, co-digestion could be a cost-effective method to remedy ammonia inhibition. Ammonia is inhibitory to methanogens at concentrations of 100 to 150 mg/l [82], but depends on the degree of adaptation of the methanogens [83]. Furthermore, co-digestion of these substrates resulted in conditions that were favourable for methane production in the leach bed reactor, and the optimisation of this process could be considered in future research.

The two-stage system in this work proved to be a versatile system, enabling the digestion of mussels with shells and seaweed with sand. The shells and sand can be easily removed after hydrolysis and the hydrolysate circulated for methane production. The accumulation of sand in a CSTR has been reported in the digestion of the green algae *Ulva* [84], while shell removal may be costly if the mussels were to be digested in a CSTR.

The two-stage system was efficient for easily hydrolysable materials such as seaweed and mussels and this can permit faster methane production in methane high rate reactors, thus, exploring its full capacity when several leach bed processes are in operation [81]. The one-stage process, on the other hand, was efficient for the digestion of reeds which are slowly degradable, and for manure, which has a high buffering capacity. Co-digestion of easily hydrolysable materials such as seaweed and manure can enable their digestion in a one-stage process, hence reducing the capital and operating costs. The need of little of no pretreatment is an advantage of the leach bed reactor. However, the structure of the materials could reduce liquid percolation. For example, the structure of the leach bed was improved when seaweed was co-digested with manure (Paper I). In addition, long digestion times would be disadvantageous in the case of slowly degrading organic materials such as solid manure and reeds. Another problem observed was floatation of the reeds, which would result in dry zones, leading to poor digestion (Paper II). Ensuring a constant supply of feedstock to a biogas plant, as in the case of mussels, could be problematic due to unforeseen weather conditions. Despite the technical advantages offered by the one-stage dry digestion and the two-stage systems, economic analyses of the entire process are required to investigate the economic feasibility of these processes.

According to the International Energy Agency, the deployment of new and efficient biofuel conversion technologies and the exploration of new types of biomass are both important measures to decarbonise the transport sector [85]. Seaweed and algae have a high biomass yield per hectare, do not compete with cultivation on arable land, can grow in fresh, brackish, saline and wastewater, and also have the potential of CO_2 and nutrient recycling from polluted streams [85]. Another important aspect in the use of marine biomass is the recycling of nutrients from the sea and other wastewaters. Today, predictions of phosphorus depletion in the next 50 years and also increased extraction cost from non-renewable phosphorus rocks is a very controversial topic [86]. Phosphorus is a vital component of fertiliser, and the sustainable recycling of nutrients is important for the future of the agricultural sector.

Therefore, improvement of the two-stage and one-stage dry digestion systems and the utilisation of reeds, mussels, manure and seaweed have potential for the augmentation of biogas production.

5. Biogas production and the removal of heavy metals from seaweed

Methane production from seaweed was studied in batch mode and in the two-stage system consisting of a leach bed reactor and a UASB reactor. The efficiency of seaweed hydrolysis in a leach bed reactor alone was evaluated. Post-treatment of the seaweed residue after hydrolysis in the leach bed reactor was also investigated with regard to the release of soluble organics. Another reason for hydrolysing seaweed was to release the heavy metals into a liquid so that they could be removed using IDA Cryogel carriers. Zn ion mobilisation from the solids in the leach bed and also after the post-treatment of the residue was investigated. The heavy metals removal from these hydrolysis liquids, and the effect on methane production was evaluated.

5.1. Hydrolysis

Seaweed hydrolysis was performed in leach bed reactors and the recalcitrant residue that was not hydrolysed was post-treated (Paper III).

In the hydrolysis experiments in the leach bed reactors, the effects of mesophilic (35 °C) and thermophilic (55 °C) temperatures, alkaline addition and dilution with water were evaluated (Table 2). The results showed that leach bed hydrolysis at 22 °C with hydrolysate withdrawal and dilution with water (H3 in Table 2) led to comparable organic matter solubilisation to hydrolysis at 55 °C and NaOH addition without hydrolysate withdrawal and water dilution (H2). Organic matter solubilisation was higher in both H2 (0.46 g sCOD/g VS_{added}) and H3 (0.43 g sCOD/g VS_{added}) than in H1 (0.31 g sCOD/g VS_{added}).

Comparisons have been made of the degree of acidogenesis expressed as the ratio $COD_{VFA}/sCOD$, which represents the amount of VFAs in the soluble organics [87]. In the present work, this ratio was generally higher in H3, mainly due to the dilution of the reactor contents with water, but it was generally lower in H1 and H2. This indicates that most of the soluble organics in H3 were converted to VFAs, while in H1 and H2 hydrolysis and acidogenesis were inhibited by the low pH, of about 5.0 to 5.5, and VFA accumulation, as evidenced by the fairly constant VFA profile. A retention time of 10 days may be appropriate for the hydrolysis of seaweed in a leach bed reactor since 91% of the process yield in H2 was solubilised during this period. Agar gel formation was observed in thermophilic leach bed hydrolysis (55 °C). It may, therefore, be necessary to dissolve this gel to improve the circulation of the hydrolysate in the pipelines of full-scale processes. The agar gel may also limit enzyme access, leading to poor solubilisation of the organics present in the seaweed [65].

Table 2. COD solubilisation, methane yield and Zn mobilisation % during seaweed hydrolysis.

	Process yield (g sCOD /g VS_{added})	Methane yield (l CH_4/g VS_{added})	Zn mobilisation (%)
Leach bed hydrolysis of seaweed			
H1 (37 °C without dilution)	0.31 ± 0.01	0.15 ± 0.01	13-21
H2 (55 °C + NaOH without dilution)	0.46 ± 0.01	-	40
H3 (22 °C with water dilution)	0.43 ± 0.01	-	-
Post treatment of seaweed residue			
C1 (Control 1)	0.09 ± 0.01	-	-
C2 (Control 2)	0.21 ± 0.02	-	-
P1 (Dilute H_2SO_4 + autoclaving)	0.47	-	-
P2 (Dilute NaOH + autoclaving)	0.78 ± 0.10	0.11 ± 0.02	33
Combined H1 and P2	0.86 ± 0.11	0.34 ± 0.02	54

C1 control: seaweed soaked for 1 hour in distilled water; C2 control: seaweed soaked for 1 hour in 0.25% w/v NaOH

Post-treatment of the seaweed residue was performed by soaking in acid (P1) or base (P2), followed by autoclaving. The alkaline treatment was more effective than the acid treatment, and about 78% of the organics present in the residue was solubilised (Figure 8). Insoluble Ca^{2+} alginate gel has been reported to be soluble at increasing Na^{+}/Ca^{2+} ion ratios [88].

In summary, leach bed hydrolysis of seaweed followed by alkaline post-treatment and autoclaving resulted in about 86% solubilisation of the organic matter. A period of 10 to 15 days may be sufficient for the combined treatment procedure, and the hydrolysates can then be treated in high-rate methane reactors.

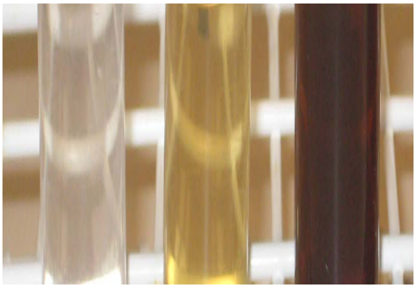

Figure 8. Results of post-treatment of seaweed residue. Control C1 (left), acid treatment and autoclaving (middle) and alkaline treatment and autoclaving (right).

In conclusion, post-treatment of seaweed and subsequent biogas production from the hydrolysate may provide a method of disposing of the digestate, which could contain high heavy metal concentrations. However, the fate of the heavy metals after treatment in a UASB reactor was not studied, and could be an area for further research.

5.2. Mobilisation and removal of heavy metals

The mobilisation of Zn ions was evaluated under the conditions in H1, H2 and P2 (Table 2). Zn ions were studied as they were present at high concentrations. The objective of improving heavy metal mobilisation is to increase the concentration in the liquid and thus maximise the amount that can be complexed and removed by the IDA Cryogel carriers. The initial Zn ion concentration in the seaweed was 460 µg/g VS; and 61 ± 7 µg/g VS and 184 ± 11 µg/g VS were mobilised under the conditions in H1 and H2, respectively. Hence leach bed hydrolysis at 55 °C and NaOH addition with water dilution was more effective than hydrolysis at 37 °C.

The combination of leach bed treatment with alkaline and autoclave treatments of seaweed (H1 and P2) resulted in 54% Zn mobilisation. The mobilisation was 21% in H1 and 33% in P2. The mobilisation of Zn ions was low, despite the prevailing low pH of 5.0-5.5 in the leach bed reactor. It was suspected that this low Zn ion mobilisation was due to sulphide precipitation of the metals and also possibly to the presence of chelating groups, which have been reported in seaweed [89-90]. Zn ion mobilisation of 56% has previously been reported in maize hydrolysis in a leach bed reactor [91].

Heavy metal removal was performed on the seaweed hydrolysate from the leach bed reactor (H1) (Paper IV). The IDA Cryogel was efficient in removing the heavy metals, and the percentages removed were 79% Cd, 59% Cu, 70% Ni and 41% Zn ions. Although toxic metals such as Cd were removed, the IDA Cryogel also removed Ni, which is a key nutrient for methanogenesis [31]. It may, therefore, be advantageous to use specific ligands (for example, the molecular imprinting technique) in order to target particular heavy metals in the seaweed hydrolysate.

5.3. Effect of pretreatment and heavy metal removal on methane yield

The combination of leach bed hydrolysis and post-treatment with an alkali and autoclaving resulted in a significant improvement in the methane yield: 0.34 l/gVS_{added}, which was 2.8 times higher than that of untreated seaweed. This was achieved when the hydrolysate from the leach bed reactor and the post-treatment hydrolysate were co-digested at a ratio of 1:1 based on g total COD. Their separate digestion resulted in lower methane yields. Co-digestion may have balanced the nutrients and diluted any toxic compounds produced during pretreatment. For instance, the ratio of COD/SO_4^{2-} in the post-treatment hydrolysate was higher than that in the leach bed hydrolysate. Higher ratios of COD/SO_4^{2-} have been reported to favour methane production, while lower ratios favour the domination of SRB [92]. Methane production from the digestion of seaweed hydrolysates from the leach bed was more rapid than from the raw or unhydrolysed seaweed.

A significant reduction in methane potential resulted when the IDA Cryogel was used to remove heavy metals from the seaweed hydrolysate. This is thought to be due to the removal of key nutrients, such as Ni, needed for methane production. Furthermore, the inhibition of methanogens due to H_2S toxicity would also increase, as the metals were not available to precipitate with the high levels of H_2S present during the digestion process.

Based on the results of this research, it is recommended that the heavy metal removal step be included after AD. As the seaweed already contains sulphate, some metals may be precipitated in the leach bed and UASB reactors. The capture of metals by extra-polymeric substances used to stabilise the granular bed may result in additional reduction in the metal concentration. Finally, IDA Cryogel can be used as a polishing step after two-stage AD. Exploring all the possibilities of metal removal in this process may keep the level of the Cd below the permitted limit and this could be considered for further investigation.

6. Biogas and biohydrogen production from wheat straw

This section describes the studies carried out on biogas and biohydrogen production from wheat straw. Pretreatment of wheat straw was performed in order to release the bound sugars into the liquid.

6.1. Steam and enzyme pretreatment

The wheat straw was pretreated with steam and an enzyme (Paper V). Figure 9 shows the wheat straw before and after pretreatment. Dilute phosphoric was used as a catalyst in the steam pretreatment instead of the commonly utilised sulphuric acid. Sulphuric acid is efficient, but can be problematic in the AD process due to the formation of H_2S and competition with SRB [93]. Another modification of the pretreatment was the use of a more efficient Celluclast enzyme, Cellic C Htech (Novozymes, Denmark). The pretreatment was efficient, and 95% of the sugars bound in the lignocellulose of the wheat straw were released, which is comparable to results in previous studies [94]. Enzyme treatment improved the C/N ratio from 47.7 in the wheat straw to 20.1 in the wheat straw hydrolysate, and is comparable to the recommended ratio of about 16 for AD [95]. Steam pretreatment also led to sterilisation of the organic material, which can be an added advantage when using a monoculture for biohydrogen production.

Figure 9. The original wheat straw and the sugar-rich liquid obtained after steam and enzyme pretreatment.

However, the pretreatment resulted in the production of compounds such as HMF and furfurals which are toxic to methanogens [95]. HMF and furfurals can be degraded in anaerobic systems for biogas production [96]. Inhibition can be reduced by dilution of the high-strength hydrolysate before being used in biogas and biohydrogen production. Another drawback of this pretreatment was the loss of organic compounds, especially during the soaking step in the dilute acid, but also during steam pretreatment and the enzymatic hydrolysis, which was performed for 3 days at 50 °C. Volatilisation of organics that could have been converted to hydrogen and methane would have been lost in these stages. Soaking also resulted in the loss of important nutrients and buffer substances needed for efficient AD. Spraying of dilute acid, therefore, appears to be better than soaking. Loss of nutrients can be costly, as there is then a need to add nutrients, as has been reported previously in a techno-economic evaluation of a combined biohydrogen and biogas process based on potato peels [97]. Pretreatment with steam and enzymes has been reported to be effective in the hydrolysis of lignocellulosic material [63, 94].

6.2. Effect of steam and enzyme pretreatment on methane potential

The effect of pretreatment methods such as cutting, grinding, steam pretreatment and combined steam and enzyme pretreatment on wheat straw were evaluated with regard to methane potential (Paper V). Cutting the straw to lengths of 1-2 cm and grinding had no significant effect. However, a substantial improvement in the methane yields was obtained with the steam/enzyme pretreated straw, 0.32 l CH_4/gVS_{added} compared to 0.18 l CH_4/gVS_{added} for the untreated wheat straw. Comparable results have been reported for wheat straw in previous research [98]. The biogas potential of the wheat straw hydrolysate was 11.9 MJ/kg TS, which can be compared to the lower heating value of 16.3 MJ/kg TS of the sugars bound in the wheat straw. The low energy content of the hydrolysate is due to the loss of organics during pretreatment. The separated hydrolysate can be treated in high-rate methane reactors, while the lignin, which yielded 4.2 MJ/kg TS, can be used for the generation of energy to supplement the heat and power supply of the entire process.

Agricultural and forestry residues, which are mainly of lignocellulosic origin, have great potential for biogas production. The potential in Sweden has been estimated to be about 74 TWh/year, which is equivalent to about 10% of the total energy consumption in the country [99]. Hence, expanding biofuel production from wheat straw and other lignocellulosic materials would help attain national goals and also EU objectives regarding the substitution of fossil fuels with renewable fuels.

6.3. Treatment of seaweed, wheat straw and dark fermentation hydrolysates in UASB reactors

The hydrolysates of seaweed, seaweed/wheat straw, wheat straw and the DF effluent were efficiently treated in a UASB reactor, resulting in high methane production rates, high removal of organic compounds and stable operating conditions (Papers IV, V and VI). Table 3 presents the results of the treatment of the hydrolysates at high OLRs. The methane production rates ranged from 0.99 to 3.04 l CH_4/l·day, and depended on the OLR and HRT. However, the methane production rate from seaweed was lower than expected, and this was suspected to be due to the toxic compounds, especially sulphate, present in the seaweed [84, 100]. Sulphate can be converted by SRB to H_2S, which is toxic to methanogens, and SRB also compete with methanogens for organic compounds [93]. This effect was also reflected in the low methane yield of 0.21 l CH_4/g COD at an OLR of 4.9 g COD/l·day obtained from seaweed hydrolysate, compared with that from wheat straw hydrolysate (Paper V) and the DF effluent (Paper VI). No significant improvement was seen in the methane yield in the co-digestion of seaweed and wheat straw hydrolysate. However, the process can be operated at a high OLR of 6.59 g COD/l·day. Previous studies have shown that low ratios of seaweed to steam-pretreated wheat straw resulted in improved methane yields [101]. Further investigations using UASB reactors are therefore recommended.

Indeed, the reactors were very stable despite the high OLR rates applied. The features of stable operation were high organic matter or COD removal, neutral effluent pH, low total VFA in the effluent, and high buffering capacity measured as the partial alkalinity. This confirms the versatility of the UASB reactor, which has also been reported in the treatment of a wide range of wastewaters [53, 102-103]. The high settling, and the protective and adaptive nature of the anaerobic granules also allow high methane productivity under these stable operating conditions [4, 96].

Table 3. Treatment of the hydrolysates of seaweed, seaweed/wheat straw and dark fermentation in a UASB reactor in comparisons with other feedstocks.

	Seaweed hydrolysate (Paper I)	Seaweed hydrolysate (Paper I)	Seaweed/wheat straw hydrolysate (1:1 gCOD) (Paper V)	Seaweed/wheat straw hydrolysate (1:1 gCOD) (Paper V)	Wheat straw hydrolysate (Paper V)	Wheat straw hydrolysate (Paper V)	Dark fermentation effluent (Paper VI)	Dark fermentation effluent (Paper VI)	Palm oil mill effluent [34]	Slaughter-house waste [106]
Nutrient addition	-	-	-	-	AD medium	AD medium	AD medium	AD medium	AD medium	-
COD of influent (g/l)	10.6	10.6	9.4	18.4	27.7	27.7	15.3	15.3	97	9.0-10.3
HRT (days)	2.1	0.5	2.68 ± 0.48	2.68 ± 0.29	8.0 ± 0.8	2.7 ± 0.3	2.5 ± 0.2	1.5 ± 0.2	-	1.0
OLR (g COD/l·day)	4.9	20.6	3.63 ± 0.51	6.59 ± 1.38	3.5 ± 0.2	10.4 ± 1.2	6.0 ± 0.5	10.5 ± 1.2	5.8	10.0
GPR (l CH_4/l·day)	0.99 ± 0.02	3.04 ± 0.16	0.82 ± 0.20	1.47 ± 0.43	0.89 ± 0.12	2.70 ± 0.53	1.64 ± 0.12	2.64 ± 0.34	2.56	1.52
Methane yield (l CH_4/g COD)	0.21 ± 0.02	0.16 ± 0.02	0.22 ± 0.07	0.22 ± 0.04	0.28 ± 0.04	0.26 ± 0.04	0.28 ± 0.03	0.26 ± 0.04	0.44^a	0.25^b
COD removal (%)	80.7 ± 2.4	80.6 ± 0.9	95	96	94	94	95	94	93	-
Effluent pH	7.52 ± 0.05	7.37 ±0.13	6.91	6.93	7.34	7.47	7.50	7.53	-	-
tVFA (g/l)	0.02 ± 0.01	0.02 ± 0.02	>0.01	>0.01	0.14 ± 0.08	0.19 ± 0.01	<0.01	0.06 ± 0.03	-	0.31
PA (g/l)	3.4 ± 0.1	2.9 ± 0.1	1.4 ± 0.3	1.2 ± 0.4	3.9±0.4	5.3 ± 0.2	5.8 ± 0.2	5.4 ± 0.1	-	-

AD medium: anaerobic basic medium.
a: methane yield based on g VS
b: methane yield based on g VS
PA: partial bicarbonate alkalinity

Nutrient and buffer supplementation were necessary for the treatment of wheat straw and DF hydrolysates in the UASB reactor. Signs of early process failure occurred in the treatment of both wheat straw and the DF effluent. The addition of the AD basic medium, described earlier, was therefore needed to sustain treatment in the UASB reactors [104]. Seaweed may be a suitable co-substrate, as well as manure, due to its rich nutrient and buffer content. The treatment of seaweed alone without the addition of nutrients or buffer capacity (AD basic medium) was possible in these investigations. In addition, seaweed may constitute a cheap source of nutrients and buffer capacity in the treatment of wheat straw hydrolysate. Manure is also a suitable co-substrate, however, there is increasing competition for this material due to the increasing number of biogas plants [105]. In comparison, the treatment of palm oil effluent [34] and methanol condensate [103] in a UASB reactor required the addition of AD basic medium, while the treatment of slaughter house waste in a similar system did not [106]. The cost of adding nutrients to AD systems treating potato peels has been found to be significant. Hence, the use of suitable co-substrates could be important in reducing the cost of biogas processes [97].

6.4. Co-production of biohydrogen and biomethane

The wheat straw hydrolysate was used for biohydrogen production in a CSTR, and the effluent was subsequently treated in a UASB reactor for methane production. Hydrogen production in the CSTR was performed with a monoculture of the extreme thermophile, *C. saccharolyticus* (Paper VI).

The objective of DF for H_2 production was to investigate the optimum sugar concentrations and the effect of sparging with biogas (60% CH_4 and 40% CO_2) at different dilution rates. In the laboratory experiments, methane was replaced by nitrogen due to safety reasons. Pre-trails showed that replacing methane with nitrogen had no significant effect on H_2 production, whereas the use of CO_2 had a negative effect and this effect has been reported previously [41].

High H_2 yields were obtained at 10% hydrolysate concentration (11.4 g/l total sugar); fermentation was not possible at higher hydrolysate concentrations. The reason for this could be the presence of inhibitors such as HMF and furfurals, as this inhibitory effect has been reported previously [107]. Another reason could be the high osmolarity of the 20% v/v hydrolysate (0.26 osmol/kg of H_2O), which was around the critical level of 0.22-0.26 osmol/kg of H_2O, reported for *C. saccharolyticus* [79]. Hydrolysate at 10% concentration was then used to investigate the effect of sparging with N_2 and CO_2 at different HRTs in the CSTR. High H_2 production rates of 1.8 to 3.5 l/l·day were obtained at HRTs of 0.3 to 0.8 days with N_2 and CO_2 sparging of 6 l/h. For comparison, a low H_2 production rate, 0.8 l/l·day has been reported for wheat straw hydrolysate (containing about 3 g/l sugars in the feedstock) in a UASB reactor [108-109]. Furthermore, comparable, high H_2 productivities have also been reported in the DF of sugar beet molasses using *C. saccharolyticus* [110]. The ability of *C saccharolyticus* to tolerate high osmotic and high partial hydrogen pressures and also to utilise complex sugars, makes it attractive for industrial applications (Paper VI [41]). Additionally, higher substrate concentration reduces the handling cost and the reactor volume [97]. It is also beneficial from a life-cycle perspective as it reduces the volume of process water required.

The DF effluent was efficiently treated in a UASB reactor, resulting in high methane production rates of 2.6 to 4.0 l/l·day, indicating that biogas production is suitable for harnessing the remaining energy from the DF effluent. A total treatment time of approximately 7 days (3 days' pretreatment of straw, 1 day of H_2 production and 3 days' methane production) is required for the conversion of wheat straw to biohydrogen and biogas. This is shorter than the treatment time of 30 days usually employed for the digestion of organic waste in conventional CSTRs [22].

Considerably higher energy yields were obtained in the co-production of biohydrogen and biogas (9.9 MJ/kg TS) than in the production of H_2 only (1.2 MJ/kg TS), indicating the need for a biogas production step in residual energy recovery. Comparable energy yields were obtained in the production of biohythane and biogas from the wheat straw hydrolysates (Table 4). The energy recovery of the biohythane process was 83% of the energy content of the wheat straw hydrolysate and 61% of that in the wheat straw, implying that the biohythane process was efficient in recovering the energy of the wheat straw. The remaining energy in the straw was contained in the lignin fraction, which can replace about 86% of the energy consumption of the biohythane process. Therefore, extra energy is needed to supplement the remainder of the energy.

Table 4. Energy yields of the biohythane and biogas process.

	Case 1: DF + $AD_{OLR\ 10.5\ g\ COD/l\cdot day}$ (Paper VI)	Case 2: $AD_{OLR\ 104\ g\ COD/l\cdot day}$ (Paper V)	Units
Dark fermentation	1.2	-	MJ/kg TS
AD	8.7	10.0	MJ/kg TS
Total	9.9	10.0	MJ/kg TS
Biogas potential of straw hydrolysate	11.9	11.9	MJ/kg TS
Energy in wheat straw	16.3	16.3	MJ/kg TS
Products / biogas potential of straw hydrolysate	83	84	%
Products / Energy in straw	61	61	%

The kinetic model of the biohythane process was validated with the experimental data showing high productivities and energy recovery efficiencies. The model also suggested even higher H_2 and CH_4 productivities, while maintaining a high COD reduction of 90%. However, further investigations on the stability of the fermentation processes should be considered before full-scale implementation.

Techno-economic analyses revealed that the biohythane process was not economically feasible. One reason was the small scale of the process (2 tonne/h), which resulted in a high capital cost of the steam pretreatment and gas upgrading units. Also, the nutrient cost constituted about 55% of the total production cost, showing that nutrient cost reduction is important for a feasible process. Replacement of expensive additives such as yeast extract in fermentation can significantly reduce the cost of nutrient supplementation. Future research should include the study of alternative cheap nutrient additives, as reported in a similar biohythane process based on potato steam peels [97]. In addition, omission of gas sparging in DF fermentation can reduce the cost of the biohythane process. Gas sparging improved the H_2 yield and productivity, but it is an energy-demanding process. Improving amine recovery can also reduce the operating cost since it accounts for about 60% of the operational cost. In a previous study, the high cost of feedstock, 52-67% of the total production cost, was found to be a major contributing factor to the high co-production cost of bioethanol, biogas and CHP from industrial hemp [62]. In the present work, the cost of the feedstock ranged from 8-18% of the total production cost. Therefore, the use of agricultural residues such as wheat straw could lead to a feasible biohythane process. Low risk of contamination of the thermophilic monoculture is an advantage of DF, since only a small group of microbes can grow at this temperature (70 °C). In addition, the wheat straw hydrolysate that results from pretreatment (190 °C for 5 minutes) can be used in the biohythane process, as it is sterile. In summary, biohythane production from wheat straw has the potential of high yields, productivities and energy conversion efficiencies. However, more research is needed to reduce the process costs.

7. Conclusions and outlook

The utilisation of new biomass such as mussels, manure, reeds, seaweed and cheap lignocellulosic agricultural residues, such as wheat straw, and their combinations, is vital to increase biogas production so as to attain objectives set by the EU for replacing fossil fuels with biofuels. Also, the employment of efficient techniques such as co-digestion, two-stage AD systems, one-stage dry digestion and pretreatment is important in harnessing the energy contained in biomass. The two-stage AD system allowed the reduction of the heavy metal content in seaweed using IDA Cryogel carriers. However, conditions in the leach bed reactor did not favour the efficient solubilisation and subsequent removal of the metals to levels below those acceptable for biofertilisers.

This research has demonstrated the suitability of the UASB reactor in the treatment of a range of hydrolysates (seaweed, wheat straw, DF effluent) under stable operating conditions, and that it was possible to attain high methane production rates and treatment efficiencies. In addition, seaweed hydrolysate supplemented the nutrient and buffering capacity needs and could, therefore, constitute a cheap nutrient supplement. High biohydrogen and methane production rates were achieved in the two-stage system consisting of a CSTR combined with a UASB reactor. The use of the thermophile, *C. saccharolyticus*, which displayed interesting characteristics, such as its ability to grow under high hydrogen partial pressure, utilisation of complex carbohydrates and growth under high osmotic pressures, was important in attaining the high hydrogen productivities.

Further optimisation of the operating conditions of the one-stage dry digester, the two-stage system and their techno-economic analyses are recommended as topics for future studies. In addition, the development of ligands that can target specific toxic heavy metals could be investigated. Studies of the growth of *C.*

saccharolyticus on cheap nutrient sources and increasing the biomass concentration in the hydrogen reactor could be strategies to further improve the present hydrogen productivity.

In conclusion, the use of new feedstocks, two-stage and one-stage dry digestion, pretreatment and high-rate bioreactors can improve the production of biofuels such as biogas and biohydrogen. However, economic evaluation of the processes studied in this thesis is needed before their full scale applicability.

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Paper I

Co-digestion of seaweed and manure: An evaluation of dry anaerobic digestion configuration

Valentine Nkongndem Nkemka, Jorge Arenales-Rivera and Marika Murto

Department of Biotechnology, Lund University, P.O. Box 124, SE-221 00, Lund,
Sweden.
Telephone: +46 46-222 83 47
Fax: +46 46-222 47 13
Corresponding author: valentine.nkemka@biotek.lu.se

ABSTRACT

Two-stage, dry anaerobic co-digestion of seaweed and solid cow manure was studied on a laboratory-scale. The results showed that it was beneficial to operate the second stage methane reactor, which produced 83% of the methane, while the remainder was produced in the first leach bed reactor. Also, the two-stage system was more stable for the co-digestion for these materials when compared to their separate digestion. In addition, the initial ammonia inhibition observed for manure digestion, and the acidification of the leach bed reactor in seaweed digestion, were both avoided when the materials were co-digested. Co-digestion of these materials also resulted in improved leach bed structure, which facilitated better liquid circulation in the leach bed than when seaweed was digested alone. Alternatively, a one-stage dry digestion process will suffice for the co-digestion of seaweed and manure, since conditions in the leach bed reactor favour methane production. In conclusion, the co-digestion of seaweed and manure in dry anaerobic digestion systems provides several benefits. However, the fate of Cd and other heavy metal contaminants from seaweed in the effluent were not studied and care should be taken in the application of the digestate as a fertiliser.

Key words: co-digestion; leach bed reactor; manure; seaweed; two-stage process; UASB reactor.

1. Introduction

Anaerobic digestion is a technology employed for waste treatment and also for the conversion of biomass into renewable energy. The technology satisfies both EU objectives of (i) a 65% reduction in the landfilling of organic wastes by 2016 (EC, 1999) and (ii) an increase in the proportion of gross domestic renewable energy consumption to 20% by 2020 (EC, 2006). These policies, together with the environmental benefits of biogas production, have stimulated biogas markets in the EU and also worldwide (Weiland, 2010). Hence, there is a need to increase biogas production to meet the set goals and also to satisfy growing market demands. Improved process technology and the exploration of new biomass types are strategies reported to augment biogas production (Gunaseelan, 1997).

In the Scania region of Sweden, large quantities of solid animal manure are available, due to intensive animal farming, which have an estimated biogas potential of about 115 GWh/year. In connection to this, the pipeline exchange of manure and digestate (fertiliser) between a centralised biogas plant and animal farms has also been investigated, and plays a vital role in transport cost reduction, since manure has a high water content (Björnsson and Lantz, 2010). Seaweed is another abundant organic material in this region, which could be treated together with the manure. Marine eutrophication results in seaweed deposits with an estimated biogas potential of 103 GWh/year, depending on the collection season and the area (Anissimoff, 2009). The anaerobic digestion of these materials therefore represents great potential as a source of renewable energy production and also as a waste treatment technique.

Manure is an abundant waste material that can be efficiently treated by anaerobic digestion. This technique leads to improved fertiliser quality, odour and pathogen reduction, and a decrease in greenhouse gas emissions, while producing renewable energy in the form of biogas (Holm-Nielsen et al., 2009). During biogas production, manure is sometimes used as a co-substrate to supplement nutrients and improve the buffer capacity of the process. However, ammonia inhibition has been associated with the digestion of manure (Angelidaki and Ahring, 1994).

Seaweed, on the other hand, can be a good material for biogas production, with a biomass yield greater than any land-based source and which also does not compete with agricultural land. It is easily hydrolysable and has a low lignin content (Yanagisawa, 2011). The C/N ratio reported for seaweed ranges between 7 to 31 (Habig et al., 1984), which is within the ratio of about 25 needed for efficient anaerobic digestion (Sialve et al., 2009). Seaweed adsorbs nutrients from the sea and has been used to stabilise a pilot-scale anaerobic process digesting milk waste (Matsui, 2010). However, seaweed also contains compounds, such as NaCl, sulphate, heavy metals and tannins, which are potential inhibitors of methanogens (Cecchi et al., 1996; Peu, 2011; Rigoni-Stern et al., 1990). The anaerobic digestion of seaweed may, therefore, be restricted by these compounds.

