

Biogas Research Center (BRC) Report
2014:2

IMPROVEMENT OF THE BIOGAS PRODUCTION PROCESS

Explorative project (EP1)

Anna Karlsson, Annika Björn, Sepehr Shakeri Yekta, Bo H. Svensson

Biogas Research Center

www.liu.se/brc

Linköping University
SE-581 83 Linköping
Sweden

Biogas Research Center

BRC is a center of excellence in biogas research funded by the Swedish Energy Agency, Linköping University and a number of external organizations with one-third each. BRC has a very broad interdisciplinary approach, bringing together biogas-related skills from several areas to create interaction on many levels

- between industry, academia and society
- between different perspectives
- between different disciplines and areas of expertise.

BRC's vision is:

Resource-efficient biogas solutions are implemented in many new applications and contribute to a more sustainable energy supply, improved environmental conditions, and good business opportunities.

BRC contributes to the vision by advancing knowledge and technical development, as well as by facilitating development, innovation and business. Resource efficiency is central, improving existing processes and systems as well as establishing biogas solutions in new sectors and enabling use of new substrates.

For BRC phase 1, the first two year period from 2012-2014, the research projects were organized in accordance with

Table 1, showing important challenges for biogas producers and other stakeholders, and how these challenges were tackled in eight research projects. Five of the projects had an exploratory nature, meaning that they were broader, more future oriented and, for example, evaluated several different technology paths (EP1-EP5). Three projects focused more on technology and process development (DP6-DP8)

Table 1. Challenges, exploratory (EP) and technology and process development (DP) projects for BRC phase 1, 2012-2014.

Challenges	More gas from existing systems	New feedstock	New sectors	Co-operation for improved performance	Relevant societal conditions
Explorative projects	EP1 - Improvement of the biogas production process	EP2 - Systematic assessment of feedstock for an expanded biogas production	EP3 - Biogas in new industries	EP4 - Collaboration for improved economic and environmental performance	EP5 - Municipalities as system builders in energy systems
Technology and process development projects	DP6 - Increased methane production and process stability in biogas reactors	DP7 - Enzymatic increase of sludge digestibility		DP8 - Systems and technology for effective use of biofertilizers	

This is a report from the EP1 project – Improvement of the biogas production process. Please, observe that there are reports and/or scientific papers for the other projects as well.

During phase 1 of BRC, the following organizations participated in the center: Linköping University, Biototal AB, InZymes Biotech AB, Kemira OYJ, Lantbrukarnas Riksförbund,

Lantmännen, Linköpings kommun, Nordvästra Skånes Renhållnings AB, Scandinavian Biogas Fuels AB, Svensk Biogas i Linköping AB, and Tekniska Verken i Linköping AB.

- The work presented in this report has been financed by the Swedish Energy Agency and the participating organizations.



Summary

There are several ways to improve biogas production in anaerobic digestion processes and a number of strategies may be chosen. Increased organic loading in existing plants will in most cases demand the introduction of new substrate types. However, to substantially increase the Swedish biogas production new, large-scale biogas plants digesting new substrate types need to be established.

Better utilization of existing digester volumes can be linked to:

- Increase of organic loading rates and/or reduced hydraulic retention time
 - Optimizing the anaerobic microbial degradation by identifying rate-limitations, its causes and possible remedies such as:
 - Nutrient and trace element balances
 - Needs and availability of trace element
 - Process design aiming at an increase of the active biomass (e.g. recirculation of reactor material, two stage processes)
 - Process inhibition (enzymatically regulated product inhibition and toxicity)
 - Improved pre-treatment to increase degradation rates and VS-reduction
 - Mixing and rheology
 - Better monitoring and control
 - Co-digestion with more high-potential substrates

The present report reviews a number of fields that are linked to improvements in the biogas production process as based on the bullets above.

A well-working, active biomass is a prerequisite for efficient biogas production processes, why factors affecting microbial growth are crucial to obtain stable processes at the highest possible organic load/lowest possible hydraulic retention time.

The microorganisms need nutrients, i.e. carbon, nitrogen, phosphorus, calcium, potassium, magnesium and iron as well as trace elements such as cobalt, nickel, manganese, molybdenum, selenium and tungsten for growth. The need of nutrients and trace elements varies with the substrate digested, the organic loading rate, the process design (e.g. the reactor configuration, the degree of recirculation etc). In addition, the complexity of the chemical reactions controlling the bioavailability of the trace metals is wide, why optimal addition strategies for trace elements needs to be developed.

Substrates as food wastes, sewage sludge, cattle manure, certain energy crops and algae are good bases to obtain processes with good nutrient- and trace element balances. These kinds of substrates can often be implemented for “mono-substrate” digestion, while substrates dominated by carbohydrates or fats needs to be co-digested or digested in processes modified by e.g. nutrient- and trace element additions, sludge recirculation, etc. Protein-rich substrates often include enough nutrients, but can give other process problems (see below).

Iron, cobalt and nickel are the nutrients/trace elements given most attention so far. However, molybdenum, selenium and tungsten have also, among others, been shown

effective in different AD applications. The effects have, however, mainly been shown on turnover of VFAs and hydrogen (resulting in increased methane formation), while just a few studies have addressed their direct effect on rates of hydrolysis, protein-, fat- and carbohydrate degradation. Selenium- and cobalt-containing enzymes are known to be involved in amino acid degradation, while selenium and tungsten are needed in fat- and long chain fatty acid degradation. Enzymes active in hydrolysis of cellulose have been shown to be positively affected by cobalt, copper, manganese, magnesium and calcium. This implies that trace element levels and availability will directly affect the hydrolysis rates as well as rates and degradation pathways for digestion of amino acids, long chain fatty acids and carbohydrates. However, their effect on hydrolysis seems neglected, why studies are needed to map the metals present in active sites and co-factors of enzymes mediating these primary reactions in AD. Further investigations are then needed to elucidate the importance of the identified metals on the different degradation steps of AD aiming at increased degradation rates of polymeric and complex substrates. It should also be noted that the degradation routes for amino acid degradation in AD-processes, factors governing their metabolic pathways, and how ATP is gained in the different pathways seem unknown. The different routes may result in different degradation efficiencies, why a deeper knowledge within this field is called for.

Trace metals added to biogas reactors have positive effects on the process only if they are present in chemical species suitable for microbial uptake. Interaction of biogenic sulfide with trace metals has been identified as the main regulator of trace metal speciation during AD. Fe, Co and Ni instantaneously form strong sulfide precipitates in biogas reactors but at the same time show very different chemical speciation features. The soluble fraction of Co widely exceeded the levels theoretically possible in equilibrium with inorganic sulfide. The high level of soluble Co is likely due to association with dissolved organic compounds of microbial origin. Fe and Ni speciation demonstrated a different pattern dominated by low solubility products of inorganic metal sulfide minerals, where their solubility was controlled mainly by the interactions with different dissolved sulfide and organic ligands. To our knowledge, the information about chemical speciation of other trace metals (Se, Mo, and W among others) and its effects on the bioavailability in anaerobic digestion environments is rare. Providing information on the metal requirements by processes linked to their bioavailability in biogas reactors is identified as a key knowledge needed for maximizing the effect of metals added to biogas reactors. Further research is also needed for development and design of proper metal additive solutions for application in full scale biogas plants. A practical approach is to supplement trace metals in specific chemical forms, which are either suitable for direct bio-uptake or will hamper undesirable and bio-uptake-limiting reactions (e.g. mineral precipitation).

Recirculation of reactor material as a way to enrich and maintain an active microbial biomass (and, thus, an increase in the substrate turnover rate) in tank reactors has been tested for digestion of fat within BRCs project DP6. The methane yield increased from 70 to 90% of the theoretical potential at a fat-loading rate of 1.5 g VS/L and day. The same strategy has been successful during digestion of fiber sludge from the pulp and paper industry, i.e. the recirculation has been crucial in establishment of low hydraulic retention times. Also degradation of sewage sludge (SS) would likely be improved by recirculation as the retention

time of the solid SS is prolonged in such a system. However, this remains to be tested. The recirculation concept also needs to be evaluated in larger scale reactors to form a base to include extra costs and energy consumption vs. the benefits from increased yields.

To divide the anaerobic digestion process into two phases, where the hydrolytic/acidogenic and the syntrophic/methanogenic stages of anaerobic digestion are separated, might be a way to enhance degradation of lignocellulosic materials as the hydrolysis of these compounds may be inhibited by the release of soluble sugars. It should be noted that the natural AD of ruminates is phase-separated and improvements in AD can likely be achieved using these natural systems as a starting point. Also the degradation of aromatic and chlorinated species is likely enhanced by phase separation. One way to obtain such systems is to combine a leached bed for hydrolysis of insoluble material with a methanogenic reactor treating the leachate. Plug flow reactors might be another possibility as well as membrane reactors, which physically separates the hydrolyzing and methanogenic phases.

Inhibition caused by toxic levels of ammonia (protein- and ammonia rich substrates), fat-rich substrates and long chain fatty acids (LCFAs), aromatic compounds, salts etc. have been reported in many cases and some remedies are suggested. Ammonia can be stripped off as a measure to overcome too high levels. Another option is to adjust pH of the reactor liquid by addition of acid shifting the ammonia-ammonium balance in the system towards less free ammonia. A decrease in alkalinity by acid addition might also affect the availability of trace elements as solubility of trace metal mineral phases is generally higher at lower pH. LCFA degradation has been shown to benefit from periodic additions of fat and is, thus, an effective strategy to minimize inhibition by the release of the LCFA. Adsorption to zeolites has also been shown to abate the inhibition by LCFA. The best way to avoid inhibition is, however, to keep the processes nutritionally well balanced and using concepts suitable for the actual substrate mix digested (i.e. sludge recirculation, phase separation etc.) in order to obtain the highest possible degradation rate for problematic compounds, thus, avoiding accumulation of inhibitory components such as LCFA and aromatics. High ammonia and salt levels can often be regulated by the substrate mix.

The hydrolysis is often reported as rate limiting in digestion of complex polymers in balanced anaerobic digestion systems, while the methanogenesis is regarded as rate-limiting for more easily degraded substrates. As mentioned above the effect on methane formation rates by the addition of trace elements have been shown in numerous studies, while their effect on the hydrolysis and acidogenic AD steps are much less studied. Thus, the effects of the trace elements on the early steps in the AD-chain need to be investigated further.

To obtain high-rate hydrolysis, effective and energy efficient pre-treatment methods are crucial for a large number of substrates. The rate of hydrolysis is to a large extent dependent on the properties of the organic compounds in the substrate e.g. carbohydrates, proteins, fat or lignocellulosic material as well as particle size and pre-treatment methods applied. The establishment and colonization by sessile microorganisms and biofilms is highly important for efficient and high rate hydrolysis. Microbial formation of organic compounds and the availability of surfaces are factors influencing these key processes, which in turn are tightly

coupled to the growth conditions for the hydrolyzing microorganisms. This is an area recently brought up as an issue for detailed research.

Mixing is mostly needed for effective high-rate biogas production, but too extensive mixing can destroy the syntrophic interactions necessarily taking place during AD. However, the efficiency of the mixing system design in relation to colonization, presences of dead zones, changes in viscosity/rheology, etc. seem unclear and this area thus calls for further attention.

In high-loaded efficient processes a monitoring program following parameters e.g. organic loading rate, gas-production, VS-reduction, pH and VFA-levels is needed. This can be achieved through sampling and analysis off line, but there are of course benefits with on-line monitoring. A number of different methods have been suggested and tested, and some titration- and spectroscopic methods are applied, but none seems commonly in use. The reasons for the low interest to apply these methods may be the need for expertise on calibration, validation and multivariate analysis of most on-line methods, high maintenance demands (cost and time), and functional problems related to fouling, gas bubbles, sensor location, disturbing particles etc.

New substrates with the highest potential for use in existing or new biogas plants seem to be forestry-based biomass, certain energy crops and macro-algae. Both the energy crops and the macro-algae can be chosen to give nutritionally well balanced AD-processes, while AD on forestry biomass demands nutrient supplements. For both the energy crops and the macro-algae sustainable cultivation systems need to be developed. Crop rotation systems should be employed to minimize tillage as well as fertilization- and pesticide utilization at highest possible TS-yields. System analyses aiming at sustainability and economy of TS and methane yields per ha including needs of nutrient supplements should therefore be performed.

In all three cases (forestry biomass, energy crops and algae) pre-treatment methods to create high internal surface areas are needed. However, the pre-treatment methods chosen need to be highly energy- and resource efficient to obtain sustainable systems (a positive energy balance). New plants will for profitability likely need to be large with highly developed infrastructure for substrates supply and distribution of the produced biogas/electricity nearby. Process concepts aiming at highest possible loading rates at shortest possible retention time will be needed, which likely are met by including both phase-separated process systems and systems for sludge recirculation.

It should also be noted that the lignin in substrates from forestry biomass needs to be used for production of e.g. polymeric materials or as a fuel to obtain reasonable energy balances for AD of lignocellulose. Pre-treatment methods obtaining separation of lignin is therefore needed. A substantial research and development is in progress within this field.

The possibilities for AD within the pulp and paper industry are interesting, especially if specific effluents within the pulp- and paper production units are selected and the raw material for the pulp and paper production is chosen considering the biogas yields of the residues.

Preface

The aim of work behind the present report was to examine possible improvements of existing biogas production units in Sweden in order to support the further development of process-related issues within the Biogas Research Center (BRC) hosted by Linköping University. This activity formed one of the exploratory projects within the BRC and is the outcome from a strong interaction among the representatives of the BRC partner companies and scientists at Linköping University. The partner company representatives and the scientists formed a very active working group which strongly cooperated in a set of workshops to initialize the project and follow ups during the process of compilation of the report. Thus, the report very much rely on and is supported by the demands within the biogas-producing actors in Sweden and a review of the past and ongoing international research for identification of possibilities to improve the biogas production in current systems as well as when it comes to future options. Not the least has the knowledge from in house research and development among the biogas-producing companies together with their experience of practical applications of such results been instrumental. Thus, as the group of main responsible researchers working with the report at Linköping University, we express our great gratitude to all contributors.

The outcome has been used as a support to identify new possible core projects within BRC phase 2 in close cooperation between academy, the industry and other organizations involved.

The members of the working group included: Irene Bohn (NSR, Helsingborg), Jan Moestedt and Erik Nordell (Tekniska Verken i Linköping AB), Jörgen Ejlertsson (Scandinavian Biogas Fuels, Linköping), Mats Söderström and Jonas Ammenberg (IEI, LiU) and Martin Karlsson (IFM, LiU).

Linköping 14-11-02

Annika Björn, Anna Karlsson, Sepehr Shakeri Yekta and Bo H. Svensson

Table of contents

PREFACE	0
SUMMERY	3
1 INTRODUCTION	11
2 RESEARCH STRATEGY AND FOCUS	11
2.1 STEPS – ACTIVITIES - OUTCOME	11
2.2 PRIORITIZED RESEARCH AREAS	13
3 AD OF ORGANIC MATERIAL / SUBSTRATE UTILIZATION	14
3.1 NUTRIENTS AND TRACE ELEMENTS	15
3.2 THE SWEDISH BIOGAS POTENTIAL	15
3.3 THE ANAEROBIC DEGRADATION CHAIN	16
3.4 HYDROLYSIS	17
3.5 FAT- AND PROTEIN-RICH MATERIALS	17
3.5.1 PROTEIN DEGRADATION	18
3.5.2 LIPID DIGESTION	20
3.6 LIGNOCELLULOSIC MATERIAL	24
3.6.1 FACTORS AFFECTING THE DEGRADATION OF LIGNOCELLULOSE	26
3.6.2 ANAEROBIC DIGESTION OF ENERGY CROPS AND STRAW	30
3.6.3 ANAEROBIC DIGESTION OF WOOD	31
3.7 ANAEROBIC DIGESTION OF HOUSEHOLD/FOOD WASTE	35
3.8 ANAEROBIC DIGESTION OF SEWAGE SLUDGE	38
3.9 SUBSTRATES FROM THE SEA	38
3.9.1 ALGAE	39
3.9.2 COMMON REEDS	41
3.9.3 MUSSELS	42
3.9.4 FISH INDUSTRY WASTES	43
4 PRE-TREATMENT	44
4.1 EFFECTIVENESS AND ASSESSMENT OF PRE-TREATMENTS	44
4.2 PRE-TREATMENT METHODS	45
4.2.1 PHYSICAL PRETREATMENTS	45
4.2.2 PHYSIOCHEMICAL PRE-TREATMENTS	46
4.2.3 CHEMICAL PRE-TREATMENTS	47

4.2.4	BIOLOGICAL PRE-TREATMENTS	48
5	MIXING AND RHEOLOGY	49
5.1	MIXING	49
5.1.1	PROCESS TECHNOLOGY AND MIXING TECHNIQUES	50
5.1.2	EFFECTS OF MIXING ON THE BIOGAS PROCESS	51
5.1.3	FOAMING	51
5.1.4	HYDRODYNAMICS	52
5.2	RHEOLOGY	53
5.2.1	RHEOLOGICAL CHARACTERIZATION OF SUBSTRATE AND REACTOR SLUDGE	53
5.2.2	RHEOLOGY AND MIXING PERFORMANCE	54
		55
6	THE BIOCHEMICAL DYNAMICS OF TRACE ELEMENTS DURING ANAEROBIC DIGESTION PROCESSES	56
6.1	METALS AND ORGANIC MATTER INTERACTIONS	58
6.2.	THEORETICAL DETERMINATION OF TRACE METAL SPECIATION AND BIOAVAILABILITY	59
6.3.	BIOCHEMICAL ENGINEERING OF TRACE METAL BIOAVAILABILITY	59
7	MONITORING	61
7.1	TITRATION	62
7.2	GAS CHROMATOGRAPHY	62
7.3	SENSORS	63
7.3.1	FLUORESCENCE SPECTROSCOPY	63
7.3.2	INFRARED SPECTROSCOPY	63
7.3.3	NEAR INFRARED SPECTROSCOPY	63
7.3.4	CHEMICAL MULTISENSORY SYSTEMS (ELECTRICAL TONGUES AND NOSES)	64
7.3.5	ACUSTICCHEMOMETRICS	64
7.3.6	EVALUATION OF COMMERCIAL SYSTEMS	64
APPENDIX 1		67
ÖVERSIKT FÖR TILLSTÅNDSPROCESSER FÖR BYGGNATION OCH DRIFT AV BIOGASANLÄGGNINGAR I SVERIGE		68
ÖVERSIKT ÖVER STYRMEDEL SOM RÖR BIOGASPRODUKTION		69
STYRMEDEL		69
STYRMEDEL PÅ NATIONELL NIVÅ		70
AKTÖRER		71
APPENDIX 2		72
EDUCATION, LITERATURE AND STANDARD METHODS		72

EDUCATION AND COURSES	72
LITERATURE	72
8 REFERENCES	73

1 Introduction

Anaerobic digestion (AD) of organic matter to biogas has developed from a method for treatment of waste mainly within the sewage area, to a process aiming at production of methane as an energy carrier. This is a trend, which has emerged in Sweden and internationally during the last 15 years leading to a refocus of the economics to now include commercial profit aspects. This has initiated efforts to maximize the methane production at currently operating and planned biogas plants and a considerable research and development to optimize AD has been done by academia and in the industrial sector, not the least among members of the Biogas Research Center (BRC).

Factors affecting the economy of a biogas plant are linked to substrate issues related to supply, price, needed transports, digestibility and pre-treatment needs. The substrate mix mainly governs the choice of process design, including possible organic loading rate (OLR) and hydraulic retention time (HRT) and downstream measures (sludge treatment, fertilization value etc.).

The aim of explorative project EP1 is to identify ways to enhance the biogas production in existing plants and to investigate possible future implementations for new biogas production units. The results will be used to support the identification of new possible core projects within BRC in close cooperation between academy, the industry and other organizations.

The approach to reach these goals has included possible bottlenecks and process problems related to degradation of fats, proteins and carbohydrates (mainly lignocellulose substrates), pre-treatment, nutrient deficiencies, rheology of reactor liquids (causes and effects), mixing, possible and less-investigated “new”/non-established substrates as well as established but “problematic” substrates (i.e. substrates with inherent risk for process disturbances or giving lower yields than expected etc.), monitoring and control.

Many of the factors above are to various extents affected by political directives and drivers, permissions and legislations. This is not dealt with in depth in this report. However, an overview is given in Appendix 1 (in Swedish).

2 Research Strategy and Focus

Steps – activities - outcome

The project was initiated with a workshop, in which EP1-project participants contributed with their experiences of challenges and improvements to reach higher methane yields in their biogas reactors. Prior the workshop, the participants were asked to deliver a short abstract and prepare for oral presentations and discussions. A gross list of specific problems, bottlenecks and improvement potentials related to nutritional status, trace element speciation, mixing, pre-treatments, post digestion, pollution, reactor design, process control, the use of process waste fractions and of new substrates was the main outcome at the

workshop. Examples from this list are sedimentation of “heavy grit” in buffer tanks and reactors, contamination by plastics, and fast shifts in viscosity. Among the improvements listed were: additions of nutrients/trace elements, allowing for increased organic loads of N-rich substrates at stable conditions, rebuilding of receiving tanks, feeding loop and mixing systems, post-digestion for increased biogas yields and reduced methane slip.

A specific seminar discussing a suitable method for categorization and prioritization of identified process problems, bottle-necks and optimization procedures was conducted. This resulted in a framework modified after Feizaghaei *et al.* (2014) for collection, classification, and assessment of potentials for improvement of the biogas process as presented in table 1.

Table 1. Overview of the framework for collection and assessment of potential improvement of biogas production processes.

Steps	Activities	Outcome/Results
1: Collection	Workshop with BRC EP1-project members Questionnaire to biogas producers Literature review Conferences, Seminars, Workshops	Gross list Data from previous reactor screenings State-of-the-art Synthesis
2: Classification	Categorization scheme	Experience of process improvements for increased methane yield Specific process problems Bottlenecks for process optimization New ideas for improvements
3: Improvement assessment	Improvement potentials	Substrate availability/New substrates; Nutrient status/Trace element speciation/Nutrient dosage; Microflora dynamics & pathways; Pretreatment; Reactor design; Mixing/Rheology; Process control; Pollutants; Post-treatment; Laws & Regulations
4: Results and analysis	Workshop with biogas plants construction group First draft of EP1-report Workshop with BRC-members Second draft of EP1-report Conclusive discussion	Compilation and concluding remarks Core-projects Feedback Final report from EP1 Project charters for BRC phase 2

Apart from the knowledge within the EP1 participant group, we have drawn on experiences and, to some extent, results from an earlier extensive survey performed at WES. 15 full-scale biogas plants were sampled at 2-4 occasions over a period of three years and analyses of microbial composition and activity, nutrient status, trace metals speciation and rheology have been made. The initial results from this survey show a large difference in process performance, microbial composition, organic matter characteristics, as well as chemistry and speciation of trace metals when comparing plants treating sewage sludge and co-digestion plants respectively (Shakeri Yekta *et al.* 2012a, Sundberg *et al.* 2013, Shakeri Yekta *et al.* 2014a; Björn *et al.*, 2012b). A questionnaire focusing on process changes, improvements and challenges was prepared and distributed to the operators of the 15 plants as a follow up of the earlier investigations. We have received replies from 10 of the 15 plants. The survey

response indicated mainly process disturbances linked to the digestion of household waste and mixing which are discussed further in Chapter 3.7 and Chapter 0 respectively.

Additional seminars, national workshops and attendance at the World Congress of Anaerobic Digestion (AD13) and Green Gas Research Outlook (GGROS) also provided input and state-of-the art knowledge for the project. A second workshop with the BRC project participants was conducted to discuss the first draft of the present report focusing on conclusions and potential core-projects for the BRC phase two. A synthesis of the discussions from the workshop identified additional challenges to be solved in order to explore the Swedish biogas production potential e.g. needs for prioritizing of optimization measures, for investigations of enzymes and trace elements important for the optimization of the hydrolysis step, use of energy crops, plastic sorting techniques, and separation of heavy metals.

Prioritized research areas

The collection, classification and assessment resulted in a number of main priorities:

- New/non-established substrates such as lignocellulose materials and biomass from the sea
- Pre-treatment and digestion methods linked to the substrate characteristics
- Needs of nutrients (deficiency vs. inhibition) and availability of trace metals
- Rheology of reactor liquids and mixing systems linked to the biogas production process
- Monitoring methods to control high-loaded biogas processes

These areas will be addressed in the following sections linked to process problems, bottlenecks and optimization opportunities. Figure 1 presents an overview of topics identified as important.