The co-digestion of manure and seaweed can reduce the negative effects of the inhibitory compounds present in the latter and also balance nutrient composition. Co-digestion of organic materials has been reported to balance nutrients, improve the synergistic effects of microbes, reduce inhibition by ammonia or other compounds and also increase the buffering capacity in biogas reactors (Bouallagui et al., 2009; Lehtomäki et al., 2007).

In the present investigation, the solid cow manure used had a total solids (TS) content of 20.6%, while the TS of the seaweed was 31.2%. Consequently, these substrates were appropriate for treatment in a dry anaerobic digestion system. Such systems are becoming popular at a farm-scale, especially in Germany, for organic material with high dry matter contents of 15–30% (Nizami and Murphy, 2010). Utilisation of the one-stage dry digestion (leach bed) system is less expensive and easy to operate at a farm-scale, due to its simple construction and handling. Furthermore, most feedstocks can be used in their original form without the need for size reduction.

In a two-stage anaerobic digestion system, hydrolysis/solubilisation of solid organic materials mostly occurs in the first (leach bed) reactor, which generates a leachate with low pH and soluble organic compounds, mainly as volatile fatty acids (VFA). This leachate can be subsequently treated in a controlled manner in a high-rate methane reactor, in order to achieve high methane productivities under stable operational conditions. Alternatively, methane production can also occur in the leach bed reactor, especially during the latter part of the digestion phase, when hydrolysis becomes rate-limiting, or in the digestion of organic materials, which can generate sufficient buffer capacity (Nkemka and Murto, 2013). Hence, only a one-stage dry digestion process is needed for the digestion of such organic materials, representing a lower investment and reduced operational costs (Karagiannidis and Perkoulidis, 2009).

The main advantages of using a two-stage dry digestion system include high methane yields, low energy demands, the application of high organic loading rates (OLR), process stability, less foaming and the fact that the methane reactor is less sensitive to toxic shocks and variations in the feedstock (Bouallagui et al., 2009;

Lehtomäki et al., 2008; Nizami and Murphy, 2010; Parawira et al., 2008). However, the high initial investment required is the major disadvantage of using two-stage systems. Continuous stirred tank reactor (CSTR) is a one-stage anaerobic digester, widely-used because of its simple construction and operation, and low initial investment cost (Mata-Alvarez et al., 2000). The CSTR is designed for the treatment of waste streams with low TS content (TS <10%), such as sewage sludge and liquid manure (Angelidaki and Ahring, 1994; Nizami and Murphy, 2010). Conversely, the disadvantages of the one-stage CSTR are the high energy demands and handling costs of dealing with large volumes of liquid (Nizami and Murphy, 2010).

The current laboratory-scale investigation evaluated the dry anaerobic digestion of seaweed and solid cow manure separately, in addition to their co-digestion. The two-stage system used was a leach bed reactor combined with an upflow anaerobic sludge blanket (UASB) reactor. The leach bed reactor alone (without the UASB reactor) was used to digest the solid cow manure. Biochemical methane potential (BMP) batch tests were also performed on these materials for comparison.

2. Materials and methods

2.1. Cow manure

Solid cow manure was collected from a farm in the east of Scania, Sweden, in February 2009 and stored at -20°C until use. The TS of the manure was 20.6%, the volatile solid (VS) proportion was 82.4% of the TS, and the NH_4^+-N content was 1.3 g/l.

2.2. Seaweed

Seaweed was collected from a beach near Trelleborg, Sweden, in May 2008. It was reduced by grinding to 2–3 cm pieces and stored at -20°C until use. The TS of the seaweed was 31.2%, the VS was 30.0% of the TS, and the NH_4^+-N content was 0.02 g/l.

2.3. Dry anaerobic digestion systems

Anaerobic dry digestion was performed using a one-stage leach bed reactor, or a two-stage system combining a leach bed reactor with a UASB reactor. Seaweed and seaweed/solid cow manure combined were digested using a two-stage system. Solid cow manure on its own was digested using only a leach bed reactor. Duplicate digestion systems were used in the experiments.

The leach bed reactor was a 1.2 l plastic reactor, 30 cm in height, with an internal diameter of 7.5 cm. The UASB reactor had a volume of 1 l, with an active liquid volume of 0.85 l. Both reactors were operated under mesophilic (37ºC) conditions. The remaining reactor set-up and operation were similar to that previously described (Nkemka and Murto, 2013). Internal recirculation of the liquid reactor content was achieved using peristaltic pumps at 5 ml/min for both the leach bed and UASB reactors. The recirculation was performed from bottom to top for the leach bed reactor, but from top to bottom for the UASB reactor. Liquid exchange between the reactors of the two-stage system was accomplished using a multi-channel peristaltic pump and a timer switch. The effluent from the UASB reactor was also recirculated into the leach bed reactor. Prior to the start of the experiments, the leach bed reactors were flushed with nitrogen to create anaerobic condition.

The volume and composition of the gas, total chemical oxygen demand (tCOD) and pH were monitored throughout the course of the experiments.

2.3.1 Methane production from solid cow manure using a one-stage leach bed reactor

At the start of the experiment, 94 g (16.0 g VS) solid cow manure and 200 ml tap water were placed in the leach bed reactor. The leachate was recirculated over the hydrolysis bed as described in Section 2.3. The experiment was conducted for a period of 60 days.

2.3.2 Methane production from the co-digestion of seaweed and solid cow manure using an anaerobic two-stage process

Seaweed and solid cow manure were co-digested using a mixture ratio of approximately 1:1 grams VS. Consequently, 84 g seaweed (7.9 g VS), 46 g cow manure (7.8 g VS) and 200 ml tap water were added in the leach bed reactor. The experiment lasted 36 days, with a constant organic loading rate (OLR) of 1.5 g COD/l.day applied to the UASB reactor, and corresponded to a varying hydraulic retention time (HRT) of 1–9 days. The objective of the operation was to avoid organic overload of the UASB reactor, especially during the initial phase when a rapid hydrolysis rate was expected.

2.3.3 Methane production from seaweed using an anaerobic two-stage process

At the start of the experiment, 200 g (15.5 g VS) seaweed (TS = 24.1%; VS = 32.1% TS) and 200 ml tap water were added in the leach bed reactor. Care was taken when adding the seaweed to the leach bed, so as not to pack the bed and cause clogging. The experiment was conducted for 24 days at a constant HRT of 3 days. The OLR decreased progressively from 3.2 to 0.3 g COD/l.day, as the hydrolysis and solubilisation became rate-limiting the soluble organics were transferred and converted into methane in the UASB reactor. The liquid transfer from the leach bed reactor into the UASB reactor was initiated after 1 day of hydrolysis.

2.4. Biochemical methane potential tests

Biochemical methane potential tests (BMP) tests were performed in batches in order to validate the methane potentials obtained in the two-stage system and the leach bed reactor. Methane production from seaweed, solid cow manure and seaweed/solid cow manure combined (1:1 based on grams VS) was evaluated. The experimental set-up matched that previously described by (Nges and Liu, 2009). The methane potential tests were performed in 0.5 l E-flasks, in triplicate, for 38 days under mesophilic conditions (37°C). The inoculum/substrate ratio was set at 2:1, based on grams VS. The volume and composition of the biogas were analysed and the temperature was measured throughout the course of the experiments.

2.5. Analytical methods

TS and VS were analysed according to standard methods (APHA, 1998). The biogas composition and volume, tCOD and NH_4^+-N were analysed as previously described by (Nkemka and Murto, 2010). Elemental analyses of seaweed and solid cow manure were performed by LMI AB, Helsingborg, Sweden. Elemental analyses of nitrogen and carbon were undertaken, from which C/N ratios were calculated. Fe, Al, B, Cu, P, S, Zn, Mn, Na, Mg, Ca, K and Si were analysed by means of inductive coupled plasma atomic emission spectrometry (ICP-AES), while Mo, Cr, W, Se, Ni, Cd, Co, As, Hg and Pb were analysed using inductive coupled plasma mass spectrometry (ICP-MS). Kjeldahl-nitrogen (Kj-N) analyses were performed on the solid cow manure. Gas volumes were normalised to 0°C and 1 atmosphere. Free NH_3 concentrations were calculated using the formula previously described by (Hansen et al., 1998):

$$\frac{[NH_3]}{[NH_4{}^+ - N]} = \left(1 + \frac{10^{-pH}}{10^{-\left(0.09018 + \frac{2729.92}{\mathrm{T(K)}}\right)}}\right)^{-1}$$

Where $[NH_3]$ is the concentration of free NH_3 in mg/l, $[NH_4^+\text{-}N]$ is the concentration of ammonium-nitrogen in mg/l and T the temperature in Kelvin.

3. Results and discussion

3.1. Elemental composition of solid cow manure and seaweed

The nutrient compositions of the solid cow manure and seaweed were comparable, as demonstrated in Table1. Key micronutrients, such as Fe, Co, Ni, Mo, Se and W, were all present in concentrations suitable for biogas production (Schattauer et al., 2011). The concentration of Fe was in excess in the seaweed, being about five times higher than in the manure. The C/N ratio for the seaweed was low, although co-digestion with manure can improve this ratio, increasing it to a level close to that recommended for biogas production (Sialve et al., 2009).

The concentration of P in the seaweed was also lower than in the manure. The manure satisfied Swedish fertiliser quality guidelines (100 mg Cd/kg P), while the seaweed exceeded the maximum value by 7.5 times (Otero et al., 2005). As a consequence, the seaweed is not suitable as a fertiliser in its present form. The digestion of seaweed and the removal of organic compounds can result in even higher Cd concentrations. Hence, there is risk of surpassing this limit when seaweed digestate is applied as a fertiliser. Further research concerning the fate of Cd and other toxic heavy metals after anaerobic digestion is required before the application of seaweed digestate as a fertiliser.

The amount of S in the seaweed, 1, 841 mg/kg was very high when compared to the manure, 630 mg/kg. Although S is an important element for anaerobic digestion, excess amounts are inhibitory, since it results in competition between sulphate-reducing bacteria and also the production of H_2S, which inhibits methanogens (Peu, 2011). The concentration of Na^+ ions was higher in the seaweed (1.87 mg/kg) than in the manure (1200 mg/kg), which could also inhibit the biogas process. However, both substrates can be diluted to some extent, in order to attain the required TS content prior to anaerobic digestion. Dilution can therefore reduce concentrations below inhibitory levels, favouring an efficient biogas process.

Comparison of the chemical compositions of the seaweed and manure suggested that seaweed may be an alternative co-substrate for anaerobic digestion. However, care must be taken not to exceed heavy metal concentration limits when using the digestate as a fertiliser. Furthermore, the utilisation of both land- and marine-based biomass for anaerobic digestion, and the subsequent return of the digestate to farmland, is crucial for the sustainability of the agricultural sector. In fact, since key nutrients, such as P, are near depletion, research efforts should not only be focused on nutrient recycling from land biomass but also from marine biomass, where a greater amount of nutrients are lost.

Table 1. Elemental composition of solid cow manure and seaweed.

Metal	Solid cow manure (mg/kg)	Seaweed (mg/kg)
Fe	110	530
Al	40	178
B	5.2	13.1
Mo	0.39	0.14
Hg	0.007	0.012
Cu	4.7	1.4
Cr	0.23	0.78
As	0.04	0.87
W	0.01	0.02
Se	0.24	7.49
Co	0.33	0.20
Pb	0.12	0.99
P	830	265
S	630	1841
Zn	27	14
Cd	0.04	0.20
Mn	34	5.3
Ni	0.31	1.21
Na	1200	1872
Mg	930	562
Ca	2000	5616
K	5800	1092
Si	206	79
C	105	52
N		5.6
N-kj	0.62	
C/N	16.9	9.3
TS (%)	20.6	24.1-31.2
VS (% of TS)	82.4	30.0-32.1

3.2. Methane production from solid cow manure in a one-stage leach bed reactor

A methane yield of 0.14 l/g VS_{added} (0.09 l/g TS_{added} or 0.02 l/g_{added}) was obtained when solid cow manure was digested in a leach bed reactor for 77 days (Table 2). The methane yield was low, whether expressed per gram TS or per gram wet weight, due to the high water content of the solid manure. Hence, efficient transportation of the raw materials is vital if biogas production costs are to be kept low. The methane yield of 0.12 l/g VS_{added} obtained after 38 days in the leach bed reactor was similar to that acquired after 38 days from the BMP test. This indicates that digestion for an extended period would result in higher methane yields.

Methane represented 48% of the total gas produced in the leach bed reactor. The methane production rate was insignificant during the first 6 days of digestion, but increased subsequently, to reach a maximum on day 20 (Figure 1). A gradual decrease in the methane production rate was then observed

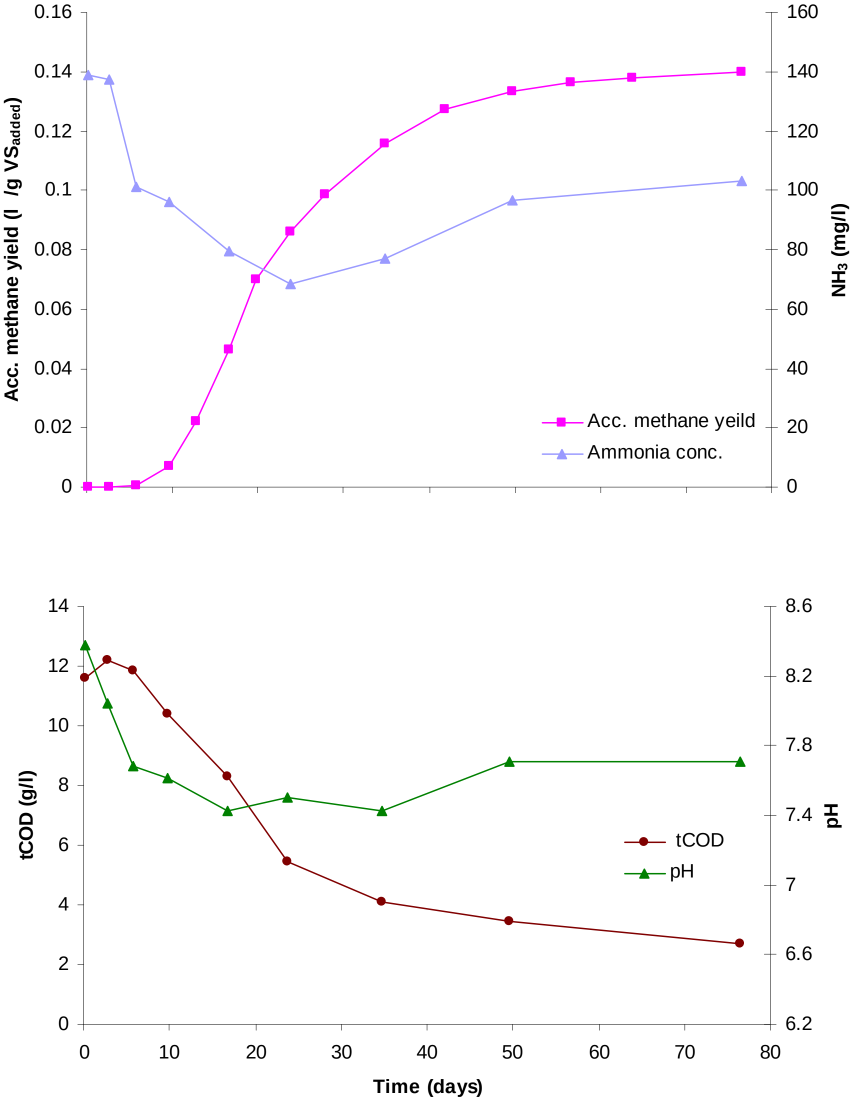

Figure 1. (a) Cumulative methane production and ammonia concentration in the anaerobic digestion of solid cow manure in leach bed reactors, (b) pH and tCOD variation in the leach bed reactor.

until the termination of the experiment on day 77. Furthermore, 83% of the methane was produced during the first 35 days of digestion. Macias-Corral et al., (2008) reported a methane yield of 0.08 l/g VS from dairy cow manure in a two-stage anaerobic digestion lasting 70 days, which is similar to the results presented here. In another study, the digestion of cow manure in a CSTR resulted in a high methane yield of 0.21 l/g VS, with a HRT of 30 days and OLR of 1.3 g VS/l.day (Alvarez and Lidén, 2008). No starter culture or inoculum was added during the present study. A faster start-up time and a shortened digestion period can be achieved by recycling part of the digestate to act as an inoculum for the next batch (Kusch et al., 2008).

The pH was 8.4 at the start of the experiment and therefore above the neutral pH that represents suitable conditions for methanogens during efficient biogas production (Moosbrugger et al., 1993). The initial concentration of free NH_3 (139 mg/l) was high and thereby close to the inhibitory range of 150 to 200 mg/l reported for methanogens (Angelidaki and Ahring, 1994; Gerardi, 2003). However, NH_3 inhibition is also dependent on methanogenic sludge adaptability (Braun et al., 1981). In this study, there was a correlation between initial inhibitory levels of free NH_3 and the 6-day period of insignificant biogas production, indicating ammonia inhibition. Subsequently, pH and NH_3 concentrations decreased as the solid cow manure was solubilised, indicated by an increase in tCOD, which reached a maximum of 12.2 g/l on day 3. In turn, the soluble organic matter was efficiently converted into biogas as conditions became favourable for its production, reflected by the low tCOD concentration (2.7 g/l) at the termination of the experiment. The pH increased during the latter period, resulting in a progressive increase in free NH_3 concentrations from 68 mg/l (day 24) to 103 mg/l (day 77). This may indicate the possibility of long-term NH_4^+-N accumulation and ammonia inhibition following several rounds of operation.

The adjustment of pH with dilute acid or co-digestion with a carbon-rich organic material are recommended strategies to avoid lengthy start-up times and the expected long-term ammonia inhibition (Lehtomäki et al., 2007). Moreover, Kusch et al., (2008) reported that start-up times can be shortened by the addition of 20–30% of inoculum, which introduces buffering compounds and microorganisms.

3.3. Methane production from the co-digestion of solid cow manure and seaweed using an anaerobic two-stage process

The methane yield obtained after 30 days of solid cow manure and seaweed co-digestion in a two-stage anaerobic process was 0.11 l/g VS_{added} (0.06 l CH_4/g TS_{added} or 0.02 l/g_{added}), which was similar to the yield of 0.13 l/g VS_{added} obtained after 38 days in the BMP test (Table 2). The methane yield obtained was low. A longer digestion time or post-treatment of the slowly-degradable fraction would be required to extract the remaining energy bound in the material. A total of 83% of the methane was collected from the UASB reactor, while only 17% was obtained from the leach bed reactor, indicating that the second stage methane reactor was required for the rapid conversion of the soluble organics into biogas (Figure 2). Recycling the effluent from the UASB reactor into the leach bed reactor resulted in the transfer of some buffering species and microorganisms from the former into the latter. As a result, the pH of the leach bed reactor increased to about 7 after 7 days, providing pH conditions suitable for methanogens, and leading to the onset of biogas production after about 10 days.

During the experiment, tCOD concentrations in the leach bed reactor increased to a maximum of about 10 g/l and then gradually decreased to about 1 g/l by the end of the experiment as the organics were converted into biogas, mainly in the UASB reactor (Figure 2). At the start of the experiment, leachate transfer to the UASB reactor was not as efficient as under the operational conditions due to clogging. Consequently, the initial methane production rate in the pH-neutral UASB reactor was low. Interestingly, conditions in the leach bed reactor were favourable for methane production at this time. It is suggested, therefore, that co-digestion of seaweed and solid cow manure in the correct proportions can lead to efficient biogas production in a one-stage leach bed reactor, without the need for a second methanogenic reactor. Hence, the investment and operating costs of having two reactors can be avoided (Karagiannidis and Perkoulidis, 2009). Briand and Morand, (1997) reported a methane yield of 0.17 l CH_4/g VS_{added} and content of 55% when a 1:1 ratio of algae and manure were co-digested.

Table 2. Methane production in batch tests (BMP), leach bed reactor and anaerobic two-stage processes.

Substrate	Process	Duration (days)	Methane yield (l/g VS_{added})	Methane content (%)
Seaweed	BMP	38	0.17 ± 0.01	49
Solid cow manure	BMP	38	0.09 ± 0.01	38
Seaweed/solid cow manure	BMP	38	0.13 ± 0.01	48
Seaweed	Two-stage process	24	0.16 ± 0.02	41[a], 65[b]
Seaweed/solid cow manure	Two-stage process	30	0.11 ± 0.01	42[a], 43[b]
Solid cow manure	Leach bed process	77	0.14 ± 0.01	48

a: the methane content in the leach bed reactor
b: is the methane content in the UASB reactor
Ratio of substrate mix: 1:1 based on g VS

The tCOD concentration in the UASB reactor was about 1 g/l, due to the efficient conversion of the transferred soluble organics into biogas (results not shown). The pH ranged from 7.4 to 7.7, presenting good conditions for methanogenesis. The concentration of NH_4^+-N was fairly constant (323 mg/l; corresponding to 21 mg/l of free NH_3 at pH 7.3 and 37 °C) throughout the entire experimental period.

The co-digestion of seaweed and solid cow manure provided better leach bed structure than was observed during the digestion of seaweed alone, thereby improving liquid percolation.

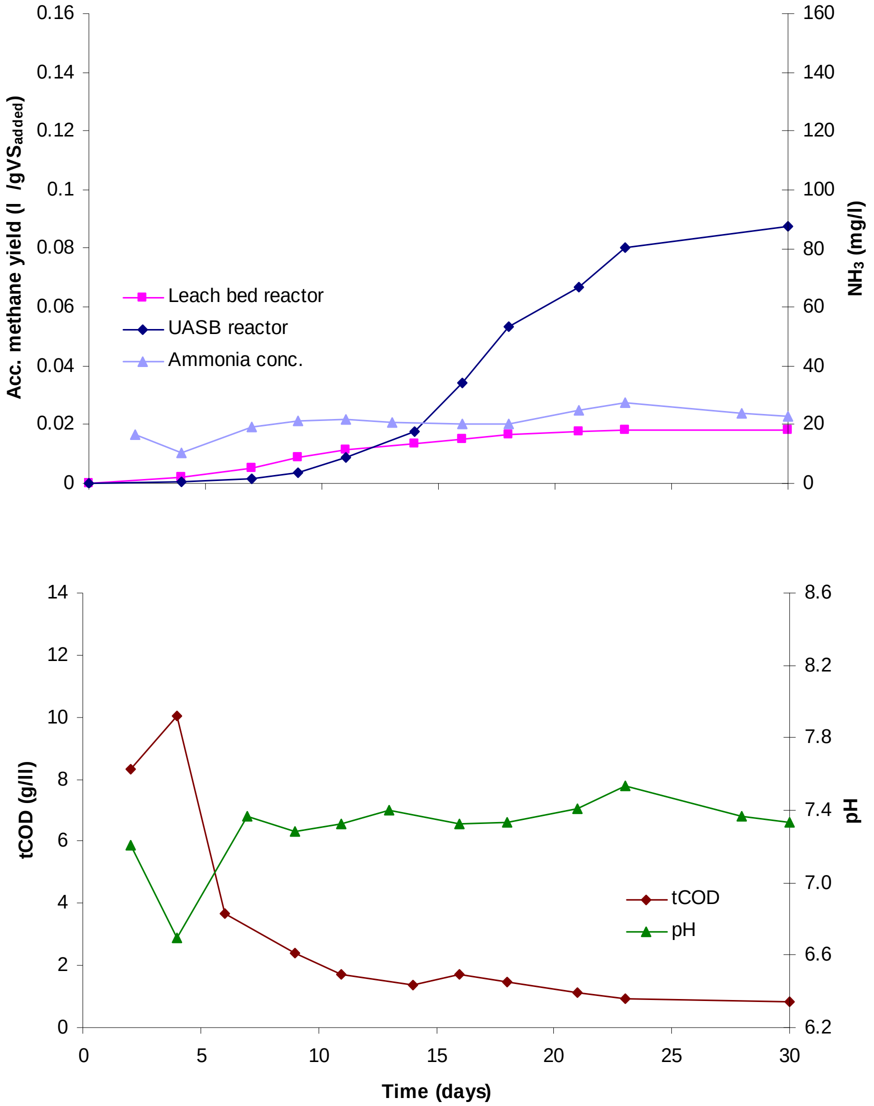

Figure 2. (a) Cumulative methane production in two-stage anaerobic digestion of seaweed and solid cow manure and the ammonia concentration in the leach bed reactor, (b) pH and tCOD variation in the leach bed reactor. Seaweed/solid cow manure 1:1 based on g VS in the digestion.

3.4. Methane production from seaweed in an anaerobic two-stage process

A methane yield of 0.16 l CH_4/g VS_{added} (0.04 l CH_4/g TS_{added} or 0.01 l CH_4/g_{added}) was obtained from the anaerobic digestion of seaweed in a two-stage system over 24 days (Table 2), which is similar to that obtained in the BMP test. This demonstrates that the two-stage system had a suitable process configuration for the dry digestion of seaweed. The methane proportion of the total gas produced during the process was 41% in the leach bed reactor and 65% in the UASB reactor. The soluble organics transferred into the UASB reactor were in a more reduced state, resulting in a higher methane content than in the leach bed reactor, where hydrolysis was dominant. Higher pH increases the solubility of CO_2, thus the higher pH in the UASB also contributed to the higher methane content in the gas phase.

Approximately 75% of the total methane produced in the two-stage system was collected within the first10 days (Figure 3). In other words, the two-stage process produced methane yields comparable to the seaweed BMP test in a short time. In addition, 80% of the methane was produced in the UASB reactor and only 20% in the leach-bed reactor. Moreover, the methane production rate in the UASB reactor attained a maximum on day 3 and then decreased gradually as the organics were converted into biogas.

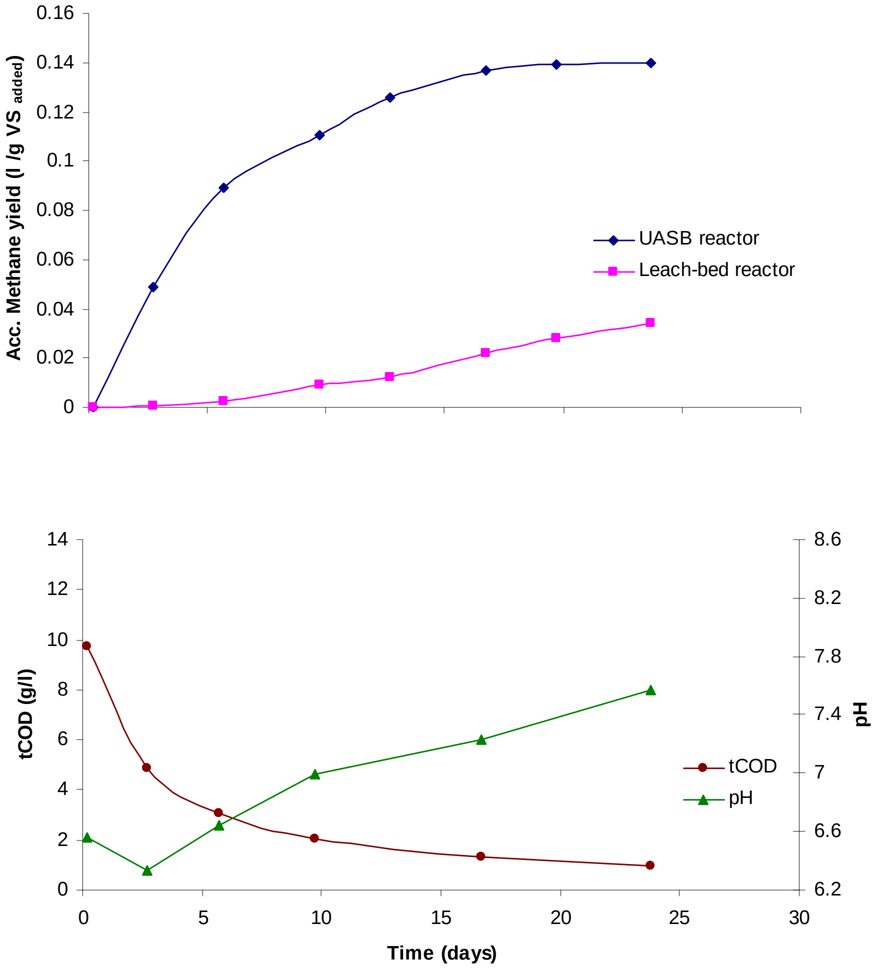

Figure 3. (a) Cumulative methane production in two-stage anaerobic digestion of seaweed, (b) pH and tCOD variation in the leach bed reactor.

Acidification of the leach bed was reported when seaweed was digested alone. Consequently, methane production would take longer if only a leach bed reactor was used (Nkemka and Murto, 2010). It is, therefore, advantageous to include a second (UASB) reactor, combined with liquid recycling, in a two-stage digestion of seaweed under the operating conditions applied in this study.

Comparable methane yields of 0.13–0.15 l CH_4/gVS_{added} and contents of 65–80% were obtained when water hyacinth was digested in a two-stage anaerobic reactor (Chanakya et al., 1992; Kivaisi and Mtila, 1998). In another study, this time concerned with the anaerobic digestion of marine algae in a two-stage system, 30% of the biogas was produced from the acidogenic and 70% from the methane reactor (Vergara-Fernández et al., 2008), comparable with the results of the present study. High methane yields have also been reported in the anaerobic two-stage process due to phase separation (Ghosh et al., 2000; Lehtomäki et al., 2008). The reactors in a two-stage process are optimised to suit the condition of the acidogens and methanogens (Ghosh et al., 2000; Nizami and Murphy, 2010).

Seaweed was efficiently solubilised and hydrolysed in the leach bed reactor, producing a leachate with a high organic content (9.7 g tCOD/l) during the initial phase of the experiment (Figure 3). The soluble organics were then efficiently converted into biogas in the UASB reactor, as reflected in the low tCOD concentration (1.2 g tCOD/l) and neutral pH in the effluent from this reactor (results not shown). The digestion of seaweed in the two-stage process was incomplete and thus more time or post-treatment of the recalcitrant fraction would be needed to recover the remaining energy bound in the material. (Vergara-Fernández et al., 2008) reported similarly-high initial COD concentrations (5.3–6.8 g/l) in the hydrolytic reactor prior to methane production in an upflow anaerobic filter during the anaerobic digestion of marine algae. Furthermore, (Lehtomäki et al., 2008) also described comparable COD concentrations (<1g/l) in the effluent of a batch two-stage anaerobic digestion of grass silage.

In the current study, the digestion of seaweed in a two-stage process was stable, owing to the neutral pH and low tCOD concentrations in the UASB reactor. Blockage of the leach bed reactor was experienced during the experiment, which could represent a serious problem in large-scale implementation of these processes, since it may limit contact between the bacterial biomass and the substrate. Although the seaweed was digested under stable operational conditions in the two-stage system, not all the material was digested. Efficient seaweed hydrolysis strategies, such as dilution of the leach bed reactor content and alkaline and autoclave treatments, which thus improve the overall efficiency of methane production, have been described previously (Nkemka and Murto, 2012). In addition, several leach bed reactors could be operated in parallel, in order to provide a sufficient, constant leachate supply to the UASB reactor, thus maximising its optimum OLR capacity and also maintaining constant methane production. In a previous study, the treatment of seaweed leachate in a UASB reactor at a high OLR of 20.6 g tCOD/l.day has been reported (Nkemka and Murto, 2010). However, the resultant biogas quality could be poor, due to high H_2S concentration, which can be corrosive to engines if not removed, especially when the biogas is upgraded and used as a fuel.

4. Conclusions

Seaweed and manure were co-digested in a dry anaerobic two-stage system using a leach bed reactor for hydrolysis and a UASB reactor for methane production. The benefits of co-digestion of these materials in a two-stage system were stable operational conditions at neutral pH, low tCOD and low NH_3 concentrations in the digestion system liquids. Although most of the methane was produced in the second stage UASB reactor, the process can also be optimised such that the co-digestion can be performed in only a one-stage leach bed dry digestion system, since methanogenic conditions also prevailed in the latter. Another aspect to consider is the Cd concentration in seaweed, which is close to the maximum levels permissible in fertiliser in Sweden. Thus, application of the digestate as a fertiliser would need to be performed with caution in order to comply with the recommended limits.

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Paper II

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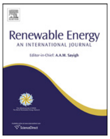

Two-stage anaerobic dry digestion of blue mussel and reed

V.N. Nkemka*, M. Murto

Department of Biotechnology, Lund University, P. O. Box 124, SE-221 00 Lund, Sweden

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ABSTRACT

Blue mussels and reeds were explored as a new biomass type in the Kalmar County of Sweden to improve renewable transport fuel production in the form of biogas. Anaerobic digestion of blue mussels and reeds was performed at a laboratory-scale to evaluate biogas production in a two-stage dry digestion system. The two-stage system consisted of a leach bed reactor and an upflow anaerobic sludge blanket (UASB) reactor. The two-stage system was efficient for the digestion of blue mussels, including shells, and a methane yield of 0.33 m^3/kg volatile solids (VS) was obtained. The meat fraction of blue mussels was easily solubilised in the leach bed reactor and the soluble organic materials were rapidly converted in the UASB reactor from which 68% of the methane was produced. However, the digestion of mussels including shells gave low production capacity, which may result in a less economically viable biogas process. A low methane potential, 0.22 m^3/kg VS, was obtained in the anaerobic two-stage digestion of reeds after 107 days; however, it was comparable to similar types of biomass, such as straw. About 80% of the methane was produced in the leach bed reactor. Hence, only a leach bed reactor (dry digestion) may be needed to digest reed. The two-stage anaerobic digestion of blue mussels and reeds resulted in an energy potential of 16.6 and 10.7 GWh/year, respectively, from the estimated harvest amounts. Two-stage anaerobic digestion of new organic materials such as blue mussels and reeds can be a promising biomass resource as land-based biomass start to be limited and conflict with food resources can be avoided.

1. Introduction

"Biogas – new substrate from the sea" is an ongoing project that aims to explore new biomass types from the sea for biogas production in the Kalmar County, Sweden [1]. The project aims to completely substitute fossil transport fuel by 2030 and also reduce greenhouse gas emissions and eutrophication. Biogas production from food wastes, slaughterhouse wastes and manure are not sufficient to replace the fossil transport fuel in this region [2]. It is therefore paramount to explore new biomass types such as fish waste, macroalgae, blue mussels and reeds as new resources to improve biogas production. The present research focused on the utilisation of blue mussels and reeds for biogas production. Eutrophication has been reported to be a serious problem in the Baltic Sea, resulting in algal blooms and oxygen-depleted zones [3]. Trails of cultivation of blue mussels are present in the Kalmar Strait. Mussels are filter feeders, feeding on plankton and being able to take up nutrients from the water they filtrate. This can be taken advantage of as a means to recover lost nutrients from the sea and thus, reduce the eutrophication effect. The mussels in this region are too small to be used as food by humans and hence, can be utilised as an interesting biogas feedstock. Eutrophication also leads to extensive growth of reeds, leading to inaccessible shores and overgrown bays. There is an interest in harvesting reeds to improve beach accessibility for recreational purposes, which can also generate income. Thus, these types of biomass can be collected and subsequently used for renewable energy production in the form of biogas. Biogas, composed of methane and carbon dioxide, can be upgraded and used as a transport fuel. The digested residue from the biogas process containing recovered nutrients can in turn be recycled to the farmland as a fertiliser. Therefore, the benefits of collecting marine biomass for renewable energy production purposes are many.

It has been estimated that about 65,000 tons wet weight (ww) per year of blue mussel can be harvested from the east coast of Sweden. Annual collection of 6360 tons ww per year of reed has been reported in the Kalmar region [4]. The production of biogas from blue mussels and reeds could significantly contribute to the replacement of fossil transport fuel.

Selection of a process configuration for the digestion of a biomass is greatly dependent on substrate characteristics [5]. Organic materials that are not suitable for digestion in a conventional continuous stirred tank reactor (CSTR) have to be adapted to

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* Corresponding author. Tel.: +46 46 222 83 47; fax: +46 46 222 47 13.
E-mail address: valentine.nkemka@biotek.lu.se (V.N. Nkemka).

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suit this reactor configuration. Digestion of blue mussels in a CSTR could be problematic due to the presence of shells and sand, which can result in the accumulation of inert material in the reactor. Mechanical problems of pumping mussels with shells and clogging can be other difficulties. Digestion of blue mussels in a CSTR entails the removal of shells and sand, which could be expensive. A strategy to remedy this problem is to digest blue mussels in a two-stage anaerobic process. In this system, blue mussels are hydrolysed in the leach bed reactor and then the hydrolysate is transferred into the methane reactor for biogas production. Hence, the shells can be easily removed from the leach bed reactor after digestion.

Total solid (TS) content of the reeds in the present study was about 82% and the digestion in a CSTR will require dilution of the reeds with other organic materials with high water content such as liquid manure. In the absence of a suitable co-substrate, the reeds have to be diluted with water in order to reduce the TS content to about 10%, which is a suitable concentration for a CSTR [6]. Addition of water will increase production costs due to a rise in energy demands for heating, mixing and pumping. Moreover, large liquid volumes can increase handling costs. The two-stage system has been reported to be suitable for the digestion of dry organic waste such as municipal solid waste and straw bedding [7,8]. Hence, two-stage anaerobic processes can be appropriate process configuration for treating reeds with a high TS content.

This laboratory-scale study examined two-stage anaerobic dry digestion of blue mussels and reeds separately. There are few research studies reporting the separate two-stage anaerobic digestion of these two biomass types.

2. Materials and methods

2.1. Blue mussels and reeds

Blue mussels (*Mytilus edulis*) and reeds (*Phragmites australis*) were collected from the coast in Kalmar, Sweden, in October and August 2010, respectively. Some initial characteristics of blue mussels and reeds are presented in Table 1. Blue mussels (TS of 41.2% of ww, VS of 18.8% of TS and carbon to nitrogen ratio (C/N) of 7.4) were frozen until use. Reeds (TS of 81.9% of ww, VS of 93.3% of TS and C/N ratio of 32.5) were frozen, and then thawed and cut to about 2–4 cm before use.

2.2. Methane potential batch tests

Methane potential batch tests were performed on blue mussels with shells and reeds. The inoculum used in these tests was collected from a mesophilic full-scale biogas plant, Söderåsens Bioenergi AB, Sweden, co-digesting industrial food waste and energy crops. The TS of the inoculum was 3.7% of ww and VS was 63.9% of the TS. The batch tests were performed in 0.5 l E-flasks under mesophilic conditions (37 °C) and in duplicates. Inoculum to substrate ratio was set at 2:1 on the basis of g VS. The duration of the experiment was 31 days for mussels and 107 days for reeds. Avicle cellulose (crystalline cellulose) was used as a control and also to assess the cellulose activity of the inoculum. The experimental set-up was as described earlier by Kreuger et al. (2011) [9]. Gas-tight bags used for biogas collection were made from aluminium packaging material transofoil, Flextrus AB, Sweden. Gas volume and composition were analysed and the temperature recorded during the course of the experiment.

Table 1
Some initial characteristics of blue mussel and reed.

	Mussel	Reed
TS (% of ww)	41.2	81.9
VS (% of TS)	18.8	93.3
C/N	7.4	32.5

2.3. Two-stage anaerobic process

Anaerobic digestion of blue mussels and reeds was performed in duplicate reactors in a batch anaerobic two-stage process. The process comprised of a leach bed reactor and a UASB reactor (Fig. 1).

The leach bed reactor was a 1–l glass reactor of height 38 cm and internal diameter of 6.5 cm. A small plastic cylinder and a plastic mesh were placed at the bottom of the reactor to suspend the solids and avoid blockage in the tubings. The top of the reactor was sealed with a rubber stopper to which a gas-tight bag was connected. The temperature of the reactor was maintained at a mesophilic condition (37 °C) by circulation of water from an external thermostat through a water jacket of the reactor. The liquid reactor content was recirculated over the leach bed by a peristaltic pump set at a flow rate of 5 ml/min and the flow rate was adopted from previous experiments.

A glass UASB reactor (height 41 cm and internal diameter of the head space 7 cm) with a volume of 1 l and an active liquid volume of 0.85 l was used. A funnel was installed at the upper part and used as a gas separator. A butyl rubber stopper was used to secure the top of the reactor, which was in turn connected to a gas-tight bag. The temperature of the reactor was maintained at mesophilic (37 °C) conditions. Granular anaerobic sludge used as inoculum in the UASB reactor was collected from a pilot-scale UASB reactor treating sewage at Hammarby Sjöstadsverk, Sweden. The TS content of the granular sludge was 7.0% of ww and VS content of 71.0% of the TS, and was inoculated with 300 g of the granules. Background methane production from the granules was reduced by incubation at 37 °C for 10 days prior to the start of the experiment.

Anaerobic basic nutrient solution was added to make up the liquid volume of the reactor [10]. The liquid reactor content was recirculated from top to bottom at a flow rate of 5 ml/min and the upflow velocity was 0.08 m/h. At this flow rate, the sludge bed was stable and gas removal from the sludge bed facilitated.

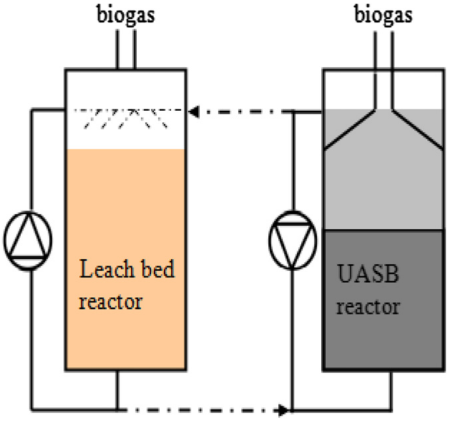

Fig. 1. Schematic diagram of two-stage anaerobic digestion. Liquid exchange between reactors was performed manually with a syringe.

At the start of the experiment, the liquid content of the reactor had a pH of 7.36 and a chemical oxygen demand (COD) of 0.16 g COD/l.

Prior to the start of the experiment, 200 g of blue mussel and 464 g of the anaerobic nutrient solution was added into the leach bed reactor. The amount of VS added was 15.4 g and the TS content in the leach bed reactor was 15% of ww. The liquid in the reactor was recirculated for one day. Thereafter, the leachate was manually transferred daily into the UASB reactor using a 100 ml syringe. The organic loading rate (OLR) applied was about 1 g COD/l.d. The same volume of liquid was transferred back from the UASB reactor to the leach bed reactor. The liquid transfer between the reactors was stopped after 10 days. Transfer of the liquid was stopped when the pH in the leach bed reactor was neutral and the COD concentration was low, favouring methane production in this reactor. On day 31, 150 ml of liquid from the UASB reactor was transferred into the leach bed reactor to wet the topmost part of the blue mussel bed that was not submerged. Liquid transfer enabled contact between the microbial biomass and the substrate. The extra water added was already available in the UASB reactor and not from an external source. The experiment was performed for 44 days.

The leach bed reactor was filled with 65 g of reed and 306 g of the anaerobic nutrient at the start of the experiment. The amount of VS added was 50 g and the TS content in the leach bed reactor was 15% of ww. The liquid in the leach bed reactor was recirculated for a day before the daily manual transfer started as described in the previous section. The liquid exchange between the reactors was stopped on day 13, when the conditions became favourable for biogas production in the leach bed reactor. On day 15, 400 ml of liquid from the UASB reactor was transferred into the leach bed reactor to wet the topmost part of the reed bed that was not submerged as the reed was floating in the reactor. The experiment was performed for 107 days.

The volume and composition of the gas, NH_4^+-nitrogen, COD and pH were monitored in the reactors during the experiments.

2.4. Analytical methods

The TS and VS contents were analysed according to standard methods [11]. The gas volume and composition, pH, COD, and NH_4^+-nitrogen were determined according to Nkemka and Murto (2010) [12]. The C/N ratio of blue mussel and reed was analysed by Eurofins (Linköping, Sweden). The temperature around the gas bags was recorded when measuring gas production. Methane volumes were normalised to 0 °C, assuming constant pressure 1 atm and expressed as m^3.

3. Results and discussion

3.1. Two-stage anaerobic digestion of blue mussels

The total methane yield obtained in the two-stage system from blue mussels was 0.33 ± 0.01 m^3/kg VS corresponding to 0.06 ± 0.01 m^3/kg TS and 0.03 ± 0.01 m^3/kg ww after 44 days of digestion (Table 2). Methane production in the UASB reactor was rapid when the leachate from the leach bed reactor was transferred into the UASB reactor. About 85% of the methane was produced in 23 days in the two-stage system and most of the methane production (68%) occurred in the UASB reactor (Fig. 2a). The methane content of the total gas produced was 58% in the leach bed reactor and 67% in the UASB reactor. High methane content is important as the biogas can be upgraded and used as a transport fuel. Fig. 3 shows the accumulated methane yields of the batch test of blue mussels. A methane yield of 0.29 ± 0.01 m^3/kg VS was obtained after 31 days of digestion in the batch test, showing that the digestion in the two-stage system was very efficient. Methane production in the batch test was also rapid when compared to the two-stage system and about 83% of the methane was produced in 13 days. Although the treatment period of the two-stage process was 44 days at an OLR of 1 g COD/l.d in the UASB reactor, a short treatment time was possible when operating the UASB reactor at a high OLR. There are few published results on the digestion of blue mussels. Johansson (2009) [13] obtained a high methane yield, 0.42 m^3/kg VS, in the digestion of the flesh fraction of blue mussels. Methane yields of blue mussels are expected to be lower when digested with shells due to the mass transfer limitations in the leach bed. The composition of blue mussels could also influence the variation in methane yields.

The meat fraction of the blue mussels was easily hydrolysed into soluble organic compounds. This is reflected in the initial increase in the concentration of COD in the leach bed reactor. The maximum concentration of COD attained was 14.0 g COD/l (day 3). The concentration of COD subsequently decreased to 4.4 g/l (day 10) due to the transfer of the leachate from the leach bed to the UASB reactor. The COD concentration in the leach bed reactor then decreased progressively to less than 1 g/l at the end of the experimental period (Fig. 4a). The decrease in the COD concentration in the UASB reactor was due to the efficient conversion of soluble organic compounds into biogas. Lim et al. (2008) [14] reported that mussels can be easily solubilised, with a soluble COD to total COD solubilisation of 67% in 4 days.

Optimal biogas production in a two-stage system can be achieved by efficient solubilisation and hydrolysis of the solid organic material to obtain soluble organics in the leachate that are in turn transferred to the methane reactor for biogas production. In the present study, the mussels were hydrolysed efficiently, as reflected in the high COD concentration in the leachate. Furthermore, 68% of the methane was produced in the methane reactor indicating that there was an efficient transfer of organic compounds from the leach bed reactor to the UASB reactor. The exchange of liquid between the reactors also led to the transfer of some buffer capacity and microorganisms from the UASB to the leach bed reactor, and this initiated the rapid onset of methanogenesis in the leach bed reactor. A small increase in biogas production in the leach bed reactor was observed due to the transfer of liquid on day 31 from the UASB reactor, completely submerging the mussel bed. It is therefore important for the entire blue mussel bed to be wet, so that no dry zones occur in order to explore the full biogas potential

Table 2
Methane yield, methane content, and energy potential of batch and two-stage anaerobic digestion of blue mussel and reed.

Substrate	Process type	Duration of experiment (days)	Methane yield (m^3 CH_4/kg VS added)	Methane content (%)	Energy potential (GWh/year)
Mussel	Batch	31	0.29 ± 0.01	50	16.6
	Two-stage process	44	0.33 ± 0.01	58[a], 67[b]	
Reed	Batch	31	0.19 ± 0.01	42	10.7
	Batch	127	0.33 ± 0.03	47	
	Two-stage process	107	0.22 ± 0.02	48[a], 60[b]	

[a] Methane content in total biogas produced in leach bed reactor.

[b] Methane content in total biogas produced in UASB reactor.

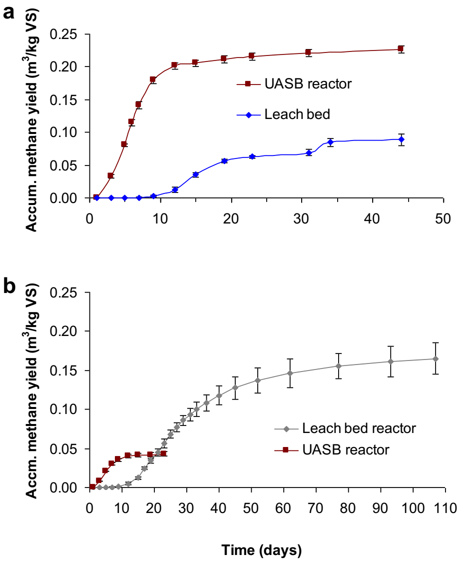

Fig. 2. Accumulated methane yields of the anaerobic two-stage digestion of blue mussel (a), and of reed (b).

of the material. In addition, a long digestion time is required to fully harness the biogas potential of the remaining organic materials in the leach bed reactor. Typical problems reported in leach bed reactors are poor soaking of the bed, channel formation and arid zones that can be significant at a larger scale [15].

The pH in the UASB reactors was between 7.2 and 7.7, within optimal limits for methane production [16]. The NH_4^+-nitrogen concentration also increased in the UASB reactor from 0.3 to 1.1 g/l due to the mineralisation of organically bound nitrogen in the protein fraction of blue mussels. The COD concentration out of the UASB reactors was less than 1 g/l when they were in operation, demonstrating that the degradation was effective and occurred under stable conditions. The decrease in concentration may also partly be due to dilution during the transfer of liquid from both reactors.

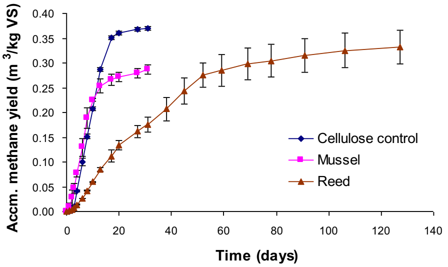

Fig. 3. Accumulated methane yield of batch test of avicel cellulose control, blue mussel, and reed.

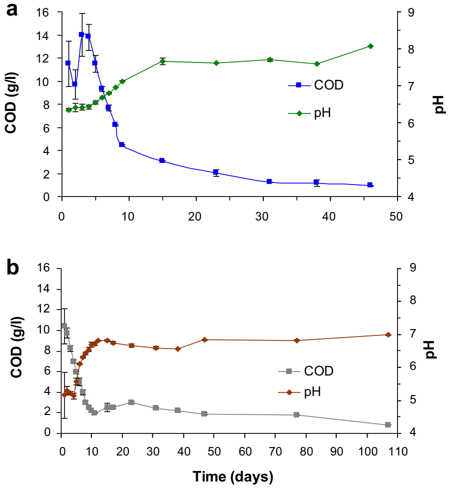

Fig. 4. pH and COD concentration in the leach bed reactor in the anaerobic two-stage digestion of mussel (a), and of reed (b).

NH_4^+-nitrogen concentration increased from 0.01 g/l to 1.1 g/l in the leach bed reactor due to the mineralisation of the proteins as previously described. NH_4^+-nitrogen is a nitrogen source for microorganisms as well as a buffering compound. One factor to consider in the digestion of blue mussels is the accumulation of NH_4^+-nitrogen and risk of ammonia inhibition of methanogens that might occur after several rounds of digestion. The C/N ratio of the mussels was 7.4; the reported ratio that is suitable for anaerobic digestion is about 25 [17]. Therefore, it may be advantageous to co-digest blue mussels with a carbon-rich material that reduces the nitrogen content. Another option can be to utilise a biogas system where multiple leach bed reactors are in use combined with a UASB reactor, treating different substrates with varied nutrient content. Thus, treatment of a favourable mixture of leachates can be beneficial for the entire two-stage system. The digestion of blue mussels in a two-stage process was efficient due to the rapid hydrolysis of the material. However, gas productivity (per reactor volume) was low due to the shells. In a previous study, no net energy balance was reported when blue mussels were used for biogas production, mainly due to the high harvesting cost when compared to algae and reeds [18]. Furthermore, mussel cultivation was instead the most efficient method for nutrient removal. Application of the digestate as a fertiliser, however, depends on the concentration of toxic compounds, such as heavy metals and other pollutants. Further investigations are required as mussels are filter feeders and might accumulate these compounds.

3.2. Two-stage anaerobic digestion of reeds

A total methane yield of reeds of 0.22 ± 0.02 m^3/kg VS was obtained after 107 days of digestion in the two-stage system (Table 2). This corresponded to a methane yield of 0.20 ± 0.02 m^3/kg TS and 0.16 ± 0.01 m^3/kg ww. Fig. 2b shows the cumulative methane production in the leach bed and UASB reactors. In the two-stage system, 80% of the methane was produced in the leach bed reactor. Hence, it is important to collect the biogas from this reactor and one-stage dry digestion can suffice in the digestion of reeds. Methane production ceased after a few days in the UASB reactor when the liquid transfer was stopped on day 13. The methane content in the total gas produced was 48% in the leach bed reactor and 60% in the UASB reactors. A higher methane yield of 0.33 ± 0.03 m^3/kg VS was obtained in the batch test after 127 days when compared to the two-stage system, indicating that the digestion of reeds was incomplete in the two-stage system (Fig. 3). The methane yield of 0.32 ± 0.03 m^3/kg VS was obtained in the batch test after 107 days and about 83% of the methane was produced in 52 days. However, the methane yield (0.19 ± 0.01 m^3/kg VS) of reeds in the batch tests was low in the short period of 31 days. The methane yield obtained in the batch test from the cellulose control was 0.37 m^3/kg VS, which can be compared to the theoretical yield of 0.415 m^3/kg VS calculated using Bushwell's formula [19]. Hence, the inoculum contained microorganisms that possessed cellulose degrading activity.

The pH in the leach bed reactor increased from 5.2 to 6.7 and the COD concentration decreased from 10.4 g/l to 2.0 g/l during the first 11 days (Fig. 4b). After stopping leachate transfer from the leach bed to the UASB reactor (day 13), the digestion proceeded in the leach bed reactor since conditions became favourable for biogas production. The COD concentration decreased to 0.8 g/l at the end of the experimental period. The onset of methanogenesis in the leach bed reactor was due to the removal and degradation of the easily hydrolysable compounds, and their conversion into methane in the UASB reactor. Furthermore, the leachate transfer also added some buffering species and microorganisms into the leach bed reactor. After this period, methane production was gradual since the hydrolysis in the leach bed was rate-limiting due to the slowly degradable lignocellulose fraction of the reeds. The concentration of NH_4^+-nitrogen was below 0.3 g/l throughout the experiment. The C/N ratio of the reeds was 34.2, which was at the upper limit of the recommended ratio suitable for anaerobic digestion [17].

During the 13 days that the UASB reactors were in operation, the pH ranged from 7.1 to 7.3 and the COD content was below 0.6 g/l, indicating that the degradation was efficient and the UASB reactors were operated under stable conditions.

A slight increase in the concentration of COD occurred on day 15 when 400 ml of liquid was transferred from the UASB to the leach bed reactor. The slight increase in COD was reflected in a slight drop in pH as a result of increased solubilisation and hydrolysis. This again shows that the entire bed was not in contact with the bacterial biomass and a completely soaked bed is important for the efficient digestion of the material. The floating of reed in the leach bed reactor can be problematic at full-scale and measures that ensure submergence of the reeds are thus of importance.

The composition of reeds has been reported to contain 28–34% of TS cellulose, 22% of TS hemicellulose and 14% of TS lignin. This is comparable to the composition of straw of 35% of TS cellulose, 25% of TS hemicellulose and 18% of TS lignin [21]. The similarity in the composition of reeds and straw is also reflected in the reported methane yields. Jagadabhi et al. (2011) [22] reported a methane yield of 0.26 m^3/kg VS when fresh reed was first hydrolysed in a leach bed reactor for 31 days followed by methane production in a UASB reactor of the pressed liquid. Methane yields from reeds are comparable to rice straw, which was reported to 0.239 m^3/kg VS during 89 days of digestion in a pilot-scale [23]. Reeds have a lower methane yield than the energy crop maize, which has 0.27–0.40 m^3/kg VS [24]. Reeds contain lignocellulose, which is slowly degraded and a long digestion time is needed to obtain the maximum biogas potential. The residence time plays an important role in the design of a full-scale plant. The present study showed that the digestion time should be long in a two-stage system, 70–90 days, in order to explore the full potential of the material. The solubilisation of organic compounds of reeds was low and was indicated by the low COD concentration in the leachate. This shows that the hydrolysis of reeds became rate-limiting faster than during the digestion of mussels. Furthermore, the onset of methane production was also rapid in the leach bed reactor containing reeds and 80% of the total methane produced was from the leach bed reactor. This means that the two-stage system was not as efficient as in the case of mussels. Thus, it might be possible to digest reeds in a one-stage leach bed reactor (dry digestion). In this dry anaerobic digestion system, digested residue from a previous batch that is rich in anaerobic microorganisms can be used to inoculate the fresh material, thereby providing both microorganisms and buffering species for a faster start-up and a stable operational process. Stable operational pilot-scale dry anaerobic digestion processes of municipal solid waste operating at an OLR of 11.6 kg VS/m^3.d [25] and full-scale dry digestion of energy crops at an OLR of 16–17 kg VS/m^3.d [26] have been previously reported. Although reeds with high TS can be a suitable material for combustion, freshly harvested reeds could be ensiled as a preservation method while pre-hydrolysing the material for easy use in a biogas reactor, especially when reed supply is low in winter. In addition, steam pretreatment of reeds, for instance, could be another option that may accelerate the degradation and obtain faster biogas production [27]. Reeds can be a suitable material for biogas production and has been reported to have the highest energy balance when compared to algae and mussels due to its high energy density [18].

3.3. *Energy potential of two-stage anaerobic digestion of blue mussels and reeds*

An estimated amount of 65,000 tons/year ww of blue mussels and 6360 tons/year ww of reeds can be collected in the Kalmar region, Sweden. The VS content of blue mussels was 7.7% of ww and the VS of reeds 76.4% of ww. In the estimation of energy potential, the lower heating value for methane, 9.97 kWh/m^3, was used. The calculated energy potential of two-stage anaerobic digestion of blue mussels and reeds were 16.6 and 10.7 GWh/year, respectively (Table 2). The biogas potentials obtained from sewage sludge and household waste have been estimated to be 19 GWh/year and 17 GWh/year, respectively [28]. Hence, blue mussels and reeds can be a substantial biomass resource for renewable transport fuel production.

4. Conclusions

The anaerobic digestion of blue mussels in a two-stage system was efficient as the meat fraction of blue mussels was easily degraded. The utilisation of a high rate methane reactor (UASB reactor) is beneficial for faster biogas production. The digestion of blue mussels with shells resulted in a low methane yield per volume wet weight of whole blue mussels. Hence, the methane production capacity per reactor volume was low, which will affect the economy of a plant. There is also the risk of ammonia accumulation and inhibition after long-term operation due to the mineralisation of proteins. Co-digestion with suitable substrates containing low levels of nitrogen can minimise the risk of ammonia inhibition.

The methane yield of reeds can be compared to that of straw. The digestion of reeds was slow and depended on the content of lignocellulose. Pretreatment might be an option to accelerate the digestion and lead to faster production of biogas. The digestion of reeds in the two-stage system was not as necessary as for blue mussels. Reeds can possibly be digested in a one-stage dry anaerobic digestion process in a leach bed reactor alone, with digestate recycling as inoculation for the process.

This study demonstrated the potential of anaerobic dry digestion of blue mussels and reeds for the production of renewable energy. It enables the utilisation of biomass that cannot be easily digested in their present form in conventional biogas systems. Furthermore, the conflict of food material used for energy production is avoided by the use of these types of biomass.

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Paper III

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Short communication

Exploring strategies for seaweed hydrolysis: Effect on methane potential and heavy metal mobilisation

V.N. Nkemka*, M. Murto

Department of Biotechnology, Lund University, P.O. Box 124, SE-221 00, Lund, Sweden

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ABSTRACT

Energy-rich methane can be harnessed from seaweed deposits by anaerobic digestion. However, the high heavy metal content in the seaweed and its digestates limits their use as fertilisers. Heavy metal contaminants from solid seaweed can be removed by, mobilisation into a liquid phase and subsequent metal ions adsorption. In this laboratory-scale study, pretreatment strategies for enhancing seaweed hydrolysis in relation to metal ions mobilisation were evaluated. Methane potential batch tests were also performed on the resulting treatment leachates. The results show that about 86% of the soluble organic compounds were hydrolysed/solubilised in a leach bed reactor followed by alkaline/autoclave post-treatments. However, Zn ion mobilisation was only 54% from the combined treatments. A 2.8-fold higher methane yield was obtained when the seaweed hydrolysis leachate and the post-treatment leachate were co-digested, compared to raw seaweed. This study demonstrated the efficient utilisation of seaweed for biogas production, and the partial heavy metals mobilisation to enable the metal removal for improved fertiliser quality.

1. Introduction

Large amounts of seaweed are deposited on beaches as a result of marine eutrophication, creating a significant waste handling problem [1–3]. This is of peculiar concern in countries around the highly eutrophied Baltic Sea. Anaerobic digestion has the potential as a waste treatment method for seaweed, and for use in renewable energy recovery in the form of methane. The use of seaweed and its digestate as a fertiliser is however, restricted due to high heavy metal contamination. Seaweed can accumulate heavy metals, forming metal coordination complexes with groups, such as carboxyl, carbonyl, hydroxyl, phenol and sulphonate groups that are contained on their cell wall. In fact, because of this reason, seaweed has been used in heavy metal bioadsorption [4].

Reduction of heavy metal concentration can be performed in a two-stage anaerobic system. In the first hydrolytic stage, solid organics are hydrolysed into organic acids that decreases pH, hence favouring the metal ions mobilisation [5]. Subsequent treatment of the liquid to precipitate or to adsorb the heavy metals can be performed. The resulting leachate with low heavy metal content can then be treated in a second stage methane reactor to produce biogas. An imminodiaacetic acid (IDA) polyacrylamide cryogel carrier, which is a supermacroporous adsorbent material, has been used to remove divalent metal ions such as Cd, Cu, Ni and Zn in seaweed leachate [6] and maize [7], and aimed at improving fertiliser quality. Hence, toxic heavy metals (especially cadmium) present in seaweed leachate could be reduced using two-stage anaerobic digestion combined with an IDA-cryogel adsorbent.

The present study investigated strategies for enhancing seaweed hydrolysis and heavy metals mobilisation. In particular, Zn ion mobilisation was studied as it occurred in high concentration. The methane potential from the resulting treatment leachates was also evaluated.

2. Materials and methods

2.1. Seaweed

The seaweed was composed of a mixture of brown and red seaweed, and was collected from Trelleborg, Sweden, in November 2006 and in May 2008. The seaweed was ground to a particle size of 2–3 cm and thereafter stored at −20 °C until use. The seaweed collected in November 2006 had total solids (TS) of 21.7 of wet weight (ww); volatile solids (VS) was 71.1% of TS. The seaweed collected in May 2008 had a TS of 24.6% of ww and a VS content of 30.4% of TS.

2.2. Seaweed hydrolysis in leach bed reactor

Hydrolysis experiments with and without batch dilutions with water were performed under different conditions on the seaweed collected in November 2006. The experiments were conducted in 1.2 l duplicate plastic reactors and with an active volume of 0.9 l. The leachate was recirculated over the hydrolysis bed using a pump at a constant flow rate of 10 ml/min. For all experiments, 0.3 kg of seaweed and 0.6 l of distilled water were added into the reactor. No inoculum was added, and

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* Corresponding author. Tel.: +46 46 222 83 47; fax: +46 46 222 47 13.
E-mail address: valentine.nkemka@biotek.lu.se (V.N. Nkemka).