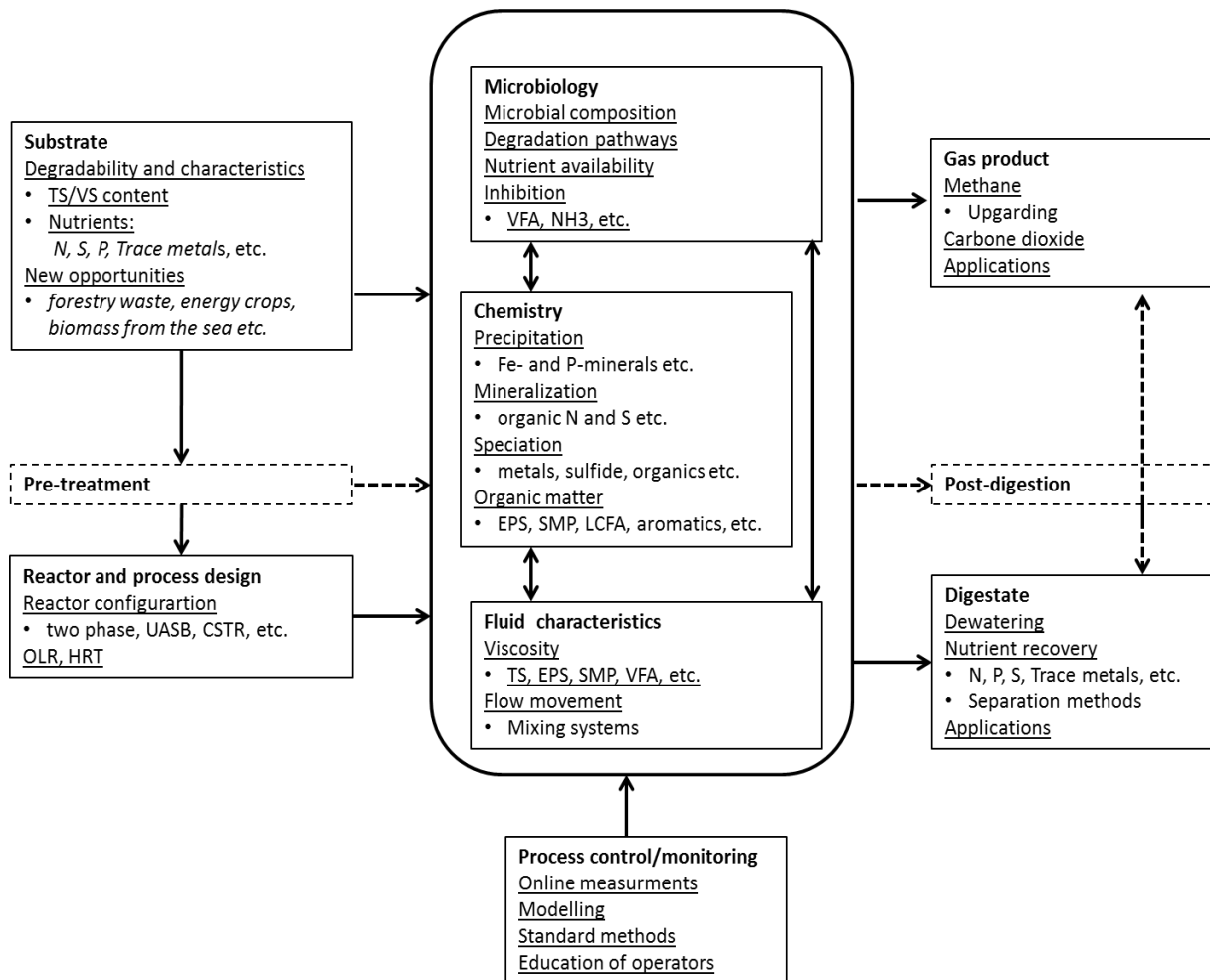


Figure 1. Overview of the identified important areas for future research. Economy, energy balances and legislations/regulation will affect the system on all levels. Abbreviations: CSTR - completely stirred tank reactor; EPS - extracellular polymeric substances; HRT - hydraulic retention time; LCFA -long chain fatty acids; OLR - organic loading rate; SMP - soluble microbial products; VFA - volatile fatty acids; UASB - upflow anaerobic sludge blanket.

3 AD of organic material / Substrate utilization

During an efficient biogas production, a maximized reduction of volatile solids (VS) and methane production should ideally be obtained at the highest possible organic load. This means an optimization of the utilization of both the organic material and the reactor volume available. To achieve these goals key process parameters such as pH and concentrations of fermentation products (mainly addressed by following the volatile fatty acids VFAs) need to be maintained within appropriate ranges. The pH of the biogas process is mainly governed by carbonate/bicarbonate buffering, which, in turn, to a great extent is affected by the release, formation and consumption of ammonia and VFAs, and the release of sulfide formed from sulfate and sulfite (Anderson and Yang 1992). Thus, the nitrogen and sulfur contents of the substrate can be important factors to obtain the buffering capacity required to maintain a stable pH at varying substrate composition and VFA concentrations but at the

same time avoiding process disturbances due to interactions of high concentrations of ammonium and sulfide.

Nutrients and trace elements

The anaerobic digestion process is dependent on the growth of microorganisms. Thus, there is a necessity to supply nutrients in sufficient amounts and at right proportions to sustain an optimal growth of the bacteria and archaea to obtain an efficient biogas production from a given substrate. The carbohydrates and lipids of an organic substrate mostly provides carbon, oxygen and hydrogen, while nitrogen and sulfur are supplied via proteins and phosphorus from e.g. nucleic acids phospholipids. Together with these elements, most organic substrates provide potassium, sodium, magnesium, calcium and iron and to some extent trace metals (micro-nutrients). The trace elements are often found in the active sites of enzymes essential for AD. The proportions and availability of the nutrients in a given substrate will to a certain extent be reflected in the microbial community and also determine the growth rate. In other words, the nutrient balance will govern the degree of degradation of the substrate and therefore the biogas production efficiency at a given hydraulic retention time and organic loading rate. This means that a limitation of any of the nutrients will lead to a limitation of the biogas production efficiency. Several of the enzyme systems needed for the anaerobic utilization of organic matter for biogas production have demands for specific trace elements, e.g. the methanogens need Ni and Co to a greater extent than bacteria while e.g. W and Se are required by the latter e.g. during fat and long fatty acid degradation (c.f. Gustavsson et al. 2013).

From the above it is clear that optimization of biogas production from any substrate should be based on an analysis of the nutrient composition to investigate the need for complements. Such needs may be taken care of by co-digestion of a combination of substrates leading to the right proportions and amounts of nutrients. As is discussed in detail below special concern should also be given to the presence and bioavailability of the trace metals.

The Swedish biogas potential

Linné et al. in 2008 wrote a report on the Swedish biogas potential from domestic residues, which was used for a categorization by Dahlgren et al. (2013). The main substrate categories (the theoretical methane potential in GWh/year is given in parenthesis) were: 1) food waste from households, restaurants, grocery stores etc. (1300 GWh), 2) industrial residues, including meat and dairy industry, breweries, bakeries etc. (2000 GWh) 3) sewage sludge (700 GWh), 4) agricultural residues except straw (800 GWh), 5) straw (1300 GWh), 6) manure (4200 GWh), 7) energy crops (7200 GWh, assuming cultivation on 10% of the Swedish arable land). The list should be complemented with the annual production of forest biomass residues ("GROT") amounting to 60 TWh. It should also be noted that the annual growth of the Swedish forest corresponds to 400 TWh, hence giving a substantially larger bio-energy and lignin potential than what is listed above.

Below we have chosen to give an overview of established but problematic substrates as well as of new/unestablished substrates. Protein- and fat-rich substrates, household waste, lignocellulosic substrate (energy crops and wood) and substrates from the sea are discussed. A rough estimation shows that household/restaurant waste, sewage sludge and cattle manure are substrates characterized by good nutrient balances while imbalanced processes are likely obtained if industrial residues (often with high contents of proteins and/or fats) or agricultural residues/energy crops (often mainly carbohydrates/lignocellulose) as well as swine and poultry manure (high ammonia content) are digested as single substrates. Most of the above substrates are, however, well established and knowledge on how to obtain good nutrient balances in high loaded, efficient processes exist, but are not always applied on site. Among new and/or non-established substrates with high availability/potential energy crops, forest residues and algae still needs to be investigated from a nutritional aspect to be fully explored.

Related to the Swedish biogas potential, discussions with actors within the biogas business gives a picture of a lot of high cost projects within the municipalities, where money are invested in unsuitable equipment and processes. This problem needs to be abated to obtain a resource effective and profitable biogas production. The uncertainty within the legislation linked to CO₂-taxes on biogas and other drivers within the area is off course also negative for the development for increase biogas production in Sweden.

The anaerobic degradation chain

The anaerobic degradation chain can be divided into a number of stages: 1) the hydrolysis of complex polymers, 2) fermentation of amino acids and sugars, 3) oxidation of long chain fatty acids and alcohols, 4) oxidation of intermediary fermentation compounds (mainly VFAs), 5) homoacetogenesis and 6) methanogenesis (Pavlostathis and Giraldo-Gomez et al. 1991).

The first step, hydrolysis, is often reported as rate limiting in digestion of complex polymers for balanced anaerobic digestion systems, while the methanogenesis is regarded as rate-limiting for more easily degraded substrates (Vavilin et al. 2010). It should however be noted that there are large variations in reported values for the rates within each of the above steps. These variations depend on differences in microflora composition and microbial activity of the different steps (reviewed by Ma et al. 2013) caused by type of substrate, particle size, available surface area, type of digestion system applied etc. However, these differences are likely to some extent also related to the lack of standard procedures for determination and expression of biokinetic coefficients (Pavlostathis and Giraldo-Gomez et al. 1991 and Donoso-Bravo and Mairet 2012). To improve an AD-process, the rate-limitation, its causes and possible remedies should ideally be identified. Rate-limitations of the AD are, apart from slow hydrolysis, often found to be due to nutrient deficiencies, lack of syntrophic interaction (due to e.g. too strong shear stress) and inhibitory substances (metals, ammonia, fermentation products and other organic compounds). These items are discussed in the sections below.

Hydrolysis

The hydrolysis of particulate organic matter in AD to a large extent seems to be dependent on the properties of its organic components (e.g. carbohydrates, proteins, fat or lignocellulosic material). Furthermore, the presence of external microbial products as a part of the organic material (sessile microorganisms and biofilms) is of importance, since the hydrolysis is mainly performed on available surfaces of the macromolecules (Jensen et al. 2009 and reviewed by Song et al. 2005). This is highlighted by Vavilin et al. (1996), who showed that modeling of the hydrolysis needs to be divided into: 1) the colonization of the biomass and 2) the enzymatic hydrolysis of colonized surfaces in order to fit experimental data.

Fat- and protein-rich materials

Substrates with high protein and fat contents often originate from the food industry (slaughter houses and food-processing industries). These residues are desirable substrates for biogas production as they have high methane potentials (up to 500-600 dm³/kg VS; Salminen and Rintala 2002; Hejnfelt and Angelidaki 2009). However, they are often associated with process disturbances. The amounts of ammonia and sulfide in a reactor liquid depend on the substrate composition, e.g. processes in which protein-rich material or manure from swine and poultry are used tend to yield high ammonia levels (Pechan et al. 1987; Poggi-Varaldo et al. 1997; Hansen et al. 1998). In addition to raising the pH, and affecting the buffering capacity (NH₂-groups are set free as NH₄⁺/NH₃), high NH₄⁺/NH₃ levels have been shown to have a negative impact on AD with NH₃ likely giving the toxic effects (Koster and Lettinga 1984; Gallert et al. 1998; Hansen et al. 1998). The amount of NH₃ present is dependent on the total N-NH₄⁺ levels in combination with pH and temperature, i.e. the higher the pH and/or temperature, the more NH₃. Acclimatization to high ammonia levels is possible and has been shown (van Velsen 1979). This includes a change in the pathway for acetate conversion to methane by applying mainly syntrophic acetate oxidation to CO₂ and H₂ coupled to hydrogenotrophic methane formation (Schnürer et al. 1994, Schnürer and Nordberg 2012).

High-strength lipid wastes such as fat, oil and grease (FOG or grease trap wastes, GWT), may be problematic during AD, since these substrates can give rise to a row of operational challenges: inhibition of the methanogenic archaea, substrate- and product transport limitations, sludge floatation, foaming, blockage of pipes and pumps and clogging of gas collectors and gas transport systems (see review by Long et al. 2012). Sludge floatation seems mainly to be a problem when applying upflow anaerobic sludge (UASB) techniques, where the floatation lead to losses of the active bed. For completely stirred tank reactors (CSTRs) foaming is often reported to cause problems. LCFA produced in FOG hydrolysis, are claimed to limit the anaerobic digestion process by inhibiting the activity of syntrophic acetogens and methanogenic archaea (Hwu et al. 1998, Pereira et al. 2005, Palatsi et al. 2010).

3.1.1 Protein degradation

Proteins are polymers of amino acids linked together by peptide bonds and their hydrolysis is mediated by extracellular proteases giving rise to polypeptides and free amino acids. Structural proteins present in bacterial cell walls, hair, nails, and feathers etc. are harder to hydrolyze and therefore needs pre-treatment before digestion (cf. Salminen et al. 2003). Most protein degraders in anaerobic digesters seem to be gram-positive Clostridia which are active both in the hydrolysis and in the subsequent amino acid degradation (reviewed by Ramsay and Pullammanappallil 2001)

Examples of protein rich materials are slaughter house- and some other food industry waste. However, in Sweden most/all of these are already used for biogas production. New sources of protein rich materials so far not explored may be found in algae, fishery/fish industry wastes and certain energy crops.

Aldin et al. (2011) showed that reduced particle size increased the rate of protein degradation. An increase of the specific surface area from 0.01 to 0.192 m²/g of casein increased the rate of methane production from 6 to 14 mL per g COD per day due to increased hydrolysis rates. Tommaso et al. (2003) performed a test with a lab-scale horizontal-flow anaerobic immobilized biomass reactor (HAIB), showing that the presence of carbohydrates or carbohydrates and lipids decreased the rate of protein hydrolysis of bovine serum albumin (BSA). No explanation to this observation was given, but it might be linked to thermodynamics as starch is a more favorable substrate. A more stable AD process was obtained with the mixed substrate (starch, glucose, BSA and lipids) then with BSA only (Tommaso et al. 2003). Elbeshbishy and Nakhla (2012) showed in batch tests that a ratio of 20:80 of BSA and starch gave the highest methane yield and the highest methane production rate. Substrate mixes of 100:0, 80:20, 50:50, 20:80 and 0:100% BSA:starch at an organic load of 5 g COD/L were investigated. The C/N ratio was about 13 in the 20:80 case and VFA analysis showed that BSA gave propionate as main VFA, while degradation of starch generated mainly butyrate. Again, no explanation to the results were given, but might, as above, be linked to thermodynamics.

The amino acids produced from the hydrolysis are a structurally diverse group and their degradation is therefore performed by a number of different routes/enzymes. They can be categorized as 1) non-polar: glycine, alanine, valine, leucine, isoleucine, proline and methionine, 2) polar uncharged: serine, threonine, cysteine, asparagine, glutamine, 3) polar charged: aspartate, glutamate, lysine, histidine and arginine and 4) aromatic: phenyl alanine, tyrosine and tryptophane. The degradation of amino acid can be performed in Stickland reactions, where a pair of amino acids are degraded, one of them acting as an electron acceptor and the other as an electron donor and or in single amino acids degradation. The latter is performed in syntrophy with hydrogen-utilizing organisms. The Stickland degradation normally provides the degrading organism with 0.5 ATP per transformed amino acid and are faster than the single amino acid degradation. Ramsay and Pullammanappallil (2001) showed about 40% of the amino acid degradation in a laboratory scale CSTR treating artificial wastewater was degraded through Stickland degradation. That not more of the degradation is routed through Stickland seems surprising and more efficient

amino acid degradation might be obtained if this was the case. However, digestion of peptone as the only source of energy and carbon (Örlygsson et al. 1994, 1995) were not able to observe any Stickland reactions, while low hydrogen partial pressures was needed to maintain balanced degradation of the amino acids. In additions have the amino acids been shown to be more or less easy degradable. Park et al. (2014) showed that glycine, lysine, α -alanine, histidine and arginine were degraded within three days in batch incubations while degradation of β -alanine took five days and cysteine, leucine and methionine was only degraded to 50-60% within the incubation period (25 days). The degradation of each amino acid was studied separately and, thus, no Stickland reactions could take place. This fact was argued to be the main reason for the poor degradation rates for cysteine, leucine and methionine. Many details in the anaerobic degradation of individual amino acids and its links to ATP-formation seems however unclear. Many studies on amino acid degradation have been performed especially with members from the *Clostridium* genus and B12-dependent aminomutases and selenium-containing oxidoreductases active in amino acid degradation have been found (reviewed by Fonknechten et al. 2010). Fonknechten et al. (2010) sequenced the genome of *Clostridium sticklandii* and the results indicate earlier unknown possibilities for chemiosmotic ATP-generation linked to amino acid degradation. The authors also found a “new” selenoprotein active in the degradation of proline.

As mentioned above the degradation of proteins will release $\text{NH}_4^+/\text{NH}_3$ -into the AD-liquid in concentrations depending on the protein content of the substrate. Protons are consumed during the amino acid degradation resulting in an increase in alkalinity and pH of processes with protein-rich substrates. Reactors with high ammonia levels have been reported to run in an “inhibited steady state” (Hansen et al. 1998; Angelidaki et al. 1993), since such processes are characterized as stable, but with low methane yields combined with high VFA-levels in the effluents. Although stable, these processes are undesirable, as the methane potential of the substrate is partially wasted by the high levels of fermentation products e.g. organic acids in the effluent. This means suboptimal utilization of the substrate and, thus, a suboptimal economic production of biogas. Furthermore, the organic matter of the effluent is likely unstable and odorous giving rise to undesired down-stream problems.

There are, however, means to overcome process problems related to anaerobic degradation of substrates with high protein and/or fat content. Adaptation of the microflora has been shown successful to some extent. Cuetos *et al.* (2008) studied the digestion of solid slaughterhouse waste alone and together with municipal solid waste (MSW). A first set-up with OLRs of 1.7 and 3.7 kg VS $\text{m}^{-3} \text{ day}^{-1}$ for the single substrate and co-digestion processes, respectively, failed. In a second experiment, lower OLRs were applied and the HRT was increased from 25 to 50 days, which resulted in a stable process. The authors were then able to slowly increase the OLR and decrease the HRT to the original settings aimed at. Also Salminen and Rintala (2002), Edström et al. (2003) and Palatsi et al. (2011) have shown reversible inhibition effects during digestion of protein-rich slaughterhouse wastes.

Ammonia stripping by addition of CaO, NaOH, or KOH to the substrate can reduce ammonia levels in AD (Zhang and Jahng; 2010). However, the stripping only applies on substrates that

already contain ammonium/ammonia (i.e. manure) and, thus, likely have minor effects on protein-rich materials, where the nitrogen is bound in the organic material.

Another way to increase the tolerance to protein-rich wastes might be to adjust the pH of the reactor liquid by addition of acid, and, thus, shift the ammonia-ammonium balance of the system and reduce the levels of free ammonia. The process pH also affects the carbonate and sulfide systems in the reactor liquid, i.e. an increase in pH also increases the levels of CO_3^{2-} and S^{2-} in the reactor liquid, and these species might negatively affect the availability of trace metals that are essential for bacterial growth (Callander and Barford 1983b). Hence, in processes, where substrates generating high N-NH_4^+ levels and a pH at about 8, addition of acid may improve the process performance in several ways (Karlsson and Ejletsson 2012).

Protein rich material is also known to cause foaming, see further information in chapter 5.1.3.

Conclusions on *Protein degradation*

- Reports on problems with hydrolysis efficiency of protein rich materials seems scarce indicating that this is not a main problem in AD (structural proteins not considered).
- Protein degradation increases the NH_4^+ and NH_3 levels in the reactor liquid, which above certain levels can affect the AD negatively.
 - Co-digestion with other substrates or slow adjustment of the microflora to high ammonia levels in most cases solve the problem.
 - Ammonia stripping and pH-adjustments have also been suggested as remedies of ammonia inhibition.
- There seems to still be knowledge-gaps in how the amino acid degradation is performed in biogas producing systems.
- Se and Co seems to be important trace elements in degradation of amino acids as enzymes containing these metals have been shown active in their degradation

Priorities on *Protein degradation*

- Investigate anaerobic digestion of protein-rich algae.
- What are the degradation routes for amino acid degradation? How is energy/ATP generated?

3.1.2 Lipid digestion

Lipids, which mostly are referred to as fat, oils and/or greases (FOG) in the AD context, are mainly glycerol esters with three long chain fatty acids (LCFA) forming triglycerides. FOG are parts of many waste streams of domestic sewage and industrial effluents including food-processing, wool-scouring and food oil production (Alves et al. 2009, Long et al. 2012) Today

there are relatively small amounts of fats (used frying fat etc.) available for AD in Sweden. Most of the FOG is regenerated and used again. New sources for energy rich lipid substrates are as for proteins to be found in algae, certain energy crops (oilseeds like rapeseed) and possibly residues from the fish industry. The lipid contents in used substrates varies from $<0.1 \text{ g L}^{-1}$ for domestic wastewaters to $10\text{-}25 \text{ g L}^{-1}$ in wastewaters from wool and edible oil preparation, while dairy industry wastewaters are reported within a range of $1.5\text{-}4.7 \text{ g L}^{-1}$ (Alves et al. 2009). There are two recent important reviews available on this topic: Sousa et al. (2009), which mainly addresses the use of high-rate biogas reactors of UASB concept and Long et al. (2012) that mainly deals with the challenges of co-digesting lipids with sewage sludge. It should be noted, that AD of lipids is also common during digestion of e.g. slaughterhouse-, fish-rinsing- and household wastes, as such or together with specific lipid fractions and sometimes also in combination with manure (cf. Sundberg et al. 2013).

The co-digestion of FOG by supplementing sewage sludge biogas reactors has been investigated since long (Long et al. 2012). Especially CSTRs during laboratory conditions show a high potential by up to 200% more biogas produced from an ordinary sewage AD set up. In some cases more biogas even seems to be produced from the sewage sludge fraction. This leads to less sludge at a quality which is more easily dewatered than the control material. It should be noted that these reactors were run at long HRTs. The laboratory CSTRs mainly showed a linear increase in methane production of 400 ml methane per gram FOG VS at loadings of FOG up to approximately 40% of VS based on the sludge load (cf. Long et al. 2012).

In the case of granular sludge systems approached e.g. by the group of Alves above a introduction of sludge floatation as a part of the reactor design improved the conditions substantially and are now further investigated in a pilot scale set up (Alves et al. 2009). This secures a maximal contact between the active biomass and the LCFA. The reactor design is noted as an inverted anaerobic sludge bed (IASB).

The digestion of FOG involves fermentative bacteria hydrolyzing lipids into glycerol and LCFA. Over 90% of the methane potential from lipids is maintained in LCFA after hydrolysis (Sousa et al. 2009) and they are converted to acetate and H_2 by syntrophic acetogenic bacteria, and finally to methane by acetate-utilizing (aceticlastic) and hydrogen-utilizing (hydrogenotrophic) methanogenic archaea. The glycerol is fermented via propionate and further to hydrogen and acetate, which are converted to methane. The syntrophic acetogenic bacteria utilize β -oxidation to degrade even-numbered LCFA to acetate and hydrogen, while uneven-numbered LCFA at the end give rise to one mole of propionate per mole of LCFA. Favorable thermodynamic energetics of LCFA degradation allowing for growth of the syntrophs are accomplished by maintaining low concentrations of reducing equivalents, i.e. H_2 and formate and of acetate (Schink, 1997). However, in some cases, e.g. for oleic acid, it is saturated before being β -oxidized and bacteria able to degrade the saturated LCFA are mostly poor degraders of the corresponding unsaturated acids but not

vice versa (Sousa et al. 2009). However, in other cases other pathways seem to take place (Lalman and Bagley, 2000). This is likely coupled to the hydrogen partial pressure needed for the saturation metabolisms in relation to that needed for the β -oxidation.

FOG can, as said above, be a problematic substrate in AD. Both the FOG as such and the LCFA produced during hydrolysis might accumulate in the system. Periera et al (2004) observed that LCFA contents above a threshold level of 1 g COD_{LCFA}/g VSS resulted in significantly decreased specific methanogenic activities. The primary mechanism of LCFA inhibition is likely adsorption onto cell walls, hindering nutrient and metabolite transport (Periera et al (2004, 2005). This has been partly verified in studies on inhibition of methanogens by FOG and LCFA on granules, which when subjected to kinetic studies with acetate and hydrogen gas as the substrate demonstrated fast recovery (Sousa et al. 2009). The response by the hydrogenotrophs was higher than for the acetoclastics, which strengthens the idea that FOG and the LCFAs act as physical barriers rather than directly inhibiting the methanogenic production pathways. However, as reviewed by Long et al. (2012), there are several contradicting and inconclusive results concerning the specific inhibition of the methanogens, when AD is exposed to FOG or LCFA.