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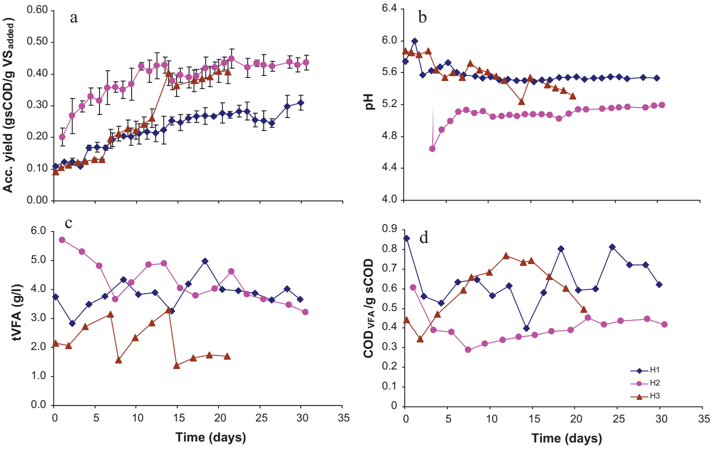

Fig. 1. (a) Accumulated process yield, (b) pH, (c) total VFA and (d) the ratio of COD_{VFA}/sCOD during seaweed hydrolysis. Hydrolysis of raw seaweed in leach bed without dilution H1: 37 °C; H2: 55 °C + NaOH. The initial pH in H2 was 12.4 and was due to NaOH addition. Hydrolysis of seaweed in leach bed with batch dilution H3: 22 °C.

the hydrolysis was dependent on the bacteria naturally present in the seaweed. The volume and composition of the evolved gas was analysed in all the experiments.

The reactors in H1 were operated at 37 °C; while H2 reactors were operated at 55 °C with 0.3 g NaOH/g VS added. The NaOH was added at the start of the hydrolysis. The liquid content in H2 was recirculated for two days and the pH was adjusted from 12.37 to 8.53 by adding equivalent amount of HCl. The reactors were operated for 30 days without withdrawing the leachate or diluting with water.

The reactors in H3 were operated at 22 °C and batch dilutions were performed weekly: by withdrawing 0.5 l of leachate and replacing it with an equivalent amount of distilled water [8]. This procedure was performed twice (on day 7 and day 14).

2.3. *Post-treatment of seaweed hydrolysis residue*

The seaweed used in these experiments was collected in May 2008. Seaweed residue (34.8% TS of ww and 27.5% VS of TS) remaining after the hydrolysis of seaweed in a leach bed reactor for 7 days at 37 °C was post-treated using different methods. Alkaline post-treatment was performed in a 0.5 l plastic container with an active liquid volume of 0.3 l. Acid post-treatment (P1) was performed using 10% weight by volume (w/v) of the seaweed residue with 2% volume by volume (v/v) H_2SO_4, and by autoclaving for 20 min at 121 °C and 3 bar. Post-treatment was also performed by pre-soaking 10% (w/v) of the seaweed residue in 0.25% (P2) (w/v) NaOH for 1 h [9], followed by autoclaving for 20 min at 121 °C and 3 bar. In the experimental control C1 and C2, 10% (w/v) of the seaweed was pre-soaked for 1 h in distilled water and 0.25% (w/v) NaOH respectively, and without autoclaving.

2.4. *Methane potential batch tests*

The seaweed used in these experiments was collected in May 2008. Methane production was performed in triplicates for 35 days under mesophilic conditions (37 °C), as earlier reported [10]. Methane production was evaluated on the following fractions: raw seaweed; hydrolysis leachate (HL) from the leach bed reactor operated at 37 °C; alkaline post-hydrolysis treatment leachate (PHL) P2; and mixture of HL and PHL (1:1 g total chemical oxygen demand (tCOD)/g tCOD). The concentration of tCOD in HL was 21.9 g/l with a pH of 5.5, and the concentration of tCOD in PHL was 12.2 g/l with a pH of 12.

2.5. *Analytical methods*

Analyses of TS, VS, soluble chemical oxygen demand (sCOD) and tCOD were performed according to standard methods [11]. In addition, analyses of sCOD, tCOD, volatile fatty acids (VFAs), biogas composition and volume and Zn ions were performed as previously reported [6]. For Zn ion analysis, 8 g of solid seaweed was digested in 100 ml 4.9 M HNO_3 and autoclaved (121 °C for 30 min) in acid washed bottles. The resulting liquid was filtered (0.45 μm Minisart, Satorius AG, Göttingen, Germany) and Zn ions were analysed. The ratio of COD_{VFA}/sCOD was calculated based on the conversion factor of VFA to COD as previously reported [12]. Samples for SO_4^{2-} were filtered (0.45 μm) and Dr. Lange test kit was used to analyse SO_4^{2-} (LCK 353) using a Lasa 100 spectrometer (Hach Lange GmbH, Germany).

3. Results and discussion

3.1. *Seaweed hydrolysis in leach bed reactor*

Temperature and NaOH addition effects were investigated in seaweed hydrolysis without dilution in leach bed reactors. The overall process yield was higher in the hydrolysis operated at 55 °C with NaOH addition (H2) (0.46 g sCOD/g VS_{added}), when compared to hydrolysis operated at 37 °C (H1) (0.31 g sCOD/g VS_{added}) (Fig. 1). The process yield obtained at 55 °C without NaOH addition was 0.37 g sCOD/g VS_{added} (experiment not described). After 10 days of hydrolysis in H2, the process yield was 0.42 g sCOD/g VS_{added}, corresponding to 91% of the final process yield. Hydrolysis was then very slow for the remaining 20 days, indicating that 10 days of hydrolysis was sufficient to release soluble organic matter under these conditions. The naturally occurring hydrolytic bacteria in H2 were active as evidenced by increased CO_2 production despite the alkaline treatment. NaOH treatment of green *Ulva* was reported to improve the degradation degree by 35% [3]. Rapid hydrolysis and acidogenesis resulted in VFAs accumulation in H1 and H2, and with a fairly constant low pH during the rest of the hydrolysis. Reported pH for optimal acidogenic activity is about 6.0 [8,13]. The VFA concentration varied during the hydrolysis (Fig. 1C). The fairly stable VFA concentration in the latter hydrolysis phase indicates acidogenesis and acetogenesis inhibition. The ratio of COD_{VFA}/sCOD is an indicator of the extent of acidogenesis, and a wide range from 0.4 to 0.9 g COD_{VFA}/sCOD has been reported for this ratio in previous studies [12]. The extent of acidogenesis in H1 was high, and the final ratio was 0.62 g COD_{VFA}/sCOD; implying that majority of the sCOD was present in the form of VFAs, although the system was still inhibited by its own product. The extent of acidogenesis was lower in H2 (0.42 COD_{VFA}/sCOD) when compared to H1. One reason for the lower extent of acidogenesis in H2 may be due to gelation observed in these experiments. These experiments were operated at 55 °C and the polymer formation may have made

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Table 1
Post-treatment of seaweed hydrolysis residue.

Experiments	pH	Process yield (g sCOD/g VS_{added})	SO_4^{2-} (g/l)
C1	6.2 ± 0.01	0.09 ± 0.01	0.05 ± 0.01
C2	12.46 ± 0.01	0.21 ± 0.02	0.07 ± 0.01
P1	0.62	0.47	–
P2	12.05 ± 0.02	0.78 ± 0.10	0.62 ± 0.01

C1 control: seaweed soaked for 1 h in distilled water; C2 control: seaweed soaked for 1 h in 0.25% (w/v) NaOH; P1 seaweed soaked for 1 h in 2% (v/v) H_2SO_4 and autoclaved; P2 seaweed soaked for 1 h in 0.25% (w/v) NaOH and autoclaved.

it difficult for the enzymes to access their substrates; an effect reported previously for brown seaweed digestion [2].

The effects of both temperature and dilution of the reactor content with water on seaweed hydrolysis in leach bed reactors was evaluated (Fig. 1). Final process yield of batch dilution strategies operated at 22 °C (H3) was 0.43 g sCOD/g VS_{added} and it was comparable to that obtained in H2. Similar final process yield of 0.39 g sCOD/g VS_{added} was also obtained at 37 °C and with batch dilution (experiment not described). A greater fraction of soluble organics 0.20 g sCOD/g VS_{added}, was released during the first week of hydrolysis in H3, and in the second week, the accumulated yield increased to 0.40 g sCOD/g VS_{added}. Hence, the first batch dilution in H3 was sufficient to release a greater fraction of the soluble organics. Dilution of the reactor content with water improved the VFA production and the release of soluble organics [8,14], by decreasing the concentration of VFAs that are less inhibitory to acidogens and acetogens. The dilution also prevented the low pH as observed in H1 and H2 (Fig. 1b). Suitable pH of about 6 was reported for acidogens [13].

3.2. Post-treatment of seaweed residue

Post-treatment was performed to evaluate the solubilisation of organic compounds that remained in the hydrolysed seaweed residue. Autoclave treatment of the seaweed residue with 2% (v/v) H_2SO_4, 0.25% (w/v) NaOH in experiments P1 and P2 resulted in process yields of 0.47 and 0.78 g sCOD/g VS_{added}, respectively (Table 1). Alkaline/autoclave treatment of the seaweed residue was more effective at the release of soluble organics than the acid/autoclave treatment. Almost all the soluble organics (86%) were released under the combined H1 and P2 conditions. It has been reported that, increasing the amount of Na^+ ions in insoluble Ca^+ ions alginate gels, leads to formation of soluble sodium alginate gels [15]. Alginates are extracted from brown seaweed and are the most abundant structural component of the cell wall and intercellular matrix [16]. Dissolving the gels can be important in large-scale operations, hence avoiding clogging of pipelines and pumps. The COD_{VFA}/sCOD ratio for P2 was lower since the post-treatment was physicochemical and not microbial. Post-treatment of seaweed hydrolysis residue also resulted in the release of SO_4^{2-} ions in the leachate which can be inhibitory in anaerobic processes at high concentrations [17].

A treatment time of 10–15 days would be sufficient to hydrolyse the seaweed in a leach bed reactor combined with post-treatment. In our previous study, a high methane production rate was obtained in the treatment of seaweed leachate in a UASB reactor; at an organic loading rate (OLR) of 20 g tCOD/l day and 2 days hydraulic retention time (HRT) [6]. Hence, about 10–15 days treatment time is needed to digest seaweed in an anaerobic two-stage process. HRTs of 27 to 11 days, which corresponds to OLRs of 1.1–2.6 g VS_{added}/l day, were reported when digesting green seaweed in a continuous stirred tank reactor (CSTR) [18]. Our results show that this type of two-stage anaerobic system has a very high treatment capacity when compared to the conventional CSTR.

3.3. Mobilisation of Zn ions during hydrolysis experiments

The concentration of Zn ions in the raw seaweed was 460 μg/g VS. Zn ion mobilisation during the hydrolysis of seaweed at 37 °C for 30 days (H1) was 13%, resulting in a concentration in the leachate of 61 ± 7 μg/g VS (Table 2). Further improvement was achieved when the seaweed was treated with 0.3 g NaOH/g VS and hydrolysed at 55 °C for 30 days (H2). Zn ions mobilisation under these conditions was 40% and the concentration in the leachate was 184 ± 11 μg/g VS_{added}. Zn ions mobilisation of 54% was obtained from the hydrolysis of seaweed at 37 °C for 7 days in a leach bed reactor, coupled with subsequent alkaline/autoclave treatment of the seaweed hydrolysis residue. About 21% of Zn ions were mobilised in the leach bed reactor and 33% in the alkaline/autoclave treatment. Selling et al. [7] reported about 56% mobilisation of Zn ions from the hydrolysis of maize in a leach bed reactor. Mobilisation of Zn ions was low in this study, despite the low pH range of 5.0–5.5 in the leach bed reactors. In our study, the low Zn ions mobilisation might be due to the precipitation of some of the mobilised metal ions. Consistent with this, formation of a black sulphide precipitate was observed in the leach bed reactor and in the tubings after about 5 days of hydrolysis of the seaweed. Another reason for the low Zn ions mobilisation could be due to metal ions chelation by anionic groups present in seaweed. Furthermore, pH has been shown to be an important parameter that influences the adsorption of Zn ions on *Ulva fasciata*: maximum Zn ions adsorption occurred at a pH range of 5–6; while Zn ions adsorption was low at pH outside of this range [19].

It could be recommended that, removal of heavy metals in an anaerobic two-stage process could be conducted after the high-rate methane reactor, and not between the leach bed reactor and the high-rate methane reactor. Sulphide precipitation may be important in the reduction of the heavy metal concentration in the anaerobic two-stage process. IDA-cryogel carriers could then be used as a polishing step for the removal of low concentrations of heavy metal ions from the effluent: since it enables the efficient removal of low metal ions concentrations and also offers the possibility of recovering the metal ions [20].

3.4. Methane potential batch tests

The methane yield obtained from the digestion of raw seaweed was 0.12 l CH_4/g VS_{added} (Table 3) and it was similar to the reported yield of 0.11 l CH_4/g VS_{added} from green *Ulva* [21]. A methane yield of 0.35–0.48 l CH_4/g VS_{added} was obtained in batch

Table 2
Zn ions concentration in seaweed and seaweed leachate and the degree of mobilisation.

	Initial conc. of Zn ions in seaweed (μg/g VS)	Conc. of Zn ions in seaweed leachate (μg/g VS)	Mobilisation of Zn (%)
H1	460	61 ± 7	13
H2	460	184 ± 11	40
P2	146	78	54

H1: hydrolysis at 37 °C without dilution; H2: hydrolysis at 55 °C + NaOH without dilution.

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Table 3
Methane yield obtained in methane potential batch tests.

Substrate	Methane yield (l CH_4/g tCOD$_{added}$)	Methane yield based on the initial amount of raw seaweed added (l CH_4/g VS$_{added}$)
Raw seaweed		0.12 ± 0.01
HL	0.27 ± 0.03	0.15 ± 0.01
PHL	0.17 ± 0.02	0.11 ± 0.02
HL/PHL	0.28 ± 0.01	0.34 ± 0.02

HL: hydrolysis leachate; PHL: post-treatment leachate.

digestion of green seaweed [18]. The methane yield obtained in the present study was low, and could be due to gelation [2], the presence of inhibitory compounds such as SO_4^{2-} [17], polyphenols [2], NaCl [22], heavy metals, and the seasonal variations in the composition and collection time of seaweed [23]. The methane yield obtained from seaweed leachate (HL) was 0.27 l CH_4/g tCOD$_{added}$ (or 0.15 l CH_4/g VS$_{added}$ of initially added seaweed). The methane yield obtained from HL was comparable to that of raw seaweed. However, the methane production rate from the seaweed leachate was higher when compared to that of raw seaweed (results not shown). A reported methane yield of 0.26 l CH_4/g tCOD$_{added}$ was obtained in the digestion of green seaweed *Ulva* leachate in an anaerobic fixed bed reactor [3]. Methane production from the post-treatment leachate (PHL) resulted in a methane yield of 0.17 N l CH_4/g tCOD$_{added}$ (or 0.11 N l CH_4/g VS$_{added}$ of the seaweed initially added), and it was also similar to that obtained from raw seaweed. The methane yield of the PHL was lower than that obtained from the HL, possibly due to the formation and release of inhibitory compounds during alkaline/autoclave treatment of the seaweed residue [24]. Co-digestion of HL/PHL at a 1:1 g tCOD/g tCOD ratio resulted in a methane yield of 0.28 N l CH_4/g tCOD$_{added}$ (0.34 N l CH_4/g VS$_{added}$ of raw seaweed initially added), and it was 2.8-fold higher than the methane yield of raw seaweed. A methane yield of 0.25 N l CH_4/g VS$_{added}$ was reported from autoclaved red algae in a batch test [25]. Mixing the leachates may have diluted the inhibitory compounds and also balanced the micro and macronutrients. Adjustment of the pH to 8.1 was achieved when mixing HL (pH 5.5) and PHL (pH 12) and could thus be treated in methane reactors with little pH adjustments. In addition, a neutral pH is preferred to prevent corrosion in metal pipelines in large-scale processes. However, the use of NaOH in the post-treatment may increase the likelihood of Na^+ ion inhibition. Alternative bases such as $Ca(OH)_2$ are inhibitory to methanogens at higher concentrations than Na^+ ions and could substitute for NaOH [26].

4. Conclusions

Enhanced hydrolysis of seaweed shall facilitate the utilisation of the energy bound in seaweed for methane production. Efficient hydrolysis also facilitates the removal of the high heavy metal content. Reduction of this heavy metal content is important, since it allows the use of the seaweed digestate as a fertiliser. Seaweed was efficiently hydrolysed in a leach bed reactor combined with alkaline/autoclave post-treatment. Despite the high organic matter solubilisation, Zn ions mobilisation from seaweed was partial from the combined treatments. This could be attributed to the complex binding onto chelating compounds and sulphide precipitation of the mobilised metals. Anaerobic digestion of the hydrolysis and the post-treatment leachates resulted in a higher methane yield when compared to their separate digestion and also to raw seaweed. Efficient hydrolysis of seaweed enabled the utilisation of seaweed for high methane productivity and in a methane high rate reactor. Hence, a two-stage system can be a feasible alternative for biogas production from seaweed.

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Paper IV

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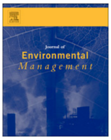

Evaluation of biogas production from seaweed in batch tests and in UASB reactors combined with the removal of heavy metals

Valentine Nkongndem Nkemka, Marika Murto*

Department of Biotechnology, Lund University, P. O. Box 124, SE-221 00 Lund, Sweden

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A B S T R A C T

Seaweed can be anaerobically digested for the production of energy-rich methane. However, the use of seaweed digestate as a fertilizer may be restricted because of the high heavy metal content especially cadmium. Reducing the concentration of heavy metals in the digestate will enable its use as a fertilizer. In this laboratory-scale study, the potential of seaweed and its leachate in the production of methane were evaluated in batch tests. The effect of removing the heavy metals from seaweed leachate was evaluated in both batch test and treatment in an upflow anaerobic sludge blanket (UASB) reactor. The heavy metals were removed from seaweed leachate using an imminodiacetic acid (IDA) polyacrylamide cryogel carrier. The methane yield obtained in the anaerobic digestion of seaweed was 0.12 N l CH_4/g VS_{added}. The same methane yield was obtained when the seaweed leachate was used for methane production. The IDA-cryogel carrier was efficient in removing Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} ions from seaweed leachate. The removal of heavy metals in the seaweed leachate led to a decrease in the methane yield. The maximum sustainable organic loading rate (OLR) attained in the UASB reactor was 20.6 g tCOD/l/day corresponding to a hydraulic retention time (HRT) of 12 h and with a total COD removal efficiency of about 81%. Hydrolysis and treatment with IDA cryogel reduced the heavy metals content in the seaweed leachate before methane production. This study also demonstrated the suitability of the treatment of seaweed leachate in a UASB reactor.

1. Introduction

Marine eutrophication results in the deposition of massive amounts of macroalgae on beaches. In Sweden, 5 tonnes of algae per square meter of beach has been recorded annually (Malm et al., 2004). If it is not collected, the huge amount of seaweed makes the beaches inaccessible for recreational purposes while its collection creates the problem of disposal. Some species of seaweed are edible, and some used for the production of animal feed and as fertilizers, due to their nutrient content. Seaweed also serves as a source of useful biomolecules such as agar, polyphenols and other active biomolecules (Chandini et al., 2008). Incineration of seaweed for energy purposes is not feasible as fuel must be added or efficient dewatering processes applied to reduce the high water content.

Seaweed has great potential as an energy crop because it contains easily hydrolysable sugars (Horn et al., 2000) and has a low lignin content (Stewart, 1979). The lignin content reported for sugar beets and maize was about 4.5 and 15.1 g/g dry matter respectively (Martinez-Pérez et al., 2007), while that reported for seaweed *Ulva lactuca* was lower and was about 0.03 g/g dry matter (Ventura and Castañon, 1998). Anaerobic digestion not only solves the problem of disposal, but also provides renewable energy in the form of methane (Hansson, 1982; Hanssen et al., 1987; Cecchi et al., 1996). However, the anaerobic digestion of seaweed is not straightforward. Some of the problems encountered are high concentrations of SO_4^{2-} (Cecchi et al., 1996), NaCl (Feijoo et al., 1995) and heavy metals. If these heavy metals could be removed, the digestate could be used as a fertilizer. The problem with the high concentration of SO_4^{2-} and hence sulphide in the digester would be increased when removing heavy metals that are expected to precipitate with H_2S in the digester. The subject of this study was thus the removal of heavy metals from the seaweed leachate and subsequent biogas production.

Seaweed contains high amounts of ionizable groups, e.g. carboxyl groups and sulphonate groups, on their cell wall polysaccharides, which contribute to the biosorption of heavy metals (Lodeiro et al., 2005). High concentrations of cadmium can be accumulated in seaweed originating from the Baltic Sea as the exchange of water is low. In Sweden, seaweed is classified as a toxic

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* Corresponding author.
E-mail address: Marika.Murto@biotek.lu.se (M. Murto).

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waste, due to its high content of Cd and the potential to use the digestate as a bio-fertilizer, is restricted. McLaughlin (2001) among others reported that, cadmium is toxic to humans at low concentrations that is not toxic to plants.

The accumulation of sand which reduces the HRT was reported as one of the problems in the anaerobic digestion of the seaweed species *Ulva* in a continuous stirred tank reactor (CSTR) (Briand and Morand, 1997; Morand and Briand, 1999). Seaweed can be applied in the anaerobic digestion two-stage process. In such a process, solid organic matter is first solubilized/hydrolysed in a leach-bed reactor to produce a leachate with soluble organic compounds mainly in the form of VFA. The leachate can be subsequently circulated into a high-rate methanogenic reactor for biogas production. In this study, seaweed was first hydrolysed separately and a UASB reactor was used in the treatment of the leachate. UASB reactors and other reactors with attached biomass have been reported to withstand higher concentrations of dissolved H_2S than reactors based on suspended biomass (Omil et al., 1995).

The heavy metal ions present in seaweed leachate are mobilized at the low pH prevailing during hydrolysis, and can be removed using an adsorbent. Lehtomäki and Björnsson (2006) reported an improvement in the solubilization of metals at low pH of about 4 in the anaerobic two-stage digestion of energy crops (willow, sugar beets and grass silage). The resulting leachate, with lower heavy-metal content, can then be used for biogas production in the second phase. The heavy metals are removed using a polyacrylamide cryogel, onto which an iminodiacetic acid (IDA) ligand has been introduced to produce an IDA cryogel (Plieva et al., 2006), by the formation of complexes between the divalent metal ions and the IDA groups. IDA cryogel can be used to treat wastewater contaminated with particulate matter containing heavy metals because of its supermacroporous structure. The use of IDA cryogel in Kaldnes plastic carriers reduces the risk of clogging, and is a novel method of heavy metal removal.

In this study, the production of methane from raw seaweed was evaluated in batch tests. Similar tests were then performed to evaluate the effect of removing the heavy metals from seaweed leachate. The heavy metals considered in this study were Cd, Cu, Ni and Zn. Finally, the suitability of treating seaweed leachate in a UASB reactor to produce methane, and the effect of heavy metal removal from the seaweed leachate were evaluated.

2. Materials and methods

2.1. Inoculum

The inoculum used in the batch experiments was collected from Ellinge wastewater treatment plant in Eslöv, Sweden. The inoculum used in the UASB reactors was mesophilic granules from a full-scale UASB plant in Denmark treating lactose wastewater.

2.2. Seaweed

The seaweed was collected from the beach at Trelleborg, on the southern coast of Sweden, on September 2007. The seaweed was ground with a GRINDOMIX GM 200 blender at 4500 rpm for 20 s to reduce the particle size to 2–3 cm after which it was stored at −20 °C until use. The raw seaweed had total solids (TS) of 21.7% of wet weight, volatile solids (VS) of 71.1% of TS, Cd concentration of 4.3 μg/g VS and Zn concentration of 460.3 μg/g VS.

2.3. Production of hydrolysis leachate from seaweed

The leachate was produced by hydrolysing seaweed using a 20 l plastic leach-bed hydrolytic reactor. The active reactor volume was about 14 l, and approximately 7 l of distilled water was added to 7 kg seaweed at the start of each batch hydrolysis. The liquid in the reactor was circulated at a flow rate of 15 ml/min over the seaweed to obtain efficient contact between the bacterial biomass and the seaweed. The reactor was operated at 37 °C for 10–14 days, and 8 batches of hydrolysis were performed. The leachate was collected after each batch and the total chemical oxygen demand (tCOD) ranged from 17 to 21 g/l.

The leachate was centrifuged at 6000 rpm for 10 min at 4 °C or filtered (mesh size: 125 μm particle size) to remove sand and other large particulate matter. Samples were analysed with regard to pH, soluble chemical oxygen demand (sCOD), tCOD and the concentration of VFAs. This leachate was then used to determine the methane production in batch tests and with a UASB reactor.

2.4. Removal of heavy metals from seaweed leachate

One fraction of the leachate was treated with IDA cryogel to remove heavy metals. IDA cryogel carriers were prepared as reported elsewhere (Plieva et al., 2006). The monomer concentrations of the IDA cryogel used was 10% of the gel. The IDA cryogel carriers were regenerated with 0.1 M ethylenediaminetetraacetic acid (EDTA) at a pH 7 to 8.

Removal of the heavy metals was performed by treating 0.6 l of the seaweed leachate in a 1-L acid-washed plastic container containing 0.09 l IDA cryogel carriers. Treatment was performed for 10 h at 23 °C with the aid of a magnetic stirrer set at 150 rpm. The untreated fraction was allowed to stand next to the treated one for the same length of time. Samples were taken continuously to evaluate the efficiency of heavy metal removal. The metal ions analysed were Cd, Cu, Ni and Zn.

2.5. Methane production in batch tests

The methane produced from raw seaweed, untreated and treated seaweed leachates was investigated. The experimental set-up was the same as described previously (Parawira et al., 2004). The methane potential batch tests were performed for 30 days in triplicates under mesophilic conditions (37 °C). Inoculum substrate ratios (ISRs) of 1:1 and 1:2 g VS/g VS were used in the batch tests of raw seaweed while the ratio of 1:1 g VS/g tCOD was used for the batch tests on seaweed leachate. $NaHCO_3$ (1.4 g/g COD_{added}) was used to buffer any VFA present in the seaweed leachate that might cause inhibition of the methanogens. The pH, tCOD, sCOD, and total VFA (tVFA) are presented in Table 1 and the heavy metal concentration of Cd, Cu, Ni and Zn are presented in Table 2. The volume and composition of the gas, pH and VFA were analysed during the course of the experiments.

2.6. Methane production from seaweed leachate in UASB reactors

Untreated seaweed leachate and the leachate treated with IDA cryogel to remove the heavy metals were digested in UASB reactors. The experiment was performed under mesophilic conditions (37 ± 1 °C) in UASB reactors with a working volume of 0.8 l, as

Table 1
Characteristics of the seaweed leachates digested in the UASB reactors.

	Treated leachate	Untreated leachate
pH	5.09 ± 0.09	5.06 ± 0.11
sCOD (g/l)	6.3 ± 1.1	7.3 ± 1.1
tCOD (g/l)	9.3 ± 0.6	10.6
Total VFA (g/l)	4.6 ± 0.8	5.29 ± 0.5
NH_4^+-N (mg/l)	22 ± 7	24 ± 5
SO_4^{2-} (g/l)	3 ± 2	4 ± 3
Dissolved H_2S (g/l)	0.06 ± 0.01	0.07 ± 0.01

Average of 8 hydrolysis batches.

Table 2
Removal efficiency of heavy metals from seaweed leachate using IDA cryogel carriers.

	Cd (μg/l)	Cu (μg/l)	Ni (μg/l)	Zn (μg/l)
Untreated leachate	19.0	211.7	36.6	1567.3
Treated leachate	4.0	87.4	10.9	920.6
Removal efficiency (%)	79	59	70	41

previously described (Parawira et al., 2006). Two UASB reactors were used: one was fed with the untreated leachate, and the other was fed with the treated leachate. The untreated leachate and the treated leachate were diluted to about 10.6 g tCOD/l. The leachates were then stored in 1-L acid-washed plastic containers at −20 °C until use. Table 1 presents the characteristics of the diluted seaweed leachates treated in the UASB reactors. The OLR was increased step-wise from 1.1 to 20.6 g tCOD/l/day, by decreasing the HRT from 8.8 to 0.5 days. Both reactors were operated for at least three times the HRT at each OLR applied and the total treatment period was for 107 days. Samples were taken from the reactors to determine pH, partial alkalinity (PA), tCOD, VFAs, NH_4^+-N, dissolved H_2S, and the composition and volume of the gas, to evaluate the performance of the UASB reactors.

2.7. *Analytical methods*

The PA and VFAs (acetate, propionate, i-butyrate, n-butyrate, i-valerate and n-valerate) were determined according to Murto et al. (2004). The sCOD and tCOD were measured on filtered (0.45 μm Minisart, Sartorius AG, Göttingen, Germany) and unfiltered samples, respectively, according to standard methods (APHA, 1998). Heavy metals samples were preserved by acidifying the samples with 65% concentrated HNO_3 to pH less than 2, stored in acid-washed plastic sample tubes and at 4 °C. A graphite furnace atomic absorption spectrometer (GBC932 AA, Dandenong, Australia), was used to determine the concentration of heavy metals. For the analysis of Cd^{2+} ions, 4% ammonium dihydrogen phosphate was used as the modifier matrix.

The TS and VS were analysed according to standard methods (APHA, 1998). The biogas composition was determined using a gas chromatograph (6890N Agilent Technologies, CA, USA), equipped with a Hay sep N 80/100, a molecular sieve column (5A 60/100,) and a thermal conductivity detector. Helium was used as carrier gas at a flow rate of 12 ml/min. The injector, oven and detector temperatures were 105 °C, 60 °C and 150 °C, respectively. The gas volume was measured using a 100 ml glass syringe (furtuna®, Germany). Methane yields were normalized by correcting the gas volume to STP and the results expressed as N l CH_4/g $tCOD_{added}$ for liquids or N 1 CH_4/g VS_{added} for solids. The gas production rate (GPR) was also normalized and expressed as N l CH_4/l/day. Dissolved H_2S was measured immediately after sampling. Analysis of dissolved H_2S and SO_4^{2-} ions was performed using a standard turbidometric method (APHA, 1998).

A Dr. Lange test kit (LCK 303) was used to analyse NH_4^+-N in the filtered (0.45 μm filter) samples using a Lasa 100 spectrometer (Dr. Bruno Lange GmbH, Germany).

The means of the methane yields were analysed using the independent sample test with SPSS 16.0. The equality of variance was tested with the Levene's test and the normality of the variables was tested with the normal Q–Q plot.

3. Results and discussion

3.1. *Removal of heavy metals from seaweed leachate*

The IDA cryogel was efficient in removing Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} ions from seaweed leachate, as can be seen in Table 2. The removal efficiency ranged from 41 to 79% and it was dependent on the metal. Removal of heavy metals is also dependent on the solubilisation in the hydrolysis of the seaweed.

The concentration of Cd^{2+} ions in the raw seaweed was 4.3 μg/g VS, which corresponds to 3.2 μg/g TS. Only a small fraction of the Cd^{2+} ions, (0.6 μg/g TS) was mobilized in the leachate, giving a degree of mobilization of 18%. This implies that 2.7 μg/g TS of Cd^{2+} ions remained in the seaweed residue. Selling et al. (2008) obtained about 29% of Cd and 56% of Zn mobilized in the liquid when hydrolysing maize in a leach-bed reactor. The corresponding total metal removal was 25% of Cd and 40% of Zn using the IDA-cryogel packed in a column. In the anaerobic two-stage digestion of willow, sugar beets and grass silage, the cadmium concentration in the residue was 4.66, 1.52 and 0.56 μg/g TS respectively (Lehtomäki and Björnsson, 2006).

Although the pH of the leachates at the end of the hydrolysis experiment was low, 5.54 at 37 °C, the degree of mobilization of heavy metals was low. This is probably due to the fact that only about 50% (in terms of VS) of the seaweed was solubilized/hydrolysed. Another reason may be that the heavy metals were mobilized, but were precipitated, e.g. with sulphide, or captured by other chelating compounds, and were therefore not present as ions in the liquid phase. The precipitation of heavy metals as sulphides has been reported to increase with increasing pH above pH 5.5 (Rodríguez et al., 2009). The high content of ionizable groups on the cell wall polysaccharides, suggests that mobilization was affected by changes in the pH of the solution. The dominating functional groups are carboxyl groups (Lodeiro et al., 2005; Karthikeyan et al., 2007). In a study performed by Lodeiro et al. (2005) on the biosorption of Cd using brown macroalgae, the pK_a for different seaweeds was estimated to be 3.5–4.0. This implies that, at pH values lower than pK_a, the carboxyl groups are mainly protonated, resulting in low Cd uptake. At pH values higher than pK_a, the functional groups are negatively charged and the positively charged Cd ions will bind to them, increasing the Cd uptake. Therefore, in this study, the functional groups will bind the heavy metals due to the prevailing pH (>5), thus resulting in low mobilization.

Greger et al. (2007) suggested that macroalgal compost could be used in small amounts on agricultural soils as a valuable nutrient for non-food crops. In the present study, the Cd content was still high in the digested material after the treatment with IDA cryogel, and exceeded the Swedish limit for fertilizers. In future studies, more acidic conditions should be used during hydrolysis or more efficient degradation of the seaweed to achieve more efficient mobilization of heavy metals. In this way, the level of Cd could perhaps be reduced making the digested material suitable as a fertilizer. Studies are being performed to increase the solubility and to investigate the fate of the heavy metals during hydrolysis.

3.2. *Methane production in batch tests*

Batch tests were conducted to evaluate the methane production from raw seaweed, and treated and untreated seaweed leachate. A summary of the results are shown in Table 3. A methane yield of 0.12 N l CH_4/g VS_{added} was obtained when digesting raw seaweed material at an ISR of 2:1 g VS/g VS. The methane yield was slightly lower when the ISR was 1:1 g VS/g VS, which may indicate inhibition. The inhibition may be due to a high substrate concentration (Raposo et al., 2009). Another reason may be due to the dilution effect of inhibitory compounds such as H_2S, heavy metals and NaCl that were present in the seaweed when a larger fraction of the inoculum was used. Ninety percent of the methane was produced within the first 14 days of digestion, and the methane content was 44% when using an ISR of 2:1 g VS/g VS. The pH was about 7.6 and the concentration of VFAs was low. The results obtained in this study are comparable to that in a study by Briand and Morand

Table 3
Summary of the results of the methane potential batch tests.

	ISR[a] (g VS/g VS)	pH	Methane yield (N l CH_4/g VS_{added})	Methane content (%)	VFA (g/l)
Raw seaweed	1:1	7.57 ± 0.01	0.09 ± 0.01	49.7 ± 0.5	–
Raw seaweed	2:1	7.64 ± 0.03	0.12 ± 0.00	44.4 ± 1.3	–
	(g VS/g tCOD)		(N l CH_4/g $tCOD_{added}$)		
Untreated leachate	1:1	8.03 ± 0.02	0.24 ± 0.01	61.7 ± 0.3	0.25 ± 0.02
Treated leachate[b]	1:1	7.99 ± 0.02	0.20 ± 0.01	61.3 ± 0.1	0.21 ± 0.03

[a] ISR inoculum substrate ratio.

[b] Treated with IDA cryogel to remove heavy metals.

(1997), who reported a methane yield of 0.11 l CH_4/g VS_{added} after 23 days of batch digestion of *Ulva* sp. Hansson (1982), reported a higher methane yield, of 0.35–0.48 l CH_4/g VS_{added}, when digesting green algae in batch experiments.

The reason for the low methane yield, compared with that obtained by Hansson (1982), may be due to a recalcitrant fraction remaining after the utilization of the easily hydrolysable organics. The degradation of seaweed may also release other inhibitory compounds. Tannins, which are present in brown seaweed could be released and are potent inhibitors of methanogens (Moen et al., 1997). The seaweed in this study contained high amounts of sulphate, which can be reduced under anaerobic conditions by sulphate-reducing bacteria (SRB) to H_2S, with the expense of organic matter (O'Flaherty et al., 1998). Hence, not all the available organic matter in the seaweed was converted into biogas. The dilution effect of adding the inoculum may have made it difficult to observe any possible inhibition by inhibitory compounds. Seaweed collected in March usually contains high amounts of proteins and alginic acid, and low amounts of carbohydrates. As photosynthetic activity increases during spring, the carbohydrate content increases, while the ash, protein and alginic acid contents decrease (Rosenberg and Ramus, 1982). Variation in the composition and the species of seaweed may affect the methane yield. The time at which the seaweed is collected from the beach may also influence the methane yield. If the seaweed is left on the beach for a long time, waves may wash away the easy degradable fraction.

There was a decrease in the methane yield obtained from the treated seaweed leachate (0.20 ± 0.1 N l CH_4/g $tCOD_{added}$) and the untreated leachate (0.24 ± 0.1 N l CH_4/g $tCOD_{added}$) ($P < 0.05$, at 95% confidence interval). This corresponds to the methane yield of 0.12 ± 0.1 N l CH_4/g VS_{added} of the seaweed initially added. The methane content obtained from seaweed leachate was 61% compared to 44% from raw seaweed, which is beneficial if the biogas is to be upgraded. Most of the methane (90%) was produced from the seaweed leachate during the first 9 days of digestion. The methane production from *Ulva* sp leachate in a packed-bed reactor was reported by Morand and Briand (1999) to be 0.26 l CH_4/g $tCOD_{added}$. Generally, anaerobic digestion is inhibited by high concentrations of heavy metals (Wong and Cheung, 1996), but they must be bioavailable to cause inhibitory effects. On the other hand, supplementation of micronutrients such as Co^{2+}, Ni^{2+} and Zn^{2+} where they are deficient has been shown to have stimulatory effects (Speece et al., 1983; Bruce et al., 2001). In this case, the micronutrients present after treatment with IDA cryogel carriers might have not been sufficient for efficient biogas production or to precipitate H_2S that can be inhibitory to methanogens. The inoculum would have provided a source of micronutrients during the batch digestion. No inhibition resulting from the high concentrations of heavy metals was observed during the present experiments. Batch digestion was not sufficient to investigate the effect of the removal of heavy metals on anaerobic digestion of seaweed leachate in this study. Hence, a further experiment in a UASB reactor for the continuous digestion of the treated and untreated seaweed leachate was investigated.

The methane yield obtained from seaweed leachate was 0.24 N l CH_4/g $tCOD_{added}$ or 0.12 N l CH_4/g VS_{added} of raw seaweed initially added. The methane yields obtained in the anaerobic digestion of seaweed and seaweed leachate were the same in terms of g VS_{added}, but the time needed to produce biogas from leachate was shorter than that with seaweed. When seaweed is hydrolysed to produce the leachate, the remaining residue still contains a substantial amount of organic matter. This residue can be utilized, but further treatment is needed to hydrolyse the recalcitrant fraction. The resulting leachate can then be also subjected to anaerobic digestion. Co-digestion with other materials is another strategy for improving methane production from seaweed. Co-digestion may reduce the effects of some inhibitory compounds and allow the digestion of seaweed in pre-existing biogas plants. Experiments concerning these strategies are currently being evaluated.

Table 4
Values of HRT, OLR, methane yield, methane content and GPR during steady state at each OLR level. UASB 1 digested treated seaweed leachate using IDA cryogel to remove heavy metals and UASB 2 digested untreated leachate.

	OLR (g tCOD/l/day)	HRT (days)	Methane yield (N l CH_4/g $tCOD_{added}$)	Methane content (%)	GPR (N l CH_4/l/day)
UASB 1	1.1	8.6	0.18 ± 0.03	75.1	0.19 ± 0.02
	2.5	3.7	0.20 ± 0.01	71.2	0.44 ± 0.01
	4.4	2.1	0.18 ± 0.01	70.9	0.77 ± 0.01
	7.1	1.3	0.17 ± 0.02	71.1	1.14 ± 0.03
	11.0	0.8	0.15 ± 0.02	66.1	1.83 ± 0.04
	18.1	0.5	0.14 ± 0.02	63.2	2.60 ± 0.19
UASB 2	1.2	8.8	0.21 ± 0.03	73.7	0.22 ± 0.01
	2.9	3.7	0.23 ± 0.03	69.3	0.57 ± 0.01
	4.9	2.1	0.21 ± 0.02	68.9	0.99 ± 0.02
	8.1	1.3	0.19 ± 0.01	69.1	1.38 ± 0.04
	12.5	0.8	0.20 ± 0.02	65.4	2.37 ± 0.12
	20.6	0.5	0.16 ± 0.02	62.9	3.04 ± 0.16

3.3. *Methane production from seaweed leachate in UASB reactors*

3.3.1. *Methane yield and gas production rate*

Experiments were performed to evaluate the suitability of the treatment of seaweed leachate in a UASB reactor. Values of some process parameters analysed during steady state at each OLR level are presented in Table 4. One of the UASB reactors was fed with the leachate treated with IDA cryogel carriers to remove the heavy metals (UASB 1), and the other was fed with the untreated leachate (UASB 2).

The methane yield was used to compare the efficiency of UASB 1 and UASB 2. The highest methane yield obtained in UASB 1 was 0.20 ± 0.01 N l CH_4/g $tCOD_{added}$, at an OLR of 2.5 g tCOD/l/day, while the highest yield in UASB 2 was 0.23 ± 0.03 N l CH_4/g $tCOD_{added}$, at an OLR of 2.9 g tCOD/l/day. There was a significant difference ($P < 0.05$, at 95% confidence interval) of the effect of removal of heavy metals from seaweed leachate when treating it in the UASB reactors during the entire treatment period. Hence, the removal of heavy metals from seaweed leachate may have increased the inhibitory effects of SO_4^{2-} and H_2S as less heavy metals were present to allow for precipitation and reduce the concentration of H_2S.

The methane yield decreased as the OLR was increased. In UASB 1, the methane yield decreased to 0.14 ± 0.02 N l CH_4/g $tCOD_{added,}$ at an OLR of 18.1 g tCOD/l/day and in UASB 2, the methane yield decreased to 0.16 ± 0.02 N l CH_4/g $tCOD_{added}$ at an OLR of 20.6 g tCOD/l/day. A decrease in the methane yield was expected since the HRT was very short (12 h), and thus the microorganisms had very little time to convert the organics into biogas. This was reflected in the decrease in the reduction of tCOD as the OLR was increased (Table 5). The methane yields obtained in the UASB reactors were in the same range as those obtained in the batch tests, showing that biogas production in UASB reactors was at least as efficient.

Very little work has been performed on the anaerobic digestion of seaweed leachate with high-rate reactors. A methane yield of 0.24 N l CH_4/g $tCOD_{added}$ was obtained at an OLR of 2.53 g tCOD/l/day when digesting the leachate from green seaweed species *Ulva* with an anaerobic packed-bed reactor (Morand and Briand, 1999). A methane yield of 0.23–0.32 N l CH_4/g VS_{added} has also been reported in anaerobic digestion of seaweed under mesophilic conditions in a CSTR (Hansson, 1982). The methane yields were normalized to STP assuming 25 °C for easy comparison.

Although the methane yield decreased in both cases as the OLR was increased, the treatment capacity was high, and the GPR can also be used as a measure of the performance of the reactor. Generally, the GPR increased with increasing OLR during the entire treatment period. The GPR was 2.60 N l CH_4/l/day in UASB 1 and 3.04 N l CH_4/l/day in UASB 2 at the highest OLR applied to the reactors. Hence, it was beneficial to digest seaweed leachate in a UASB reactor since high methane productivity was attained. The GPR reported by Morand and Briand (1999) was 0.66 l CH_4/l/day at an OLR of 2.5 g COD/l/day using an anaerobic packed-bed reactor. A GPR of 5.5 l CH_4/l/day at an OLR of 15.8 g COD/l/day has been reported by Shin et al. (2001) when treating VFA-rich leachate from food waste in a UASB reactor.

There was a decrease in the methane content in both reactors from around 73 and 75% to 64 and 66% over the treatment period which was expected. The pH decreased in both reactors from about 7.7 to 7.3. The solubility of CO_2 decreases at higher pH. Values of bicarbonate PA increased as the OLR was increased and decreased subsequently at higher OLR (Table 5). The decrease in the PA was due to the decreased solubility of CO_2. This then led to an increase amount of CO_2 in the gas phase hence, a lower methane content of the biogas.

The main fraction of soluble organic compounds was produced during the first 10 days of hydrolysis of seaweed. The total treatment period required for two-stage treatment would thus be 10 days for hydrolysis in a leach-bed reactor and 2 days in the UASB reactor, giving a total treatment time of 12 days. Hanssen et al. (1987) reported a decrease in the HRT from 24 to 16 days in a CSTR with an increase in the OLR corresponding to 1.7 to 2.6 g VS_{added}/l/day. A decrease in the HRT from 27 to 11 days, which corresponds to an increase in the OLR from 1.1 to 2.6 g VS_{added}/l/day in a CSTR, has also been reported (Hansson, 1982). The higher treatment capacity of the two-stage anaerobic digestion system implies that a smaller reactor volume would be required compared with the conventional CSTR, and would thus be economically superior. Evaluation of the integrated two-stage process combining a hydrolytic leach-bed reactor and a UASB reactor is being planned.

3.3.2. *Total COD reduction*

The total COD reduction decreased from 90 to 80% as the OLR was increased from 1.1 to 18.1 g tCOD/l/day in UASB 1. In UASB 2, the tCOD decreased from 90 to 81% as the OLR was increased from 1.2 to 20.6 g tCOD/l/day (Table 5). The reactors reached a high volumetric COD degradation rate of around 14 g tCOD/l/day at the highest OLR applied. A high ORL of about 20 g tCOD/l/day was possible in both reactors as the seaweed leachate contained soluble organic matter mainly in the form of VFAs, which are readily converted into biogas. Furthermore, the mesophilic UASB granules were constantly growing, as evidenced by the increase in height of the sludge blanket during the period of the experiments.

An OLR of 15.8 g COD/l/day with a COD removal efficiency of 96% was achieved by Shin et al. (2001) when treating hydrolysed food waste leachate in a UASB reactor. Fang et al. (1994) also reported an

Table 5
Values of other process parameters in the UASB reactors during steady state at each OLR level.

	OLR (g tCOD/l/day)	pH	tCOD reduced (%)	Volumetric degradation rate (g $tCOD_{degraded}$/l/day)	Total VFA (g/l)	PA (g $CaCO_3$/l)
UASB 1	1.1	7.73	89.6 ± 1.7	1.0 ± 0.1	n.d.	2.2 ± 0.1
	2.5	7.52 ± 0.03	81.5 ± 1.4	1.7 ± 0.1	0.07 ± 0.10	2.3 ± 0.0
	4.4	7.55 ± 0.05	85.2 ± 2.2	3.7 ± 0.1	n.d	2.6 ± 0.0
	7.1	7.46 ± 0.05	80.8 ± 2.1	5.4 ± 0.7	n.d.	2.2 ± 0.1
	11.0	7.43 ± 0.05	79.2 ± 2.2	9.3 ± 1.2	0.02 ± 0.01	2.4 ± 0.0
	18.1	7.30 ± 0.06	78.9 ± 1.3	14.3 ± 0.3	0.03 ± 0.02	2.0 ± 0.1
UASB 2	1.2	7.70 ± 0.02	89.7 ± 2.2	1.1 ± 0.2	n.d.	3.0 ± 0.1
	2.9	7.54 ± 0.02	84.0 ± 1.9	2.2 ± 0.4	n.d.	3.2 ± 0.0
	4.9	7.52 ± 0.05	80.7 ± 2.4	3.9 ± 0.3	0.02 ± 0.00	3.4 ± 0.0
	8.1	7.49 ± 0.04	82.5 ± 0.8	6.0 ± 0.2	0.01 ± 0.01	3.0 ± 0.1
	12.5	7.45 ± 0.02	78.7 ± 4.1	9.1 ± 1.0	0.01 ± 0.01	3.2 ± 0.0
	20.6	7.37 ± 0.13	80.6 ± 0.9	14.7 ± 1.1	0.02 ± 0.02	2.9 ± 0.1

n.d. – not detected.

OLR of 32 g COD/l/day with a COD removal efficiency of 84% when treating hydrolysed protein wastewater in a UASB reactor.

3.3.3. *VFAs, alkalinity and pH*

The tVFA concentration and PA were affected in both reactors by changes in the OLR (Table 5). After the first 20 days, the tVFA concentration in UASB 1 decreased below the detection limit of 5 mg/l. During this period, the concentration of tVFA in UASB 2 was consistently below the detection limit. Both reactors were very stable in terms of VFA degradation even at high OLRs. When the OLR was increased the concentration of some VFAs increased, but these returned to low levels in both reactors as they stabilized at the new OLR level. Individual VFAs found in the reactors were acetic, propionic, n-butyric and n-valeric acids (results not shown). The total concentration of VFAs in the effluent was low, and was about 0.03 and 0.02 g/l at an OLR of 18.1 and 20.6 g tCOD/l/day in UASB 1 and 2, respectively. This indicates that an HRT of 12 h was sufficient for the methanogens to convert most of the VFAs into biogas. Some reactors are considered to be overloaded when the concentration of VFAs is above 0.2 g/l (Björnsson et al., 1997), but the level may depend on the material being digested and the type of reactor. In the anaerobic digestion of manure and co-digestion with other materials a high concentration of VFAs can be tolerated, due to the presence of buffering species, mainly NH_4^+-N (Murto et al., 2004). However in this case, the granules probably provided a good microenvironment for the sensitive methanogens. A concentration gradient will be formed in the granule due to the consumption of VFAs by acidogens and acetogens, and hence the methanogens are exposed to a lower concentration of VFAs than in the incoming leachate.

Alkalinity is a measure of the buffering capacity or the ability of a system to resist pH changes that may be caused by the accumulation of VFAs. According to Moosbrugger et al. (1993) the pH in the reactor should be maintained at about 7, as this is suitable for methanogens. The pH in the reactors was fairly stable during the present experiments. At OLRs of 18.1 and 20.6 g tCOD/l/day in the respective reactors, the pH was about 7.3 compared with a pH of about 5.1 in the incoming seaweed leachate. The main buffering species was bicarbonate, which is formed mainly by the dissolution of CO_2 produced during anaerobic digestion.

The concentration of dissolved H_2S was about 0.3–0.5 g/l in both reactors, in comparison with 0.07 g/l in the incoming leachate (results not shown). The incoming leachate had a high concentration of SO_4^{2-} which was converted, to some extent, to H_2S and HCO_3^- by SRB, at the expense of organic compounds. The concentration of dissolved H_2S that inhibits methanogens has been reported to be between 0.2 and 1.1 g/l. However, granular biomass was also reported to be able to withstand higher concentrations, of about 0.7–1.0 g/l, than suspended biomass (Omil et al., 1995). SRB compete with methanogens for H_2 and the competition is dependent on the COD/SO_4^{2-} ratio of the substrate. Methanogens have an advantage of growth at higher ratios than SRB. In this study, the ratio of COD/SO_4^{2-} was about 3 which favoured the growth of SRB more than the methanogens. The SRB can suppress the hydrogenotrophic pathway more than the aceticlastic pathway. The hydrogenotrophic pathway contributes to about 30% of the produced methane. Hence, the SRB cannot totally out compete the methanogens (O'Reilly and Colleran, 2006). The possibility of direct inhibition of methanogens by H_2S (pKa 7.2) was low since the pH was over 7.3 during the entire treatment period and the prevailing compound was HS^- which cannot enter the negatively charged bacterial wall. The inhibitory effect of H_2S is more severe at low pH (Gerardi, 2003). The HCO_3^- ions produced during the reduction of SO_4^{2-} to H_2S also contributed to increasing the buffering capacity of the reactors.

NH_4^+-N was also fairly constant and was approximately 56 mg/l in both reactors, compared with an average of 23 mg/l in the incoming seaweed leachate (results not shown). The high bicarbonate alkalinity together with dissolved H_2S contributed considerably to the alkalinity in the reactors, and hence the overall stability of the reactors, even at very high OLRs.

4. Conclusions

Two-stage anaerobic digestion of seaweed allows the removal of heavy metals from seaweed prior to biogas production. The reduction of high heavy metal content of seaweed will enable the use of the digestate as a fertilizer. The removal of heavy metals was performed with IDA cryogel carriers and these were efficient in removing Cd^{2+}, Cu^{2+}, Ni^{2+} and Zn^{2+} ions from seaweed leachate. The removal efficiency ranged from 41 to 79% and it was depended on the type of heavy metal. However, the mobilization of heavy metals from seaweed was low and needs to be improved to achieve a high total removal and a high quality digestate. The removal of heavy metals from the seaweed leachate had a significant effect and reduced the methane yield with about 17% in batch tests and about 15% in experiments with the UASB reactors compared to non-treated leachate. Therefore, post-treatment with heavy metal removal might be a more suitable option to avoid the loss of methane potential. Comparable methane yields were obtained in the anaerobic digestion of seaweed and its leachate however 14 days were required to produce 90% of the methane from seaweed compared to only 9 days with seaweed leachate, thus, shorter treatment time is required in a two-stage digestion system. Efficient treatment of seaweed leachate was achieved in a UASB reactor with a high GPR of 3.0 N l CH_4/l/day at a short HRT of 12 h. Hence, a smaller reactor volume is required reducing the cost of a treatment system. This study demonstrates that two-stage anaerobic digestion is promising as a waste handling method for seaweed.

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Paper V

Biogas production from wheat straw in batch and UASB reactors: the roles of pretreatment and seaweed hydrolysate as a co-substrate

Valentine Nkongndem Nkemka and Marika Murto

Department of Biotechnology, Lund University, P. O. Box 124, SE-221 00, Lund, Sweden
Telephone: +46 46-222 83 47
Fax: +46 46-222 47 13
Corresponding author: valentine.nkemka@biotek.lu.se

Abstract

This research evaluated biogas production in batch and UASB reactors from pilot-scale acid catalysed steam pretreated and enzymatic hydrolysed wheat straw. The results showed that the pretreatment was efficient and, a sugar yield of 95% was obtained. The pretreatment improved the methane yield (0.28 m^3/kg VS_{added}) by 57% compared to untreated straw. Treatment of the straw hydrolysate with nutrient supplementation in a UASB reactor resulted in a high methane production rate, 2.70 m^3/m^3.d at a sustainable OLR of 10.4 kg COD/m^3.d and with a COD reduction of 94%. Alternatively, co-digestion of the straw and seaweed hydrolysates in a UASB reactor also maintained a stable anaerobic process and can thus reduce the cost of nutrients addition. We have shown that biogas production from wheat straw can be competitive by pretreatment, high methane production rate in UASB reactors and also by co-digestion with seaweed hydrolysate.

Key words: anaerobic digestion; seaweed; steam pretreatment; UASB reactor; wheat straw

1. Introduction

The objective of the European Commission on Renewable Energy Road Map is to increase the gross domestic energy consumption from renewable energy sources, which amounted to 12.4% in the EU in 2010 (EurObserv'ER, 2011), to 20% by 2020. Sweden, however, already produces about 30% of its total energy from renewable sources, due to its large renewable energy assets and an active engagement in energy policies (Swedish-Energy-Agency, 2011). Biogas currently contributes 0.4% (3 TWh/year) of energy consumed in Sweden, and this could be increased to 74 TWh/year by using natural waste and forest residues. The use of new biomass resources, improved process technology, and energy, agricultural, environmental and waste-handling policies that promote sustainable development (Lantz et al., 2007) are all important to maximise the use of the energy bound in biomass resources and to attain the goal that have been set.

Biogas production can be increased by using abundant lignocellulose materials such as agricultural and forest residues (Zeng et al., 2007). However, the complex lignocellulose structure limits the accessibility of the sugars in cellulose and hemicellulose. This means that pretreatment is necessary to gain access to the sugars bound in lignocellulose, and several efficient pretreatment methods for lignocellulose material have been developed (Alvira et al., 2010). One example of such a method is steam pretreatment in the presence of dilute acid, which results to efficient lignocellulose hydrolysis and sterilisation. Building a steam pretreatment unit requires a huge initial investment, and the operation of such a plant consumes energy. Both of these factors are major drawbacks. However, process integration with other heat and power plants that produce waste heat, and the use of heat exchangers in the processing steps can substantially reduce the requirement for energy input (Ljunggren & Zacchi, 2010). Today, there are many operational demonstration ethanol plants that employ steam pretreatment and enzyme hydrolysis techniques for lignocellulose degradation (Gnansounou, 2010). The use of sulphuric acid during steam pretreatment in the presence of dilute acid is another drawback. Pretreatment is very efficient when sulphuric acid is used, but the acid creates problems downstream due to high sulphate concentrations. These can compromise methane production as a result of competition between sulphate-reducing bacteria (SRB) and methanogens. Moreover, biogas produced in this way and subsequently upgraded to be used as a transport fuel would contain high concentration of H_2S, which is corrosive to engines. We have here used phosphoric acid as an alternative to sulphuric acid, hence avoiding the problems of high downstream sulphate concentration.

It has been reported that celluclast enzymes, which hydrolyse polymers to release sugars after steam pretreatment, increase the cost of producing biofuel due to low efficiency and the high purchase costs of the enzymes. More efficient enzymes are now being produced by several companies, and we have here used a new and more efficient celluclast enzyme (Cellic C Htech from Novozyme, Denmark).

Poor buffer and nutrient content of wheat straw is a further hindrance to the use of this material in a biogas process. Co-digestion is a common method used to remedy nutrient deficiency and poor buffer capacity in biogas reactors, through the synergistic effects that co-digestion can introduce. Treatment of wheat straw hydrolysate with manure improves the performance of the upflow anaerobic sludge blanket (UASB) technique (Kaparaju et al., 2009). As manure becomes scarce due to its increasing use in biogas production, other co-substrates will be required. Seaweed generally has a high nutrient content, and is an underexplored marine resource. The seaweed *Ulva lactuca* was not suitable for combustion for energy production due to the high ash content (Bruhn et al., 2011). In addition, *Ulva lactuca* is alkaline, and this means that it can be used as a buffer in biogas reactors. Seaweed hydrolysate has been used in a UASB reactor at a high OLR, 20.6 g tCOD/l.day, without adding nutrients or alkaline agents (Nkemka & Murto, 2010).

The work presented here has evaluated dilute acid catalysed steam pretreatment and enzyme hydrolysis of wheat straw and subsequent biogas production in batch and continuous UASB processes. The investigation has evaluated the pretreatment of wheat straw and the effect of pretreatment on the methane potential in batch tests on several starting materials: wheat straw (pieces of lengths 1-2 cm), ground wheat straw, dilute acid catalysed steam pretreatment of wheat straw, and such pretreated wheat straw subsequently hydrolysed by enzymes. We have also evaluated whether wheat straw hydrolysate is suitable for methane production in a UASB reactor. Finally, we have examined the use of seaweed hydrolysate as a co-substrate in the treatment of wheat straw hydrolysate in a UASB reactor.

2. Materials and methods

2.1 Wheat straw

Wheat straw was collected from a farm in Lunnarp, Sweden, and was harvested in August 2009. It had a total solid (TS) content of 96%, of which 94% was volatile solids (VS). The wheat straw was cut with a grinding mill (Retsch GmbH, Germany), and sieved through a Vibro-screen (SWG Process Engineering Ltd., England) to obtain pieces of lengths 1-2 cm. The material was stored at room temperature until use. Table 1 presents the composition of the wheat straw.

The wheat straw was pretreated by steam pretreatment using an acid catalyst, followed by enzymatic hydrolysis. The resulting material was separated into a liquid fraction and a solid fraction. Figure 1 shows the pretreatment process, and Sections 2.2 and 2.3 will describe the pretreatment conditions.

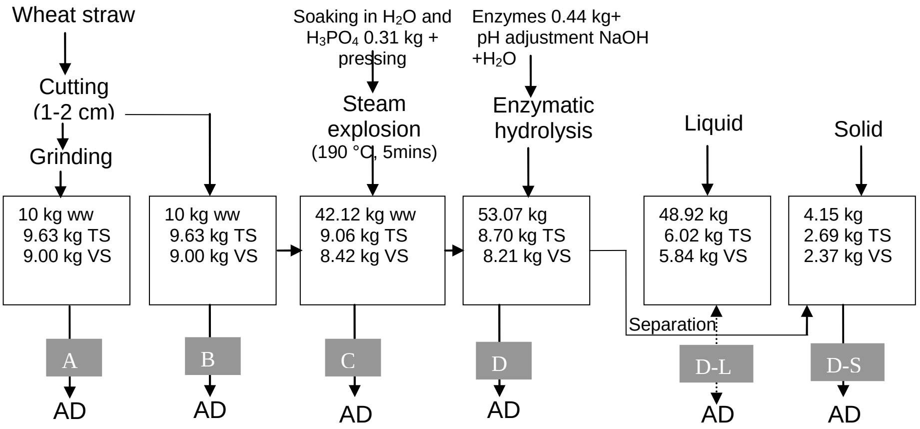

Figure 1. Schematic diagram and material flow for biogas production from pretreated wheat straw. AD is the fraction used for anaerobic digestion.

2.2 Acid catalysed steam pretreatment of wheat straw

Ten kilograms of wheat straw (in pieces of lengths 1-2 cm) was pre-soaked for one hour in 0.5% H_3PO_4 of TS. In a pre-trial, steam pretreatment of straw with 0.2% phosphoric acid was not efficient and as such, the acid concentration was increased to 0.5%. The material was then pressed in batches for about 2 minutes at 3.0×10^7 Pa using a press (Tinkturenpressen HP5M, Fischer Maschinenfabrik, Germany). The pressed wheat straw was subsequently steam pretreated in a pilot-scale reactor of volume 10 l at 190 °C for 5 min, in batches of 0.7 kg. The procedure used for the steam pretreatment has been previously described (Linde et al., 2008).

2.3 Enzymatic hydrolysis of pretreated wheat straw

The steam-pretreated wheat straw was then enzymatically hydrolysed using the celluclast enzyme Cellic C Htech (95 FPU/g, 590 IU β-glucosidase/g enzyme) (Novozyme, Denmark). The amount of enzyme added to the steam-pretreated wheat straw was calculated by assuming a content of water insoluble solids (WIS) of 10%, and a desired enzyme load of 15 FPU/g WIS. The hydrolysis was performed at 50 °C for 72 hours in a reactor of volume 45 l, with continuous stirring. The active reactor volume was 25 l. Two batches of enzymatic hydrolysis were performed. The pH of the steam-pretreated material was adjusted to about 4.8 with 50% NaOH before the enzymatic hydrolysis, since this is the optimal pH for the activity of Cellic C Htech. The slurry obtained from enzymatic hydrolysis was centrifuged (Jouan Centrifugeurs, Switzerland) and then filtered through Munktel filter paper grade 5.

Samples for methane potential measurements were taken after each processing step and stored at –20 °C until use (Figure 1).

2.4 Methane potential batch tests

The inoculum used in the batch tests was collected from a mesophilic full-scale biogas plant, Söderåsens Bioenergi AB, Sweden, co-digesting industrial food waste and energy crops. The total solid content of the inoculum was 3.7%, of which 63.9% was volatile solids. Methane potential tests were performed on the fractions (A to D-S) from the process steps shown in Figure 1. All tests were performed in triplicate and under mesophilic conditions (37 °C). The experiment was performed during 31 days except for the ground and cut wheat straws, which were digested further for 127 days. Avicel cellulose (crystalline cellulose) was used as a control to assess the cellulase activity of the inoculum, and the methane potential of the celluclast enzyme Cellic C Htech was also determined. The inoculum:substrate ratio was 2:1, based on VS content. The experimental set-up has been previously described (Kreuger et al., 2010). Air-tight bags used for biogas collection were produced from the aluminium packaging material transofoil (Flextrus AB, Sweden). The volume and composition of the gas produced were measured and the temperature recorded during the experiment.