Several studies have reported an adaptation of the AD process to LCFA exposure (Palatsi et al. 2009; Cavaleiro et al. 2008; Nielsen et al. 2006; Barserba et al. 2012; Souza et al. 2007). The reason for the adaptation is currently not clear, but anaerobic communities acclimated to continuous LCFA digestion have been shown to be dominated by methanogenic archaeal communities from the genus *Methanosarcina* (Barserba et al. 2012; Souza et al. 2007; Palatsi et al. 2010), of which several species are capable of both acetoclastic and hydrogenotrophic methanogenesis. After acclimatization of a reactor to LCFA degradation, Salvador et al. (2013) observed a stable methanogenic community dominated by *Methanosaeta*- and *Methanobacterium*-like archaea. According to Souza et al. (2013) methanogens responds differently to the types of LCFA they are exposed to, i.e. whether they are saturated or not. Furthermore, LCFA degradation through β -oxidation is thought to occur through a highly specialized community of syntrophic bacteria belonging to the families *Syntrophomonadaceae* and *Syntrophaceae* within the phyla *Firmicutes* and *Deltaproteobacteria*, respectively (Sousa et al. 2009). Populations of *Syntrophomonadaceae* have been observed to become predominant in biogas systems exposed to LCFA (Sousa et al. 2007). However, limited changes in bacterial community structure during acclimation to LCFA have also been shown (Palatsi et al. 2010) but it should also be noted that changes in specialized syntrophic bacteria may be easily overlooked by the molecular analysis employed (Palatsi et al. 2010). In this context it should be mentioned that the bacteria performing β -oxidation likely are strongly depend on the nutrient conditions. This is furthered by the comprehensive work by Worm et al. (2009) showing the pathway dependence on whether selenium or tungsten was present. Furthermore, Goncalves et al. (2012) in their presentation of the ISAB concept concluded that a substantially increase in the methane production was obtained by nitrogen addition.

Operational strategies have subsequently been proposed to minimize inhibition by intermittent batch feeding LCFA to anaerobic co-digestion systems (Palatsi et al. 2009; Cavaleiro et al. 2008). For instance, periodic loading of LCFA was shown to be an effective strategy to minimize inhibition, resulting in shorter acclimation times with subsequent LCFA pulses (Palatsi et al. 2009). Repeated pulses of oleic acid to a manure biogas system were also shown to increase the tolerance of methanogens to LCFA (Nielsen et al. 2006). These studies, however, investigated intermittent feeding strategies with pure LCFA and not FOG or lipid waste streams, and were operated for relatively short time periods. One strategy suggested for lipid-containing slaughterhouse wastes was presented by Nordell et al. (2013), where Zeolites were shown to abate the problem of inhibiting LCFA by adsorption. However, additional studies are needed to investigate the mechanism of process acclimation through long-term lipid exposure in order to develop feeding strategies and process indicators to optimize stability and methane output from anaerobic digesters.

Research and development is under way to enable high-rate AD of lipid-containing wastewaters as well as including FOG in co-digestion with other substrates (cf. Alves et al. 2009 and Long et al. 2012) e.g. sewage sludge (DP6 results in progress), manure etc. Obstacles to overcome are the adsorption of the FOG, and the LCFA released by the hydrolysis of the FOG, to the active microbial biomass, which leads to problems with nutrient exchange and sludge properties causing loss of biomass via the effluent in UASB-based systems (Alves et al. 2009), but also the formation and activity of lipases, which initially hydrolyse the FOG into glycerol and LCFA. Lipases are very hydrophobic and will adsorb to FOG structures and display their activity. However, they may adsorb to any other hydrophobic structure as well, which, thus, may interact with the lipases and, hamper its FOG-solubilizing role (Alves et al. 2009; Long et al. 2012) Preliminary results from the BRC DP6-project show that sludge recirculation in an AD process treating waste vegetable oil and sewage sludge improved the digestibility of the oil and the increased degradation rate could be linked to increased numbers of beta-oxidizing, syntrophic bacteria.

Pre-treatment methods for FOG are covered in chapter 4.

Conclusions on Fat degradation

The two main reviews (Alves et al. 2009 and Long et al. 2013) referred to above emphasize the high potential of methane production from lipids and indicate potential to use AD in UASB and CSTR based techniques. There are however several issues, which needs to be further investigated to obtain an optimal methane production from lipids or lipid-containing materials. Some of these are:

- Elucidation of the possible inhibition mechanism: Specific acids, effect of acid mixtures, saturated vs. unsaturated acids, etc.
- An understanding of the effects of microbial community structure on the acclimation and performance of lipid digestion. Shifts in key microbial communities, such as methanogenic archaea and syntrophic β -oxidizing bacteria, during exposure to lipids needs particular attention due to their direct involvement in the degradation of the problematic LCFA.
- Develop feeding/digestion strategies to allow for an optimal fat degradation. This includes sludge re-circulation, intermittent feeding, stirring options, use of surface active components in CSTR applications etc.
- Pre-treatment in a pre-reactor, where the main hydrolysis of the lipid materials is conducted. This may be combined with a pre-heating, which results in a higher solubility of the FOG components as well as the LCFA.
- The possibility to achieve priming effects by the FOG or enhance the FOG degradation by co-digestion together with e.g. carbohydrate rich materials

Priorities on Fat degradation

- Evaluate the effect of sludge recirculation on AD of fat in a pilot plant, including costs and energy consumption in the process.
- Evaluate the possibility to produce fat-rich substrate from energy crops (rapeseed etc.) for biogas production.

Lignocellulosic material

About 50% of the carbon globally fixed by photosynthesis is found in the lignocellulose of plant cell walls. Lignocelluloses are therefore abundant, possible substrates for anaerobic digestion to biogas. However, the lignocelluloses are often recalcitrant and energy-intensive pre-treatment is therefore often used prior to AD, why costs and energy balances needs to be considered. General information on lignocellulose composition and properties as well as problems and solutions in conjunction with anaerobic digestion of its different fractions are presented below.

The main components of lignocellulose are cellulose, hemicelluloses and lignin, but the proportions among the three depend on plant species (reviewed in Zhao et al. 2012). The

holocellulose fraction (cellulose and hemicellulose) is the main organic fraction constituting between 63 and 78% of the dry weight of wood, while lignin is reported to comprise 15-38% of the dry weight (reviewed by Zehnder 1988). The lignin and cellulose content is normally higher in woody biomass, while grass biomass holds a higher proportion of hemicelluloses, extractives and inorganics (Zhao et al. 2012).

Cellulose is the most abundant organic carbon compound on earth and is found in primary and secondary plant cell walls. It consists of long, unbranched chains of D-glucose units linked together at the β -(1-4) position. The chains are held together by hydrogen bonds forming cable-like micro-fibrils. A partial hydrolysis of cellulose produces cellobiose, while a complete hydrolysis gives mainly glucoses. The cellulose exists both in water-insoluble crystalline forms and amorphous (soluble) structures. The natural (native) crystalline structure of cellulose is denoted cellulose I. This structure can, however, be converted to cellulose II, III and IV by different treatment methods. The amorphous cellulose holds shorter, more bended and twisted chains which differ in the hydrogen-bound-pattern compared to the crystalline structures. Micro-crystalline cellulose contains a mix of crystalline and amorphous regions (reviewed by Monlau et al. 2013)

Carboxymethyl-cellulose (CMC) and methyl-cellulose (CM) are soluble derivatives of cellulose (reviewed by Miron et al. 2001).

The bacterial degradation of crystalline cellulose is complex and involves a number of enzymes:

- The superfamily of glycoside hydrolases (GH; Berlemont and Matriny 2013) including:
 - Endo-cellulases that split internal β -1,4 glucosidic bonds
 - Exo-cellulases that degrades the polymers from its ends
 - β -glucosidases that degrades cellobiose to glucose
- Carbon-binding modules or domains (CBM or CBD) are important during the degradation of crystalline cellulose as they bind and position the bacterial enzymes onto the substrate (Miron et al. 2001, Wilson 2008). However, the CBM does not seem to have any effect on the degradation of dissolved cellulose.

Anaerobic cellulolytic clostridia degrade cellulose through the action of an extracellular multi-enzyme complex - a cellulosome (Schwarz et al. 2001). The cellulosome binds to the substrate and performs the complete degradation. The advantages of the cellulosome are according to Schwarz (2001): 1) an optimized synergy as the correct ratio between the needed components is guaranteed, 2) non-productive adsorption is prevented as the spacing between the working components is optimal, 3) competition for the limited number of available binding sites is avoided, 4) stops of the hydrolysis due to missing components needed for the degradation is avoided as all enzymes are present in the same complex.

The **hemicellulose** fraction of lignocelluloses (about 30% of the dry weight; Barakat et al. 2011) is diverse and characterized by substituted, branched, short-chained carbohydrates (~70-200 units) with a 1,4-linked β -D-hexosyl backbone. They vary in composition (glycosidic linkages, side-chain composition and degree of polymerization) depending on the type of cell

tissue and species (Zhao et al. 2012) but xylose is the major constituent. Pre-treatment of lignocelluloses for bio-ethanol production often gives a liquid hydrolysate containing hemicelluloses and its degradation products xylose, arabinose, furfural and 5-hydroxymethylfurfural) and lignin residues (syringaldehyde and vanillin among others).

Xylanases are key enzymes for the breakdown of hemicelluloses as they randomly break the β 1,4 backbone of the xylan polymers (Collins et al. 2005) but also esterases and other enzymes for the hydrolysis of various substituted xylans are needed (Saha 2003). The xylanases exist in diverse forms differing in action mechanisms, substrate specificities, hydrolytic activity etc. (Collins et al. 2005). Several reports show that xylanases are present in the cellulosomes (reviewed by Schwarz et al. 2001) and, thus, can assist in the bacterial lignocellulose degradation performed by clostridia and the rumen microflora.

Lignin is the second most abundant biopolymer on Earth (after cellulose). It is mainly built from p-hydroxyphenyls (H), guaiacyls (G) and syringyls (S). The proportions among the units vary with plant species and cell types. Hardwood mainly contains G and S units, while G is the main building block of softwood lignin (reviewed in Zhao et al. 2012). Lignin is highly recalcitrant and its decomposition is considered rate limiting in the turnover of biospheric carbon. Its degradation takes place under oxic conditions as mediated by fungi and bacteria (reviewed in Bugg et al. 2011). However, oligolignols and monoaromatic lignin derivatives from the hydrolysis can be transformed and degraded under anaerobic conditions (Zehnder 1988).

Lignin has a higher energy content than cellulose and hemicellulose (30% higher than that of cellulose; Novaes et al. 2010) and separated from the cellulose and hemicellulose it may be used as fuel or as a basis for replacement of petroleum-based components in a variety of composite materials (Doherty et al. 2011). Such measures are necessary to support the economy of AD based on forest products and should be targeted in conjunction with the exploration of the digestion biotechnology within this sector.

3.1.3 Factors affecting the degradation of lignocellulose

Most literature available within this research area seems linked to the production of bio-ethanol, why many articles focus on saccharification of lignocellulose and this is reflected in the literature reviewed below.

The surface area available for enzymatic attack of lignocellulose is related to the crystallinity of cellulose and to its lignin- and hemicellulose contents (Taherzadeh and Karimi 2008). In a review on "Substrate-related factors affecting enzymatic saccharification of lignocelluloses: our recent understanding" Leu and Zhu (2013) emphasize that the pore size and internal surface area are very important for the enzymatic saccharification of lignocellulosic materials, why size reduction as such has a limited effect on the cellulase activity. Leu and Zhu (2013) also divide the mechanical pre-treatment into class I and class II, where class I increases the external fiber surface area, but do not significantly affect the cell walls, whereas class II reduces the particle size to less than individual fibers with a significant destruction of the microfibril cross-links and the cell walls (ball milling and wet-disk milling are examples of Class II processes). Class I-treatments have a limited effect on untreated

lignocellulolytic materials (approximately 10%), while class II pretreatments (stone grinder in this case) result in a gradual destruction of cell walls to a final, near complete, saccharification. The energy consumption for such a complete mechanical treatment is, however, high (see further discussion on pre-treatment in chapter 4).

Leu and Zhu (2013) stresses the importance of removing hemicelluloses before lignin to obtain an efficient cellulase activity. For most low-level-lignin lignocelluloses (e.g. cornstover and switchgrass) it seems sufficient to remove the hemicelluloses, while for high-level lignin lignocelluloses approximately 20% of the lignin content needs to be removed together with as much as possible of the hemicelluloses. The advantage of removing the hemicelluloses is due to the formation of large pores which facilitates the cellulase activity. The same authors points out that lignin removal is always more expensive than removal of hemicellulose.

Another phenomenon that might prevent an efficient saccharification of lignocelluloses is the unspecific binding of the cellulase to lignin and hemicelluloses. Sulfonation of lignin has been proven to have a positive effect as it hinders the binding of cellulase to lignin, and e.g. Wang et al. (2012) reports a 75% enhancement in enzymatic saccharification. The sulfonation can be achieved by sulfite pre-treatment (SPORL), but will likely give problems with high S concentration in the biogas process but no evaluations of its effect in AD have been made as far as we know as the SPORL-treatment is mainly applied for the ethanol industry.

pH will affect surface charges and thereby surface hydrophobicity and thus the binding of cellulase both to cellulose and to lignin. The optimal pH for degradation of cellulose in lignocelluloses will differ depending on the composition of the specific lignocellulose. Lan et al. (2013) showed that a pH between 5.2 and 6.2 was needed to obtain maximal saccharification of lignocelluloses, while hydrolysis of pure cellulosic substrates should, according to cellulase purchasing companies, be performed between pH 4.5 and 5.

3.1.3.1 Possibilities to increase hydrolysis rates

As for all other complex polymers, the bacterial hydrolysis/solubilisation of cellulose is mostly regarded as the rate-limiting step during AD (Bonne et al. 1993; Noike et al. 1985 and reviewed by Jensen et al. 2009).

Clostrida have been shown to be the most common bacteria degrading cellulose in landfills and anaerobic digesters, while *Flavobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavifaciens* are dominant cellulose degraders in the rumen (reviewed by Jensen et al. 2009) and there is research showing that inoculation with rumen fluid improves AD in artificial systems (below). Also fungi and protozoa likely have an effect on the degradation of cellulose/lignocellulose in the rumen and rumen-inoculated systems.

Apart from pre-treatment techniques (chapter 4), there are a number of studies on how to enhance the hydrolysis rate of cellulose in artificial systems. Jensen et al. (2009) investigated the effect of inoculum source and microbial biomass concentration on the solubilisation kinetics of cellulose. Rumen fluid and leachate from a leach bed reactor treating municipal solid waste (MSW) was used as inocula and the effect of the inoculum source as well as the concentration of sessile and planktonic (free-living) biomass was followed. The hydrolysis

kinetics showed that the rate was limited by the colonization of the cellulose rather than by the cellulose concentration. The hydrolysis rate was correlated to the concentration of sessile biomass protein for both rumen fluid and leachate, which indicates that the hydrolytic capacity was similar independent of the source of the microbial biomass. The correlation between sessile biomass and hydrolysis capacity will however only be valid as long as the binding sites on the substrate is not limiting in the system (Weimer 1998 and Sanders et al. 2000).

Song et al. (2005) showed that the peak of hydrolysis occurred much faster with microcrystalline cellulose (day 1 vs. day 3) in a rumen-inoculated system compared to a leachate-inoculated system. As in the study by Jensen et al. there was likely a difference in biomass concentration with higher cell density in the rumen-inoculated system. Song et al. also showed that the methanogens resided within the biofilms formed on the cellulose.

In a study by O'Sullivan et al. (2005) the cellulose solubilisation rate was shown to mainly be attributed to surface area and not linked to the microbial species (only Clostridia however). The complete colonization of the substrate (a mix of newsprint, office paper and food waste in a leach bed reactor) took four days after that a first order hydrolysis kinetics was established. The rate of solubilisation appeared mainly to be linked to the rate of surface area colonization and the biofilm architecture. The inoculum came from a reactor system degrading microcrystalline cellulose.

Baba et al. (2013) tested to pretreat waste paper with rumen fluid for 6 or 24 hours before anaerobic batch digestion. The best effect was obtained with 6 hours soaking, which increased the methane yield 2.6 times compared to untreated waste paper and resulted in a production corresponding to ~75% of the theoretical methane potential.

Investigations with reactor approaches mimicking animal digestion tracts have been reported: Contreau et al. (2013; AD) constructed two lab-scale systems, based on the cow and termite digestion systems, respectively, with waste activated sludge as the substrate. The cow reactor system included a pre-CSTR with solids recycling followed by an acid hydrolysis, neutralization before digestion in the main digester. The termite process contained two pretreatment steps: an enzymatic treatment followed by an alkali treatment at pH 9 and the effects of different incubation periods and temperatures were here evaluated in batch tests (BMP). The effect of micro-aeration of the WAS was tested as a way to mimic the termite digestive tract. The cow system revealed high positive effects on both methane yields and VS-destruction by 80 and 55%, respectively, while the batch tests with the termite conditions gave increases of 35 and 26%, respectively compared to reference systems.

Also nutrients and trace elements likely affect the hydrolysis efficiency. Studies within this area seem far from covering all enzymes and metals possibly involved, but some results have been reported. Ko et al (2011) showed that a bi-functional endo-exocellulase isolated from bovine rumen was stimulated by Co^{2+} , Cu^{2+} and Mn^{2+} , while Hg^{2+} , Zn^{2+} , Ni^{2+} and Mg^{2+} decreased its activity. Also Bao et al. (2011) studied effects of metals on endo- and exocellulase activities and found that the endocellulase was stimulated by Co, Ni and Mg,

while Ca had a negative effect. Ca^{2+} has also been shown essential for cellulose degradation in several studies (reviewed by Schwarz et al. 2001 and Demain et al 2005).

To divide the anaerobic digestion process into two phases, where the hydrolytic/acidogenic and the syntrophic/methanogenic stages of AD are separated, might be a way to enhance hydrolysis of lignocellolytic material. However, the hydrolysis of lignocellulose can be inhibited by the release of soluble sugars (mainly cellobiose), as the cellulase production and possibly also cell growth of *Clostridium cellulolyticum* and *Clostridium thermocellum* are affected by the sugar levels (reviewed by Desvaux 2005 and Demain 2005) and a two-phase AD might therefore be a way to prevent product inhibition from soluble sugars.

One way to obtain such a system would be to combine a leached bed for hydrolysis of insoluble material with a methanogenic reactor digesting the leachate. Busch et al. (2008) partly describes such a system for degradation of food waste and maize, respectively. The authors suggest an open (aerobic) leached bed combined with a methanogenic filter reactor. Part of the leachate is digested in the methanogenic reactor and then recirculated back to the leached bed, while a part of the leachate is recirculated without passing the methanogenic reactor to sustain an active hydrolyzing microflora in the bed. The treatment time in the leachate bed was 5-8 days for food waste and 14-18 days for maize. The hydraulic retention time in the methanogenic filter was around 20 h. However, the study did not provide information on the extent of aerobic degradation of the hydrolysis products or on the levels of soluble sugars recirculated to the leached bed. To minimize losses of methanogenic substrate, an anaerobic two-stage reactor, likely similar to the systems described by Parawira et al. (2007) and Nizami et al. (2010), would be suitable. Parawira et al. reported on the use of a leached bed combined with a methanogenic filter reactor during AD of sugar beet leaves and potatoes (peeled and unpeeled), respectively. The experiments were run from 21 to 36 days, the loading of the methanogenic filter was between 2 and 20 kg COD/(m³ day) and the concept seemed to work well for the short times tested. No comparison to a one stage process was, however, presented and the levels of soluble sugars was not measured in the leachate. To be able to obtain continuous supply of substrate for the methanogenic reactor, application of two or more leached beds were needed. Nizami et al. (2010) have modelled a system with six leached beds, where one bed is fed every 7 days (42 days retention time). The modelling showed a consistent 4 g/L COD leachate supply for the methanogenic reactor. The two stage system described by Nizami et al. was shown to be more efficient than a one-phase system treating the same rye-grass substrate.

Ghosh et al (2000) investigated applications of two-stage digestion combining a “pseudo - plugg-flow” hydrolyzing-acid stage reactor (APD) with a methanogenic filter that treated the liquid effluent from the APD. Household waste was digested and the performance of the two-stage system was compared with a one-stage process. Both the VS-reduction and the methane yield was improved in the two-stage process (around 20% increase), but the VS-reduction was still only around 30%. The APD-reactor was tilted and mixed with a double ribbon mixer and an overflow from this reactor was pumped to the methanogenic filter. The authors did not consider possible product inhibiting effects. This design might be of interest also for pre-treated energy crops/wood material as a possible way to avoid product

inhibition in hydrolyzing systems by keeping the levels of soluble sugars at a low level in the hydrolyzing step, while running a continuous process.

A physical separation of the hydrolyzing and methanogenic phase can be obtained by an introduction of a membrane reactor. Such systems, with the main purpose just to keep the levels of soluble sugars at a low level are described by Andric et al (2010) and have also been tested (Knutsen and Davids 2004 among others). These systems are designed for ethanol production by fungal cellulases, but could possibly be applied during AD of lignocellulosic materials. However, this remains to be tested. Highlighted drawbacks of membrane reactors are low flux, membrane fouling, high capital- and operational costs, which need to be considered in such setups.

In general two-phase systems can reduce the risk for growth imbalances in the AD system, as differences in activity and growth rates of the microbial community, digestion rates and optimum pH of the different stages can be controlled. However, the possible benefits from a phase separation depend on the type of substrate, and reactor system used etc. and that two-stage processes often result in higher investment costs. Fox and Pohland (1994) highlighted the disruption of syntrophic relationships as one major drawback for two phase digestion, where the thermodynamics of the degradation reactions demand low levels of hydrogen/formate concentrations. Fats, aromatic structures and some amino acids will, thus, not be degraded in an acidogenic phase lacking syntrophic activity, while carbohydrate-rich substrates should be suitable for two-phase digestion. Transformations demanding reductive capacity, e.g. conversion of phenol to benzoate, de-chlorinations and reduction of double bonds etc., might also, according to Fox and Pohland, be benefited in an acidogenic phase. Thus, very likely this first stage would exert a detoxification of certain substrates, before they enter the methanogenic phase.

According to Ke et al. (2005) the two-phase digestion concept has been applied for AD of many wastewaters and solid wastes, and a number of different two-phase systems are listed in review: two CSTRs, UASB-UASB, CSTR-anaerobic filter, CSTR-UASB leached beds combined with methanogenic filters/UASB systems but also with membrane and hybrid reactors. The phase separation can be kinetic (HRT and OLR) or physical (membrane). In many unmixed/low- mixed systems (plug flow reactors, UASBs etc.) a “semi-two-phase” can develop with lower pH, higher concentrations of VFA etc. at the inlet (Fox and Pohland 1994).

3.1.4 Anaerobic digestion of energy crops and straw

Today about 2% of the Swedish arable land potential is used for cultivation of energy crops, while 10% was assumed in the calculation of the Swedish biogas potential (Linné et al. 2008). In comparison Germany, the largest biomass-for-energy producer in EU, uses 16% of its arable land for energy crop cultivation of which about half is used for biogas production with maize as the dominating crop http://www.biodeutschland.org/tl_files/content/dokumente/biothek/Bioenergy_in-Germany_2012_fnr.pdf).

The Swedish project “Crops 4 Biogas” have done system analyses on the use of industrial hemp, sugar beet, maize, triticale (rågvete), ley crops (vall) and winter wheat (reference

crop) for biogas production (Björnsson 2012 and Björnsson and Lantz 2013). The efficiency regarding energy input/energy balance, biogas per hectare, cost and greenhouse gas emissions was evaluated.

The methane yields ($\text{Nm}^3/\text{ton TS}$) were for hemp 230, for beet (including tops) 350, for maize 310, for triticale 340, for ley crops June 270 and for ley crops August 220 and for winter wheat kernel 370. These values were, together with crops yields, used to estimate the area efficiency. Beets, with or without tops, were shown to give the highest biogas yields per ha (161 and 134 GJ/ha), followed by corn (103), tritocale (91) and winter wheat kernel (88), ley (80) and finely hemp (75). However, also the energy input varied widely among the crops. A calculation of the ratios between energy in and energy out (biogas production) distinguished two groups: triticale, corn and beet with energy inputs at 14-16% of the possible energy yields and wheat kernel, hemp and ley crops at 19-20%. As fertilizing stands for a substantial part of the energy input for growing these crops, strategies involving both bio-fertilizers (digestion residues) and mineral fertilizers were evaluated. The energy input was shown to decrease and even out between the two groups, when bio-fertilizers was used instead of mineral fertilizers, i.e. a mean value of 11% for all crops.

The highest cost for production of biogas from energy crops demonstrated by the “crops-4-biogas”-project was the production of the biomass, making up for about 2/3 of the total cost, while the capital cost in these cases were 16-18% of the total. This means that economical investment supports have very little effect on the viability of the production from energy crops. Whole crop silage from triticale and wheat kernels was found to have the lowest production cost per kWh biogas (0.45 SEK). The total production cost was here also lower than for digestion of cow manure. The cost for digestion of manure is mainly linked to capital and operating costs. All crops except industrial hemp could likely be used for a viable biogas production if a production subsidiary of 0.02-0.2 SEK/kWh was introduced. The process limits used were a max of 3 kg of digested TS/ m^3 reactor volume and day, a max TS of 9.5% in the reactor and a max HRT of 46 days.

Furthermore, preliminary results from the “crops-4-biogas”-project showed that the type of fertilizer (bio vs. mineral) did not affect the crop yields, however, this findings need further evaluation. The investigators stressed the importance of choosing crops with low negative environmental impact and the importance of including crops rotation for a sustainable production of biomass for biogas production.