2.5 Methane production in UASB reactors

The wheat straw hydrolysate (D-L) was treated in duplicate in UASB reactors. Granular anaerobic sludge was obtained from a mesophilic full-scale plant in the Netherlands and used as inoculum in the UASB reactors. The TS content of the granular sludge was 6.4%, the VS content was 43.5% of TS, and the reactors were initially loaded with 250 g of the granules. The UASB reactors had an active liquid volume of 0.8 l and were operated under mesophilic (37 °C) conditions as previously described (Nkemka & Murto, 2010). A modified anaerobic basic nutrient solution was used to dilute the wheat straw hydrolysate as previously reported (Angelidaki et al., 2009). Urea (100 kg/m^3) was used instead of ammonium chloride in the basic anaerobic medium. Urea is a cheap source of nitrogen and is also a buffering species.

Methane production and the treatment capacity were determined by increasing the feedstock concentration and by decreasing the hydraulic retention time (HRT). The organic loading rate (OLR) was increased by first increasing the concentration of the feedstock from 10.0, 19.3, 27.7 and finally to 47.7 kg COD/m^3 (where "COD" is an abbreviation for "chemical oxygen demand") at a constant HRT of about 8 days. The increase in the feedstock concentration corresponded to an increase in OLR of 1.2 to 6.1 kg COD/m^3.d (Table 4). The next stage of the increase in OLR was a decrease in the HRT from 8.0 to 2.7 days, carried out when the concentration of the feedstock was 27.7 kg COD/m^3. This corresponded to an increase in the OLR from 3.5 to 10.4 kg COD/m^3.d. The total treatment period was 145 days.

The wheat straw hydrolysate was also treated by co-digestion with seaweed hydrolysate (SWH) (in a ratio of 1:1 based on kg COD/m^3), instead of adding the anaerobic basic medium as previously described. The OLR was increased by increasing the concentration of the mixed hydrolysates from 9.5 to 18.4 kg COD/m^3, which corresponds to an increase in the OLR from 3.6 to 6.6 kg COD/m^3.d at a constant HRT of 2.7 days. The total treatment time was 47 days. The seaweed hydrolysate was produced by seaweed hydrolysis, described in Section 2.6.

The processes were allowed to run for at least three times the HRT at each OLR level, to reach a semi-steady state condition. The composition, temperature and volume of the gas produced were monitored during the experiments, as were the levels of ammonium-nitrogen, COD, pH and the concentration of volatile fatty acids (VFA).

2.6 Production of seaweed hydrolysate from seaweed

The seaweed used to produce leachate was collected from the beach at Trelleborg, on the southern coast of Sweden in May 2008. The TS level was 22.3%, of which 32.9% was VS. The seaweed was ground to produce pieces sized

2 to 3 cm, which were stored at -20 °C until use. Seaweed leachate was produced by hydrolysing the seaweed using four Schott bottles of volume 5 l as hydrolytic reactors and were placed in a ventilated hood. At the start of the hydrolysis, 6.3 kg of seaweed and 6.3 kg of water were added to the four reactors. No inoculum was added and the hydrolysis depended on the natural bacterial floral of the seaweed (Nkemka & Murto, 2010). Consequently, only the buffer and nutrient contribution from the seaweed can be evaluated in the co-digestion of seaweed and the straw hydrolysate in the UASB reactor. The reactors were operated at 37 °C for 12 days. The leachate was subsequently filtered through a sieve of mesh size 125 μm to remove sand and other large particles. The pH of the leachate was 5.76, and it contained 0.21 kg/m^3 NH_4-N, 2.78 kg/m^3 total alkalinity (TA), and 26.4 kg/m^3 COD. The leachate (SWH) was subsequently used as a co-substrate during methane production in batch tests and in UASB reactors.

2.7 Analytical methods

TS and VS were analysed using standard methods (APHA, 1998). Dr. Lange test kits LCK 114 or LCK 914 were used for COD analyses and the analyses were carried out in a Lasa 100 spectrometer (Dr. Bruno Lange GmbH, Germany). Analyses of NH_4^+-N, partial (PA) and total alkalinity, pH, gas volume and gas composition were carried out as previously reported (Nkemka & Murto, 2010; Parawira et al., 2006). Acetic acid, ethanol, formic acid, furfural, hydroxylmethyl furfural (HMF), lactic acid and sugars were analysed after the pretreatment using a high-performance liquid chromatograph (HPLC) (Shimadzu, Kyoto, Japan) equipped with a refractive index detector (Shimadzu) and as previously reported (Linde et al., 2008). Samples for VFA determination in the effluent of the UASB reactors were analysed by HPLC (Jasco, Tokyo, Japan) and as earlier reported (Kreuger et al., 2011). The elemental analyses presented in Table 2 were performed by LMI AB, Helsingborg, Sweden. Levels of nitrogen and carbon were determined and used to determine the C/N ratio. Levels of Fe, Al, B, Cu, P, S, Zn, Mn, Na, Mg, Ca, K and Si were determined by inductively coupled plasma atomic emission spectrometry (ICP-AES). Levels of Mo, Cr, W, Se, Ni, Cd, Co, As, Hg and Pb were determined by inductively coupled plasma mass spectrometry (ICP-MS). Levels of total organic carbon (TOC) and Kjedahl-nitrogen (Kj-N) in the liquid samples were determined. Methane volumes were corrected to 0 °C and 1 atm.

2.8 Statistical analyses

The effects of the pretreatment on the methane yield in the batch tests were analysed using the statistical software SPSS 16.0. Univariate analyses of variance and *post hoc* (Tukey) tests were performed, and 95% confidence intervals determined.

3. Results and discussion

3.1 Wheat straw composition

Table 1 presents the sugar and lignin contents of the wheat straw, which were in the same range as previously reported (Linde et al., 2008).

3.2 Acid catalysed steam pretreatment and enzymatic hydrolysis of wheat straw

Steam pretreatment in the presence of dilute H_3PO_4 followed by enzymatic hydrolysis using the celluclast enzyme Cellic C Htech was an efficient pretreatment of the wheat straw (Table 1). The yield of D-glucose was 35.2 g/100 g TS and the yield of xylose was 21.4 g/100g TS. The total sugar yield was 95%, and the yield of D-glucose was 95% and that of xylose was 94% of the theoretical yield. The result of this study was comparable to the total sugar yield obtained following steam pretreatment/enzymatic hydrolysis with 0.2% H_2SO_4 at 190 °C for 5 min (Linde et al., 2008). Hence, steam pretreatment with an acid catalyst gave a high sugar yield. Sulphuric acid is an efficient acid catalyst for the steam pretreatment of lignocellulose materials. However, as earlier mentioned, the high concentration of SO_4^{2-} ions in the hydrolysate in a biogas reactor can be problematic in the subsequent biogas process. SRB compete with methanogens for H_2 and use organic carbon, which may further reduce the methane potential of the material. Furthermore, H_2S produced under anaerobic conditions can be toxic to methanogens at neutral pH and can precipitate valuable micronutrients that are needed for anaerobic digestion (Hulshoff Pol et al., 1998). We have used H_3PO_4 acid in the steam pretreatment, as a novel method to circumvent these problems. We have also used a more efficient enzyme in the enzymatic hydrolysis than those previously used, Cellic C Htech (95 FPU/g enzyme), and this contributed to the efficient release of sugars. It was possible to obtain high sugar yields with the enzyme that was commonly previously used, Celluclast 1.5 L (60.9 FPU/g enzyme), but larger amounts were required due to its low specific activity

(Sipos et al., 2010). Enzymes are expensive, and the use of small amounts of an efficient enzyme can reduce the process cost.

Table 1. Composition of wheat straw and concentrations and yields of sugars, lactic acid, acetic acid, ethanol, HMF and furfural in the liquid after the steam pretreatment and subsequent enzymatic hydrolysis of wheat straw.

Wheat straw		Wheat straw hydrolysate (WSH)		
	Content (% of TS)		Yield (g/100g TS)	Concentration in wheat straw hydrolysate D-L (kg/m^3)
Glucan	37.0	Cellobiose	3.9	6.97
Xylan	22.8	D-glucose	35.2	62.37
Galactan	0.9	Xylose	21.4	37.84
Arabinan	2.8	Arabinose	2.0	3.47
Mannan	2.0	Lactic acid	0.21	0.37
Acid-insoluble lignin	16.4	Glycerol	3.6	6.34
Acid-soluble lignin	1.2	Acetic acid	2.7	4.79
Water extractives	12.4	Ethanol	0.2	0.29
Ethanol extractives	1.0	HMF	0.1	0.23
		Furfural	0.6	1.14
		COD		196
		pH		4.41
		TS (%)		12.3
		VS (%) of TS		98.4
		NH_4-N (kg/m^3)		0.04

Other compounds, such as acetic acid, ethanol, formic acid and lactic acid, were produced in small amounts during the steam pretreatment in the presence of dilute acid/enzymatic hydrolysis. Furthermore, furfural and HMF, both of which may inhibit methanogens, formed during the steam pretreatment. The concentration of furfural that inhibits methanogens ranges from 2.4 to 3.0 kg/m^3 (Raj, 2009). The hydrolysate produced by the pretreatment, however, must be diluted before digestion in a methane reactor due to its high COD content, and this reduces the risk of furfural inhibiting the methanogens. In addition, Torry-Smith et al. have reported that furfural and HMF are degraded in UASB reactors (Torry-Smith et al., 2003).

The pH of the hydrolysate was low, 4.41, and treatment of the hydrolysate for biogas production required pH adjustment to a neutral range. The COD content was 196 kg/m^3, which was higher than the sum (123.8 kg/m^3) of all the compounds present in the D-L fraction. This shows that other compounds were present in the hydrolysate that were not identified and that could contribute to methane production. The TS content of the liquid hydrolysate, D-L, was 13.3% and the VS content was 98.2% of TS. This liquid hydrolysate is suitable for anaerobic digestion in a continuous stirred tank (CSTR) reactor since a TS concentration of about 10% is required for processing in a CSTR. Alternatively, the hydrolysate may be treated in a methane high-rate reactor, which allows higher rates of methane production, and we evaluated this process.

3.3 Mass flow and nutrient variation during steam pretreatment and enzyme hydrolysis

Variation in the total mass during the pretreatment process was caused mainly by the addition of process water (Figure 1). Estimation of the volume of process water needed is important for subsequent cost estimations. Water is added in the process at various stages, such as soaking the wheat straw in dilute H_3PO_4, as steam during the steam pretreatment, and during the enzymatic hydrolysis. Water is, on the other hand, lost during the pressing step prior to steam pretreatment. This resulted in the loss of organic matter in process steps B (9.00 kg VS) and C (8.42 kg VS) that could have been used for methane production. In addition, 10.13 l of water was lost or evaporated during the enzymatic hydrolysis (50 °C for 72 hours), and volatile compounds that may contribute to the methane potential were also lost at this stage. Moreover, other volatile compounds would have been lost during steam pretreatment: it was difficult to account for these losses in the present study.

Table 2. Compositions of wheat straw, wheat straw hydrolysate (D-L) and seaweed hydrolysate (SWH).

Units (mg/l)	Wheat straw	Wheat straw hydrolysate (D-L)	Seaweed hydrolysate (SWH)	Anaerobic digestion requirement
Fe	59	1.5	17	1-200[a,b]
Mo	1.2	0.026	0.02	0.001-50[a,b]
Mn	15.00	0.61	0.93	0.005-55[a,b]
Ni	0.62	0.10	0.13	0.005-30[a,b]
W	0.42	0.01	0.00	0.018-18.3[a,b]
Se	0.11	0.04	3.40	0.008-0.35[b]
Co	0.037	0.010	0.020	0.001-20[a,b]
Na	170	1200	930	
Mg	620	20	240	67-4800[a,b]
Ca	2200	59	670	0.54-89[a,b]
K	4400	78	620	0.22[a]
Si	1100	67	65	
P	890	390	95	0.34[a]
S	610	94	290	0.32-13 000[a]
N	8610	-	-	-
C	411 000	-	-	-
C/N	47.7	20.1	7.3	16-25[c]
Kj-N	5800	460	670	-
TOC	-	-	4900	-

a: (Hinken et al., 2008), b: (Schattauer et al., 2011), c: (Bouallagui et al., 2009)
D-L: wheat straw hydrolysate 196 kg COD/m^3, SWH: seaweed hydrolysate 26.4 kg COD/m^3

The C/N ratio is an important macronutrient parameter in achieving stable anaerobic digestion. The C/N ratio varied during the pretreatment (Table 3), and the final ratio obtained with wheat straw, 47.7, was higher than the value of about 25 that is suitable for anaerobic digestion (Bouallagui et al., 2009). In contrast, the C/N ratio of the D-L fraction, 20.1, was low due to the enzyme addition, and this fraction was suitable for methane production. The wheat straw contained low concentrations of certain micronutrients (Fe, Mo, W, Se, Ni, Co) that are necessary for anaerobic digestion (Hinken et al., 2008; Schattauer et al., 2011), and the concentrations of these nutrients became much lower due to dilution with process water. Hence, it was necessary to add these nutrients to achieve a more balanced anaerobic process.

The concentrations of Mg (620 mg/l), Si (1100 mg/l), K (4400 mg/l) and Ca (2200 mg/l) ions were high in the wheat straw, and these ions, together with carbonate, are components of ash. The carbonates of these ions are important as they provide the buffer capacity that is needed in a biogas reactor. In a previous study, the high ash content of the seaweed *Ulva lactuca* gave a high alkalinity in the reaction vessel (Bruhn et al., 2011). The ash component in the present study, however, was lost during the soaking and pressing steps before steam pretreatment. The amounts of nutrients lost were determined from the metal ion concentrations (Table 2) and the volume changes that occurred due to the addition of process water (Figure 1). In large-scale steam pretreatment in the presence of dilute acid, spraying of phosphoric acid may be preferred to soaking, since the latter requires a large vessel and large volumes of water.

The concentration of sodium ions in the wheat straw was low, 170 mg/l. This is to be compared with the range 3,000 to 16,000 mg/l that is required to inhibit the anaerobic digestion process by 50%, in the absence of other nutrients or salts (Feijoo et al., 1995). The concentration of sodium, however, increased during pH adjustment in the enzymatic hydrolysis step, and its concentration in the D-L fraction was 1,200 mg/l. The concentrations of metals that are toxic to anaerobic digestion were generally low in the D-L fraction. Some metals, however, are toxic in a very narrow range of concentration. The toxicity of a metal, further, depends on several factors, such as metal bioavailability, synergy, and antagonism with other metal ions (Chen et al., 2008).

The nutrient contents of the wheat straw hydrolysate D-L, 196 kg COD/m^3, and of the seaweed hydrolysate (SWH), 26.4 kg COD/m^3, are compared to the anaerobic nutrient requirements (Table 3). The SWH contained a high concentration of Fe (17.0 mg/l) and of other micronutrients, such as Mo, Se, Ni, and Co, that are needed for anaerobic digestion. The SWH also contained high concentrations of Mg and Ca, which can combine with carbonate to provide buffer capacity during the anaerobic digestion of the hydrolysate. High concentrations of heavy metals, in contrast, might inhibit the anaerobic process. The concentration of sulphate ions was high when using SWH as a co-substrate, which is another major drawback of using SWH. The high sulphate concentration may lead to a high concentration of SRB, which has a negative effect on methane production, as earlier mentioned. Co-digestion of D-L and SWH can reduce the sulphate concentration and in this way lead to a stable anaerobic process. The anaerobic digestion of wheat straw hydrolysate, therefore, requires nutrients and alkalising agents to be added, or digestion with a suitable co-substrate, in order to achieve stable operation.

3.4 Methane potential batch tests

The methane yields obtained in batch tests after 31 days of digestion (except otherwise stated) were determined. Figure 2 presents the methane yields of the different materials after the processing steps (processes A to D-S). Volatile compounds in the liquid samples from the processing steps have been included when calculating methane yields. It has been reported that volatile compounds evaporate when measuring TS and VS by oven drying, leading to overestimation of the methane yields (Kreuger et al., 2011).

The methane yield from wheat straw pieces of length 1-2 cm (process B) was 0.18 $m^3/kg\ VS_{added}$. Grinding wheat straw into a powder (process A) did not result in a significant ($p > 0.05$) improvement in the methane yield, 0.21 $m^3/kg\ VS_{added}$. Steam pretreatment in the presence of dilute acid of the wheat straw (process C) resulted in a 39% increase ($p < 0.05$) in the methane yield to 0.25 $m^3/kg\ VS_{added}$. The methane yield increased further, by 57% from baseline to 0.28 $m^3/kg\ VS_{added}$, ($p < 0.01$) following steam pretreatment in the presence of dilute acid and enzymatic hydrolysis (process D). The biodegradability and rate of methane production from the materials produced in processes C and D were higher than those of processes A and B. This was reflected in that about 90% of the methane was produced after 17 days in processes C and D. Material from processes A and B was digested for 127 days and the methane yields were 0.31 $m^3/kg\ VS_{added}$ for process A ("A-127 days" in Figure 2) and 0.28 $m^3/kg\ VS_{added}$ for process B (results not shown). The methane yield obtained from wheat straw in the present study was similar to that reported by Dererie et al. (Dererie et al., 2011). The slow rate of methane production from the material after processes A and B was expected, since wheat straw contains lignocellulose, which degrades slowly. Steam pretreatment in the presence of dilute acid and enzymatic hydrolysis have a significant effect on the biodegradability, and thus also the rate of methane production. This effect has been earlier reported for lignocellulose hemp (Kreuger et al., 2010).

The methane yield from the liquid fraction (D-L) was 0.32 m^3/kg VS_{added} (0.27 m^3/kg COD_{added}). This value was as expected, since the fraction contained easily hydrolysable sugars and soluble organic substances (Section 3.2). This confirms that treatment by steam and dilute acid followed by enzymatic hydrolysis is efficient. The rate of methane production was high, and about 95% of the methane had been produced after 17 days. The methane yield from the solid fraction (D-S) was 0.14 m^3/kg VS_{added}, which was higher than expected. The solid fraction contained lignin, which degrades slowly. However, some sugars would have been trapped in the slurry after the separation, and these contributed to the methane potential. Therefore, digestion of the process fractions C and D may give a high methane production. We suggest that the D-S process fraction should be washed in a large-scale process in order to recover the remaining organic substances from the solid fraction. These substances can be useful for methane production. The liquid process fraction D-L can be used to produce methane in high-rate methane reactors, enabling in this way faster treatment. We have investigated this idea further.

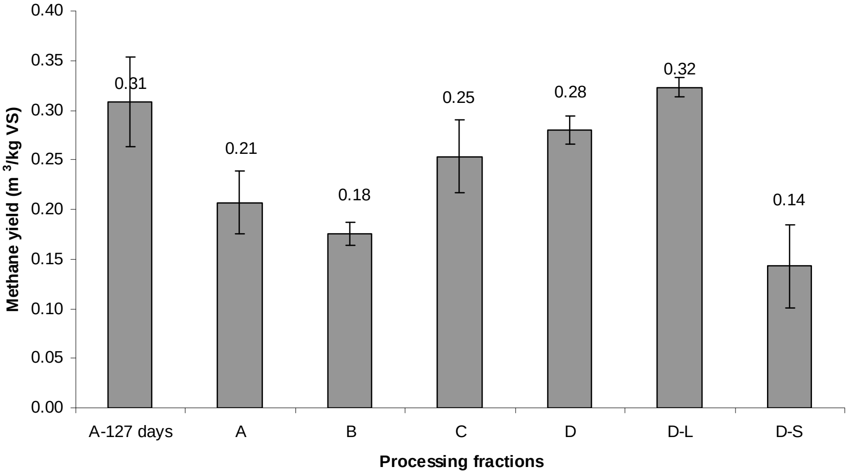

Figure 2. Methane yield after 31 days' digestion of different processing fractions in the steam pretreatment in the presence of dilute acid of wheat straw

The inoculum possessed cellulase activity, as was shown by the fact that the methane yield from avicel cellulose, 0.37 m^3/kg VS_{added}, was about 89% of the theoretical yield (0.415 m^3/kg VS_{added}). The methane potential of the celluclast enzyme was 0.12 m^3/kg VS_{added} after 31 days, and 0.29 m^3/kg VS_{added} after 59 days. About 90% of the methane was produced between days 20 and 59. We suspected that ammonia was inhibiting the process as the NH_4^+-N concentration was high in the inoculum, and it was 4.8 kg/m^3 at a pH of 8.28 after 31 days of digestion. Mineralisation of the protein (celluclast enzyme) caused the increase in the concentration of NH_4^+-N. More experiments are needed to investigate the inhibition of the digestion by the celluclast enzyme during the methane potential batch test. It may be possible to use another source of inoculum to obtain inoculum that contains a low concentration of NH_4^+-N. The pattern of methane production achieved when the celluclast enzyme is used allows us to deduce that most of the enzyme flows through a methane high-rate reactor untreated, due to the short operational HRTs of 2 to 4 days. Hence, the contribution of the enzyme to the methane potential of the entire process would be low in such a reactor. We estimated that the energy potential of the enzyme added during the work presented here was 1.6 to 4.1% of the energy content of the wheat straw (16.3 MJ/kg TS). A previous study reported that adding a different enzyme, Celluclast 1.5 L from Novozyme, contributed to the overall energy yield (Kreuger et al., 2010).

The methane potential of wheat straw was lower than the methane potentials of milk and fully ripened maize (0.27 to 0.37 m^3/kg VS), which are most often used for biogas production (Amon et al., 2007). However, steam pretreatment in the presence of dilute acid of lignocellulose crop residues such as wheat straw, followed by enzymatic hydrolysis, can result in comparable methane potentials.

3.5 Energy yields of batch methane production from wheat straw

Figure 3 presents the energy yields from biogas from the materials, taking into account the material losses from the different processing pretreatment steps (A to D-L). The lower heating value (LHV) of the sugar fraction of wheat straw dry matter (TS) was 16.3 MJ/kg TS: the rest of the energy is contained in the lignin fraction. The use of cut wheat straw (process B) and powdered wheat straw (process A) gave similar energy yields: 7.8 and 9.2 MJ/kg TS, respectively. Higher energy yields were obtained from the material after process C, 10.7 MJ/kg TS, and after process D, 11.9 MJ/kg TS, than those obtained after processes A and B. Digestion of the wheat straw for 127 days (“A-127” in Figure 3) resulted in a high energy yield of 13.6 MJ/kg TS. Steam pretreatment in the presence of dilute acid and enzymatic hydrolysis gave significantly higher energy yields, and the pretreatment method can be recommended for wheat straw.

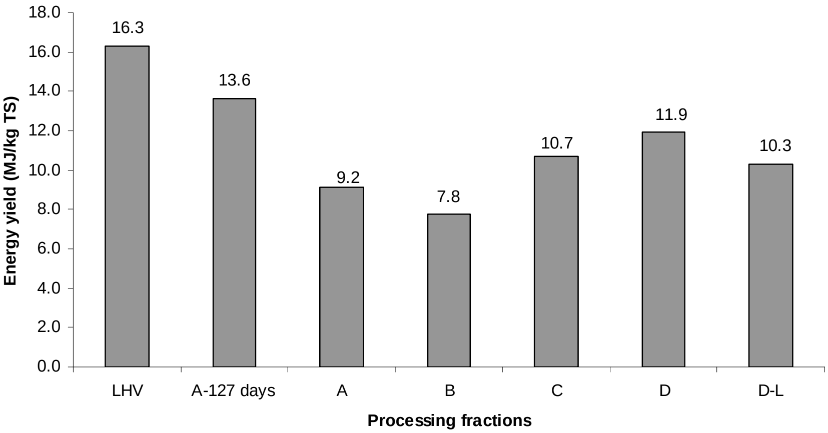

Figure 3. Energy yield after 31 days' digestion of different processing fractions in the steam pretreatment in the presence of dilute acid of wheat straw.

Anaerobic digestion of the solid residue (D-S), which contained mainly lignin, gave an unexpectedly high energy yield of 2.5 MJ/kg TS. Soluble compounds trapped in the solid fraction may be the cause of this. It is difficult to degrade lignin under anaerobic conditions, and the energy potential of the D-S fraction was therefore added to the energy potential of the D-L fraction. The lignin may, as an alternative, be combusted to produce heat and electricity, in which case the estimated energy yield from lignin is 4.2 MJ/kg TS. The energy produced can then supplement the heat and power for the biogas process.

Material losses occurred during the processing steps, especially in processes C, D and D-L and these losses resulted in lower energy yields. Material was lost in process C in the form of organic compounds during the soaking and pressing steps and during the steam pretreatment step, as mentioned in Section 3.3. The material loss in process D may have been due to evaporation of volatile compounds during the enzymatic hydrolysis. Further material was lost in the separation process that gave materials D-L and D-S. It is clearly crucial to minimise material losses during the processing steps in order to harness as much as possible of the energy bound in the wheat straw.

3.6 Methane production from wheat straw hydrolysate in UASB reactors

There were early signs of process failure when the wheat straw hydrolysate (D-L) was treated in a UASB reactor without adding nutrients or buffer. The pH decreased from 7.17 to 6.21 at an OLR of 1.68 kg COD/m^3.d after 10 days of treatment (data not shown). It was evident that it was necessary to supplement the hydrolysate with nutrients and buffering agents, and these were added with the anaerobic basic medium described in Section 2.5.

Table 3 presents the results of treating the D-L fraction in the UASB reactors. The COD concentration of the D-L increased from 10.0 to 47.7 kg/m^3, and the concentration of total monomeric sugars from 5.46 to 26.03 kg/m^3. Adding nutrients and buffering agents to the anaerobic basic medium resulted in an increase in the pH of the hydrolysate from 4.14 to 7.01-7.14, which is suitable for methane production (Gerardi, 2003).

The methane production rate (MPR) from the D-L fraction increased from 0.32 to 2.70 m^3/m^3.d when the OLR increased from 1.2 to 10.4 kg COD/m^3.d. The methane yield, in contrast, decreased from 0.30 to 0.26 m^3/kg COD as the OLR was increased. This is approximately the same methane yield as that obtained in the methane potential batch test, 0.27 m^3/kg COD. Organic material may be washed out at very short HRTs and high OLRs, since the microbes have less time to convert the organic compounds into biogas. The methane content of biogas ranged from 49 to 55% during the entire treatment period. Kaparaju et al. have reported a similar methane yield from the treatment of wheat straw hydrolysate in a UASB reactor: 0.27 m^3/kg COD at an OLR of 2.8 kg COD/m^3.d (Kaparaju et al., 2009). In the present study, the energy yield decreased from 11.56 to 10.02 MJ/kg TS as the OLR increased. The energy yield obtained when the D-L fraction was treated in the UASB reactor was comparable also to the batch methane potentials. The high MPR achieved at an OLR of 10.4 kg COD/m^3.d may result in a high rate of energy production, in which case the treatment of the D-L fraction in a high-rate UASB reactor is beneficial. We have obtained methane and energy yields similar to those of Kaparaju et al. (Kaparaju et al., 2009), and one factor in this is the treatment efficiency of granular anaerobic sludge in the treatment of diluted waste streams. Further, the methanogens (which are more sensitive than acidogens) are protected inside the granules while the acidogens are concentrated around the periphery of the granules (Torry-Smith et al., 2003).

Table 3. Summary of process data in the treatment of wheat straw hydrolysate (D-L) and its co-digestion with seaweed hydrolysate (SWH) in UASB reactors. The data were obtained under stable operating conditions.

Parameter	D-L	D-L	D-L	D-L	D-L	D-L/SWH	D-L/SWH
Duration of operation (days)	62	20	18	25	20	33	14
pH	7.14	7.14	7.14	7.01	7.14	5.79	5.50
Influent COD (kg/m^3)	10.0	19.3	27.7	47.7	27.7	9.5	18.4
Monomeric sugars (kg/m^3)	5.46	10.53	15.12	26.03	15.12	-	-
HRT (days)	7.5 ± 0.7	7.9 ± 1.4	8.0 ± 0.8	7.8 ± 0.7	2.7 ± 0.3	2.7 ± 0.5	2.7 ± 0.3
OLR (kg COD/m^3.d)	1.2 ± 0.05	2.5 ± 0.1	3.5 ± 0.2	6.1 ± 0.6	10.4 ± 1.2	3.6 ± 0.5	6.6 ± 1.4
MPR (m^3 /m^3.d)	0.32 ± 0.01	0.67 ± 0.05	0.89 ± 0.12	1.52 ± 0.02	2.70 ± 0.53	0.82 ± 0.20	1.47 ± 0.43
Methane yield (l CH$_4$/g COD)	0.30 ± 0.01	0.29 ± 0.04	0.28 ± 0.04	0.27 ± 0.02	0.26 ± 0.04	0.22 ± 0.07	0.22 ± 0.04
Methane content (%)	55 ± 2	55 ± 3	51 ± 2	49 ± 2	49 ± 2	56 ± 2	52 ± 4
Energy yield (MJ/kg TS)	11.56	11.18	10.79	10.41	10.02	-	-
pH of effluent	7.26	7.31	7.34	7.51	7.47	6.91	6.93
Effluent COD (kg/m^3)	0.37 ± 0.03	0.51 ± 0.02	0.87 ± 0.11	1.69 ± 0.16	1.08 ± 0.06	0.46 ± 0.11	0.82 ± 0.13
COD reduction (%)	96	95	94	94	94	95	96
Partial alkalinity (PA) (kg/m^3)	2.4 ± 0.1	4.1 ± 0.1	3.9 ± 0.4	7.5 ± 0.2	5.3 ± 0.2	1.4 ± 0.3	1.2 ± 0.4
VFA of effluent (kg/m^3)	0.03 ± 0.02	0.05 ± 0.02	0.14 ± 0.08	0.12 ± 0.08	0.19 ± 0.01	>0.01	>0.01
NH_4^+-N (kg/m^3)	0.38 ± 0.03	0.58 ± 0.02	0.66 ± 0.09	1.04 ± 0.08	0.70 ± 0.11	0.07 ± 0.01	0.04 ± 0.02

Treatment of the D-L fraction in the UASB reactors was performed under stable operation conditions. Evidence for this comes from stability of the pH, which ranged from 7.26 to 7.47 in the period during which the OLR was increased. This neutral pH is suitable for anaerobic digestion. The COD concentration in the effluent was low, and ranged from 0.37 to 1.08 kg/m^3 when the OLR was increased from 1.2 to 10.4 kg COD/m^3.d, corresponding to a reduction in COD that ranged from 94 to 95%. Torry-Smith et al. reported that the COD decreased to 84% at an OLR of 14 kg COD/m^3.d when treating lignocellulose effluent formed during ethanol production in a UASB reactor (Torry-Smith et al., 2003). The PA in the UASB reactor was maintained at a high level, as required, during the treatment period, and ranged from 2.4 to 7.5 kg/m^3, while the NH_4^+-N concentration ranged from 0.38 to 1.04 kg/m^3. The OLR was initially increased from 1.2 to 6.1 kg COD/m^3.d by increasing the concentration of the feedstock. The OLR was further increased by decreasing the HRT from 8.0 to 2.7 days, which increased the OLR from 6.1 to 10.4 kg COD/m^3.d. The reason for this increase was to maintain a low concentration of NH_4^+-N in the reactor: this concentration decreased from 1.04 kg/m^3 to 0.7 kg/m^3 due to the increase in the OLR. Ammonia inhibition can be avoided by maintaining a concentration of NH_4^+-N lower than 1 kg/m^3 and a concentration of free ammonia lower than 0.05 kg/m^3. However, Calli et al. have reported that UASB granular sludge can adapt to high levels of NH_4^+-N (1 to 6 kg/m^3) after long-term treatment (Calli et al., 2005). It is also possible to lower the concentration of NH_4^+-N by reducing the concentration of urea. Sodium hydrogen carbonate and urea were the main buffer compounds in the anaerobic basic medium: urea is a cheap source of nitrogen and buffer compound, the use of which can reduce operating costs.

The concentration of VFAs was below 0.2 kg/m^3 during the treatment of the D-L hydrolysate, and the VFAs consisted mainly of acetic and propionic acids. A VFA concentration lower than 0.1 kg/m^3 has been reported at an OLR of about 10 kg COD/m^3.d in a UASB reactor used to treat VFAs and alcohol produced from the dark fermentation of food waste (Han et al., 2005). Furthermore, increase in the OLR results in the accumulation of VFAs in the reactor. Murto et al. have reported well-functioning biogas processes in manure treatment with high VFA concentrations, where a high buffering capacity in the reactor ensured satisfactory function (Murto et al., 2004). We have controlled the buffer capacity by adjusting the feedstock, which enabled the UASB reactor to be operated at a much higher OLR. Other difficulties that prevent UASB reactors achieving very high OLRs are granule shearing and granule bed disruption due to vigorous gas production, which may eventually lead to process failure (Mahmoud et al., 2003).

The granular bed in the reactor grew, and the level of granules in the UASB reactor increased from 8.4 to 27.4 g VS during the treatment period. Efficient biomass growth allowed sugars and VFAs to be degraded at the high OLR of 10.4 kg COD/m^3.d.

3.7 Methane production from wheat straw and seaweed hydrolysate in UASB reactors

The mixture of wheat straw (D-L) and seaweed hydrolysate (SWH) (1:1 based on kg COD/m^3) was co-digested in a UASB reactor without addition of the anaerobic basic medium.

Methane production from the mixture of D-L and SWH in the UASB was rapid, with an MPR that ranged from 0.82 to 1.47 m^3/m^3.d. The OLR increased from 3.6 to 6.6 kg COD/m^3.d (Table 3). The methane yield, 0.22 m^3/kg COD, remained almost constant as the OLR increased. The methane yield obtained from the co-digestion of D-L and SWH in the UASB reactors was similar to that obtained in the methane potential batch test. It has recently been reported that synergistic effects arise that improve batch methane yields from seaweed (*Saccharina latissima*) pretreated with steam at 210 °C for 10 minutes and wheat straw (Vivekanand et al., 2011). The methane yield was 0.241 m^3/kg VS for a seaweed:straw ratio of 50:50, and 0.270 m^3/kg VS when the ratio was 75:25 (Vivekanand et al., 2011). We have obtained a methane yield of 0.19 m^3/kg VS from the treatment of SWH alone, at an OLR of 8.1 kg COD/m^3.d, with an MPR of 1.38 m^3/m^3.day (Nkemka & Murto, 2010). Treatment of wheat straw and seaweed hydrolysate may improve methane productivity and yield, and further studies are required to determine optimum mixture ratios.

The pH increased slightly during co-digestion of D-L and SWH, from 4.14 to lie in the range 5.50 to 5.79 in the mixed liquid hydrolysates. The mixture of hydrolysates, however, contained some alkalinity that was contributed from the SWH, even though the pH was low. The inherent total alkalinity of the SWH was 2.78 kg/m^3, while that of D-L was almost zero.

Treatment of the mixture of D-L and SWH in the UASB reactors was highly stable. The pH in the reactor was about 6.9, which is within the range recommended for anaerobic digestion (Gerardi, 2003). The COD concentrations in the effluent were low and the reduction in COD was comparable to that obtained in the treatment of the D-L hydrolysate. The PA was lower than that obtained during the treatment of the D-L in the UASB reactor, since no buffering compounds were added. The VFA/PA ratio is an important parameter used to assess the stability of anaerobic digesters, with a recommended range of 0.07 to 0.08 (Gerardi, 2003). A ratio greater than 0.5 shows that the process is imbalanced and that the process may fail. The VFA/PA ratio in this study was

0.01, well below the recommended range. This indicates that the process was stable, and that the inherent alkalinity of the SWH was sufficient. We conclude that the co-digestion of D-L and SWH in a UASB reactor is a viable process that makes it avoids the addition of nutrients or buffering agents and may be important in the reduction of the operational costs.

Combustion is a simpler and faster method of producing energy from wheat straw than anaerobic digestion. It suffers, however, from several disadvantages: problems with ash disposal, emission of NO_x, and corrosion in the boilers due to the high KCl content of straw. Anaerobic digestion, in contrast, allows nutrients of the wheat straw digestate to be recycled to farmland as fertiliser. Competition between use for food and use for biofuel production is also avoided. Furthermore, biofuel produced from cheap lignocellulose raw material can compete more readily with fossil fuel. In Sweden, the lowest CO_2 emissions are achieved when short-rotation forest, straw and forest residues are used to produce renewable energy (Börjesson, 1996). Hence, increasing the share of renewable fuel in the transport sector that comes from wheat straw may have a significant effect in reducing greenhouse gas emission.

Producing multiple biofuels (ethanol, hydrogen and methane) is a recent trend in the production of biofuels. Such co-production ensures the efficient utilisation of the energy content of biomass (Dererie et al., 2011; Kreuger et al., 2010). Further benefits of multiple fuel production are the production of high-value fuels and chemicals, energy security, and stability (both for the environment and for society). The high operating cost of multiple fuel production, however, may be a limitation. A techno-economic analysis that compared the co-production of biohydrogen and biogas with biogas production alone showed that the initial investment costs and nutrient requirements are the major contributors to the high production cost (Ljunggren & Zacchi, 2010). Moreover, the sole production of biogas uses resources efficiently and has several environmental benefits over the production of biodiesel and bioethanol (Börjesson & Mattiasson, 2008). Hence, direct biogas production from pretreated wheat straw may be an efficient method of producing renewable energy and techno-economic analyses of this process is recommended for the future.

4. Conclusions

Wheat straw is a lignocellulose residual material that does not compete with land used for producing food and can be used for biogas production. Acid catalysed steam pretreatment and enzymatic hydrolysis of the straw is efficient and gives high methane yields. A high methane production rate was achieved from the hydrolysate, supplemented with nutrients, in a UASB reactor. Co-digestion of the straw and seaweed hydrolysates in a UASB reactor was efficient and does not require the addition of nutrients or buffering agents. The biogas production from wheat straw may thus compete economically with production from other non-lignocellulose feedstocks.

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Paper VI

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Design of a novel biohythane process with high H_2 and CH_4 production rates

Karin Willquist [a,*], Valentine Nkongendem Nkemka [b], Helena Svensson [a], Sudhanshu Pawar [c], Mattias Ljunggren [a], Hans Karlsson [a], Marika Murto [b], Christian Hulteberg [a], Ed W.J. van Niel [c], Gunnar Liden [a]

[a] Department of Chemical Engineering, Lund University, Getingevägen 60, 221 00 Lund, Sweden
[b] Department of Biotechnology, Lund University, Getingevägen 60, 221 00 Lund, Sweden
[c] Applied Microbiology, Lund University, Getingevägen 60, 221 00 Lund, Sweden

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A biohythane process based on wheat straw including: i) pretreatment, ii) H_2 production using *Caldicellulosiruptor saccharolyticus*, iii) CH_4 production using an undefined consortium, and iv) gas upgrading using an amine solution, was assessed through process modelling including cost and energy analysis. According to simulations, a biohythane gas with the composition 46–57% H_2, 43–54% CH_4 and 0.4% CO_2, could be produced at high production rates (2.8–6.1 L/L/d), with 93% chemical oxygen demand (COD) reduction, and a net energy yield of 7.4–7.7 kJ/g dry straw. The model was calibrated and verified using experimental data from dark fermentation (DF) of wheat straw hydrolysate, and anaerobic digestion of DF effluent. In addition, the effect of gas recirculation was investigated by both wet experiments and simulation. Sparging improved H_2 productivities and yields, but negatively affected the net energy gain and cost of the overall process.

1. Introduction

Hydrogen is an interesting energy carrier due to its potentially high efficiency of conversion to usable power and its low emission of pollutants, but it is currently used mainly as a reducing agent in the chemical and food industries. Most of the H_2 produced today is derived from fossil fuels, and efforts are therefore made to produce it from renewable resources.

The biological production of H_2 is a promising alternative since it does not require elevated temperature and pressure and agricultural, forest and industrial waste can be used as substrates [1]. However, a number of challenges must be overcome before biohydrogen can become economically feasible. The primary challenge is the low substrate conversion efficiency. In a conventional dark fermentation (DF) process only about 7.5–15% of the energy contained in glucose is converted to H_2 (corresponding to a yield of 1–2 mol H_2 per mole glucose the rest of the energy is contained in volatile fatty acids (VFA)) [2]. Fortunately, recent progress in the characterisation of new potential organisms has revealed a number of organisms providing better H_2 yields [3]. *Caldicellulosiruptor saccharolyticus*, is one such organism, and is able to produce almost the theoretical maximum yield of 4 mol H_2 per mole glucose [4]. In addition, *C. saccharolyticus* can grow on hemicellulosic waste [5–9], and can grow and produce H_2 at partial H_2 pressures (P_{H2}) of up to 0.67 bar [3]. However, 65% of

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* *Corresponding author*. Tel.: +46462228271; fax: +46462224526.
E-mail address: Karin.willquist@tmb.lth.se (K. Willquist).

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the energy contained in the glucose still remains in the liquid as acetate. Consequently, acetate must be converted into a suitable product or energy carrier.

There are three main strategies for the conversion of acetate to energy: conversion to H_2 employing light or electricity, or reduction to CH_4 through anaerobic digestion (AD) [2]. This paper focuses on the latter alternative as AD is a faster and simpler process than the other two [2]. In addition, it has been shown to be an energy-efficient strategy for the production of a mixture of H_2 and CH_4, known as hythane [10–12]. Hythane can be used as a chemical, or as an energy carrier in gas combustion engines.

However, before hythane can be used, the CO_2 must be removed. In this study, the removal of CO_2 is achieved by an amine solution consisting of a mixture of 40% *N*-methyldiethanolamine (MDEA), 10% piperazine (PZ) and 50% water, by weight. This is a solvent commonly used in industry for the removal of CO_2 in various mixtures of gases, including biogas. However, there are also other methods of removing the CO_2 using e.g. biological means where the CO_2 is converted to sought after product such as diesel [13] or glycerol [14]. In addition to CO_2, sunlight is required for the production making the process complex, which is why this process is not assessed in this study.

In this study, biohythane production from wheat straw using a four-step process (pretreatment, DF, AD and gas upgrading) was analysed using an adapted kinetic model. The model was calibrated and evaluated against wet-experimental data. In addition, the effect of gas recirculation from the AD step to the DF step was experimentally investigated, as was the omission of sparging in the DF step. The model was used to simulate the DF, AD and gas upgrading process steps to maximise either hythane productivity or net energy yield, while maintaining high H_2 yields, COD conversion efficiency and CO_2 removal.

2. Materials and methods

2.1. System analysed in simulations

The process investigated consists of four steps, as illustrated in Fig. 1, namely: i) pretreatment based on steam explosion and enzyme hydrolysis, ii) H_2, CO_2 and organic acid production using DF, iii) CH_4 production through AD of the acids produced in the DF step, and iv) gas upgrading using an amine solution for CO_2 removal. The rate of feed used in simulations was 2 ton of straw per hour (equivalent to 32 TJ/h or 8.8 MW), based on the dry weight of the material. The gas produced in the AD reactor and 97% of the gas from the DF reactor were assumed recirculated and used in the DF reactor as a sparging gas. The residence times in the two reactors were varied to achieve optimal productivity and energy yield, while maintaining 93% COD conversion efficiency.

2.2. Experimental set-up

The wheat straw was pretreated using steam explosion with phosphoric acid and subsequent enzyme hydrolysis (Cellic C Htec, 95 FPU/g, 590 IU β-glucosidase/g enzyme, Novozymes, Bagsværd, Denmark), as described previously [15]. The hydrolysate contained (g/L): cellobiose (6.97), D-glucose (62.3), xylose (37.8), arabinose (3.47), glycerol (6.34), lactate (0.37), acetate (4.79), ethanol (0.29), hydroxymethylfurfural (HMF) (0.23) and furfural (1.14). The traces of ethanol and lactate found in the hydrolysate were probably a result of biological activity of a contaminant during or after the pretreatment process.

The DF process for the production of H_2 was performed using *C. saccharolyticus* (DSMZ 8903), which was cultivated in a controlled continuous stirred-tank reactor with a working volume of 1 L (Applikon, Schiedam, the Netherlands) at 70 °C, pH 6.9. The protocols for medium preparation, cultivation and calculations of DF with and without sparging, have been described previously [16]. The process differed in this study in that a (60:40) N_2:CO_2 gas mixture was used as sparging gas. N_2 was used instead of CH_4 for safety reasons. This was possible since both N_2 and CH_4 are inert gases not affecting the fermentation profile in *C. saccharolyticus* (Pawar S, Nkemka V, Zeidan A, Murto M, van Niel EWJ. Biohydrogen and biogas production from wheat straw hydrolysate in a two-step uncoupled process: A proof-of-concept study, manuscript in preparation). In addition, the hydrolysate from wheat straw was diluted 10-fold to give a final sugar concentration of 11 g/L and used as substrate (Pawar S, Nkemka V, Zeidan A, Murto M, van Niel EWJ. Biohydrogen and biogas production from wheat straw hydrolysate in a two-step uncoupled process: A proof-of-concept study, manuscript in preparation), giving a total COD concentration of 19.6 g/L.

The AD process for CH_4 production was performed in an upflow anaerobic sludge bed (UASB) reactor with a working volume of 0.8 L, at 37 °C, as described elsewhere (Pawar S, Nkemka V, Zeidan A, Murto M, van Niel EWJ. Biohydrogen and biogas production from wheat straw hydrolysate in a two-step uncoupled process: A proof-of-concept study, manuscript in preparation). The upflow velocity was between 0.08 and 0.09 m/h with a recirculation rate of 7.2 L/day. Granular anaerobic sludge used as inoculum in the UASB reactors was collected from a mesophilic full-scale plant in Netherlands. Two different kinds of medium were used: i) DF effluent from wheat straw hydrolysate containing (g/L): glucose (3.88), xylose (1.24), acetate (4.03), lactic acid (0.24), propionic acid (0.02) and ethanol (0.11), and ii) a semi-defined medium intended to resemble the DF effluent, containing the same sugar, acid and ethanol contents. To mimic the DF effluent containing *C. saccharolyticus* cells (cell dry weight of 1.1 g/L), yeast extract (1 g/L) was added to the semi-defined medium. This is the same amount of yeast extract as was used in the DSM 640 medium for DF (http://www.dsmz.de/microorganisms/medium/pdf/DSMZ_Medium640.pdf) In addition, to mimic the DF effluent containing NH_4^+, 0.11 g/L $(NH_4)_2SO_4$ was added to the semi-defined medium giving a final ammonium concentration of 0.1 g/L. The COD contents of the semi-defined medium and the DF effluent were 11.3 and 15.2 g/L, respectively, and the pH was 7.52 ± 0.08.

Both the semi-defined medium and the DF effluent were further supplemented with nutrients from a basic anaerobic medium, as previously described [17], with the exceptions that the vitamin solution was replaced with yeast extract (1 g/L), and NH_4Cl (1 g/L) was replaced with urea (1 g/L). See Nkemka

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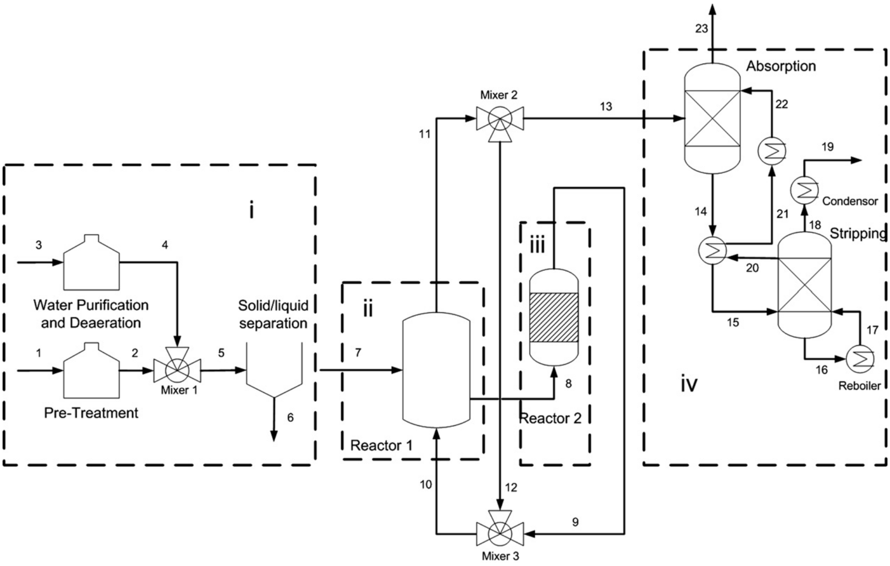

Fig. 1 – Process flow sheet of the proposed biohythane process: i) pretreatment of 2 ton/h wheat straw hydrolysate, ii) DF using *C. saccharolyticus*, iii) AD using an undefined mesophilic consortium, and iv) gas upgrading using an amine solution.

and Murto for more information [15]. Furthermore, the DF effluent was not treated with the reducing agents Na_2S and cysteine-HCl since the effluent had already been reduced by cysteine-HCl (1 g/L) prior to DF and by dissolved H_2 produced during DF. Cysteine-HCL (0.5 g/L) and Na_2S (0.25 g/L) were added to the semi-defined medium just before use. The effluent and semi-defined medium were stored at −20 °C before use, replaced regularly (every second day) and kept cold (4 °C) during use to avoid contamination.

2.3. *Analyses*

The hydrolysate composition was analysed as described previously [15]. The sugars, ethanol, VFAs, H_2, CO_2 and cell mass after DF were monitored and analysed regularly, as described by Willquist et al. [16]. The pH, CH_4, COD, NH_3, sugars, ethanol and VFA contents were also monitored and analysed regularly during AD, as described previously [15].

2.4. *Kinetic model and simulations of the DF and AD steps*

The DF step was simulated with a model developed specifically for *C. saccharolyticus* [18]. The AD step was simulated with a model based on AD model 1 (ADM1) [19], but adopted for the biohythane process (see Appendix 1 for more information).

Two additional reactions were added to the AD model accounting for lactate oxidation and fermentation. These were assumed to occur by two groups of microorganisms ($X_{lac,o}$ and $X_{lac,f}$; Eqs. (1)–(5)) according to Oyekola et al. [20]. The annotation of the parameters is consistent with that used by Batstone et al. [19].

$$\text{Lactate oxidation}: \quad 3\,\text{Lactate}^- \rightarrow \text{acetate} + 2\,\text{propionate} + HCO_3^- + H^+ \quad \Delta G_0 - 169.7\ \text{kJ/reaction} \tag{1}$$

$$\text{Lactate fermentation}: \quad 2\,\text{Lactate} + 2H_2O \rightarrow 2\,\text{acetate} + 2HCO_3^- + 4\,H2 \quad \Delta G_0 - 7.98\ \text{kJ/reaction} \tag{2}$$

Oxidation:

$$\rho_6 = k_{mlac\_o}\frac{S_{lac}}{K_{Slac,o} + S_{lac}}X_{lac,o}I_{inlim} \tag{3}$$

Fermentation:

$$\rho_5 = k_{mlac\_f}\frac{S_{lac}}{K_{Slac,f} + S_{lac}}X_{lac,f}I_{inlim}I_{H2,lac} \tag{4}$$

Where k_{mlac_o} and k_{mlac_f} are the Monod specific uptake rates (μ_{max}/Y) for lactate oxidisers and fermenters, respectively, S_{lac} is the lactate concentration (kg COD/m^3) and I_{inlim} is the inorganic nitrogen limitation function, as previously described [19]. The H_2 inhibition function of lactate fermentation ($I_{H2,lac}$) is given by:

$$I_{H2,lac} = \frac{K_{i,h2,lac}}{K_{i,h2,lac} + S_{H_{2,aq}}} \tag{5}$$

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where $K_{i,h2,lac}$ is the 50% inhibitory H_2 concentrations for lactate fermentation and $S_{H2,aq}$ the dissolved H_2 concentration. VFA inhibition was also included in the model according to Vavilin et al. [21].

The methane production from unspecified residual reduced carbon derived from yeast extract and the wheat straw hydrolysate (corresponding to 60% in the DF effluent), which was measured by soluble COD analysis, but not specified in the sugar and acid analyses (residual reduced organic carbon, S_{CODr}), was lumped into one reaction (ρ_{15}) through the kinetic expression (ρ_{15}):

$$\rho_{15} = k_{CODr} S_{CODr} Y_{CODr} \tag{6}$$

where k_{CODr} is the rate constant for uptake of S_{CODr}, and Y_{CODr} is the yield of S_{CODr} that is converted.

Some of the reduced organic carbon will be converted to CO_2. Therefore, a stoichiometric parameter for CO_2 conversion (C_{COD}) was included in the model (Appendix A, Table A1).

2.5. Reactor design for AD

The UASB reactor was modelled as a stirred-tank reactor since the recirculation stream was 4–13 times higher than the feeding rate. Moreover, the hydraulics of large-scale UASB reactors resembles a tank more than a tubular reactor [22].

The stability of the full-scale AD process was evaluated by estimating the ratio between the loading rate of the rate-limited substrate (in this case acetate) and dividing it by the maximum uptake rate of that substrate [23]:

$$kOLR = \frac{LR}{k_m \times X} \tag{7}$$

where k_m is the Monod maximum specific uptake rate of the rate-limited reaction (kg COD_S/kg COD_X/d), X is the total biomass concentration (kg COD) and LR is the loading rate (kg COD/d) of the rate-limited substrate.

All volumetric gas productivities were normalized to 0 °C and 1 atm. The calculated COD in the feed and the residual COD were based on the measured or modelled COD concentrations. Carbon balances and the gas productivity of the DF step were calculated as described previously [24].

2.6. Design of the gas-upgrading system

A model of the gas upgrading process was developed to aid in the design of a gas-upgrading reactor to remove the CO_2 from the biohythane. A standard industrial amine solution for CO_2 removal was simulated, and the required dimensions of the absorber, stripper and heat exchangers for 99% CO_2 removal were calculated. The amine solution was assumed to consist of a mixture of 40% MDEA, 10% PZ and 50% water, by weight [25].

A reduced version of the model developed by Zhang et al. [26], consisting of only two reactions, was used to describe the reactions of CO_2 with PZ and MDEA (Eqs. (8) and (9)).

$$CO_2 + PZ \cdot H_2O \leftrightarrow PZH^+ + HCO_3^- \tag{8}$$

$$CO_2 + MDEA \cdot H_2O \leftrightarrow MDEAH^+ + HCO_3^- \tag{9}$$

The dimensions of the absorber and the stripper were determined by solving the integral balances for each unit and then the differential balances. Firstly, the integral balances for the absorption tower were solved to estimate the sizes of the incoming and outgoing gas and liquid streams and the incoming and outgoing temperatures of the gas and liquid, by solving the mass and energy balances for the system. The incoming gas flow was determined by simulation of the DF and AD steps, and used as input to the model. The maximum possible gas flow through the absorption tower, to avoid flooding of the tower, was determined by empirical relations established by the manufacturer of the packing material. This maximum gas flow gave the diameter and the pressure drop over the column. Finally, differential calculations were used to estimate the column height and the concentration and temperature profiles over the height of the column. The column height was determined from the amount of CO_2 that needed to be removed in the column. To ensure that the absorption equipment was not under-dimensioned, safety factors were introduced. The safety factors for the absorption tower were 10% for the diameter, and 100% for the height of the column.

The same principle was applied to the calculation of the stripper dimensions. Because of the difficulties associated with solving the differential balances for the stripper [27], a second short-cut method based on the theoretical plate height was also applied for the determination of the stripper height [28]. The stripper height used was the higher of the values obtained with the two methods. A safety factor of 10% was used for the diameter, while a safety factor of 100% was applied when using differential balances, and of 50% when using the short-cut method, since the latter is based on empirical relations. The dimensions of the stripper and the reboiler duty for the stripper and the cooling effect of the condenser were determined from integral balances, by solving the mass and energy balances for the system. The dimensions of the heat exchangers were determined using conventional theory [28].

2.7. Techno-economic analysis of the two process scenarios

A techno-economic analysis was carried out including all major process steps (pretreatment, DF, AD and gas upgrading) as well as auxiliary equipment (steam boiler, heat exchangers, pumps and compressor). A thorough description is given elsewhere [29,30].

2.8. Parameter estimation

Most parameter values in the DF model were adopted from Ljunggren et al. [18]. However, this model does not take into account the case of no sparging, which affects the mass transfer rate (k_La). Without sparging, the mass transfer rate is dependent on the intrinsic gas production, and was assigned a value of 3 h^{-1} (72 d^{-1}), based on model extrapolation using the reported mass transfer model [18]. This value is in accordance with the value of 5 h^{-1} or 120 d^{-1}, estimated by Batstone et al. [19].

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Most of the parameters for the AD model were taken from Batstone et al. [19], with the exception of parameters for acetate, propionate and S_{CODr} consumption, which were calibrated towards experimental data. The hydrogen inhibition coefficient for lactate oxidation ($Ki_{\_H2lac}$) was taken from literature of the 50% H_2 inhibition concentration for *Desulfovibrio vulgaris* (2% H_2; 4×10^{-5} kg COD/m^3) [31] and compared to thermodynamic considerations (Eq. (2)) according to Batstone et al. [19]. According to thermodynamic considerations, the value of $Ki_{\_H2lac}$, should be 2×10^{-4} kg COD/m^3 at acetate, lactate and HCO_3^- concentrations of 1 mM, 1 mM, and 100 mM, respectively. However, since the value of dissolved hydrogen concentration did not exceed 5×10^{-6} kg COD/m^3 in any of the simulations, this had a negligible impact. The other parameter values, for lactate oxidation and fermentation, were taken from Oyekola et al. [20].

The physical properties of the components involved in gas separation, such as density, viscosity, surface tension, diffusivity of the amines in water solution, diffusivity of gases in the amine solution, gas solubility in the amine solution and heat capacity, parameter values were taken from the literature [32–47]. However, there were no journal publications on the heat of reaction for the investigated system. The only reference found was an interim report from North Carolina State University [48]. For this reason an investigation on the heat of reaction for the system was conducted within the project. The data acquired from this investigation was used in the simulation model (Svensson H, Hulteberg C, Karlsson H.T. Heat of solution of CO_2 in aqueous solutions of N–methyldiethanolamine and piperazine, manuscript in preparation).

3. Results and discussion

3.1. *Verification of the DF model and the effect of sparging*

The soluble portion of the hydrolysate was used as a substrate for H_2 production in the DF step. Based on previous knowledge, the main concerns when designing DF using *C. saccharolyticus* are the sensitivity of this thermophile to elevated levels of H_{2aq} and osmotic pressure [3,18]. In addition, inhibitors in the wheat straw hydrolysate may affect the fermentation profile. Therefore, the DF model was used to study the effect of recirculating gas from the AD step to the DF reactor, and to investigate the effects of omitting sparging.

To verify the model, experiments were performed using the wheat straw hydrolysate, where the gas recirculation was mimicked by sparging the reactor with a N_2:CO_2 gas mixture (60:40 v/v) (see Materials & Methods), at a rate of 6 L/L/h. The experiment without sparging was performed on a modified DSM 640 medium [16] with 10 g/L glucose as carbon source. In addition, data from continuous operation on hydrolysate with N_2:CO_2 sparging (6 L/L/h) published elsewhere (Pawar S, Nkemka V, Zeidan A, Murto M, van Niel EWJ. Biohydrogen and biogas production from wheat straw hydrolysate in a two-step uncoupled process: A proof-of-concept study, manuscript in preparation) were used to verify the model.

Overall, the DF model was able to predict the fermentation profile with gas recirculation and in the absence of sparging with reasonable accuracy for the first 30 h (Fig. 2), verifying the ability of the model to simulate these conditions. Interestingly, based on the fermentation profile, *C. saccharolyticus* did not seem to be inhibited by the use of hydrolysate, indicating that the bacterium is able to tolerate concentrations of 11 g/L sugars together with all compounds present in the hydrolysate, such as 23 mg/L HMF and 114 mg/L furfural. According to the experimental data, the cells enter a new growth phase after 30 h, where the sugars are consumed and acetate is formed. This behaviour was not predicated by the model. Consequently, the concentration of cell mass remained the same, while the model predicted cell lysis. This finding merits more research, however, this diphasic growth would not occur in continuous mode, and it would therefore not influence the applicability of the model. The carbon balance closed at 107%, indicating that 7% of the product could originate from carbon sources other than those accounted for in the model. This C balance is consistent with previously published data on the cultivation of *C. saccharolyticus* on the same hydrolysate in continuous mode (112 ± 5%; Pawar S, Nkemka V, Zeidan A, Murto M, van Niel EWJ. Biohydrogen and biogas production from wheat straw hydrolysate in a two-step uncoupled process: A proof-of-concept study, manuscript in preparation).

Consistent with previous results [16], the lactate production rate increased, and the acetate and biomass production rates decreased in the absence of sparging (Fig. 2). This effect was captured by simulating the dissolved H_2 concentration (Fig. 2E–F). When the reactor was sparged the dissolved H_2 concentration was well below the critical value of 2.2 mM [18], while without sparging the dissolved H_2 concentration approached 2 mM, causing severe inhibition.

The model was able to predict the elsewhere reported H_2 productivity in continuous operation (Pawar S, Nkemka V, Zeidan A, Murto M, van Niel EWJ. Biohydrogen and biogas production from wheat straw hydrolysate in a two-step uncoupled process: A proof-of-concept study, manuscript in preparation) with only a slight underestimation of (4% and 8%) of the dilution rates, 0.05 h^{-1} and 0.15 h^{-1}, respectively (simulation data not shown). However, the model overestimated the sugar consumption rate by 50% in continuous operation.

3.2. *Effect of CO_2 in the recirculated gas on hydrogen production in the DF process*

The results described above, and presented in Fig. 2, illustrate the positive effect of sparging the DF reactor. However, sparging with CO_2 is known to have negative effects on H_2 productivity and the growth of *C. saccharolyticus* [49], as also illustrated by the model (Fig. 3A, B). The inhibitory effect of CO_2 is caused largely by increased osmotic stress through increased levels of bicarbonate and caustic agents [49]. This stress occurs immediately upon initiation of the experiment when the reactor is sparged with CO_2 approaching the critical value (0.28 M; [18]) as illustrated in Fig. 3C, which hinders growth and thus also inhibits H_2 productivity (Fig. 3). In contrast, when the culture is sparged with N_2, the estimated

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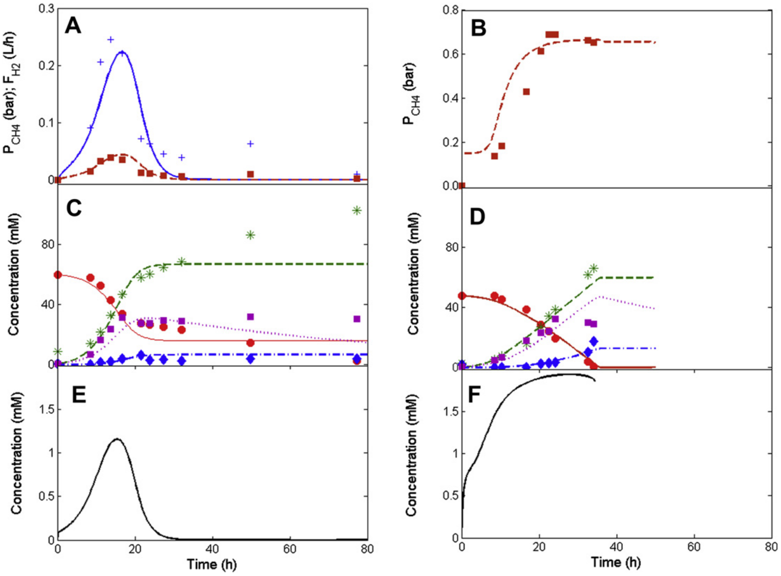

Fig. 2 – Simulated and measured effect of gas recirculation (A, C, E) and absence of sparging (B, D, F), on hydrogen production (A, B), dissolved substrate consumption and product formation (C, D) and dissolved H_2 concentration (E, F) in the DF step. The symbols indicate H_2 productivity (+), P_{H2} (■), sugar consumption (●), acetate formation (✳), biomass formation (■) and reduced product formation (lactate and ethanol; ◆). The lines indicate simulations of H_2 productivity (——), P_{H2} (--), sugar consumption (–), acetate formation (---), biomass formation (··), reduced product formation (_ · _) and dissolved H_2 concentration (-).

osmolarity is well below the critical value at the beginning of the experiment and does not approach it until the end of the experiment. This is in accordance with previous observations from wet experiments [49]. Interestingly, sparging with a mixture of CH_4 and CO_2, instead of 100% CO_2, gave an increased the H_2 productivity by a factor of 3 and the biomass concentration by a factor of 23 (Fig. 3). This is due to a dilution of the CO_2 leading to lower osmolarity. The positive effect of sparging with CH_4/CO_2 is slightly less than, but still comparable to, that of N_2 (Fig. 3). Based on these results the gas-recirculation scenario was used for further investigations of the kinetics of the combined process.