Straw is a residue available in large amounts (the theoretical Swedish potential is 1300 GWh/year). Its digestion give a methane yield of around 0.2 NL $\text{CH}_4/\text{g VS}$ (Risberg et al. 2013). These authors applied steam explosion pre-treatment without any positive effects on the yields and neither did co-digestion with manure increase the yields per g VS. Thus, Risberg et al. (2013) concluded that the yields of the stable co-digestion process was too low for viable biogas production at currently available technology and economy conditions

3.1.5 Anaerobic digestion of wood

Most of the research on degradation of wood material for bio-fuel production deals with the initial enzymatic saccharification of the cellulose fraction of lignocellulose for ethanol

production. These investigations are complemented with studies aimed for the use of the other fractions of the lignocellulose in the resulting hydrolysis liquid for biogas production. As an example Barakat et al. (2012) studied the anaerobic degradation of xylose and the lignin by-products syringaldehyde, vanillin, 5-hydroxymethylfurfural (HMF) and furfural in batch assays. Actual methane yields (% of theoretical in parenthesis) for xylose, syringaldehyde, vanillin, 5-hydroxymethylfurfural (HMF) and furfural were 0.30 (80%), 0.45 (84%), 0.11 (17%), 0.45 (78%), 0.43 (74%) L/g compound, respectively. Possible effects of the by-products on the degradation of xylose was also investigated and no inhibition on the xylose degradation was observed, demonstrating the possibility to use such hydrolyzing liquids for biogas-production.

Anaerobic digestion of effluents from the pulp and paper industry has been reported on, but due to the great differences in effluent characteristics, the potential methane formation reported varies a lot. AD of bleached/unbleached thermo-mechanical and chemical thermo-mechanical (TMP and CTMP, respectively), has been successfully implemented in full scale, as well as on neutral sulfite semi chemical (NSSC) effluents and kraft/sulphite mill condensates (Driessem and Wasenius 1994; Dufresne et al. 2001; Tielbaard et al. 2002). However, with increasing concentrations of substances such as tannins, resin acids, wood extractives or lignin in the effluents new process concepts are likely needed. Wastewaters that are known to cause problems include wood-room effluents, composite CTMP-wastewaters, spent liquors from chemical pulping and bleaching effluents (reviewed in Lacorte et al. 2003 and Sierra-Alvarez et al. 1994).

The results on TOC- (total organic carbon) and/or COD- (chemical oxygen demand) reduction during AD of the wastewater effluents are often inconsistent and in many cases hard to compare. This is likely due to the varying composition of the studied effluents, which are highly dependent on the settings at the mills, which affect the type of chemicals occurring in the wastewater, as well as the concentration of TOC/COD and its organic composition (reviewed in Rintala and Puhakka 1994). In addition, most studies so far focused mainly on toxicity reduction of the pulp- and paper industry effluents (Buzzini and Piers 2007; Latorre et al. 2007; Poggi-Valaldo et al. 1996; Vidal and Diez 2005). However, the large biogas potential associated with many of these often TOC/COD-dense effluents is rarely considered.

As a first step towards a higher degree of implementation of AD within the Swedish pulp- and paper industry, Ekstrand et al. (2013) reported on a survey of methane potentials (batch tests) of 62 wastewater effluents from ten processes at seven pulp and/or paper mills including Kraft pulp (KP), TMP, CTMP and NSSC-pulp. The effluents included material from wood rooms, cooking and oxygen delignification, bleaching (often both acid- and alkali effluents), drying and paper/board machinery as well as total effluents before and after sedimentation. The results showed a large variation in methane yields (percent of theoretical methane potential assuming 940 NmL CH₄ per g TOC) among the effluents. For the KP-mills, methane yields above 50% were obtained for cooking effluents, paper machine

wastewaters, condensate streams and composite pre-sedimentation effluents (results from at least at one mill). The composite effluents from the two NSSC-processes gave methane yields of 60% of the theoretical potential, while all effluents studied for a TMP mill gave the best average yields, ranging 40-65% of the theoretical potential. Most of the samples from the CTMP process correspondingly yielded 40-50%.

In a future application of AD within the pulp and paper industry treatment of specific effluents within the pulp- and paper production units selected in relation to the raw materials used, here considering the biogas yields from the waste residues. The introduction of AD in such plants would be beneficial for the total energy balance by reducing energy demands for aeration as well as for sludge burning. An introduction of AD into the production of ethanol from woody materials seems promising and would likely improve the energy balance of such industries.

There are some studies investigating the biogas potential from pure wood materials: Yao et al. 2013 reported a methane yield of 0.28 L/g VS from poplar processing residues after pretreatment with 5% NaOH compared to around 0.13 L/g VS for the untreated material. Alkali treatment prior to anaerobic digestion has also been investigated for spruce and birch, the maximum yield increased from 0.23 (untreated) to 0.36 L g VS for birch and from 0.03 (untreated) to 0.21 L g VS for spruce (Mohsenzadeh et al. 2011). In a test with alkali pre-treatment of pine wood the methane yield increased from 0.07 (untreated) to 0.18 L/g VS.

In a study by Vivekanand et al. (2013), where birch was steam exploded at 170-230°C before assayed for in methane potential in batch tests, gave maximum yields of 0.37 L CH₄/g VS (*but seems very high compared to the theoretical potential*) as compared to about 0.2 L/g VS for untreated birch. Similarly, the CH₄-potential from steam-exploded Salix (maximal yield was 0.24 NL/g VS; Horn et al. 2011) and spruce (0.3 L/g VS; Janzon et al. 2013) was investigated. In the spruce-case the steam-explosion was combined with a SO₂-treatment. Notably, Janzon et al. did not see any negative effect on the methane yield from the furans produced by the steam-explosion pre-treatment.

All potentials listed above were obtained from batch tests, while AD of wood in semi-continuous or continuous systems seems rare. Results from Department of Water and Environmental Studies, LiU, however show that methane yield obtained in batch assays often are higher than when the same lignocellulosic material is digested in lab-scale semi-continuous digesters. About 70% of the batch yields seem to be obtained in the reactor systems (unpublished results).

From the studies referred above, wood-based AD shows yields from 0.2-0.3 L CH₄/g VS depending on type of pre-treatment and raw material. The amount of forest residues are globally estimated to 30 EJ/year (corresponding to 8000 TWh; McKendry 2002) and in Sweden about 60 TWh of "GROT" is available yearly which would correspond to a rough maximum of 3000 Mm³ CH₄. It should, however, also be noted that the annual growth of the

Swedish forest corresponds to 400 TWh giving a substantially larger bio-energy and lignin potential.

Conclusions on AD from lignocellolytic material

- The hydrolysis seems rate limiting and pre-treatment is needed for reasonable degradation rates.
- Energy balances are important to consider and the lignin fraction must at least for wood be utilized to obtain a reasonable energy balance and economic feasibility.
- Why are methane yields in semi-continuous systems lower than those obtain in batch? Possible explanation is nutrient and /or trace element deficiencies or hydrolysis rates.
- Two-phase AD might be an interesting way to avoid product inhibition during hydrolysis.
- The methane potentials in treatment of residues from the pulp and paper industry are interesting especially if the pulp and paper production is adjusted considering the biogas yields of the residues.
- Linking of AD to the production of ethanol from woody materials seems promising and would likely improve the energy balance of the ethanol industry.
- A concern is the efficient hydrolysis occurring in the rumen, which is far from reached in biogas reactors

Priorities on AD from lignocellolytic material

- The yields of the semi-continuous lab-scale systems are often too low for a profitable biogas production, roughly only 50-60% of theoretical yields are obtained, while batch digestion give higher outputs. The reasons for these gaps need to be investigated to obtain better yields from lignocellolytic material.
 - Results within the BRC show that sludge recirculation improves digestion of fat likely as a result from increased microbial density. The same technique should therefore be tried for lignocellolytic materials.
 - Specific nutrient needs for hydrolysis should be investigated.
 - Two-phase AD as a way to avoid product inhibition should be investigated further both on microbial and a system structure levels.
- A collaboration with the pulp and paper industry with the aim to adapt the raw material for the combination of high quality products and high biogas yields from the residues
- Cultivation of energy crops as ley-crops aiming at highest possible VS-production per ha should be investigated.

Anaerobic digestion of household/food waste

The answers to the questioner sent to 15 Swedish biogas plants revealed household waste to be one of the main problematic substrates in Swedish AD today. Therefore, a separate section in this report is devoted to this area. A master thesis study by Johanna Björkmalm (Björkmalm 2013) and a report by Bernstad et al. (2013) confirm this situation by identifying several problems with pre-treatment and digestion of household waste. The Björkmalm study is part of the Waste Refinery project “Benchmarking biogas production plants of organic waste for increased energy and cost efficiency” (Kostnadseffektiv produktion av biogas och biogödsel) aiming towards efficient biogas processes with food waste as a main substrate. The study included four co-digestion plants and four pre-treatment plants. It concludes that the costs for the pre-treatment of solid waste made up for a large part of the total operation- and maintenance costs (spear parts and personnel) for the investigated plants. It also showed that there were large variations in the volumes of methane produced per ton of substrate as well as in the energy ratios ($\text{energy}_{\text{out}}/\text{energy}_{\text{in}}$). Improvements were suggested to decrease the costs for operation and maintenance by: **1)** recirculate/heat exchange the digested residue, **2)** upgrade/change the equipment for pre-treatment and alternative hygienization treatment, **3)** make dilutions with grease trap sludge instead of water, **4)** improve the monitoring of energy consumption. Improved systems for collection of source-separated household waste and product development on the pre-treatment side was also seen as areas that needed improvement.

The main problems with household/food waste highlighted in the WES survey and the above publications are:

Pre-treatment

- Poor separation of plastic and other inorganic materials
- Digestible organic material is lost with the reject
- The equipment for pre-treatment of the food waste is in some cases not originally designed for treatment of household waste and therefore not ideal from neither a function- or service point of view.

Digestion

- Mainly soft plastics accumulates in the digester and can form both floating layers and accumulate in other parts of the reactor, thus, reducing the active volume of the digester. The plastic can also get stuck in the impeller and disturb the digester mixing system.

Digestate

- The digestate is often contaminated with plastics and other in-organics.

About 150 of Sweden's 290 municipalities are today collecting the organic fraction of household waste and another 70 are about to start (Svensk avfallshantering 2013). In total

673 000 ton of household waste was treated biologically (AD or compost) during 2012. The organic fraction of household waste holds a biogas potential of 400-500 mL/g VS (reviewed by Hansen et al. (2007)) and mostly provides a well balanced mix of carbohydrates, fats and proteins easy to digest and therefore attractive substrates for AD.

A main problem with the organic fraction of household waste is the relatively large amounts of non-organics in the sorted waste. Also the plastic bags used in the collection system in many municipalities is a problem as much of especially the soft plastics passes the pre-treatment and follows the organic fraction into the reactor.

A number of different pre-treatment techniques are applied for the separation and fractionation of the organic part of the household waste. A review by Hansen et al. (2007) evaluated three pre-treatment methods used in Danish communities: 1) screw press, 2) disc screen and 3) shredding + magnetic removal of metals. The article presents chemical composition and methane potentials of the different fractions obtained by these methods. 34 and 41% of the organic material was lost during the screw press and disc screen treatments, respectively, while 98% of the material was retained during shredding. It was, however, pointed out that the shredding could only be used for well-sorted waste as no separation apart from magnetic metals is obtained with this method. Average potentials of the wastes regardless of pre-treatment method were around 450 NmL CH₄/g VS. The highest fractionation of the waste was obtained with the screw press, resulting in one wet, easily degradable and one dry, more recalcitrant fraction containing plastics and/or paper from the sorting bags etc. Bernstad et al. (2013) reports on a survey of 17 Swedish AD-plants with a large span of pre-treatment methods from simple shredding to processes involving several steps including pressing and dispersion. The study showed losses of 2-45% of the organic material and AD of household waste was often seen as problematic due to high maintenance costs and negative effects on the digestate quality. A case study including four plants, three using screw press and one using dispersion was performed and 20-40% of the incoming VS was lost in the pre-treatment, corresponding to 9-28% of the total methane potential. In addition 13-22% of the total N ended up in the rejected fraction. Björkmalm (2013) show that the screw press method gives high losses of organic material (around 50%) compared to treatment with pulper milling, separation by flotation etc. One way to reduce the losses of organic material in the pre-treatment may be to heat the material before treatment, which will solubilize a larger part of the organics. Both positive and negative effects of heating household waste before pre-treatment have, however, been reported (reviewed by Björkmalm 2013). There were also problems with both plastics in the digester and digestate, wear of equipment and high maintenance in all three cases of the Björkmalm study.

The accumulation of plastic in the reactors give decreased operational reactor volume which in turn leads to lower methane yields as well as lower HRT of the organics. One of the main reasons for the problems with the plastics was likely poor equipment, which was not originally designed for pre-treatment of household waste, but also modern installations seems to be associated with some problems with plastics, however, no studies are available for these so far. During 2013, Avfall Sverige launched an action plan to eliminate all visible contamination in AD-digestates. One of the milestones will be to investigate how well the

pre-treatment of SPCR 120 certified plants separate plastics and other visible contaminants from the organic fraction used for biogas production. Also the possible presence micro-plastics (less than 2 mm) should probably be further investigated (Leven et al. 2012).

One way to reduce the fraction of soft plastics would be to use paper-bags for collection of household waste. The paper-bags also have drawbacks as they often break during handling and can start to mold in storage awaiting treatment (reviewed by Björkmalm; 2013), but if the plastic fraction can be substantially reduced in this way it is likely preferable.

Björkmalm (2013) also listed new, alternative, treatment methods: A HC-pulper with reject separation is marketed by Cellwood Machinery in Nässjö, Sweden. The system has been installed on a plant in Denmark and also at the biogas plant in Växjö, Sweden. A “Waste food depacker” from Haarslev (Denmark) has been installed at SRV in Huddinge, Sweden, with hammer-milling to separate plastics, cloths etc. from the organic waste. BioPrePlant also markets a system for pre-treatment of food waste. The system has been installed and is in use at the co-digestion plant in Linköping, Sweden. In addition a system for enzymatic pre-treatment of food waste is commercially available from the Danish company “REnescience”, but is not in operation yet. An estimation of the economy in running the existing pre-treatments Björkmalm (2013) shows that the main costs are linked to the man-hours for maintenance, while new equipment will have a high acquisition cost but likely extensively lower maintenance costs and it might therefore in the long run be economically beneficial to invest in new equipment.

It should also be noted that food waste has been reported to have a relatively high Cd/P ratio (mean value of 37 mg/kg P; Avfall Sverige B2012/02) and this in combination with the new suggested limits for the Cd-levels in bio-fertilizers from the Swedish Environmental Agency will make the digestion of food waste in processes where the digestate is used as a bio-fertilizer highly problematic and will likely affect the economy for many biogas-producers.

Conclusions on Anaerobic digestion of household waste

- The main problem with digestion of household waste seems to be the pre-treatment
 - Parts of the organics is often lost with the reject
 - The organic fraction after pre-treatment contains plastic that accumulates in the digester and contaminates the digestate
 - High maintenance costs
 - No or only scarce information on the performance of the different pre-treatment systems for household waste seems available.
- The suggested limits for Cd-content in bio-fertilizers will affect the possibility to digest food waste.

Priorities on Anaerobic digestion of household waste

- Evaluation and development of efficient and robust pre-treatment methods for source separated household waste.

Anaerobic digestion of sewage sludge

Swedish sewage treatment plants annually generates about 800 GWh of biogas from digestion of primary and secondary sludge. The VS-reduction is, however only, 40-50%. The major reason for the low digestion potential is the low digestibility of secondary sludge (waste activated sludge; WAS). The WAS is a mix of microbial biomass and extracellular polymeric substances (EPS) containing recalcitrant compounds. Some studies report that only 30-50 % of the EPS are degradable in AD (reviewed by Carrère et al. 2010). Pre-treatment methods increase the digestibility of WAS and an overview of available methods are given in chapter 4 (pre-treatment). Biogas yields and VS-reduction has however also, in preliminary studies within the BRC, been shown to increase substantially by the action of digested sludge recirculation aiming for 4-5 % average TS in the digester and a retention time of 8-15 days. An increase in digestibility of the sewage sludge from today's 50% up to 70% would roughly increase the biogas yields with 200 GWh. In addition the total amount of sludge as well as its ash content would decrease due to the increased degree of degradation.

Priorities on Anaerobic digestion of sewage sludge

- Develop protocols for a higher efficiency in degrading the organics of WAS.

Substrates from the sea

The Swedish project "Biogas-new substrates from the sea" (financed by the European regional development fund) identified algae, common reed, mussels and fish wastes as

organic materials from the seas/shores of interest for biogas production (http://www.rfkl.se/Documents/Miljo/Nooil/examples/ex_Bioqasfolder%20engelsk%20slutversion.pdf.pdf.)

3.1.6 Algae

Algae encompasses unicellular micro- and multicellular macro algae (sea-weeds). Together with Cyanobacteria (“bluegreen algae”) they stands for a substantial amount of aquatic biomass. Both macro- and microalgae can grow at high rates (reviewed by Debrowski et al. 2013) and, thus, form large amounts of biomass in short time under favorable conditions (light, heat and nutrients). The algae biomass contains less cellulose then terrestrial plants and no lignin, which in theory would make them more easy to digest. The protein content varies among species and with age: brown macroalgae contain protein at 3-15% of the dry weight, while green and red seaweeds may contain 10-50% proteins (Fleurence 1999; Table 5 in Prajapati et al. 2013 for overview of individual species). Microalgae protein contents (ranging 5-25% of dry weight) are reported (<http://www.fao.org/docrep/003/w3732e/w3732e07.htm>). The algae biomass can be cultivated and/or harvested from natural habitats.

To harvest algae biomass from natural habitats has, however, proven difficult as the methods applied are often both expensive and energy demanding. In the case of macroalgae, harvesting of large amounts from the sea can also harm delicate ecosystems (reviewed by Hughes et al. 2012). Harvesting of macroalgae from shores by use of excavators or amphibious vehicles have been tested, but problems with high noise and high amounts of sand in the harvested biomass (Hansson 2012) as well as costs and energy demand render it no-profitable with existing techniques. An additional problem with natural grown biomass is differences in quality and amounts over the year (reviewed by Debowski et al. 2013). Also the “Biogas-new substrates from the sea” project abandoned the harvesting of algae from sea and shores due to harvesting problems and high algae Cd-content.

The cultivation of algae can be performed in more or less sophisticated open ponds or in closed photobioreactor systems. Harvesting is in these cases facilitated, but there are problems with dehydration and storage of the biomass. In addition, Zaimes and Kannan (2013) showed in a case study including 21 locations in the US, that the fossil energy return on investment ($EROI_{fossil}$) and life cycle greenhouse gas (GHG) emissions from cultivation of microalgae in open raceway pond systems span from 0.38 to 1.08 (values over 1 are desired as more biomass energy is gained than fossil fuel consumed during the production) and from -46 to +49 g CO_2 eq./MJ-biomass respectively. Given the large variations in both $EROI_{fossil}$ and GHG-emissions, the authors stressed the importance of system analysis for each specific production case. Cultivation, dewatering and harvesting are today energy intense activities and to develop renewable/more sustainable methods, therefore seems crucial for commercial production of microalgae to support biogas production. Zamalloa et al. (2011) investigated the potential from microalgae grown in raceway ponds and concluded that a production of 90 ton dry matter per ha and year together with an anaerobic digestion system standing a load of 10-20 kg COD/m³ and day combined with a 75% VS conversion efficiency was needed for the exploration of biogas production from microalgae. The authors

then assumed the use of a high rate UASB-system for the digestion, but the set-up has still not been tested in practice.

Macroalgae can be cultivated at sea. Today this is done on long line systems and China is the world's largest producer of cultivated sea-weed. Hughes et al. (2012) argued that cultivation of macro-algae on continental shelves at sea is the only way to produce the large quantities of sea-weed needed for a substantial biogas production from algae. According to these authors new cultivation- and harvesting methods will be needed for such farming.

For AD of algae a row of problems has been identified: cell walls resisting anaerobic degradation, toxic compounds produced by the algae, sodium ion toxicity and for some species high C:N ratios giving problems with high pH and NH_3 -inhibition (reviewed by Debowski et al. 2013 and Prajapati et al. 2013). This may be circumvented by co-digestion with cellulose-rich material (references within Debowski et al. 2013), while for AD of algae with low protein contents, co-digestion with more protein-rich materials is suggested (Hughes et al. 2012). Methane potentials span 200-400 L CH_4 /kg VS for microalgae and 50-300 for macroalgae (reviewed by Debowski et al. 2013). The main reason for the large differences in digestibility are the composition and, hence, the degradability of the cell walls. Algae with no cell wall or protein rich cell walls are more easily digested than species with cellulose/hemicellulose cell walls (reviewed by Prajapati et al. 2013). Jard et al. (2013) reported that pre-treatment of the red macroalgae *Palmaria palmate* with NaOH at 20°C increased the methane yield from 310 to 370 mL CH_4 /g VS. Thermal treatments up to 160°C did not have any effects and temperatures above 160°C affected the yield negatively. Other studies have, however, shown thermal pre-treatment to be effective (reviewed by Prajapati et al. 2013). The variation in the results is likely linked to the different algae species.

Zamalloa et al. 2012 digested microalgae of the species *Scenedomus obliquus* and *Phaeodactylum tricornutum* in batch and in a hybrid flow-through reactor system and reported 0.24 and 0.36 L CH_4 g VS in batch, while conversion efficiencies in the continuous system was low, 26-31% and 50% for the *S. obliquus* and *P. tricornutum*, respectively. The HRT was 2.2 days and the loading rates 2.8 g VS/L for *S. obliquus* and 1.9 g VS/ for *P. tricornutum*. The authors pointed out that algae are not easily digested in continues systems. Optimization of AD with algae as substrates is thus needed.

To use of wastewaters and/or industrial flue gas for growth of algae for AD as a form of industrial symbiosis is pointed out as a possible scenario by several authors (Zaimes and Khanna 2013 and Zamalloa et al. 2011). Prajapati et al. (2013) has written a review on the subject of growing algae for production of biogas and concludes that combining growth of algae with wastewater treatment may be a way to reduce the cost of the algae production. They also suggest the use of wet biomass in high rate AD as this would reduce the drying costs and give the possibility to recirculate the nutrients from the AD back to the algae cultivation including a reuse of CO_2 in the cultivation.

Conclusions on AD from algae

- Given the differences in growth and methane yields of algae a screening for the best species and cultivation possibilities is of need.
- CO₂ and nutrient uptake should be considered for optimal outcomes in industrial symbiosis scenarios.
- Problems with suboptimal process performances linked to possible pre-treatment needs, inhibitions, reactor design etc. needs to be solved
- The delicate energy balance, at least for microalgae cultivation in ponds/bioreactors, even in the best cases give no or little room for pre-treatments and, thus, algae with no or minor requirement of pre-treatment should be selected..

Priorities on AD from algae

- Find the best candidate for high production of a nutrient balanced, easy digestible macroalgal biomass.
- Investigate possibilities/benefits in combined wastewater treatment/growth of microalgae for production of digestible biomass.

3.1.7 Common reeds

Risén et al. (2013) have performed a system analysis on harvesting and AD of common reeds (*Phragmites australis*). The biogas was assumed to be used as biofuel and the nutrients recovered from the harvesting/AD should be returned to agricultural land. The reed used in the study was harvested during the summer in south east Sweden. An amphibian carrier equipped with a cutting shovel was used for the harvesting. The main drawback with the method was the many turns to empty the shovel, which rendered the harvesting to become a high energy sink. Harvested reed was stringed and ensiled by use of a bale press. Transportation on land was tractor bond. Fragmentation by use of the diesel-driven chopping machine was assumed, but not evaluated in practice. The harvested and ensiled reed was co-digested in a pilot scale CSTR. The main substrate was the same as used in a full scale digester in the Kalmar County and the methane yield from reed was about 220 m³/ton VS reed, when co-digested with the substrate used in the full-scale reactor. According to the authors this potential in agreement with those reported by Jagadabhi. et al. (2011; 220-260 m³ CH₄/ton VS) and from a two stage process combining a leach bed with and UASB-reactor (Nkema and Murto 2011). The leach bed was incubated for 107 days and 80% of the methane was produced there, the remaining 20% in the UASB.

The overall energy balance for the above system was found to be positive (Risén et al 2013) and was accompanied by a reduction in greenhouse gas emission of 80% compared to a fossil reference system. The energy surplus corresponded to about 40 L of petrol per ton wet weight of reed. In addition the content of N and P in the reed suggested that 1 ha of reed contains N to meet the fertilizing need for 0.5 ha and P for 0.7 ha. A project report from the “Biogas-new substrates from the sea”, which partly funded the above study, points out that the quality of the reed was crucial for an acceptable AD, and that reed harvested in the water held a poor quality. The harvest time was also important for quality and thus the AD-result.

Conclusions on AD of reeds

- It seems most efficient to harvest reed growing terrestrially
- The time of harvesting is important
- Energy balances are delicate
- The benefit of catching and recirculation of N and P to arable land should be considered in evaluations of reed as a substrate for AD.

Priorities on AD of reeds

- A project considering benefits and drawbacks in a system perspective should be performed.