3.3. *Potential and parameter calibration of the AD process*

The third step of the process is the conversion of acids and residual sugar by AD. For this purpose the ADM1 model was used and adapted to the specific conditions of this biohythane process (Appendix A). The ADM1 model is well established and has been used in similar AD processes [50]. Interestingly, a preliminary simulation, using the parameters specified previously [19], indicated that AD could be performed on DF effluent at hydraulic retention times (HRTs) as low as 0.2–0.5 days resulting in elevated CH_4 productivities (>4 L/L/d), without any significant loss in COD conversion efficiency. This implies that the reactor volume could be decreased, leading to a potential reduction in process costs. However, some parameters, such as those governing acetate uptake kinetics, are usually substrate-specific and should be calibrated for each system. Therefore, a continuous experiment was performed in a UASB reactor on a semi-defined medium (Fig. 4A, C, E, G, I). The kinetic parameters of acetate and propionate uptake, given in Table 1, were estimated from the acetate and propionate data (Fig. 4E).

The S_{COD} constituted a significant part of the reduced organic carbon in the DF effluent (60%; Materials and Methods). Since the sources of these reduced organic molecules have not been specified, they were lumped into one first-order kinetic reaction (ρ_{15}, Eq. (6)). The measured residual COD concentration was used to determine the rate and yield of the methane production from the reduced organic molecules (k_{COD} and the Y_{COD}; Table 1). The S_{COD} was in the same range as the sugar uptake and the yield was 90%. Hence a significant part of the unspecified reduced carbon was converted to CH_4. The effect of S_{COD} was illustrated by using two different models, one with and one without ρ_{15} (Eq. (6)) (Fig. 4A, I). This simulation demonstrates that ρ_{15} is essential for accurate predictions that agree with the data. C_{COD} was estimated from the partial CH_4 pressure (P_{CH4}) data. Finally, the CH_4

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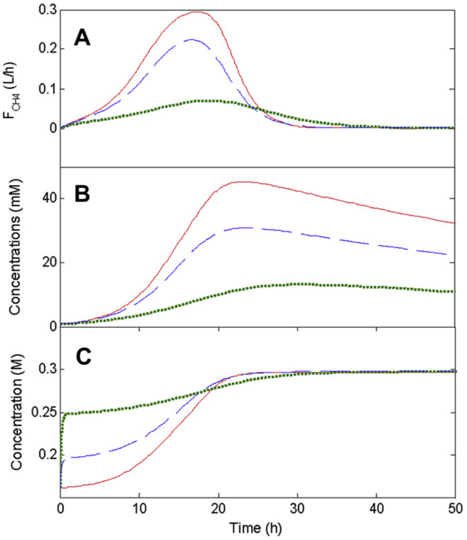

Fig. 3 – Simulated effect of sparging gas (6 L/h), N_2 (—), CH_4:CO_2 (60:40; - -) and CO_2 (···), on H_2 productivity (A), biomass formation (B) and osmolarity (C) in the DF step.

productivity and P_{CH4} were used to estimate k_La and the gas volume (Table 1). The estimated gas volume was in agreement with that predicted by the DF model [18], and k_La was in the same range as previously reported for a UASB reactor [51].

3.4. *Verification of the AD model*

To verify the model of AD in the UASB reactor using DF effluent data obtained in the present study were used together with data published elsewhere (Pawar S, Nkemka V, Zeidan A, Murto M, van Niel EWJ. Biohydrogen and biogas production from wheat straw hydrolysate in a two-step uncoupled process: A proof-of-concept study, manuscript in preparation). The predictions of the model corresponded well with the measured data, including the residual reduced carbon (Fig. 4B, D, F, H, J), although it slightly underestimated the residual inorganic nitrogen (Fig. 4H). This could be explained by the consumption of proteins present in the straw, yeast extract and *C. saccharolyticus* cells, none of which is included in the model (Appendix A). In addition, the change in productivity at different HRTs was not captured by the model since the productivity depends on k_La, which in turn depends on the gas flow rate [18], which is not accounted for in the ADM1 model [19]. Neither could the model capture the adaptation profile of the AD step at low HRT (Fig. 4B). However, the simulations gave values in the same range as the measured data, illustrating the applicability of the model to the conditions used. In contrast, when ρ_{15} was excluded, the model overestimated the residual COD after AD and underestimated the gas productivity at low HRT (Fig. 4B, J).

3.5. *Effect of increased HRT on biohythane productivity*

Simulations with the calibrated AD model and the experimental data confirmed the preliminary results that the AD step of this particular biohythane process could be operated at low HRT, resulting in a significant increase in methane productivity. The methane productivity increased with decreasing HRT, as shown in Fig. 4A, B. At an HRT of 1 day the CH_4 productivity reached 4 L/L/day (Fig. 4B). This can be compared with results from another type of biohythane process based on DF of the hydrolysis effluent of wheat straw, showing a CH_4 productivity of 2 L/L/d at the same HRT [10]. In addition, results published elsewhere have shown that the maximum H_2 productivity using wheat straw hydrolysate with *C. saccharolyticus* in the presence of N_2:CO_2 sparging (6 L/h) was 1.8–3.5 L/L/d (3–6 mmol/L/h; Pawar S, Nkemka V, Zeidan A, Murto M, van Niel EWJ. Biohydrogen and biogas production from wheat straw hydrolysate in a two-step uncoupled process: A proof-of-concept study, manuscript in preparation), i.e. 2–4 times higher than previously reported [10].

The CH_4 productivity in AD is generally limited by substrate conversion efficiency and granule stability [52,53]. Possible biological explanations of the superior gas productivity in this biohythane process are summarized in Fig. 7. Firstly, pretreatment speeds up hydrolysis, which is generally a rate-limiting step in the biogas process [19]. Secondly, the sugars are fermented by *C. saccharolyticus*, which is recognized for its abilities to give high H_2 and acetate yields, and is less inhibited by H_2 than *Thermoanaerobacter* species generally found in anaerobic sludge in biohythane processes [3,12].

Thirdly, acetate is the main dissolved fermentation product from the DF process added to the AD process, while the propionate and butyrate concentrations are low. Oxidation of these acids could be rate-limiting at high P_{H2} [19]. However, since H_2, propionate and butyrate concentrations are low in the AD process, this is not a significant problem. Fourthly, the methanogenesis from H_2 and CO_2 is avoided in the biohythane process, since DF and AD are performed in separate reactors. H_2, propionate and butyrate are produced and consumed in AD only when the sugars have not been completely consumed and/or lactate production becomes extensive in DF (Fig. 7).

Finally, acetoclastic methanogenesis generally becomes a rate-limiting step in the biogas process, if there is an imbalance between fermentation and methanogenesis. This is because during fermentation, the formation of acids is only accompanied by a fall in pH if fermentation is faster than methanogenesis [19]. In contrast, the pH during DF in the biohythane process described here was maintained at 6.9 by the addition of a caustic agent, and the pH in the AD process did not decrease below 7.4. At this pH, the fraction of the undissociated form of acetate (the form inhibiting acetoclastic methanogens [54]) is less than the dissociated form, resulting in less inhibition and hence faster acetoclastic methanogenesis. By applying the model the NH_4^+ concentration could

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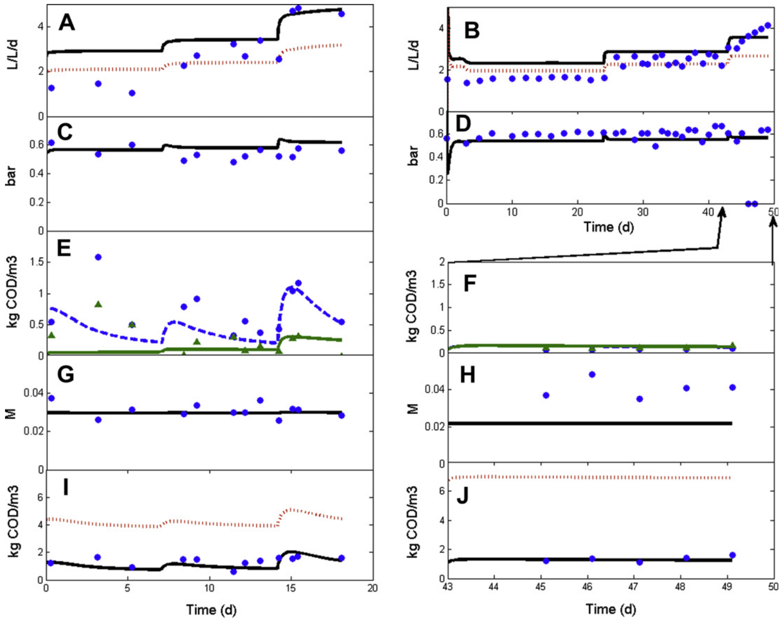

Fig. 4 – Fitted result on semi-defined medium (A, C, E, G, I) and verification of the AD model using DF effluent (B, D, F, H, J) at different HRTs, 1.5 d (0–7 d), 1 day (7–14 d) and 0.5 d (14–18 d) and on DF effluent 2.5 d (0–24 d), 1.5 d (24–43 d) and 1 day (43–49 d). A & B show normalized CH_4 productivity (0 °C, 1 atm) (L/L/d), C & D show P_{CH4} (bar), E & F show residual acetate and propionate concentrations (kg COD/m^3), G & H show residual inorganic nitrogen concentration (M), I & J show the residual COD concentration (kg COD/m^3). The symbols (●, ▲) indicate the measured data points and the lines the simulated values. The dotted lines (···) in A, B, I and J show the simulated data when ρ_{15} (Eq. (6)) is excluded from the model. Note the different scales on the x-axes.

be set to maximize growth of the consortium while minimizing the NH_4^+ inhibition on acetoclastic methanogenesis.

3.6. *Process design using the calibrated combined model*

To understand the overall process and the effect of the HRT on the overall H_2 and CH_4 output, the two models for DF and AD were combined into one. Using this combined model, the output at different HRTs of DF (HRT_{DF}) and AD (HRT_{AD}) were studied (Figs. 5 and 6).

Table 1 – Calibrated parameter values. The reference is for the benchmark value.

Parameter	Benchmark value	Estimated value	Reference
$km_{\_ac}$ (COD/COD/d)	8	4.0	[17]
$km_{\_pro}$ (COD/COD/d)	13	4.9	[17]
k_{S_pro} (COD/COD)	0.42	0.22	[17]
k_{COD} (COD/d)	–	47	–
Y_{COD} (COD/COD)	–	0.90	–
C_{COD} (M/COD)	–	0.015	–
k_La (d^{-1})	120	190	[17]
V_g (% of V_{liq})	4	5	[16]

The complexity of this simulation is due to the coupling of HRT_{DF} to the AD step. For instance, a change in HRT_{DF} will give a change in the composition of the DF effluent, which is the feed to the AD step. This will change the methane productivity, which in turn affects the composition of the DF effluent. This problem was solved iteratively.

HRT_{DF} was chosen based on either the maximum H_2 productivity, or the “maximum” energy yield, while maintaining at least a H_2 productivity of 3.2 L/L/d in the latter scenario. HRT_{AD} was chosen based on the maximum CH_4 productivity, while maintaining at least 93% COD reduction and an OLR ≤ 24 kg COD/m^3/d. The restriction on the OLR was made to avoid instability of the AD process [53]. This selection procedure resulted in two scenarios, which in turn resulted in different dimensions of the DF, AD (Table 2) and gas upgrading equipment (Table 3). Analysis of the combined process confirmed the experimental results, i.e., that the potential biohythane productivity can be high (6.1 $L/L_{(DF+AD)}$/d), which

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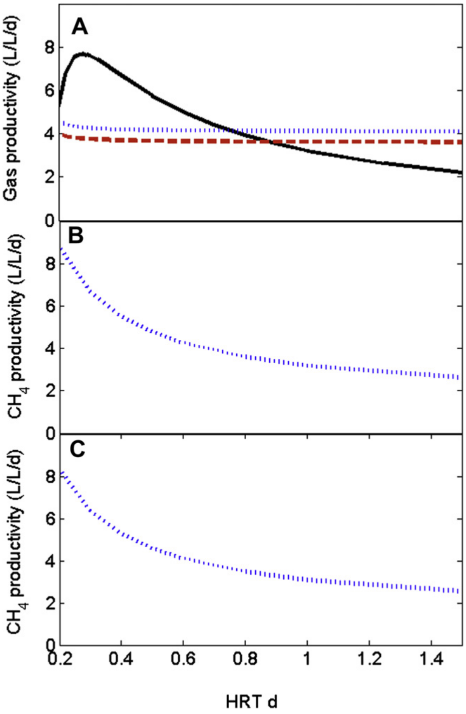

Fig. 5 – Simulated effect of HRT on DF and AD using the combined model. (A) shows the productivity of H_2 (——), CO_2(- -) and CH_4 (····) in the DF step with HRT_{AD} = 0.5 d. (B) and (C) show the CH_4 productivity in the AD process with HRT_{DF} = 0.4 d and 1 d, respectively.

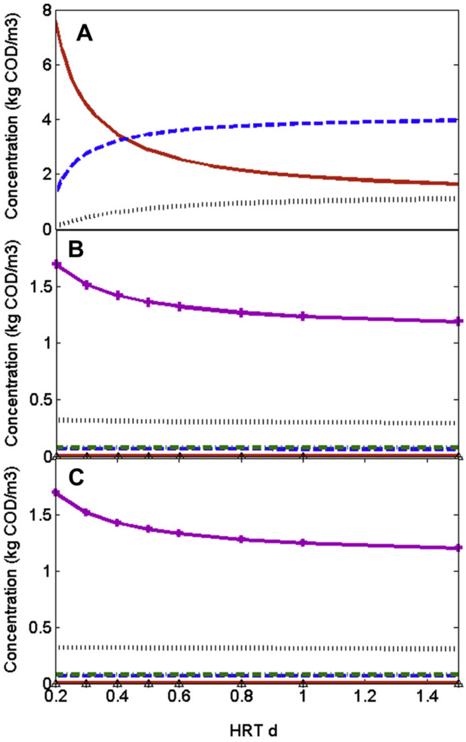

Fig. 6 – Simulated effect of HRT on acid and sugar concentration in the DF reactor. A) HRT_{AD} 0.5 d, B) AD process at HRT_{DF} 0.4 d and C) AD process at HRT_{DF} 1 d. Concentration of sugar (——), acetate (- -), lactate (···) and in B & C propionate (_·_·) and COD (——).

allows the volumes of the reactors to be decreased (Fig. 5; Table 2).

Acetate was the main dissolved product of DF in both scenarios. The lactate concentration was higher when HRT_{DF} was 1 d, and the residual sugar concentrations were, as expected, higher when HRT_{DF} was 0.28 d (Fig. 6A). In the scenario of maximum H_2 productivity (HRT_{DF} = 0.28 d), the hydrogen yield was 43% of the maximum yield (4 mol/mol) due to incomplete sugar consumption (Table 2). This yield is lower than what can be obtained for *C. saccharolyticus,* 3.6–4 mol H_2/mole C6 under optimal conditions [3]. The reason is the is due to high organic loading; 40 kg COD/m^3, which is 4–5 folds higher than the conditions when the optimal yield was achieved (1.9–4.4 g/L glucose) [55]. In addition, the CO_2 in the sparging gas 0.37–0.37 kPa contributed to increased osmolarity leading to incomplete substrate conversion (Figs. 5A and 6A). However, to put this in perspective, a value of Y_{SuH2} of 1.7–2.5 mol/mol corresponds to 0.27–0.34 L H_2/g sugar present in the substrate. This can be compared to the previously reported maximum H_2 yield of 0.19 L H_2/g sugar present in the substrate with undefined consortium [10]. To obtain higher H_2 yields, closer to the theoretical maximum, the HRT in DF must be significantly increased (Figs. 5A and 6A) or the hydrolysate concentration decreased, resulting in low H_2 productivities and hence large reactor volumes.

The effect of changing the HRT_{DF} on CH4 productivity and dissolved acid concentration in the AD process was insignificant (Figs. 5 and 6). According to the simulation, the COD conversion of the specified sugars and acids were efficient in both scenarios (Fig. 6B and C). It was mainly the unspecified organic carbon (S_{COD}) that contributed to the residual COD concentration after DF and AD treatment (Fig. 6B and C). 10% of the S_{COD} could not be converted in the AD process.

3.7. *Process stability*

Another important experimental aspect of process design is process stability. The stability of the DF process has not been studied in detail. However, an OLR of 40 kg COD/m3/d and HRT

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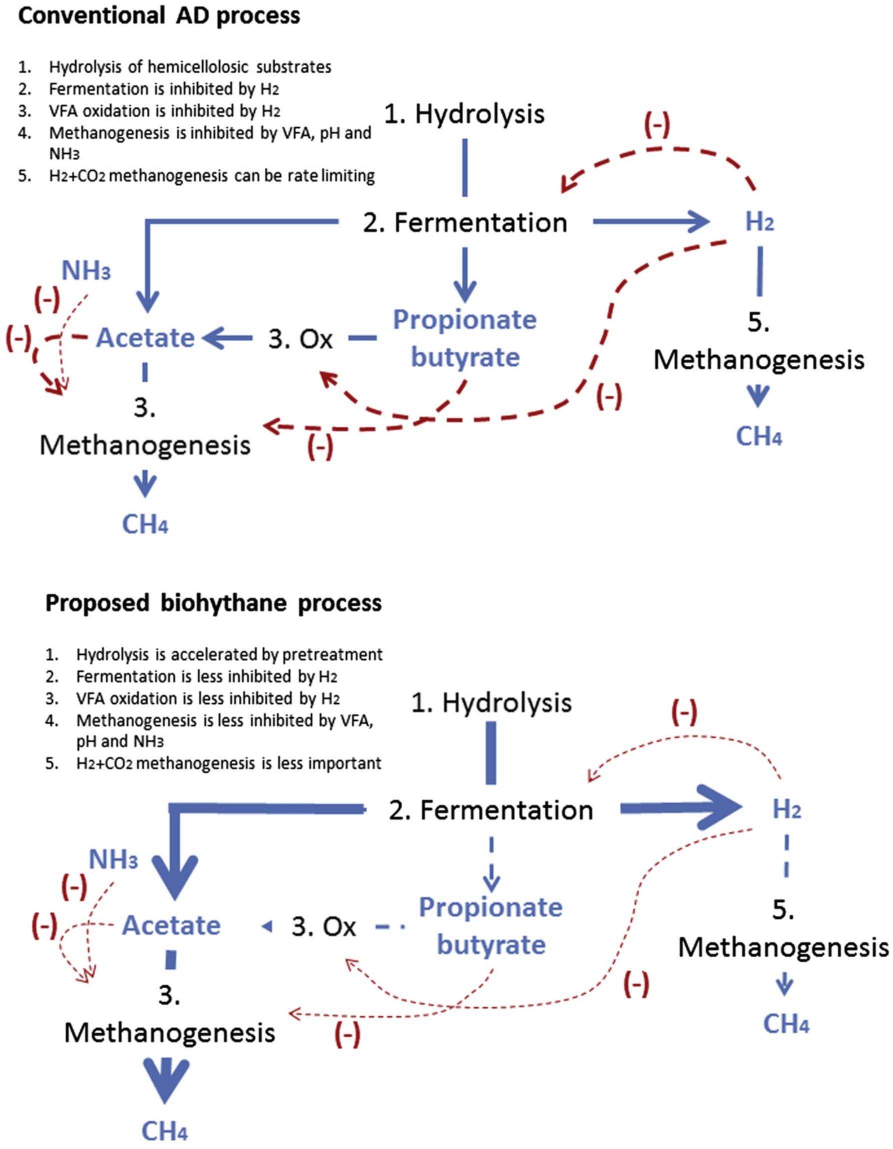

Fig. 7 – The difference between a conventional AD process and the proposed biohythane process, illustrating the arguments for the observed superior productivity of the latter.

of 0.12 days ($D = 0.35\ h^{-1}$), did not cause any process instability during wet experiments with *C. saccharolyticus* in a CSTR [55].

The stability of AD using a UASB reactor depends on four factors: i) substrate characteristics and composition, ii) organic loading rate (OLR), iii) upflow velocity, and iv) reactor volume [53]. In the current study, the substrate used had low solid, lipid and protein fractions, reducing the risk of foam and granular disruption [23,52]. For process stability it is

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Table 2 – Design of the volume of the DF and AD reactors with the criteria for maximum H_2 productivity. The volume of the AD reactor was optimised to obtain a maximum CH_4 productivity while maintaining a 93% COD conversion efficiency and an ORL ≤ 24 kg COD/d/m^3. q_{H2}, q_{CH4} and q_{gas} are the productivity of H_2, CH_4 and total gas (H_2 + CH_4 + CO_2), respectively, kOLR is the percentage of the loading rate of acetate (the rate-limited substrate) divided by the maximum uptake rate of acetate, and Y_{SuH2} is the molar yield of H_2 per unit added sugar.

Criteria	Process	HRT	OLR	kOLR	Reactor volume	q_{H2} or q_{CH4}	q_{gas}	Y_{SuH2}	COD conversion
		(d)	(kg COD/d/m^3)	(%)	(m^3)	(L/L/d)	(m^3/h)	mol/mol	(%)
q_{H2}	DF	0.28	41[a]		760	H_2: 7.8	880	1.7	
	AD	0.60	24[b]	11	1600	CH_4: 4.3			93[c]
Energy yield	DF	1.0	11[a]		2700	H_2: 3.2	1000	2.5	
	AD	0.55	24[b]	19	1500	CH_4: 4.3			93[c]

a The organic loading rate of DF was calculated based on the fermentative sugar (glucose, xylose and arabinose) content.

b The organic loading rate of AD was calculated based on the modelled effluent sugar, acid and S_{COD} contents of the DF and.

c The COD conversion efficiency was calculated based on the COD measurements in the 10-fold diluted wheat straw hydrolysate (19.8 kg COD/m^3) and the modelled organic carbon leaving AD.

recommended that the OLR should not exceed 75% of the maximum methanogenic activity [23]. In the two scenarios presented here, acetoclastic methanogenesis was the rate-limiting step, but the acetate loading rate was 11–19% of the methanogenesis rate (Table 2), hence the ORL (24 kg/COD/m^3/d; Table 2) should not cause any instability [23,53]. However, it should be noted that this model does not take into account granular washout, which is possible at high volumetric gas productivities [52]. This should be investigated before full-scale operations are undertaken.

3.8. *Upgrading of the biohythane gas*

The final step of the process was gas upgrading with a standard industrial amine solution for CO_2 removal. This solution consists of a mixture of 40% MDEA, 10% PZ and 50% water, by weight. The modelled gas separation step is designed to remove 99% of the CO_2 present in the effluent gas resulting from the biological steps. The dimensions of the system and the energy required for gas upgrading are given in Table 3.

Because the raw material used is straw, the gas produced in the process is likely to contain small amounts of H_2S, due to sulphur in the wheat straw. H_2S may also be produced from cysteine, which is one of the nutrients added to the process, which may increase the amount of H_2S in the gas. The H_2S content of the gas was not determined, but it can be assumed to be low. For instance, during the gasification of biomass, the amount of H_2S typically present in the produced gas is below 200 ppm. Absorption of H_2S in MDEA solutions is a common technique for selective removal of H_2S from CO_2-rich gases. It may therefore be assumed that no H_2S will escape the gas separation step in the modelled process. For this reason, a filter for sulphur removal was not included in the gas upgrading process.

3.9. *Energy recovery of the biohythane process*

In the scenario in which the energy yield was maximized, the net energy yield of the H_2 and CH_4 produced from the wheat straw after pretreatment, DF and AD was 7.7 kJ/g straw, corresponding to 69% recovery of the energy from the sugar fraction of the original straw (11 kJ/g straw). The scenario for maximum H_2 productivity resulted in a somewhat lower net energy yield (7.4 kJ/g straw). Of this, 48–50% of the produced energy is derived from H_2 production, and the rest from CH_4 production. This is a lower net energy yield than previously reported [10], due to lower CH_4 yield. Increased gas productivities in this study compared to those previously reported [10], could contribute to a decrease in the energy yield since there is generally a trade-off between productivity and yield [3]. However, the underestimation of the CH_4 production by the model, which overestimates the sugar conversion in DF, should be considered as a contributing factor.

The heat demand of the process is fairly high (3000 and 3300 kW) and does not differ significantly between the two scenarios (10%). The DF step requires an additional 1800 kW despite efficient heat recovery. This is mainly due to the low

Table 3 – Process design of the gas separation reactor with the criteria for minimum P_{CO2} (0.005 bar) in the effluent biohythane gas. The heat exchanger for the internal heat exchanging between stream 14 and 20 (see Fig. 1) is labelled HEX1 and the heat exchanger for the external heat exchanging of stream 21 (see Fig. 1) is labelled HEX2. Diameter (D) and height (H) are abbreviated in the table.

Criteria	Absorber		Stripper				Heat exchangers		HEX2	Effluent gas		Amine flow
	D	H	D	H	Reboiler duty	Cooling duty	Area HEX1	Area HEX2	Cooling duty	q_{gas}	P_{H2}:P_{CH4}	
	(m)	(m)	(m)	(m)	(kW)	(kW)	(m^2)	(m^2)	(kW)	(m^3/h)	bar:bar	(kg/h)
q_{H2}	0.77	3.6	0.76	4.5	870	280	480	290	460	540	0.46:0.54	16000
Energy yield	0.81	3.7	0.79	4.5	953	310	528	310	500	640	0.57:0.43	17000

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substrate concentration in the feed (11 g/L sugar), requiring large water flow rate. In addition, sparging the DF reactor leads to extensive water evaporation. In the calculations it was assumed that there was a 5 °C temperature drop in the gas recirculation which means that part of the water in the effluent gas is condensed. The pretreatment and gas upgrading demands 340 kW and about 900 kW heat, respectively. The energy available in the lignin residues separated after the enzymatic hydrolysis is 2600 kW which means that the lignin in the straw can cover only 86–87% of the heat demand of the process with the current set up and hence additional fuel is required in the burner.

The electricity demand of the process is also high, which is almost solely due to sparging. Large amount of gas is recirculated, which needs to be compressed to be used as a sparging gas in the DF step. 97% of the effluent gas from the DF step is recirculated which means that the stripping gas flow rate is more than 32-times that of the gas flow rate to the gas upgrading. The electricity demands of the compressors for the two scenarios are 1700–1900 kW; higher for the high energy yield scenario due to larger recirculated gas flow rates. If the sparging could be avoided the heat and electricity demands of the process can be significantly reduced.

3.10. *Cost analyses of the biohythane process*

The two scenarios results in similar production costs as summarized in Table 4. There are two main reasons for this i.e. i) the difference in the energy yield in the two scenarios is not large (7%), ii) the fermenters constitute a minor part of the total capital cost and a reduction of the size of fermenters has a small impact on the capital costs.

The current process is not cost-effective due a number of reasons. At the small-scale investigated herein (2 ton/h) the capital costs of the steam-explosion pretreatment unit and the gas upgrading equipment are high. The cost of the gas upgrading equipment is largely dependent on the safety margins, which are large (10–100%), due to the lack of experimental data on the absorption system employed. With more accurate data to determine the necessary parameter values the size of the safety margins can be greatly reduced, which could lead to substantial savings on the capital cost of the equipment (Svensson H, Hulteberg C, Karlsson H.T. Heat of solution of CO_2 in aqueous solutions of *N*–methyldiethanolamine and piperazine, manuscript in preparation). Other significant contributors to the overall process cost are the use of expensive nutrients such as yeast extract, which constitute the main part of the nutrient cost and the high demand of amine solution to make up losses in the gas upgrading.

Table 4 – Breakdown of the total production cost for the two scenarios (€/GJ biofuel). Operating cost includes water, electricity, straw for burning and chemicals (except nutrient addition) and other costs includes insurance, maintenance and human resources.

	H_2 productivity	Energy yield
Raw material	12	11
Capital	22	25
Nutrient addition	88	85
Operating costs (other than nutrient addition)	36	37
Other costs	1.7	1.7
Total production cost	160	157
Total production cost excluding nutrient addition	72	75

Future studies should thus focus on avoiding the addition of yeast extract. A recent publication has shown that yeast extract can be omitted from the DF medium, since *C. saccharolyticus* can produce all its amino acids in the presence of essential vitamins [56]. The yeast extract demand of the AD step has not been evaluated, but the *C. saccharolyticus* cells could possibly be hydrolysed and used as a nutrient source. These improvements would have a considerable impact on the cost of the process. If the nutrient cost could e.g. be reduced by 80%, the total cost of the process would be reduced by 44%. Another strategy to reduce the operating costs is to avoid sparging the DF reactor, which contributes to the energy demand. *C. saccharolyticus* can grow and produce H_2 at partial pressures up to 0.67 bar [16], albeit at the expense of H_2 productivity and yield. One way of improving H_2 productivities in the absence of sparging, could be to use a UASB reactor for biohydrogen production [57], using *Caldicellulosiruptor* species [3,58]. In addition, the cost of the gas upgrading unit could be reduced by avoiding amine losses (assumed 1% loss in the flow), since the amine solution accounts for almost 60% of the total operating costs. Industrial experience has shown that the need for amine make up is virtually zero in contrast to 1% assumed in the presented calculations (personal communications), indicating a potential to reduce the overall operating cost of the process.

4. Conclusions

The novel biohythane process concept, combining DF using *Caldicellulosiruptor saccharolyticus* with AD, has the significant benefit of being able to produce a high-value gas at high productivities (6.1 $L/L_{DF+AD}/d$ pure biohythane, i.e. $H_2:CH_4:CO_2$, 46:53.6:0.4) and reasonable energy efficiencies (66–70% of the energy in the sugar fraction of wheat straw).

However, the analysis of the process identified several shortcomings that require improvements for the process to be energy and cost efficient. Sparging the DF reactor was shown to be highly energy demanding. In addition, if the hydrogen is to be used as a chemical it must be separated from the methane, or the methane must be reformed into H_2, which would increase the production cost. To avoid these costs, future research should focus on improving the H_2 productivity in the absence of sparging. Nutrient addition in the DF and AD processes and amine losses during gas upgrading should also be reduced to improve the economics of the process. Moreover, by increasing the size of the process, the impact of the capital costs could be reduced. These are findings with a great potential for improvements that makes this process interesting for future analysis.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ijhydene.2012.08.092.

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