3.1.8 Mussels

There seems to be just a few studies on AD of mussels. Nkema and Murto (2013) reports a methane potential of 0.33 L/g VS in a leach bed/UASB system. The dry first stage was found suitable as the mussel meat was here easily solubilized and then further digested in the USAB, while the shells, that wear on the moving parts and clog pipes in wet digestion systems, stayed in the bed-reactor. The energy potential from two stage digestion of mussels was estimated to 16.6 GWh/year (from 65 000 wet weight of blue mussels from the Swedish east coast). The authors pointed out that accumulation of $\text{NH}_4^+/\text{NH}_3$ from the digestion of the mussel protein might be a problem, if not co-digested with substrates holding a higher C/N ratio. The methane production from digestion of only mussels was also found to be low per unit reactor volume due to the shells. A study by Gröndahl et al. (2009) showed that AD of blue mussels did not result in a positive energy balance mainly due to high energy consumption during harvesting. The mussels were, however, found efficient in removing nutrients from the Baltic Sea. Thus, if mussels are chosen for nutrient removal from the sea, AD could be included to degrade the biomass and make the nutrients available for fertilization.

Conclusions on AD of mussels

- If mussels are to be used as substrate for AD energy efficient harvesting methods needs to be used/developed.
- A two stage process might solve the problem with the shells.
- The benefits of nutrient recovery should be considered.

Priorities on AD of mussels

- A project considering benefits and drawbacks in a system perspective should be performed.

3.1.9 Fish industry wastes

Wastes from processing of fish include heads, viscera, scales and wastewater from the process. The waste and wastewaters are rich in proteins and fats. Around 45% of the fish is assumed to be waste, thus, generating over 60 million tons of fish waste per year globally (reviewed by Nges et al. 2012). The fish waste can be used for production of low value animal feed, but also products with higher values (fish oil, omega 3 fatty acids, enzymes, proteins etc. (reviewed by Nges et al. 2012). The production of oils and proteins, however, gives residues that might be used as AD substrates. They are often protein- and fat rich materials and, thus, represent a high methane potential but with risks of accumulation of ammonia and LCFA to inhibiting levels. Therefore, co-digestion with more carbohydrate rich materials will likely be needed to obtain stable, efficient processes.

Nges et al. (2012) determined the biogas potential of salmon waste and fish sludge (the product obtained after extraction of polyunsaturated fatty acids (PUFAs) and fish protein hydrolysate (FPH)) in anaerobic batch tests. The potentials were found to be high for both materials: 830 and 740 mL CH₄/g VS for the fish waste and fish sludge, respectively. Co-digestion of the fish sludge with Jerusalem artichoke was performed in batch (ratios of 1:1 and 1:3 of fish sludge and Jerusalem artichoke on VS basis, respectively). The potentials of the co-digestion corresponded well to those calculated from data on the digestion of the sludge and artichoke as single substrates indicating that no inhibition from the fish sludge took place in the batch tests. Similarly, Eiroa et al. (2012) reported methane yields of 280, 250, 350 and 260 mL CH₄/g VS of tuna, sardine, mackerel and needle-fish, respectively, thus, being much lower than found by Nges et al. (2012) for salmon. The substrate to inoculum ratios were 1.1-1.3 g VS waste/g VS inocula, which should be compared to about 0.7 for Nges et al. (assuming 4.25 of substrate VS) and the systems were therefore likely overloaded. Another factor making a comparison difficult is that Nges et al. had an inoculum acclimated to high NH₄-N levels (the concentration of N-NH₄ was 4 g and the pH 8), while the Eiroa inoculum, that gave the best result, was granular sludge from a reactor treating brewery waste water. Higher loadings in the Eiroa et al. system resulted in even poorer yields. The reason is unknown and co-digestion of tuna with gorse (a flowering plant) in order to increase the C:N ratio of the substrate did not have any positive effect on the yields, indicating that inhibition from NH₃ and/or LCFA might not have been the main problem in this case. Also Gebauer and Eikebrokk (2006) report methane yields in the range of 260-280 mL/g VS, when digesting salmon sludge (from fish farming) in a CSTR system and the low yield was related to high levels of NH₄-N and VFA.

Conclusions on AD from fish wastes

- All fish waste holds a high protein content and are mostly also rich in fat and, thus, are energy rich substrate for AD, but with risks of accumulation of ammonia and LCFA to inhibiting levels. Therefore, co-digestion with more carbohydrate-rich materials will likely be needed to obtain stable, efficient processes.

Priorities on AD from fish wastes

- Investigate the available potential of fish wastes as substrate for biogas production

4 Pre-treatment

Many solid materials needs some kind of levigation (grinding, cutting etc.) and likely also dilution to make them pumpable before fed into AD. Also wastewaters and different types of sludge from industries and municipalities might need pre-treatment before anaerobic digestion. Pre-treatment of residues from wastewater treatment plants seems to be the most studied followed by lignocellulosic materials (reviewed by Carlsson et al. 2012).

Effectiveness and Assessment of pre-treatments

A pre-treatment method should:

- result in an increased availability of degradable compounds in the substrate
- have a low energy demand or possibilities for reuse of energy or residual heat
- have a low capital- and operational cost

For lignocellulolytic materials, the pre-treatment should ideally produce only low amounts of degradation products from lignin and hemicelluloses as these might inhibit the hydrolysis and further degradation. However, this is more important during preparations for ethanol production than in the case of AD. Table 2 in Menon and Rao (2012) and in Carlsson et al. 2012 give good overviews of different pre-treatment methods. Carlsson et al. (2012) stresses the importance, but also the difficulties, of choosing the optimal pre-treatment method for a specific substrate. Yields, energy balances and possible negative effects associated with the different methods need to be evaluated for each specific substrate/substrate mix. Therefore, the success/suitability of different pre-treatment methods should be judged from the yield and rates of the formation of degradable compounds and/or desired products (methane for AD) in relation to costs and energy demand of the pre-treatment process. In this context it is important to have control on losses of organic material and formation of refractory compounds.

Pre-treatment methods

A large number of pre-treatment methods have been tested and evaluated. The methods chosen as well as their severity are related to the type of the complexity of the (biomass) material. Evaluations of pre-treatment methods focusing on lignocellulosic materials have been reviewed by Taherzadeh and Karimi 2008, Hendriks and Zeeman 2008, Monlau 2013 among others and also several pre-treatment methods to be applied for sewage sludge have been extensively studied (reviewed by Carrère et al. 2010 and Carlsson et al. 2012). There are also publications on treatment of household waste (Hansen et al. 2003; Li et al. 2013), fat, oil and grease (FOG; Li et al 2013) and slaughterhouse waste (Cavalerio 2012; Cuetos et al. 2010; Rodríguez-Abalde et al. 2011).

4.1.1 Physical pretreatments

The physical pre-treatment methods investigated include chipping, grinding, milling and irradiation.

- **Milling** is applied to reduce size, which increase the available particle surface area, and alter internal structure e.g. by reduction of the crystallinity of cellulose. Milling in colloid mills and the use of fibrillators and dissolvers are suitable for wet materials and possibly also fats, while extruder, roller-, cryogenic- and hammer milling mainly are applied for dry materials. Ball milling can be applied on both wet and dry materials (Taherzadeh and Karimi; 2008). It should, however, be noted that large reductions in size can cause significant losses in carbohydrates prior to digestion (e.g. for switchgrass; reviewed by Monlau et al. 2013). A general drawback with milling is the high energy consumption. The energy demand is higher for wet than for dry materials (reviewed in Monlau et al 2013). Milling of lignocellulosic materials does not separate lignin from cellulose/hemicelluloses and, thus, therefore often needs to be combined with chemical pre-treatment methods to obtain an efficient enzymatic hydrolysis of the latter fraction (at least for bio-ethanol production).
- **Irradiation** by microwaves, electron beaming or gamma rays can improve degradation of lignocellulosic materials to some extent. The success seems to be correlated to the amount of lignin as well as the degree of crystallinity characterizing the lignocelluloses. The irradiation methods are, according to Taherzadeh and Karimi (2008), expensive and difficult to apply in full scale applications. Microwave treatment of sludge have been performed and evaluated by Neda Mehdizadeh et al. (2013), who saw no positive effect on biogas yields compared to ordinary heat treatment, while Kuglarz et al (2013) reported benefits from using the microwave treatment compared to other heating methods.
- **Ultra-sonication** creates cavitation and the treatment also gives rise to the formation of OH, HO₂ and H radicals that in turn reacts chemically with the sludge material. Ultra-sonication treatment of sludge generated in systems for wastewater treatment has been shown to increase biogas yields with 24-140 % in batch and from 10-40 % in

continuous systems (reviewed by Carrère et al. 2010). The energy consumption is usually between 1000 and 16000 kJ/kg TS and the optimal TS-concentration for an efficient treatment is reported to be between 2.3 and 3.2%. In addition both positive and negative effects on the dewaterability of activated sludge from ultra-sonic pre-treatment have been reported (reviewed by Carrère et al. 2010).

- **Lysis centrifugation** can also be applied on sludge and has been shown to increase biogas yields with 15-25%. The method has been installed on several full-scale plants (Carrère et al. 2010).
- Sludge can be treated with high pressures: **liquid shear** leading to mechanical disruption of cells, **collision plates** and **high pressure homogenizers**, which display rapid depressurization of sludge Carrère et al (2010). The treatments increased the biogas yields and at the same time the hydraulic retention times of the processes could be reduced. Ferrer et al. (2009) however showed that most mechanical sludge treatments generated negative energy balances. The energy balances could however be improved by sludge thickening, but then this would mean increases in viscosity and, thus, increased energy consumption to operate the digestion process.
- Physical pre-treatment of **household waste** focus on removal of plastic and inorganic objects and on size reduction (see 0 for details).

4.1.2 Physiochemical pre-treatments

- **Steaming and Steam explosion** (autohydrolysis)

Here biomass is treated with high-pressure saturated steam, which combined with a quickly release of the pressure gives rise to an explosive decompression. The temperature is typically 160-260°C and the duration from seconds to a few minutes (Menon and Rao 2012). For lignocellulosic material a degradation of hemicellulose and a transformation of the lignin structure may occur by the treatment. At an elevated temperature there is an increase in the release and degradation of hemicelluloses, but also increased losses in total COD. Steaming and steam explosion seems to be one of the most investigated and applied pre-treatment methods for treating lignocellulosic materials.

The pre-treatment of poplar (*Populus nigra*) for 4 minutes at 210°C gave a cellulose recovery of 95% and a xylose recovery of 41% (reviewed by Taherzadeh and Karimi 2008). Another study showed removal of 75-90% of the xylose-content at pre-treatment of different lignocellulosic biomasses at 190-210°C for 2-8 minutes (Ballesteros et al. in Taherzadeh and Karimi 2008). Biogas production from steam-exploded wheat straw and paper tube residues have been shown to have a positive energy balance and to be economically viable (Shafiei et al 2013).

Steam explosion can also be combined with chemicals like SO₂ and H₂SO₄ (**dilute acid pre-treatment**), as well as ammonia in **ammonia fiber explosion** (AFEX). The latter method is more efficient on biomass low in lignin, but both the cost and the recovery of ammonia makes it expensive. Also a combination of steam explosion and

enzymatic treatment can be applied to obtain solubilized carbohydrates for digestion. Another method within the explosion category is CO₂-explosion that has been shown to improve the enzymatic digestibility of aspen and pine. The high pressure of CO₂ applied at supercritical conditions give higher glucose yields than the same treatment at subcritical conditions, but is likely too expensive.

Thermal hydrolysis of sludge at temperatures often at 160-180°C for 30 to 60 minutes leads to partial solubilization of the sludge VS and, thus, enhances its digestion (reviewed by Carrère et al. (2010)).

- **Liquid hot water pre-treatment:** Hot water processing of lignocellulose removes mainly hemicelluloses and higher xylan recovery has been shown compared to steam explosion (Laser et al. in Taherzadeh and Karimi 2008).
- **Pasteurization** or other, relatively low temperature, but still thermal, pre-treatment methods have also be applied. Rodríguez-Abalde et al (2011) and Cavaleiro et al (2013) reported positive effects of pasteurization of fractions of slaughterhouse waste. Rodríguez-Abalde et al (2011) suggested that the pasteurization mainly had a positive effect on materials with a low content of carbohydrates. The low effect on carbohydrates was suggested to be due to the occurrence of Maillard-reactions.
- Ferrer et al. (2009) compared three classes of sludge pre-treatment methods and concluded that mild biological/thermal pre-treatment was comparable to a single stage thermophilic digestion in terms of energy consumption and biogas yield, while high impact temperature-thermal hydrolysis techniques increased the use of thermal energy, but also increased the yields enough to sustain positive energy balances.

4.1.3 Chemical pre-treatments

- **Alkali pre-treatment** use bases such as NaOH, Ca(OH)₂ or ammonia for the removal of lignin and parts of the hemicelluloses. Relatively high concentrations of the bases are needed, while at low temperature. Alkaline pre-treatments have been shown to be more efficient on biomass low in lignin (e.g. agriculture wastes) than on wood (reviewed by Taherzadeh and Karimi 2008).
- **Alkaline peroxide** pre-treatment is a method, where lignocellulose is soaked in alkali water (pH 11-12 by NaOH) containing H₂O₂, at room temperature typically for 6-24 h. The treatment has been shown to give 81-88% lignin removal, if combined with steam explosion (wheat straw; reviewed in Taherzadeh and Karimi 2008).
- In the “**organosolv**” process lignocelluloses is mixed with an organic solvent (ethanol is common) and water, the mix is then heated. The reaction leaves cellulose in the solid phase, while dissolved lignin is extracted from the solvent. The solvent needs to be recovered. The organosolv process combined with acid hydrolysis results in separate hemicelluloses and lignin fractions.
- **Wet oxidation** combines water and air/oxygen at a temperature above 120°C. The process generates heat and is therefore partly self-heated once the reactions are

started. The wet oxidation effectively separates the cellulose from the hemicelluloses and the lignin. All polymers are however fractionated why the lignin-residues cannot efficiently be used as a product of its own.

- **Ozonolysis** can degrade lignin and parts of the hemicelluloses. The method is, however, likely expensive due to the ozon consumption. The effect on WAS prior to anaerobic digestion showed 36-90% increased TS removal in AD (Goel et al 2003; 167 in Taherzadeh and Karimi 2008).
- Treatment of lignocelluloses with **dilute acid at high temperature or concentrated acid (~35%) at low temperature (acid hydrolysis)** has been shown to improve enzymatic hydrolysis. The concentrated acid treatment is more efficient but highly corrosive. Recovery of the acid is energy demanding. For this reason the dilute acid treatment at high temperature is more frequently applied and is currently one of the most common chemical pre-treatment methods for lignocellulose. Tests with *Eucalyptus grandis* showed a 70% recovery of xylose at 210°C for 2 minutes in dilute H₂SO₄, while the maximum saccharification was seen after treatment at 200°C (90% cellulose conversion). A combination of steam explosion and mild acid treatment has been put forward as a possibility to increase the effect of pre-treatment of especially softwood that is often found more difficult to degrade compared to hardwood (Galbe and Zacchi 2007).

4.1.4 Biological pre-treatments

- Different species of **lignin-degrading fungi** can be used for pre-treatment of lignocellolytic materials. Brown rot fungi primarily degrade hemicellulose and lignin, while white- and soft rot fungi attacks both lignin and cellulose (reviewed by Sun and Cheng 2002). Biodegradation of straw and Bermuda grass was improved by 30-80% (reviewed by Sun and Cheng 2002). However, the incubation times are often long (5-6 weeks). Another problem is that the cellulose and hemicelluloses are degraded by the fungi.
- There are also many reports on **pre-hydrolysis** of sludge at aerobic or anaerobic conditions. The treatment seems mainly to be performed at temperatures of 55-70°C and with a duration of 1-7 days (reviewed by Carrère et al. 2010). Yields have been shown to be enhance by 10-100%.
- Biological treatment has also been used prior to AD to reduce the amount of compounds inhibiting organisms active in the anaerobic degradation chain (reviewed by Taherzadeh and Karimi 2008).

Conclusions on *Pre-treatment*

- It is of utmost importance to choose the right treatment method for a specific substrate and yields, energy balances and possible negative effects of the different methods needs to be evaluated for each specific substrate/substrate blend.
- Few studies evaluate pre-treatment effects in relation to energy input and good measures to compare different methods regarding energy consumption in relation to treated amounts and effects are scarce.
- The development of efficient pre-treatment methods separating the lignin fraction from the carbohydrates of lignocellulosic materials at low energy utilization is a clue to advance our substrate banks.
- Steam and steam explosion pre-treatments, often combined with a weak acid seems to be the most applied method for the treatment of lingocellulosic materials.
- Milling methods are effective for reduction of size, but to obtain a high degree of destruction (degradation of individual fibers) the energy consumption is often too high to be feasible.
- Mild heat-treatment seems to have a positive effect on fat- and protein rich materials.
- High impact temperature-thermal hydrolysis techniques have been shown effective for sludge pre-treatment.

Priorities on *Pre-treatment*

- Are the methods used today the best for the substrates treated in full scale applications?
- Are the methods used optimally from a resource perspective?
- Develop tools for better evaluation of different pre-treatment methods: energy consumption, effect, type of substrate, comparable original and improved methane potentials etc.
 - Evaluation of a number of substrates and key pretreatment methods

5 Mixing and Rheology

Mixing

The content of anaerobic digesters is mixed to ensure efficient transfer of organic material and nutrients to the active microbial biomass, an even distribution of temperature and buffering alkalinity, to release gas bubbles trapped in the reactor fluid and to prevent sedimentation of particulate material. The mixing systems are however often expensive to

install, maintain and run. Common energy inputs range from 10 to 100 W h m⁻³, determined by the frequency of stirring, the type of reactor, the type of mixing system used, and the total solids of the feedstock (Burton and Turner, 2003). Numerous experiences and literature studies indicate process disturbances that can be attributed to deficient design and/or operation of the mixing equipment. Poor mixing and grit accumulation due to dead zones have been observed in many cylindrical digesters (Ganidi *et al.*, 2009). There are however conflicting reports on the effect of mixing on the degree of degradation (Ong *et al.*, 2002) and the evaluation is complicated by difference in waste characteristics, organic loadings, mixing system, active reactor volumes etc. (Ganidi *et al.*, 2009). The formation of microbial aggregates has been shown to be of great importance in AD. It has been postulated that propionate oxidizing bacteria and methanogenic archaea lives in close proximity in aggregates with hydrogen gas and formate as electron carriers (de Bok *et al.*, 2004). For the reaction to be thermodynamically feasible, concentrations of the electron carriers need to be low and therefore the high rate of propionate conversion observed can only be explained by the short diffusion distance possible in obligate syntrophic aggregates (Ward *et al.*, 2008). Excessive mixing can disrupt the microbial aggregates, reducing the rate of oxidation of fatty acids and thus lead to digester instability (McMahon *et al.*, 2001).

5.1.1 Process technology and mixing techniques

Mixing of the digester content is carried out in a number of ways, continuous or intermittent at different frequencies. The type of mixing is partly governed by the reactor designs chosen and this choice is in turn based on the type of substrate/substrate mix to be digested, the desired loading rate etc. Plug-flow digesters are often horizontal containers fed with substrates with at a high solid content. The flow through the system is given by the feeding rate, while no mixing is applied. In a CSTR the hydraulic retention time (HRT) is equal to the solids retention time (SRT) and a HRT of at least 10 days is needed to keep an active methanogenic population within the reactor. This reactor type is suitable for materials with high TS and VS/suspended solids content and stirring is often performed by the use of impellers placed on a central axis and often on several different levels in the digester (Brehmer and Karaume, 2012). Also high pressure liquid or gas mixing by the use of nozzles is applied. It is easier to obtain a proper mixing in egg-shaped digesters, but they are more expensive to build (Meatcalf and Eddy, 2003). With gas and/or liquid mixing it is easier to avoid dead zones. The main drawback is higher energy consumption.

An anaerobic filter is a process system for treating wastewater, where the active biomass is fixed on a supporting matrix medium. An upward flow, where the wastewater comes in contact with the fixed biofilm, is applied for mixing/contact of the organics with the active biomass resulting in a high ratio between sludge- and hydraulic retention time. The upward flow of the formed gas contributes to the mixing of the system. Limitations of anaerobic filters are mainly due to destruction of the bed structure through an accumulation of non-biodegradable solids, which leads to clogging and channeling and, thus, shortcuts of the flow (poor mixing/contact). Therefore treatment of wastewater with high solids content should be avoided. An interrupted flow can result in problems with the fluidization once the system is restarted as the particles or granulated bed tends to clog together. The matrix medium packing materials are relatively expensive.

Apart from the mechanical/technical differences and difficulties linked to mixing, the shear thinning flow behavior (see 5.2) of many substrates and the high dry matter content contribute to the complexity to reach proper mixing for a specific AD-system (Brehmer and Karaume, 2012).

5.1.2 Effects of mixing on the biogas process

As said above, a certain degree of mixing is necessary to obtain a good contact between the substrate and the microorganisms, but excessive mixing can reduce biogas production by disturbing aggregation and granulation of the microorganisms. For example did Kaparaju *et al.* (2008) found that a minimal mixing improved methane production by 13% compared to continuous mixing during AD of cattle manure in lab-scale experiments. The effect of organic load and mixing intensities during the manure digestion in batch experiments showed that minimally mixing (thoroughly shaken by hand for about 1 minute every time a sample was taken) resulted in higher methane production. On the contrary, vigorous (110 times per minute with a 3.5 cm stroke) mixing resulted in delayed and low methane production (Kaparaju *et al.*, 2008).

It has been reported that low speed mixing allowed digesters to better absorb the disturbances of shock loadings compared to reactors with high speed mixing (Stroot *et al.*, 2001; Gomez *et al.*, 2006). Thus, reduction of mixing speed may be a tool for process stabilization. In comparing continuously vs. intermittently mixed high loaded processes stable conditions are more often obtained with the intermittent systems (Stroot *et al.*, 2001 and unpublished in-house results). These effects are likely the result of disturbance of the syntrophic interactions among methanogens and proton-reducing bacteria as described in the introduction above (5.1).

Extracellular polymeric substances (EPS) are complex high-molecular-weight mixtures of polymers ($M_w > 10,000$) and includes proteins, carbohydrates, lipids, nucleic acids (DNA) and humic substances (Sheng and Yu, 2006), which are responsible for the formation of microbial aggregates (Liu *et al.*, 2004). Measurement of EPS can be indicative of the degree of microbial aggregation and be linked to the mixing conditions of an AD-digester (Ward *et al.*, 2008). This has been shown in AD of cattle manure, where an increased mixing decreased the amount of EPS and, thus, likely also disturbed the microbial aggregates (Ong *et al.* 2002). This led these authors to suggest that minimal mixing results in larger aggregates as greater quantities of EPS were required to maintain their structure. The large aggregates might lead to an increase in biomass retention, which in turn could explain the increased gas production (Ong *et al.*, 2002). In fact, Ong *et al.* (2002) reported that the production of EPS was adversely affected by stirring. Evidence for the structural role of EPS in consortia has been reported for bacteria involved in oxidation of saturated fatty acids (e.g. by McInerney *et al.*, 1981) and digestion of cellulose (e.g. by Cheng *et al.*, 1984). Adhesion of cellulolytic bacteria to particles or to other cells is mediated via EPS (Weimer, 1992).

5.1.3 Foaming

EPS, temperature fluctuations, shock loads, hydrophobic substances, protein and/or fat rich material have been reported to cause digester foaming (Ganidi *et al.*, 2009). Foaming causes

significant operational problems and reduces revenues due to reduced gas yields (e.g. Kougias *et al.*, 2012; Rodriguez-Roda *et al.*, 2012). It may lead to blockages of gas mixing devices and sludge pumps, fouling in gas collection pipes and floating digester covers (Gandi *et al.*, 2009).

In sludge, proteins are found as soluble microbial products (SMP) but also as EPS. However, so far no information is available on how different proteins affect the foaming potential in anaerobic digesters and at which concentrations they may induce foaming (Ganidi *et al.*, 2009). Lipids are, due to their hydrophobicity, attached to the solid particles of the sludge and can entrap formed gas bubbles and, thus, induce foaming. Additional knowledge is needed to understand the role of loading rates, mixing requirements and other operating parameters that will reduce the risk of digester foaming due to protein and/or fat rich material (Long *et al.*, 2012).

Intense mixing was suggested to cause foaming (Ganidi *et al.*, 2009), but there is a lack of experimental evidences to support these suggestions. Also inadequate mixing is claimed to give formation of foam (Barber, 2005; Barjenbruch *et al.*, 2000). The likely cause is a solid/liquid phase separation giving accumulation of surface active compounds at the gaseous/liquid interphase resulting in increased surface activity and potentially foaming. Gas-mixed digesters accumulate more foam than mechanically-mixed digester due to the higher presence of gas bubbles in the bulk phase of a gas-mixed system (Ganidi *et al.* 2009).

Organic overloading due to the excess of compounds not being fully degraded may induce foaming (e.g. Moen, 2003; Barber 2005). Especially the accumulation of hydrophobic surface active by-products would promote foaming.

Thermophilic digestion has been reported to be more resistant to foam generation than mesophilic digestion (Dohanyos *et al.*, 2004). This could be attributed to the higher temperatures, lowering the surface tension and viscosity of the sludge and, hence, increasing foam drainage (Hayta *et al.*, 2001; Barber, 2005).

Reported solutions to suppress foaming are decrease of the organic load, adjustments of mixing speed, uniform sludge feeding, and addition of antifoaming agents, temperature change or dilution of the reactor content.

5.1.4 Hydrodynamics

The efficiency of a mixing system can be measured by hydrodynamics (Levenspiel, 1999). Hydrodynamic studies reveal dead zones or areas within a system that are not well mixed and therefore, in the case of AD, not receiving fresh substrate. Also any short-circuiting or channeling between the input and output points are detected (Ward *et al.*, 2008). Hydrodynamics is, however, hard to apply on AD, since digester sludge is opaque and the flow patterns inside the digester cannot be observed without using advanced techniques, such as detectable tracers (typically Li^+ ; Oliver *et al.*, 2005), computer automated radioactive particle tracking (CARPT), computed tomography (CT; Karim *et al.*, 2004), and computational fluid dynamics (CDF) simulation (e.g. Vesvikar and Al-Dahlan, 2005; Karim *et al.*, 2007; Mendoza *et al.*, 2011). The CARPT, CT and CDF techniques are, however, time consuming,

expensive and still need to be validated with experimental data. The hydrodynamic studies could also be complemented with granulometric- and rheological characterizations.

Rheology

Rheology describes the deformation of a body under the influence of stress. The shear force needed for proper mixing is dependent on the viscosity of the liquid, where increasing viscosity demands an increased energy input. Changes in substrate composition, as a means to obtain a more efficient utilization of existing biogas facilities may induce shifts in viscosity of the process liquid and, hence, problems with inadequate mixing, breakdown of stirrers and foaming (Nordberg and Edström, 2005; Menéndez *et al.*, 2006; Björn *et al.*, 2012a).

When considering the rheology for biogas reactor fluids, their viscosity is often estimated to correspond to the total solid (TS). This is mainly based on historical rheological data from sewage sludge digestion, and rheological studies of other anaerobic digester sludges are scarce (cf. Björn *et al.*, 2012a). Therefore, problems may arise when using the sewage sludge/TS relationships for other types of substrates, which may impose other rheological characteristics (Björn *et al.*, 2012a). Thus, increased knowledge on the rheological characteristics is needed to optimize mixing.

5.1.5 Rheological characterization of substrate and reactor sludge

The rheological behavior of AD-sludge from digestion of sewage sludge with different origins have been shown to mainly depend on the organic composition and the digestion time (e.g. Baudez and Coussot, 2001; Forster, 2002; Seyssiecq *et al.*, 2003). Eshtiagi *et al.* (2012) determined the rheological behavior of reactor material from AD of sewage sludge at different dry solid concentrations (1.8-4.9%) and different temperatures (25-80°C). At low shear stress the digested sludge behaved as a viscoelastic solid, but a shear banding behavior was also seen. Shear banding fluids develop multifluid phases with different rheological properties (Quemada and Beril, 2007). At higher shear stress, the behavior of the AD sludge indicated viscoplastic characteristics, i.e. it becomes more “fluent” with increased mixing, but needs a specific force to start flowing. The viscosity and yield stress increased with increased solid concentration, but decreased with increasing temperature, which was confirmed by studies of AD of sewage sludge by Björn *et al.* (2012b).

Björn *et al.* (2012b) determined the rheology of 12 of the 15 full-scale CSTRs (part of the WES study mentioned in Chapters 1 and 2) related to difference in substrate composition and operational temperature conditions. Reactor material from mesophilic (36-38°C) and thermophilic (52-55°C) reactors were sampled at two occasions. The digesters were fed with sewage sludge (SS) or mixtures of organic matter including slaughterhouse waste, food industry waste, fat, manure, fodder residues and/or the organic fraction of municipal solid waste. The results showed differences in viscosity for several co-digesting reactor (CD) liquids despite similar TS-contents. This shows that the TS-content of biogas reactor liquids is not a good estimator of the fluid viscosity for CD processes as the fluid characteristics vary as a result of substrate composition and process temperature conditions. A viscoplastic behavior was confirmed for the mesophilic and thermophilic SS-reactors. This was also the

case for the mesophilic CD-reactors, which also were strongly thixotropic, and thus, showed a partial structure recovery when mixing was stopped. Similar findings of thixotropy have been reported for pilot-scale mesophilic processes co-digesting maize/sugar beet silage (Nges *et al.* (2012)). The thixotropic behavior reported for mesophilic CD-processes is important to consider for biogas reactor operation, e.g. when applying mixing intervals instead of continuous mixing. The thermophilic CD-liquids differed from the mesophilic and instead showed a weak dilatant behavior, i.e. thickening with increased mixing when agitated (Björn *et al.*, 2012b). Furthermore, crop-based digestates sampled from a large number of crop-based CSTR biogas plants (mono-digestion), monitored in a German study (FNR, 2010), were glutinous (gel formation) in nature, leading to higher viscosity and potential problems with stirring, in contrast to manure-based digestate. Accordingly, Nges *et al.* (2012) argued that it is important to characterize rheological parameters such as viscosity, shear stress and shear rate to provide information regarding fluid behavior and resistance during mixing in crop mono-digestion processes. Sutherland (2011) reported that an explanation to increased gel formation and viscosity could be secretion of EPS by microorganisms. Also, when maize in a co-digestion plant (30% manure, 30% maize and 40% side streams) was replaced by grass, the dry matter content and viscosity was increased substantially (De Moor *et al.*, 2013). These elevated parameters could subsequently be reduced again by enzyme addition (Methaplus L100), although remained higher than the reference reactor receiving maize. De Moor *et al.* (2013) concluded that co-digestion of 20% grass would not pose any problem if the dry matter content and viscosity are improved by enzyme additions.

5.1.6 Rheology and mixing performance

Due to the shear thinning flow behavior of many substrates, the mixing process in substrate tanks is prone to create caverns with the effect that only the liquid near the agitators is flowing (Brehmer and Kraume, 2012).

Viscoplastic fluids (mainly AD sludge from processes digestion sewage sludge) has not been studied to any great extent, but it is known that their behavior drastically modifies the effect of and power needed for mixing (Savreux *et al.*, 2007). Tanguy *et al.* (1994) and Bertrand *et al.* (1996) studied the influence of viscoplasticity on the flow pattern and power consumption when mixing with an anchor impeller. They showed that the viscoplasticity created rigid zones, which were highly detrimental to mixing quality and power consumption. To increase mixing quality without a drastic increase in power consumption different set-ups were tested and the results indicated that a configuration with two perpendicular blades was the best for mixing the viscoplastic fluid.

Brehmer and Kraume (2012) investigated the rheological behavior of reactor liquids digesting corn silage, sewage sludge and biological waste, respectively, and their influence on the flow regime in stirred full-scale biogas plants. With the use of a pilot plant and numerical simulations, the flow regime and rheology in the respective biogas process, it was shown that even a small change in viscosity had a substantial impact on the flow profile and therefore on the process efficiency. If the viscosity was high, the initially optimized mixing effect given by several mixers at different levels was lost and contributed to the formation of

caverns. In some cases the whole mixing system even broke down. In addition, the distance of the substrate input to the walls was shown to affect the mixing as cavern formation was increased with the distance between the substrate inlet and the wall. Therefore, a sufficiently large distance should be ensured and also that the generated substrate stream reaches the intake area of the mixer (Brehmer and Kraume, 2012).

Conclusions on *Mixing and Rheology*

- Numerous experiences and literature studies indicate process disturbances that can be attributed to deficient design and/or operation of the mixing equipment.
- Excessive mixing can disrupt the microbial aggregates, reducing degradation efficiency and leading to digester instability. No or too low mixing will give inefficient degradation and likely also foaming problems.
- Characterization of rheological parameters such as viscosity, shear stress, and shear rate provides information about fluid behavior and resistance during mixing, and are useful tools for optimization of operational mixing conditions and energy savings. Shifts in viscosity with changes in substrate profiles and mixing (especially linked to thixotropic and dilatant behaviors) need to be considered.
- Hydrodynamic studies reveal the flow patterns inside a container, and methods within this field can therefore be used to identify dead zones or areas that are not well mixed. Hydrodynamic studies in combination with rheological data can be used for optimization of the mixing in AD-processes. The different behaviors of the AD-liquids, depending on substrate and temperature, also need to be considered and further research is needed within this area. It should be noted that in many cases the TS content of reactor sludge is not a good estimator of its viscosity behavior and fluid characteristics.
- Measurement of EPS can indicate the degree of microbial aggregation and, thus, possibly be used as an estimation of the mixing conditions in the reactor.
- Additional knowledge is needed to determine loading rates, mixing requirements and other operational parameters that can reduce the risk of digester foaming during co-digestion.

Priorities of Mixing and Rheology

- How will changes in substrate profiles affect the rheology/viscosity of a reactor liquid and what are the underlying causes for such changes?
- How does nutritional composition, trace metal availability, EPS, SMP, VFA interact to affect the viscosity/rheology in biogas reactors?
- Which process parameters induce a quick change in viscosity?
 - Which organic structures are typical in rapid viscosity changes?
- How much of the potential methane is lost in poorly mixed systems?
 - Investigate the effect of adjusting the position of the impeller in a full scale system.

6 The biochemical dynamics of trace elements during anaerobic digestion processes

During the last decades, a vast body of literature reported positive effects on AD by addition of trace metals to biogas reactors (e.g. Murray and van den Berg 1981; Takashima and Speece 1989; Jarvis et al. 1997; Kida et al. 2001; Zandvoort et al. 2006; Worm et al. 2009; Gustavsson et al. 2011; Pobeheim et al. 2011; Takashima et al. 2011; Banks et al. 2012; Bayr et al. 2012; Karlsson et al. 2012; Lindorfer et al. 2012; Facchin et al. 2013; Schmidt et al. 2013; Schmidt et al. 2014). For example, Pobeheim et al. (2011) demonstrated that addition of Co and Ni stimulated the metabolism of acetate and propionate enabling approximately two times increase in the OLR and more biogas production, while the deficiency of Co and Ni led to process instability and decrease in biogas production by 10% and 25%, respectively. Similarly, Karlsson et al. (2012) showed that addition of metals (i.e. Fe, Co, and Ni) improved the methane yield and conversion of acetate and propionate during anaerobic co-digestion of a mixture of slaughterhouse waste, manure, municipal solid waste, fat, and industrial food waste. Furthermore, these researchers showed that the amendment of trace metals potentially decreased the negative effect of inhibitory substances on methanogenic processes (as exemplified by phenyl acetate). Facchin et al. (2013) showed that supplementation of Mo and Se also increased the methane production from anaerobic digestion of food waste by 30-40%. Addition of a mixture of metals including Co, Mo, Ni, Se and W resulted in a more efficient process by increasing methane production up to 65%.

Thus, the addition of trace metals have in many cases been shown to increase process efficiency with respect to enhanced substrate digestion and biogas production combined with low concentrations of intermediate fermentation products i.e. volatile fatty acids. These improvements are explained by the fundamental necessity of metals as micronutrients for almost any form of life, where they are crucial for the intracellular synthesis and activity of essential metabolic enzymes and cofactors governing growth (cf. Crichton 2008). Biologically important metals commonly added to the biogas reactors include mainly, Fe, Cu, Zn, Mn, Ni, Co, Mo, Se, and W. Among these metals the necessity of Fe, Co, and Ni is widely addressed for a variety of substrates, while requirement for addition of other metals by AD process is different depending on the process specifications. For instance, Schmidt et al. (2013) demonstrated that addition Mn, Cu, Se, and Zn was not required when producing biogas from stillage, while Mo and W had to be amended at high organic loading rates in addition to Fe, Co, and Ni. However, Feroso et al. 2009 demonstrated that Zn deficiency in UASB reactors results in acidification and failure of the AD process and Okeh et al. 2014 demonstrated that addition of a solution of Ni, Cu, and Zn enhanced the biogas yield from rice husks. Furthermore, a review by Demirel and Scherer (2011) showed that the stimulatory concentration of Co, Fe, Mo, Ni and Se ranged between 0.05 and 0.19, 0 and 0.39, 0.16 and 0.3, 0.11 and 0.25, and 0.062 mg kg⁻¹, respectively, for energy crop-fed biogas reactors. Thus, owing to differences in substrate composition,

operational conditions (i.e. OLR and HRT) and reactor design of the AD, the type and quantity of required metals differ considerably.

The optimum concentrations of trace metals to obtain a balanced micronutrient supply in the digesters are also hard to estimate due to the variety and complexity of the chemical reactions controlling their availability for uptake by the microorganisms. As a result, the choice of trace metals and their concentrations are mainly based on trial and error approaches. Recent attempts on optimization of trace metal dosing have been done for example, for biogas production from stillage by metal “dilute-out” approach to identify the minimum requirements of metals (Gustavsson et al. 2013a, Schmidt et al. 2014). In these approaches a cocktail solution of metals are dosed during the startup phase of reactor operations followed by omission of single metals in separate systems. As a result of deletion of the metal addition, the content of the target metal in the reactor is diluted. The concentrations of metals at the time before VFAs start to accumulate due to trace metal deficiency are then reported as minimum metal requirements by the process.

The effects of trace metal addition have mainly been shown on turnover of VFAs resulting in increased methane formation, while just a few studies have addressed their direct effect on rates of hydrolysis, protein-, fat- and carbohydrate degradation. For example, Kim et al. (2003) studied the effect of Ca, Fe, Co, and Ni addition on hydrolysis of simulated primary sludge (i.e. dog food with carbohydrate, protein, and fat contents of 45%, 21 %, and 8 %, respectively). They concluded that addition of these minerals resulted in higher rates of hydrolysis under both thermophilic and mesophilic conditions. For some more examples see section 3.6.1.1. In a review article on the effect of heavy metals on anaerobic digestion and biogas production from biomass by Mudhoo and Kumar (2013) the lack of information particularly on the effect of metals on hydrolysis stage of AD was highlighted.

Despite the differences in operational conditions of anaerobic digesters, there is a common concept relating all previous investigations on the effect of trace metal addition, i.e. *metal bioavailability*. Trace metals needs to be present in chemical species suitable for microbial uptake. An experiment performed within the BRC framework demonstrated that metal content in the substrate even at supposedly sufficient levels might not be enough to meet the metal requirement by the process. This has been demonstrated for AD of a substrate mix of slaughterhouse and organic household wastes, where process instability occurred even at relatively high Ni concentrations in the reactor. Despite the fact that Ni was present in the digester in high concentration, addition of Ni resulted in stabilization of the process and increase in biogas production rates. Thus, it was concluded that presence of a certain level of metals in the biogas reactors might not reflect the metal surplus or deficiency for microbial activities in the system. This emphasizes that the metal species available for microbial uptake might differ considerably depending on the biological and chemical conditions of the reactors. The importance of trace metal speciation in anaerobic digesters has been

recognized by many other researchers (e.g. Callander and Barford 1983b, Gonzalez-Gil et al. 2003; Jansen et al. 2005; Aquino and Stuckey 2007; Jansen et al. 2007; Fermoso et al. 2009; Gustavsson et al. 2013a,b; Shakeri Yekta et al. 2014b). In the listed studies, interaction of biogenic sulfide with trace metals has been identified as the main regulator of trace metal speciation in particular on Fe, Co, and Ni during AD. However, there is a lack of comprehensive information, where the metal requirements by the AD process are linked to the metal speciation and bioavailability in the biogas reactors. Thus, the critical bioavailability aspects of metals in biogas reactors are particularly addressed in this report.

6.1 Metals and organic matter interactions

To understand the processes controlling the bioavailability of trace metals, particular attention should be paid to microbial processes contributing to alteration and regulation of the chemistry of the anaerobic digester liquid. Microbial degradation of complex organic compounds results in production of soluble intermediate products and residues including a wide range of organic compounds with strong metal-binding affinity such as those containing carboxyl, phenolic, amino, and sulfidic functional groups (Hering and Morel 1990; Smith et al. 2002; Wang et al. 2003; Aquino and Stuckey 2006; Shakeri Yekta et al. 2012b). Furthermore, complex organic compound moieties in anaerobic digesters are influenced by certain chemical species affecting metal speciation and bioavailability. As an example, sulfurization of organic compounds by sulfide gives rise to thiol functional groups with very high metal-binding affinity (Variamurthy and Mopper, 1987). A few studies have indicated the importance of the characteristics and quantities of soluble organic matter for the bioavailability and uptake of metals during anaerobic digestion (e.g. Aquino and Stuckey 2007; Lee et al. 2008).

Furthermore, it is well-known that microorganisms are able to implement specific strategies to cope with metal deficiency (i.e. micronutrient starvation) or excess (i.e. intrinsic toxicity) by altering the chemical speciation of metals in their environment. When metals are available in low concentrations or in less bioavailable forms (e.g. under sulfidic condition and extensive metal-sulfide precipitation), the microorganisms are able to excrete strong metal-binding organic compounds to facilitate the uptake of essential metals. These extracellular organic compounds encompass strong metal binding properties and in many cases may result in enhanced solubility of metal-bearing minerals (Banfield et al. 1999). A well-studied example is microbial Fe acquisition by excretion of Fe-chelating siderophores and further uptake by cognate receptors (Krewulak and Vogel, 2008). Similar processes are believed to be responsible for the increase in Co and Ni solubility and bio-uptake under sulfidic conditions in natural environments (Saito et al. 2003; Waldron and Robinson, 2009). Some microorganisms have developed metal efflux mechanisms, which pumps excess intracellular metals out of the cell as a strategy against metal toxicity when exposed to high concentrations of metals (Rodrigue et al. 2005). Although this topic is well studied in disciplines such as organic chemistry, biogeochemistry and medicine, only a few studies have

targeted the effect of microbial stress response under metal deficiency/excess on metal speciation in biogas processes.

6.2. Theoretical determination of trace metal speciation and bioavailability

Application of thermodynamic equilibrium theories was among the first approaches taken to assess chemical speciation and bioavailability of metals in anaerobic digesters (e.g. Callander and Barford 1983a). However, chemical reactions included in thermodynamic models (i.e. precipitation/dissolution and complexation with inorganic ligands) were unable to predict solubility and, thus, speciation of metals in complex biochemical environment of anaerobic digesters (Callander and Barford 1983b; Jansen et al. 2007). Inclusion of organic complexes such as thiol (S-containing compounds), and those involving O and N functional groups was suggested for development of a representative chemical network of processes controlling the trace metal speciation during anaerobic digestion (Callander and Barford 1983b). Using thermodynamic models is well appreciated in the field of biogeochemistry and a large number of studies have been devoted to this subject (cf. Rickard and Luther 2006). However, application and development of a thermodynamic equilibrium approach, including major metal-binding organic and inorganic ligands, to assess trace metal speciation and bioavailability in anaerobic digesters is limited (e.g. Aquino and Stuckey 2007; Jansen et al. 2007; Shakeri Yekta et al. 2014b). Therefore, a theoretical base for representation of trace metals dynamics in anaerobic digesters is lacking.

6.3. Biochemical engineering of trace metal bioavailability

The biological uptake of metals can be enhanced by supplementing them in specific chemical forms, which are either suitable for direct bio-uptake or hampering undesirable and, thus, bio-uptake-limiting reactions such as mineral precipitation. The organometallic compounds are widely used to enhance the bioavailability of metals (Haas and Franz 2009). According to Ferguson and Deisenhofer (2004), three main membrane transport processes are responsible for bio-uptake of these compounds: a) organometallic complexes with low molecular weight and lipophilic in nature can be transported directly through the lipid membrane of the cells; b) organometallic complexes with weak and/or moderate metal-binding properties can be dissociated in the vicinity of the membranes and, thus, increase the inorganic and free ion concentrations nearby the metal-binding sites of the cells; c) organometallic compounds can form ternary metal complexes with the metal-binding sites of the cells followed by internalization via specific transport systems.

In this regard, bioavailability of organometallic complexes depends on their chemical properties regulating the interaction of these compounds with membrane-bound and other available ligands in the liquid. Aquino and Stuckey (2007) demonstrated that biomass can take up Fe and Cu in the presence of nitrilotriacetic acid (NTA), while addition of EDTA

inhibited the methanogenic activity. The stimulatory effect of NTA addition was attributed to the ability of metal-binding ligands in the membrane to compete with NTA for metals, while the inhibitory effect of EDTA was related to strong stability of metal-EDTA complexes hampering the binding of metals to metal-binding ligands in the membrane. Using a set of biogas yield tests, Vintiloiu et al. (2013) demonstrated that Ni bioavailability was improved when a Ni-EDTA complex was added to an anaerobic medium in comparison with cases where Ni^{2+} ions were supplied. However, similar effects were not observed for Co-EDTA complexes. Furthermore, depending on the chemical nature of the organometallic compounds, microorganisms are able to implement (i.e. activate/deactivate) different metal transport mechanisms. For example, Zn-cysteine in the presence of strong Zn-EDTA complexes are taken up by phytoplankton, while in the absence of Zn-EDTA, Zn-cysteine uptake is limited (Aristilde et al. 2012). It was suggested that the enhanced Zn-cysteine bioavailability was related to a shift in metal transport mechanism from histidine-rich (RNH_2) to cysteine-rich (RSH) binding sites with higher affinity for metals.

Accordingly, by combining knowledge on the chemical properties of organometallic compounds and microbial metal-assimilation mechanisms, trace metal solutions can likely be designed to avoid undesirable reactions (e.g. precipitation of metal-bearing minerals) and to trigger suitable microbe-metal interactions. Development and design of proper metal additive solutions should benefit from results of studies carried out for the engineering of metal bio-uptake in pharmaceutical research (e.g. Della Rocca et al. 2011; Sun et al. 2013). The effect of organometallic compounds on the quality of digestate applied as biofertilizer in agricultural practices should also be taken into account in developing trace metal additives.

Conclusions on “The biochemical dynamics of trace elements during anaerobic digestion processes”

Addition of trace metals have been shown to increase process efficiency by enhancing substrate digestion, biogas production, and low concentrations of intermediate fermentation products i.e. volatile fatty acids. The choice of metals needed and their optimum concentrations for efficient biogas production differ considerably for different processes and therefore, are hard to estimate. Providing comprehensive information, where metal requirements by the process are linked to their bioavailability in the biogas reactors is identified as a key knowledge area needed for maximizing the effect of metals added to biogas reactors, or in other words, to effectively deliver trace metals to microorganisms.

To do this, the chemical speciation and bioavailability of metals in relation to the operational conditions of the reactors and substrate characteristics need to be elucidated. This includes an understanding on how organic matter characteristics, which depends on the substrate used, effect metal chemistry and uptake by microorganisms.

Priorities/future research

- How is the bio-uptake of metals by microbial community regulated under metal deficiency and surplus?
- How may this fundamental knowledge be implemented in practice to devise metal-dosing strategies based on operational conditions and substrate characteristics of AD processes?
- Information about the effects of metal addition on kinetics and extent of different AD stages (e.g. hydrolysis as well as protein, fat, and carbohydrate degradation) is scarce and therefore concerted efforts are needed to study these effects.

7 Monitoring

Operation of stable and efficient anaerobic digestion (AD) processes mostly calls for monitoring control protocols. The monitoring is needed to observe microbial imbalances in the system and, thus, in the best of cases predict and prevent process disturbances from occurring. The microbial imbalances can be induced by variations in substrate composition and organic loading rate (OLR), temperature, hydraulic retention time etc. The level of monitoring needed is, at least partly, governed by the complexity of the process. AD-systems digesting a single substrate with a fixed composition and with small variations in OLRs need less sophisticated monitoring programs than systems treating more complex, variable substrates at high or fluctuating OLR. Monitoring parameters typically analyzed on-line at wastewater sludge and co-digestion AD-plants are pH, gas production and composition. Some plants also measure VFAs and/or alkalinity, but the time interval for the analysis varies considerably. An improved monitoring can be a tool when aiming at an increased biogas production at plants, which today are operated below their capacity. As an example the need for monitoring will likely increase in wastewater AD-systems, when including for example sorted household waste or frying fat as substrates and thereby increasing the loading rates. It should, however, be stressed that independent of the monitoring systems available today, highly skilled personnel with process knowledge is needed for a successful management of biogas plants aiming at an efficient process, when digesting complex substrate mixtures.

Parameters often discussed in connection to and applied for monitoring purposes of AD are as mentioned above pH, gas production and consumption, partial- and total alkalinity, volatile fatty acids (VFA; both total concentration and individual species) as well as ammonium levels and TS/VS, TOC or COD. Independent of the monitoring system used, representative sampling must of course be assured.

Monitoring parameters should ideally reflect early shifts in microbial activity and/or be warning signals in the form of increased or decreased concentrations of key substances. The

parameters can be measured directly (e.g. as a level of VFAs or ammonia) or e.g. indirectly via microwave- or near infrared (NIR)-spectra. The indirect measuring methods demand extensive calibration/reference measurements. Propionate is likely of special interest. In a study by Nielsen et al. (2007) propionate was shown to be a key parameter for early indications of process disturbances in a system overloaded with meat, bone meal and lipids.

There is today a lack of commercial on-line methods for several reasons. The corrosive environment constitutes a large problem, the complex and shifting matrix is likely another, and fouling of sensors is often a problem. On top of that there seems to be a low interest from the suppliers of equipment (Nordberg et al. 2010). The market is too small in combination with the complications given above.

Titration

Titration methods are often simple, cost-effective and rather quick (reviewed by Lahav and Morgan 2004). Depending on the titration method and software applied, different resolutions are obtained. Most common seems to be to estimate the partial (bicarbonate) alkalinity (PA) by titrating with acid to pH 5.75 (the bicarbonate concentration, which gives the buffering capacity of the digester liquid). Also total alkalinity (TA) is determined. The titration to pH 4.5 apart from the total concentration of carbonates also includes volatile fatty acids (VFA) that don't contribute to the buffering capacity of the system. TA is for this reason regarded as an insensitive monitoring parameter (reviewed by Jantsch and Mattiasson 2003). Several other parameters can also be measured by titration: Moosbrugger et al. (1993). titrated to four endpoints giving total VFA-, carbonate-, phosphor-, sulfur- and ammonium levels provided that the start-pH is known (this and a number of other methods are reviewed in Lahav and Morgan 2004).

Molina et al. 2009 evaluated a system for online titration determination of alkalinity and total VFA. Concentrations ranging 13-4900 mg VFA (acetate)/L and 2.5-49.5 mEq/bicarbonate were possible to quantify, while Feitkenhauer et al. (2001) was able to titrate VFAs levelling 10-50 mM with a 1% error on line. The titration-based methods are all of course dependent on a pH probe, which needs calibration and cleaning to avoid fouling.

Gas chromatography

On-line systems for analysis of VFA in both gas- and liquid samples have been presented. Pind et al. (2003) constructed a system for *in situ* membrane filtration automatically linked to a gas chromatograph. Individual VFAs in the range of 0-3000 mg/L could be quantified.

Boe et al. (2005) presented a method with head-space gas sampling from a special sample-pretreatment cell, where the conditions were adjusted to give a good volatilization of the VFAs from the liquid sample. The gas was then automatically analyzed by GC. A later communication reports that quantification could be done at levels between 5 and 150 mM for acetic acid, 0.7 to 40 mM for propionic acid, 0.3-20 mM for butyric and valeric acids and 0.1-10 mM for iso-butyric and iso-valeric acids (Boe et al. 2007). The system was later linked

with a rule-based supervisor and alarms set at 40 mM total VFA and 10 mM propionate. The system could guide an effective biogas production as long as the system stayed within the operational range. However, the system could not separate decreases in biogas production due to substrate variation from those due to process disturbances, why a further development is needed before application in full scale.

Sensors

Technologies, where sensors are applied can roughly be divided into: 1) simple, reliable and low maintenance systems (i.e. pH, temperature and redox) and 2) advanced sensors or array of sensors that generally require maintenance and calibration by qualified personnel/consults (reviewed by Madsen et al. 2011).

7.1.1 Fluorescence spectroscopy

Fluorescence spectroscopy can be used to measure signals from NAD(P)H in the liquid and can, according to Peck and Chyoweth (1992), give early warnings to changes in bacterial activity. Probes for quantification of co-enzyme F420 are also available (present in methanogens (reviewed by Madsen et al. 2011)). More complex probes, where several excitation and emission wavelengths are monitored have, together with multivariate analysis, been shown to enable predictions of both VFA and COD with acceptable accuracy (Morel et al. 2004).

7.1.2 Infrared spectroscopy

Infrared spectroscopy can be used for determination of VFA, COD, TOC, total and partial alkalinity (Steyer et al. 2002). Ultra-filtration was needed and an extensive on-line calibration with different “constructed” operating conditions likely to occur was performed.

7.1.3 Near infrared spectroscopy

Near infrared spectroscopy (NIR) has been reported from several lab- (Nordberg et al. 2000; Hansson et al., 2002; Holm-Nielsen 2006; Lomborg et al. 2009) and pilot-scale studies (Holm-Nielsen et al. 2007). Advantages with NIR is the possibility to measure in matrixes with high dispersion or adsorption, thus, highly colored/dark solutions and also the ability to measure on mixes of solids and dissolved material. The results are obtained on a minute basis, but as the method is indirect an extensive calibration with reference methods are needed. The sensor is made of stainless steel and sapphire glass, which makes it resistant to corrosion. The most frequent parameters monitored seems to be total VFA, acetate and propionate, but also detection of ammonium- and total nitrogen and TS and VS have been reported (Holm-Nielsen et al. 2006, 2007). Holm-Nielsen et al. (2006) also tried to quantify COD with NIR, but without success. NIR has also been shown to work *in-situ* in full-scale. The sensor was calibrated for total VFA, acetate and propionate in sludge samples (Jacobi et al. 2009). Nordberg et al. (2012) reported initial investigations with a commercial TF-NIR instrument at a full scale plant at Borås Energi och Miljö AB, in Borås, Sweden. Polytec is marketing NIR for monitoring of biogas processes.

7.1.4 Chemical multisensory systems (electrical tongues and noses)

Chemical multisensors have been applied for characterization of both gas (Nordberg et al. 2000) and liquid phases (Buczkowska 2010) of AD processes. Nordberg et al. could measure methane, hydrogen gas, acetate and propionate with an array of chemical gas-sensors, while Buczkowska et al. (2010) could construct acceptable calibration models for VFA, COD and pH using an array of chemical sensors mounted in a flow-cell that could be placed in a recirculation loop. In both cases multivariate analytical methods were applied to treat the data. It should also be noted that liquid sensors often are regarded as having lower detection limits (sometimes down to ppb or ppt levels) but higher selectivity than gas sensors. Rudnitskaya and Legin (2008)

7.1.5 Acousticchemometrics

Acousticchemometrics might, according to a review by Madsen et al. (2011), become a future way to measure dry matter dynamics and particulate matter in the reactors as well as assess the corrosion and wear of equipment used. The same authors also suggest **Raman technology** (a complement to IR spectroscopy) as suitable for AD-monitoring in future applications.

7.1.6 Evaluation of commercial systems

Nordberg et al. (2010) presented results from tests with a number of commercially available sensors at the full scale plant at Sobacken in Borås. The plant co-digests a mix of organic wastes that varies over time and the goal was to be able to increase the load from 1.5 to 4.5 ton VS per m³ reactor volume and day. The project goal was to identify and evaluate robust methods for on-line monitoring.

Substrate storage tanks and reactors were identified as the most interesting to monitor and the following priorities were listed where “1” is the most desired:

Measurements in the substrate tank

1. The substrate composition (fat, protein and carbohydrates content) and dissolved organic material (e.g. as VFA).
2. Organic material as COD
3. Organic material as VS or as TOC
4. Total solids

Measurements in the reactor

1. Specific VFA (propionate most important)
2. Total VFA
3. Alkalinity (demands pH and automatic titration)
4. Ammonium nitrogen

For the **substrate tank** an analysis frequency of 4-5 hour was judged reasonable/needed at the prevailing feeding frequency including time for sample preparation (filtration, homogenization etc.) before analysis.

The tests/evaluations performed in relation to the priorities for the substrate tank was:

- TS-measurements at high VFA-levels
 - The VFA-losses were found to be high in many untreated samples (up to 100% losses), which could correspond to as much as 15-20% of the substrate TS. pH-adjustments to minimize the VFA-losses were shown to work but too time demanding for full scale applications. A report from SGC (SGC Report 2013:273) recommends a separate VFA-analysis as a complement to TS/VS analysis in samples with high VFA-content.
- NIR for determination of substrate composition (fat, protein and carbohydrates content, TS, VS, ammonium-nitrogen and VFA).
 - The results from the chemical analysis showed an underestimation of TS/VS in the samples as VFA is lost during the analysis. This affects both the carbohydrate content (which was underestimated) and the gas yields, that are often given per amount VS, and thus overestimated. The NIR-analysis was shown to be able to predict the VS-content of the substrate at a 78% accuracy.
- TOC and COD measurements, where homogenization of the substrate was shown important for on-line applications. Determination of TOC by UV-NIR (S::CAN) was tested and an instrument from LAR Process Analyzers was used for sample homogenization.
 - The correlation between VS and TOC was found to be good (R2 of 92%).
 - The correlation between VS and COD had a R2 of 71%. This lower correlation was expected as fat, protein and carbohydrates have different COD-values (2.91, 1.5 and 1.1 g COD/g VS respectively), but the results were also partly due to uncertainty in the COD-analysis compared to VS determination.
 - The sensor from S::CAN could not be used to estimate TS or TOC. The company suggested that a more extensive calibration was needed, but was not interested in a continued collaboration.
 - Three different homogenization techniques (sedimentation combined with mixing and/or milling) were tested, but none of them gave samples that could be filtered through an 800- μ m filter.
 - COD-measurements performed after sequential filtration of homogenized samples showed that a factor of 2.3 was needed to compensate the values from the Weedo-instrument in relation to reference measurements.
- The possibility to use impedance as a measure of TS was evaluated using an instrument from IMEGO and also a microwave technique from *hf sensor GmbH* was evaluated as a means to follow the TS-level in the substrate tank.
 - Results indicate that impedance can be used to determine TS, but it seems that mainly dissolved TS is caught by the method and it could therefore not be recommended for measurement of total TS, but might be used as a complement to total TS as it will include the VFA.

- TS determination by use of microwaves was promising, but problems with the sensor function only made two short measurements possible and further analysis was needed for a proper evaluation.

Techniques used in reactors

- An ion-selective electrode for determination of ammonium levels tested in lab.
 - Did not work satisfactory over time.
- NIR both in lab- and full-scale: A FT (fourier transform spectroscopy)-NIR instrument from Q-interline was used for the full-scale tests.
 - Both propionate and ammonium showed good correlation in regression analyses, where propionate had a more even frequency distribution leading to a higher predictability than for ammonium. Although promising, the tests with the FT-NIR in full-scale could not be performed as planned due to process problems. The dynamics in organic loading could therefore not be measured within the project, but continued tests were planned for.

The report gives a list of potential technical providers (Appendix B in Nordberg et al. 2010)

Conclusions on Monitoring

Why are there so few applications of the on-line monitoring techniques available (besides pH, temperature and biogas production) for full scale plant operation?

- Complex systems (NIR, e-noses etc.) often need expertise for calibration, validation and multivariate analysis.
- Problems with
 - representative sampling. This is a major problem that is rarely recognized in evaluations/reports according to Madsen et al. (2011).
 - appropriate sensor location. Holm-Nielsen (2006) recommends a recurrent loop sampling concept (sampling in up-flow vertical pipelines as an up-ward flow gives a mixing/turbulence facilitated by gravity).
 - disturbance from particles. Ultrafiltration or similar techniques are demanded for many applications.
 - gas bubbles.
 - fouling, corrosion and erosion of sensors
 - poisoning of electrochemical membranes
- Costs: the application should be both practical and inexpensive.
- The need is not seen?
- Lack of good examples?
- Hard to attract companies for development and application of possible techniques (Nordberg et al. 2012).

Priorities on *Monitoring*

- Evaluations of full scale applications
 - Can more methane be produced per m³ reactor volume (i.e. increased loading) in systems with process monitoring?
 - Do the costs match the profits?

Appendix 1

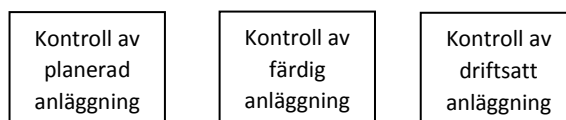
Översikt för tillståndprocesser för byggnation och drift av biogasanläggningar i Sverige

- Tillståndsprövning enligt **miljöbalken** (miljöprövning), olika prövningsnivåer (MB; Naturvårdsverket; Tabell 2)
A Tillstånd krävs, ansökan till Miljödomstolen
B Tillstånd krävs, ansökan till Länsstyrelsen
C Anmälningsskyldigt, prövas av kommunens miljönämnd
- Tillstånd enligt **Seveso**. Ansökan till Myndigheten för samhällsskydd och beredskap (MSB). Seveso är lagen och förordningen om åtgärder för att förebygga och begränsa följderna av allvarliga kemikalieolyckor.
- Tillstånd enligt **Lagen om brandfarliga och explosiva varor** (LBE) krävs både för framställning och uppgradering av gas. Ansökan till Myndigheten för samhällsskydd och beredskap (MSB).
- Bygglagen enligt **Plan och bygglagen** (PBL) Ansökan till Boverket.
- Arbetsmiljölagen**. Ansökan till Arbetsmiljöverket
- Ellagen** gäller elektriska anläggningar, elsäkerhet men också i vissa fall handel med el. Ansökan till Elsäkerhetsverket
- Lagen om Skydd mot olyckor** innehåller regler som syftar till ett tillfredställande skydd mot brand, viss farlig verksamhet mm. Ansökan till Myndigheten för samhällsskydd och beredskap (MSB)
- Förordningar om animaliska biprodukter**. Råmaterial av animaliskt ursprung måste normalt hygieniseras (70° i 1 h).

Tabell 1. Vad gäller för de olika prövningsnivåerna i tillståndsprövning enligt miljöbalken?

	A	B	C
Framställning av gasformigt bränsle (per år)	-	>150 000m ³	≤150 000 m ³
Förbränning av enbart gas (total installerad effekt)	-	-	
Förbränning av gas + annat bränsle	-	-	
Gasturbin (total installerad effekt)	-	-	
Gas avsedd som motorbränsle (per år)	-	-	>1 000 000 Nm ³
Biologisk hantering (per år)	>100 000 ton	>500 ton	>10 ton
Återvinning (rötning; avfall per år)	-	-	≤10ton

Anvisningar för vilka tillstånd som behövs för byggnation och drifttagande av en anläggning för biogasproduktion finns i BGA 2012 "Anvisningar för biogasproduktion" utgiven av "Energigas Sverige". Figur 2 ger en översikt över aktuella lagar och förordningar samt kontrollsteg.



Planering	Färdigställande	Drift	
Lag (ansvarig myndighet)			
MB (NV)	Tillstånd/Anmälan		Tillsyn
Seveso (MSB)	Tillstånd/Anmälan		Tillsyn
LBE (MSB)	Tillstånd	Avsyrning	Tillsyn
PBL (Boverket)	Bygglövsansökan	Slutbesked	
AML (AV)	Konstruktionskontroll	Anläggningskontroll	Återkommande besiktning
ELL (ELSÄK)		Elbesiktning	
LSO (MSB)		Redogörelse för brandskydd	Tillsyn

Figur 2. Översikt över lagar och förordningar som appliceras i samband med byggnation och drift av en biogasanläggning. Källa: BGA2012 "Anvisningar för biogasproduktion". **MB**=miljöbalken, **NV**=Naturvårdsverket, **LBE**=lagen om brandfarliga och explosiva varor, **MSB**=Myndigheten för samhällsskydd och beredskap, **PBL**=plan och bygglagen, **AML**=arbetsmiljölagen, **AV**=arbetsmiljöverket, **ELL**=ellagen, **ELSÄK**=Elsäkerhetsverket, **LSO**=lagen om skydd mot olyckor

Översikt över styrmedel som rör biogasproduktion

Energimyndighetens rapport "Analys av marknaden för biodrivmedel" ES 2013:08 ger en översikt över fordonsgasmarknaden och listar de styrmedel som berör biogasproduktionen.

Man konstaterar bl a att:

Den ökande produktionen av biogas för användning som fordonsgas kan härledas till politiska beslut med strävan att 1) minska oljeberoendet, 2) hantera kommunala miljö och avfallsproblem, 3) stärka jordbrukssektorn, 4) minska CO₂-utsläppen från transportsektorn (större tyngd under senare år) och att det inte skulle finnas någon fordonsgasmarknad (och sannolikt en mycket liten biogasproduktion överhuvudtaget) utan politiska styrmedel.

Energimyndigheten ser också att marknaden för fordonsgas kan komma att minska, eftersom de "bästa" substraten nu till stor del är intecknade och att de flesta nya satsningar därför kommer att kräva större investeringar. Konkurrensen om de "bästa" substraten ökar också mellan aktörerna och sannolikt av kommande direktiv på EU-nivå om begränsningar för användning av biodrivmedel framställt från mat- och fodergrödor samtidigt som man uppmuntrar användningen av avfall- och restprodukter ökar troligen på konkurrensen om de "bästa" substraten än mer. Till detta kommer att utrymmet att höja priset på fordonsgas till följd av ökande kostnader är begränsat och avgörs främst av priset på bensin och diesel.

För att undvika en minskad marknad konstaterar Energimyndigheten därför att nya styrmedel som branschen uppfattar som tillräckligt bra och långsiktiga behövs.

Styrmedel

EU-nivå

Tabell 4 i ES 2013:08 ger översikt

- **Energiskattedirektivet** anger hur medlemsstaterna ska beskatta energivaror och bränslen och direktivet har därför en stor, direkt inverkan på marknaden för fordonsgas. Nuvarande direktiv anses dock föråldrat och ett nytt är på gång. I det nya direktivet skiljer man tydligt på skatt kopplat till CO₂-utsläpp och skatt baserad på produktens energiinnehåll. För biodrivmedel innebär detta att man befrias från CO₂

skatt men betalar energiskatt. Möjligheten att behålla energiskattelättnader för biodrivmedel föreslås behållas fram tom 31 dec 2023.

- **Förnybarhetsdirektivet** har som mål att minst 10% av den slutliga energianvändningen i transportsektorn skall utgöras av förnybara energislag 2020. För att klassas som hållbara måste en energiprodukt uppfylla ett antal hållbarhetskriterier. Fordonsgas, som framställs ur restprodukter och avfall, räknas dubbelt mot %-målet.
- **Svaveldirektivet** reglerar svavelutsläpp inom sjöfarten. Direktivet styr mot lågsvavlig marin dieselolja med flytande LNG som ett möjligt alternativ. Dock är det dyrt och tidskrävande att ställa om samtligt som tillgången på LNG är begränsad.
- Tillägg (artikel 7a) i **Bränsledirektivet** föreslås ställa krav på drivmedelsleverantörer att minska växthusgasutsläppen med 6% till 2020. Tillägget kan få betydelse för biogasleverantörer. Direktivet i sin nuvarande form dock inte ett av de starkare styrmedlen för biodrivmedel.
- **Direktivet om främjande av rena och energieffektiva fordon** har i Sverige genomförts genom Lag 2011:846 som ställer miljökrav vid upphandling av bilar och vissa kollektivtjänster.
- **Infrastrukturdirektivet**, där förhandlingar pågår, är tänkt att ställa krav på att det skall finnas minimiinfrastrukturer och laddningsstationer/tankstationer för elbilar respektive naturgas (LNG och CNG) och vätgas senast 2020.

Styrmedel på nationell nivå

(Tabell 5 i ES 2013:08 ger översikt)

- **Lagen om skatt på energi** (LSE) reglerar bränsle- och elskatter och är anpassad till energiskattedirektivet. LSE inkluderar bestämmelser för energi- och CO₂-skatt samt skattebefrielse för biodrivmedel och har därigenom en stor, direkt påverkan på biogasmarknaden. Skattebefrielsen förutsätter att kraven i **lagen om hållbarhetskriterier för biodrivmedel och flytande biodrivmedel** uppfylls.
- Genom **förordningen om statligt stöd till åtgärder för produktion, distribution och användning av biogas och andra förnybara gaser** ger Energimyndigheten ett "marknadsintroduktionsstöd för ny och effektiv teknik för att öka produktionen, distributionen och användningen av förnybar biogas". Energimyndigheten stödjer också olika typer av energiforskning, man disponerar årligen ca en miljard kronor. Regeringspropositionen för forskning och innovation för ett långsiktigt hållbart energisystem (**prop 2012/13:21**) anger riktlinjer för det fortsatta forskningsstödet.
- Inom **Landsbygdsprogrammet** utgår från i år ett metanreduceringsstöd till biogasproduktion från gödsel på 20 öre/kWh producerad energibärare från stallgödsel. Mellan 2007 och 2013 delade Landsbygdsprogrammet ut investeringsstöd till gårdsbaserad biogas eller förädling.
- Fordonskattebefrielsen är ett styrmedel riktat till konsumenten och är kopplad till miljöbilsdefinitionen i vägtrafikskattelagen och har till syfte att främja inköp av bilar, lätta lastbilar och lätta bussar med låga utsläpp av koldioxid, kolmonoxid, kolväten,

kväveoxider och partiklar. Även den "**nedsetta förmånsbeskattningen**" för vissa miljöbilar, som regleras via inkomstskattelagen och **lagen om miljökrav vid upphandling av bilar och vissa kollektivtrafiktjänster** främjar bilar med låga utsläpp.

Utöver ovan listade styrmedel finns i Sverige även styrmedel på regional och kommunal nivå, ofta länkade till regionala eller kommunala färdplaner för biodrivmedel/biogas.

Aktörer

Statliga aktörer

- **Energimyndigheten** "ska verka för en kostnadseffektiv svensk energiförsörjning i kombination med en effektiv och hållbar energianvändning". Man utfärdar stöd till forskning, demonstration och investering för fordonsgas och ansvarar för hållbarhetskriterierna rörande biogasandelen i fordonsgas.
- **Energimarknadsinspektionen** har tillsynsansvar för den svenska naturgasmarknaden och "ska främja en fungerande handel med gas i konkurrens"
- **Jordbruksverket och Naturvårdsverket** har ansvar för lagstiftning som rör biogas och utfärdar även allmänna råd i ämnet.
- **Transportstyrelsen**

Regionala aktörer

- **Länsstyrelserna** har ansvar för rådgivning, tillståndsgivning och handläggning främst gällande småskalig biogasproduktion
- **Biogas "Väderstreck"** är organisationer, som sprider kunskap och erfarenheter, arbetar för samverkan och driver projekt tillsammans med länsstyrelser, kommuner, energibolag och övrigt näringsliv samt forskare inom universitet och högskolor.

Branschorganisationer

- **Energigas Sverige** är remissinstans för miljö-, energi- och försörjningsfrågor.
- **Avfall Sverige** arbetar med avfallshandlingens hela kedja. Insamling av vissa typer av substrat och rötning till biogas ingår i detta.
- **Svenskt vatten** företräder svenska vattentjänstföretag. Remissinstans och tar fram rapporter och strategier för slamhantering och slamanvändning (inkl. biogasproduktion) för slam genererat i vatten- och avloppshandlingen.
- **Lantbrukarnas Riksförbund**

Övriga

- **Swedegas** bildades av regering 1976 och ägs numer av fonder förvaltade av EQT Infrastructure. Swedegas äger sedan 2011 hela det svenska naturgasnätet.
- **Svenskt gastekniskt centrum (SGC)** ägs gemensamt av aktörer inom den svenska gasbranschen och har till uppgift att samordna och leda del av den svenska forskningen och utvecklingen inom energigasområdet. Till detta har man medel från Energimyndigheten

Appendix 2

Education, literature and standard methods

Education and courses

The polytechnic collage (yrkeshögskola) in Hallsberg offers a biennial biogas-technician education. The polytechnic collage in Varberg have a one year course for biogas-planners (biogasprojektör) and a biennial biogas and waste water treatments technician education.

WES at Linköping University gives a 5 weeks (10 weeks a “half time”) course on “The biogas process”. The course is primarily addresses students on the civil engineer programs.

Avfall Sverige offers courses related to production of biogas.

Energigas Sverige offers the following courses for personnel handling “energy gases” (natural gas, biogas, LPG (gasol), and hydrogen gas):

- Introduktionskurs energigaser
- Föreståndare biogas
- Föreståndare LNG/LBG
- Föreståndare tankstationer
- Seminarier för räddningstjänsten
- Fortbildning för behöriga Besiktningsmän
- Fortbildning för behöriga Installationsledare

Lantbrukarnas riksförbund (LRF), SGC, and regional actors (Biogas ”Väderstreck”) etc. offers shorter courses and seminars within the field of biogas production.

Literature

There are a number of Swedish handbooks within the biogas field:

Anvisningar för biogasanläggningar Energigas Sverige

Substrathandboken SGC report 200

Gårdsbiogashandbok SGC report 206

Mikrobiologisk handbok för biogasanläggningar SGC report 207

Handbok metanpotential SGC report 237

SGC have a large number of reports within the biogas field and there are also a lot of reports linking to biogas production from ”Waste refinery”.

8 References

- Aldin S, Nakhla G, Ray MB (2011) Modeling the influence of particulate protein size on hydrolysis in anaerobic digestion. *Ind Eng Chem Res* 50:10843-10849
- Alves MM, Pereira MA, Sousa DZ, Cavaleiro AJ, Picavet M, Smidt H, Stams AJM (2009) Waste lipids to energy: How to optimize methane production from long-chain fatty acids (LCFA). *Microbial Biotechnol* 2:538-550
- Anderson GK, Yang G (1992) Determination of bicarbonate and total volatile acid concentration in anaerobic digesters using a simple titration. *Water Environ Res* 64:53-59
- Andric P, Meyer AS, Jensen PA, Dam-Johansen K (2010) Reactor design for minimizing product inhibition during enzymatic lignocellulose hydrolysis. II. Quantification of inhibition and suitability of membrane reactors. *Biotechnol Adv* 28:407-425
- Angelidaki I, Ellegaard L, Ahring BK (1993) A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: Focusing on ammonia inhibition. *Biotechnol Bioeng* 42:159-166
- Aquino SF, Stuckey DC (2007) Bioavailability and toxicity of metal nutrients during anaerobic digestion. *J Environ Eng* 133:28-35
- Aquino SF, Stuckey DC (2006) Chromatographic characterization of dissolved organics in effluents from two anaerobic reactors treating synthetic wastewater. *Water Sci Technol* 54:193-198
- Aristilde L, Xu Y, Morel FMM (2012) Weak organic ligands enhance zinc uptake in marine phytoplankton. *Environ Sci Technol* 46:5438-5445
- Avfall Sverige (2012) Verkt yg f r att s kerst lla l g Cd-halt i bio-g dsel Report B 2012/02
- Baba Y, Tada C, Fukuda Y, Nakai Y (2013) Improvement of methane production from waste paper by pretreatment with rumen fluid. *Bioresour Technol* 128:94-99
- Banfield JF, Barker WW, Welch SA, Taunton A (1999) Biological impact on mineral dissolution: Application of the lichen model to understanding mineral weathering in the rhizosphere. *Proc Natl Acad Sci USA* 96:3404-3411
- Banks CJ, Zhang Y, Jiang Y, Heaven S (2012) Trace element requirements for stable food waste digestion at elevated ammonia concentrations. *Bioresour Technol* 104:127-135
- Bao L, Huang Q, Chang L, Zhou J, Lu H (2011) Screening and characterization of a cellulase with endocellulase and exocellulase activity from yak rumen metagenome. *J Molec Catal B* 73:104-110
- Barakat A, Monlau F, Steyer J, Carrere H (2012) Effect of lignin-derived and furan compounds found in lignocellulosic hydrolysates on biomethane production. *Bioresour Technol* 104:90-99
- Barber W.P (2005) Anaerobic digester foaming: causes and solutions. *Water* 21:45-59
- Baserba MG, Angelidaki I, Karakashev D (2012) Effect of continuous oleate addition on microbial communities involved in anaerobic digestion process. *Bioresour Technol* 106:74-81
- Baudez J-C, Coussot P (2001) Rheology of aging, concentrated, polymeric suspensions: Application to pasty sewage sludges. *J Rheol* 45(5): 1123-1139

Bayr S, Pakarinen O, Korppoo A, Liuksia S, Väisänen A, Kaparaju P, Rintala J (2012) Effect of additives on process stability of mesophilic anaerobic monodigestion of pig slaughterhouse waste. *Bioresour Technol* 120:106-113

Bernstad A, Malmquist L, Truedsson C, la Cour Jansen J (2013) Need for improvements in physical pretreatment of source-separated household food waste. *Waste Manage* 33:746-754

Bernstad A, la Cour Jansen J (2012) Separate collection of household food waste for anaerobic degradation - Comparison of different techniques from a systems perspective. *Waste Manage* 32:806-815

Björkmalm J (2013) Kartläggning av tekniska och processrelaterade problem och dess utvecklingsmöjligheter vid biogasanläggningar. Master thesis, Linköpings University

Björn A, Segura de La Monja P, Karlsson A, Ejlerthsson J, Svensson B.H (2012a). Rheological characterization. Chapter 3 in: *Biogas*, Editor Kumar S, In: Tech publisher, Rijeka, Croatia, ISBN 979-953-307-221-9

Björn A, Karlsson A, Shakeri Yekta S, Danielsson Å, Ejlerthsson J, Svensson B.H (2012b) Rheological characteristics of reactor liquids of 12 full-scale biogas reactors. *International Conference on Applied Energy (ICAE)*, July 5-8, 2012, Suzhou, China

Björnsson L (2012) Energigrödor för biogasproduktion Del 1 Odling och arealeffektivitet. Report no 80 from the Department of Technology and Society, Environmental and Energy System studies.'

Björnsson L, Lantz M (2013) Energigrödor för biogasproduktion Del 2 Kostandseffektivitet och styrmedel. Report no 81 from the Department of Technology and Society, Environmental and Energy System studies.

Boe K, Batstone DJ, Angelidaki I (2007) An innovative online VFA monitoring system for the anerobic process, based on headspace gas chromatography. *Biotechnol Bioeng* 96:712-721

Boone DR, Chynoweth DP, Mah RA, Smith PH, Wilkie AC (1993) Ecology and microbiology of biogasification. *Biomass Bioenerg* 5:191-202

Bremer M, Kraume M (2012) Mixing performance in biogas plants. 14th European Conference on Mixing, September 10-13, 2012, Warszawa, Poland

Buczowska A, Witkowska E, Górski L, Zamojska A, Szewczyk KW, Wróblewski W, Ciosek P (2010) The monitoring of methane fermentation in sequencing batch bioreactor with flow-through array of miniaturized solid state electrodes. *Talanta* 81:1387-1392

Bugg TDH, Ahmad M, Hardiman EM, Rahmanpour R (2011) Pathways for degradation of lignin in bacteria and fungi. *Nat Prod Rep* 28:1883-1896

Burton C.H, Turner C (2003) *Manure management treatment strategies for sustainable agriculture*, Second ed. Silsoe Research Institute

Busch G, Großmann J, Sieber M, Burkhardt M (2009) A New and sound technology for biogas from solid waste and biomass. *Water, Air, and Soil Poll: Focus* 9:89-97

Buzzini AP, Pires EC (2007) Evaluation of a upflow anaerobic sludge blanket reactor with partial recirculation of effluent used to treat wastewaters from pulp and paper plants. *Bioresour Technol* 98:1838-1848

Callander IJ, Barford JP (1983a) Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. I. Methodology. *Biotechnol Bioeng* 25:1947-1957

- Callander IJ, Barford JP (1983b) Precipitation, chelation, and the availability of metals as nutrients in anaerobic digestion. II. Applications. *Biotechnol Bioeng* 25:1959-1972
- Carlsson M, Lagerkvist A, Morgan-Sagastume F (2012) The effects of substrate pre-treatment on anaerobic digestion systems: A review. *Waste Manage* 32:1634-1650
- Carrère H, Dumas C, Battimelli A, Batstone DJ, Delgenès JP, Steyer JP, Ferrer I (2010) Pretreatment methods to improve sludge anaerobic degradability: A review. *J Hazard Mater* 183:1-15
- Cavaleiro AJ, Pereira MA, Alves M (2008) Enhancement of methane production from long chain fatty acid based effluents. *Bioresour Technol* 99:4086-4095
- Cavaleiro AJ, Ferreira T, Pereira F, Tommaso G, Alves MM (2013) Biochemical methane potential of raw and pre-treated meat-processing wastes. *Bioresour Technol* 129:519-525
- Cheng K, Stewart C, Dinsdale D, Costerton J (1984) Electron microscopy of bacteria involved in the digestion of plant cell walls. *Animl Feed Sci Tech* 10: 93-120
- Collins T, Gerday C, Feller G (2005) Xylanases, xylanase families and extremophilic xylanases. *FEMS Microbiol Rev* 29:3-23
- Conteau D, Gordon J J, Escndié R, Battimelli A, Tornjos M, Martin Ruel S, Camacho P, Steyer J P (2013) Cow and termite digestion mimicking: from animal digestive tracts to lab-scale pilot. In *Proceedings from Anaerobic digestions conference AD13*. Santiago de Compstela, Spain June 25th-28th
- Crichton RR (2008) *Biological Inorganic Chemistry An introduction*. Elsevier, Amsterdam
- Cuetos MJ, Gómez X, Otero M, Morán A (2010) Anaerobic digestion and co-digestion of slaughterhouse waste (SHW): Influence of heat and pressure pre-treatment in biogas yield. *Waste Manage* 30:1780-1789
- Cuetos MJ, Gómez X, Otero M, Morán A (2008) Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). *Biochem Eng J* 40:99-106
- Dahlgren S, Liljeblad A, Cerruto J, Nohlgren I, Starberg K (2013) Realiserbar biogaspotential i Sverige år 2030. Report from WSP
- De Bok F, Plugge C, Stams A (2004) Interspecies electron transfer in methanogenic propionate degrading consortia. *Water Res* 38: 1368-1375
- De Moor S, Velghe F, Wierinck I, Michels E, Ryckaert B, De Vocht A, Verbeke W, Meers E (2013) Feasibility of grass co-digestion in agricultural digester, influence on process parameters and residue composition. 9th International Conference on Renewable Resources and Biorefineries, June 5-7, 2013, Antwerp, Belgium
- Dębowski M, Zieliński M, Grala A, Dudek M (2013) Algae biomass as an alternative substrate in biogas production technologies - Review. *Renew Sust Energ Rev* 27:596-604
- Della Rocca J, Liu D, Lin W (2011) Nanoscale metal-organic frameworks for biomedical imaging and drug delivery. *Acc Chem Res* 44:957-968
- Demain AL, Newcomb M, Wu JHD (2005) Cellulase, clostridia, and ethanol. *Microbiol Mol Biol R* 69:124-154

Demirel B, Scherer P (2011) Trace element requirements of agricultural biogas digesters during biological conversion of renewable biomass to methane. *Biomass Bioenerg* 35:992-998

Desvaux M (2005) *Clostridium cellulolyticum*: Model organism of mesophilic cellulolytic clostridia. *FEMS Microbiol Rev* 29:741-764

Dohanyos M, Zabranska J, Kutil J, Jenicek P (2004) Improvement of anaerobic digestion of sludge. *Water Sci Technol* 49(10): 89-96

Doherty WOS, Mousavioun P, Fellows CM (2011) Value-adding to cellulosic ethanol: Lignin polymers. *Ind Crops Prod* 33:259-276

Donoso-Bravo A, Mairet F (2012) Determining the limiting reaction in anaerobic digestion processes. How has this been tackled?. *J Cheml Technol Biot* 87:1375-1378

Driessen WJBM, Wasenius C- (1994) Combined anaerobic/aerobic treatment of peroxide bleached TMP mill effluent. *Water Sci Technol* 29:381-389

Dufresne R, Liard A, Blum MS (2001) Anaerobic treatment of condensates: Trial at a kraft pulp and paper mill. *Water Environ Res* 73:103-109

Edström M, Nordberg Å, Thyselius L (2003) Anaerobic treatment of animal byproducts from slaughterhouses at laboratory and pilot scale. *Applied Biochemistry and Biotechnology - Part A Enzyme Eng Biotechnol* 109:127-138

Eiroa M, Costa JC, Alves MM, Kennes C, Veiga MC (2012) Evaluation of the biomethane potential of solid fish waste. *Waste Manage* 32:1347-1352

Ekstrand E-, Larsson M, Truong X-, Cardell L, Borgström Y, Björn A, Ejlerstson J, Svensson BH, Nilsson F, Karlsson A (2013) Methane potentials of the Swedish pulp and paper industry - A screening of wastewater effluents. *Appl Energy* 112:507-517

Elbeshbishy E, Nakhla G (2012) Batch anaerobic co-digestion of proteins and carbohydrates. *Bioresour Technol* 116:170-178

Eshtiagi N, Baudez J.C, Slatter P (2013) Rheological behavior of anaerobic sludge: impact of concentration and temperature. *World Congress on Anaerobic Digestion (AD13)*, June 25-28, 2013, Santiago de Compostella, Spain

Facchin V, Cavinato C, Fatone F, Pavan P, Cecchi F, Bolzonella D (2013) Effect of trace element supplementation on the mesophilic anaerobic digestion of foodwaste in batch trials: The influence of inoculum origin. *Biochem Eng J* 70:71-77

Feitkenhauer H, Von Sachs J, Meyer U (2002) On-line titration of volatile fatty acids for the process control of anaerobic digestion plants. *Water Res* 36:212-218

Feizaghaei R, Ammenberg J, Baas L, Eklund M, Helgstrand A, Marshall R (2014) Improving the CO₂ performance of cement, part II: framework for assessing CO₂ improvement measures in the cement industry. *J of Cleaner Production* xxx (2014) 1-10 (in press).

Ferguson AD, Deisenhofer J (2004) Metal Import through Microbial Membranes. *Cell* 116:15-24

Fermoso FG, Bartacek J, Jansen S, Lens PNL (2009) Metal supplementation to UASB bioreactors: from cell-metal interactions to full-scale application. *Sci Total Environ* 407:3652-3667

Ferrer I, Serrano E, Ponsá S, Vázquez F, Font X (2009) Enhancement of thermophilic anaerobic sludge digestion by 70° pre-treatment: Energy considerations. *Jl Res Sci Technol* 6:11-18

Fleurence J (1999) Seaweed proteins: Biochemical, nutritional aspects and potential uses. Trends Food Sci Tech 10:25-28

FNR (2010) Biogas-Messprogramm II, 61 Biogasanlagen im Vergleich. Fachagentur Nachwachsende Rohstoffe e. V., Gülzow, Germany. ISBN 978-3-9803927-8-5 (in German).

Fonknechten N, Chaussonnerie S, Tricot S, Lajus A, Andreesen JR, Perchat N, Pelletier E, Gouyvenoux M, Barbe V, Salanoubat M, Le Paslier D, Weissenbach J, Cohen GN, Kreimeyer A (2010) *Clostridium sticklandii*, a specialist in amino acid degradation: Revisiting its metabolism through its genome sequence. BMC Genomics 11

Foster C.F (2002) The rheological and physico-chemical characteristics of sewage sludges. Enzyme Microbiol Technol 30: 340-345

Fox P, Pohland FG (1994) Anaerobic treatment applications and fundamentals: Substrate specificity during phase separation. Water Environ Res 66:716-724

Galbe M, Zacchi G (2007) Pretreatment of lignocellulosic materials for efficient bioethanol production. Adv Biochem Eng/Biotech 108:41-65

Gallert C, Bauer S, Winter J (1998) Effect of ammonia on the anaerobic degradation of protein by a mesophilic and thermophilic biowaste population. Appl Microbiol Biotechnol 50:495-501

Ganidi N, Tyrell S, Cartmell E (2009) Anaerobic foaming causes – A review. Biores Technol 100: 5546-5554

Gebauer R, Eikebrokk B (2006) Mesophilic anaerobic treatment of sludge from salmon smolt hatching. Bioresour Technol 97:2389-2401

Ghosh S, Henry MP, Sajjad A, Mensinger MC, Arora JL (2000) Pilot-scale gasification of municipal solid wastes by high-rate and two- phase anaerobic digestion (TPAD). Water Sci Technol 41:101-110

Gonc¸alves MR, Costa JC, Marques IP, Alves MM (2012) Strategies for lipids and phenolics degradation in the anaerobic treatment of olive mill wastewater. Water Res 46:1684-1692.

Gonzalez-Gil G, Jansen S, Zandvoort MH, Van Leeuwen HP (2003) Effect of yeast extract on speciation and bioavailability of nickel and cobalt in anaerobic bioreactors. Biotechnol Bioeng 82:134-142

Gröndahl F, Brandt N, Karlsson S, Malmström ME (2009) Sustainable use of Baltic Sea natural resources based on ecological engineering and biogas production. WIT Transactions on Ecology and the Environment 122:153-161

Gustavsson J, Shakeri Yekta S, Sundberg C, Karlsson A, Ejlertsson J, Skjellberg U, Svensson BH (2013a) Bioavailability of cobalt and nickel during anaerobic digestion of sulfur-rich stillage for biogas formation. Appl Energy 112:473-477

Gustavsson J, Yekta SS, Karlsson A, Skjellberg U, Svensson BH (2013b) Potential bioavailability and chemical forms of Co and Ni in the biogas process-An evaluation based on sequential and acid volatile sulfide extractions. Eng Life Sci 13:572-579

Gustavsson J, Svensson BH, Karlsson A (2011) The feasibility of trace element supplementation for stable operation of wheat stillage-fed biogas tank reactors. Water Sci Technol 64:320-325

Haas KL, Franz KJ (2009) Application of metal coordination chemistry to explore and manipulate cell biology. Chem Rev 109:4921-4960

Hansen KH, Angelidaki I, Ahring BK (1998) Anaerobic digestion of swine manure: Inhibition by ammonia. *Water Res* 32:5-12

Hansen TL, Jansen JC, Davidsson Å, Christensen TH (2007) Effects of pre-treatment technologies on quantity and quality of source-sorted municipal organic waste for biogas recovery. *Waste Manage* 27:398-405

Hansson A, Tjernström E, Gardin M, Finnis P (2012) Wetlands algae biogas –A southern Baltic Sea eutrophication counter act project. Municipality of Trelleborg, Trelleborg, Sweden

Hansson M, Nordberg A, Sundh I, Mathisen B (2002) Early warning of disturbances in a laboratory-scale MSW biogas process. *Water Sci Technol* 45:255-260

Hayta M, Alpaslan M, Kose E (2001) The effect of fermentation on viscosity and protein solubility on Boza, a traditional cereal-based fermented Turkish beverage. *Euro Food Res Technol* 213: 335-337

Hejnfelt A, Angelidaki I (2009) Anaerobic digestion of slaughterhouse by-products. *Biomass Bioenerg* 33:1046-1054

Hendriks ATWM, Zeeman G (2009) Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresour Technol* 100:10-18

Hering JG, Morel FMM (1990) Kinetics of trace metal complexation: Ligand-exchange reactions. *Environ Sci Technol* 24:242-252

Holm-Nielsen JB, Andrée H, Lindorfer H, Esbensen KH (2007) Transflexive embedded near infrared monitoring for key process intermediates in anaerobic digestion/ biogas production. *J Near Infrared Spec* 15:123-135

Holm-Nielsen JB, Dahl CK, Esbensen KH (2006) Representative sampling for process analytical characterization of heterogeneous bioslurry systems-a reference study of sampling issues in PAT. *Chemometrics Intellig Lab Syst* 83:114-126

Hughes AD, Kelly MS, Black KD, Stanley MS (2012) Biogas from Macroalgae: Is it time to revisit the idea?. *Biotechnol Biofuels* 5

Jacobi HF, Moschner CR, Hartung E (2009) Use of near infrared spectroscopy in monitoring of volatile fatty acids in anaerobic digestion. *Water Sci Technol* 60:339-346

Jagadabhi PS, Kaparaju P, Rintala J (2011) Two-stage anaerobic digestion of tomato, cucumber, common reed and grass silage in leach-bed reactors and upflow anaerobic sludge blanket reactors. *Bioresour Technol* 102:4726-4733

Jansen S, Gonzalez-Gil G, van Leeuwen HP (2007) The impact of Co and Ni speciation on methanogenesis in sulfidic media-Biouptake versus metal dissolution. *Enzyme Microb Technol* 40:823-830

Jansen S, Steffen F, Threels WF, Van Leeuwen HP (2005) Speciation of Co(II) and Ni(II) in anaerobic bioreactors measured by competitive ligand exchange-adsorptive stripping voltammetry. *Environ Sci Technol* 39:9493-9499

Jantsch TG, Mattiasson B (2003) A simple spectrophotometric method based on pH-indicators for monitoring partial and total alkalinity in anaerobic processes. *Environ Technol* 24:1061-1067

Janzon R, Schütt F, Oldenburg S, Fischer E, Körner I, Saake B (2014) Steam pretreatment of spruce forest residues: Optimal conditions for biogas production and enzymatic hydrolysis. *Carbohydr Polym* 100:202-210

Jard G, Dumas C, Delgenes JP, Marfaing H, Sialve B, Steyer JP, Carrère H (2013) Effect of thermochemical pretreatment on the solubilization and anaerobic biodegradability of the red macroalga *Palmaria palmata*. *Biochem Eng J* 79:253-258

Jarvis Å, Nordberg Å, Jarlsvik T, Mathisen B, Svensson BH (1997) Improvement of a grass-clover silage-fed biogas process by the addition of cobalt. *Biomass Bioenerg* 12:453-460

Jensen PD, Hardin MT, Clarke WP (2009) Effect of biomass concentration and inoculum source on the rate of anaerobic cellulose solubilization. *Bioresour Technol* 100:5219-5225

Juang R-, Wang Y- (2003) Use of complexing agents for effective ion-exchange separation of Co(II)/Ni(II) from aqueous solutions. *Water Res* 37:845-852

Kaparaju P, Buendia I, Ellegaard L, Angelidakia I (2008) Effects of mixing on methane production during thermophilic anaerobic digestion of manure: lab-scale and pilot-scale studies. *Biores Technol* 99: 4919-4928

Karim K, Hoffman R, Klasson K, Al-Dahhan M (2005) Anaerobic digestion of animal waste: effect of mode of mixing. *Water Res* 39: 3597-3606

Karlsson A, Ejlertsson J (2012) Addition of HCl as a means to improve biogas production from protein-rich food industry waste. *Biochem Eng J* 61:43-48

Karlsson A, Einarsson P, Schnürer A, Sundberg C, Ejlertsson J, Svensson BH (2012) Impact of trace element addition on degradation efficiency of volatile fatty acids, oleic acid and phenyl acetate and on microbial populations in a biogas digester. *Jl Biosci Bioeng* 114:446-452

Ke S, Shi Z, Fang HHP (2005) Applications of two-phase anaerobic degradation in industrial wastewater treatment. *Int J Environ Pollut* 23:65-80

Kida K, Shigematsu T, Kijima J, Numaguchi M, Mochinaga Y, Abe N, Morimura S (2001) Influence of Ni²⁺ and Co²⁺ on methanogenic activity and the amounts of coenzymes involved in methanogenesis. *J Biosci Bioeng* 91:590-595

Kim M, Ahn Y-, Speece RE (2002) Comparative process stability and efficiency of anaerobic digestion; mesophilic vs. thermophilic. *Water Res* 36:4369-4385

Knutsen JS, Davis RH (2004) Cellulase retention and sugar removal by membrane ultrafiltration during lignocellulosic biomass hydrolysis. *Applied Biochemistry and Biotechnology - Part A Enzyme Eng Biotechnol* 114:585-599

Ko K-, Han Y, Choi JH, Kim G-, Lee S-, Song JJ (2011) A novel bifunctional endo-/exo-type cellulase from an anaerobic ruminal bacterium. *Appl Microbiol Biotechnol* 89:1453-1462

Koster IW, Lettinga G (1984) The influence of ammonium-nitrogen on the specific activity of pelletized methanogenic sludge. *Agr Wastes* 9:205-216

Krewulak KD, Vogel HJ (2008) Structural biology of bacterial iron uptake. *BBA - Biomembranes* 1778:1781-1804

Kuglarz M, Karakashev D, Angelidaki I (2013) Microwave and thermal pretreatment as methods for increasing the biogas potential of secondary sludge from municipal wastewater treatment plants. *Bioresour Technol* 134:290-297

Lacorte S, Latorre A, Barceló D, Rigol A, Malmqvist A, Welander T (2003) Organic compounds in paper-mill process waters and effluents. *TrAC - Trends Anal Chem* 22:725-737

- Lahav O, Morgan BE (2004) Titration methodologies for monitoring of anaerobic digestion in developing countries - A review. *J Chem Technol Biotechnol* 79:1331-1341
- Lalman JA, Bagley DM (2000) Anaerobic degradation and inhibitory effects of linoleic acid. *Water Res* 34:4220-4228
- Lan TQ, Lou H, Zhu JY (2013) Enzymatic Saccharification of Lignocelluloses Should be Conducted at Elevated pH 5.2-6.2. *Bioen Res* 6:476-485
- Latorre A, Malmqvist A, Lacorte S, Welander T, Barceló D (2007) Evaluation of the treatment efficiencies of paper mill whitewaters in terms of organic composition and toxicity. *Environ Poll* 147:648-655
- Lee D-, Li Y-, Noike T, Cha G- (2008) Behavior of extracellular polymers and bio-fouling during hydrogen fermentation with a membrane bioreactor. *J Membr Sci* 322:13-18
- Lehar O, Morgan B E (2004) Titration methodologies for monitoring of anaerobic digestion in developing countries – a review. *J Chem Technol Biotechnol* 79:1331-1341
- Leu S-, Zhu JY (2013) Substrate-Related Factors Affecting Enzymatic Saccharification of Lignocelluloses: Our Recent Understanding. *Bioen Res* 6:405-415
- Levén L, Nordberg U, Henriksson G, Yngvesson J, Enebro J (2012) Mikroplaster i biogasprocessen – Förstudie. Waste Refinery Report 48 SP Sveriges Tekniska forskningsinstitut Borås
- Levenspiel O (1999) Chemical reaction engineering, third ed. Wiley, New York
- Li C, Champagne P, Anderson BC (2013) Biogas production performance of mesophilic and thermophilic anaerobic co-digestion with fat, oil, and grease in semi-continuous flow digesters: Effects of temperature, hydraulic retention time, and organic loading rate. *Environ Technol (United Kingdom)* 34:2125-2133
- Lindorfer H, Ramhold D, Frauz B (2012) Nutrient and trace element supply in anaerobic digestion plants and effect of trace element application. *Water Sci Technol* 66:1923-1929
- Linné M, Ekstrand A, Engleson R, Persson E, Björnsson L, Lantz M (2008) Den svenska biogaspotentialen från inhemska restprodukter. Report; Avfall Sverige, Svenska Biogasföreningen, Svenska gasföreningen och Svenskt Vatten.
- Lomborg CJ, Holm-Nielsen JB, Oleskowicz-Popiel P, Esbensen KH (2009) Near infrared and acoustic chemometrics monitoring of volatile fatty acids and dry matter during co-digestion of manure and maize silage. *Bioresour Technol* 100:1711-1719
- Long JH, Aziz TN, Reyes III FLDL, Ducoste JJ (2012) Anaerobic co-digestion of fat, oil, and grease (FOG): A review of gas production and process limitations. *Process Saf Environ Prot* 90:231-245
- Ma J, Frear C, Wang Z-, Yu L, Zhao Q, Li X, Chen S (2013) A simple methodology for rate-limiting step determination for anaerobic digestion of complex substrates and effect of microbial community ratio. *Bioresour Technol* 134:391-395
- Madsen M, Holm-Nielsen JB, Esbensen KH (2011) Monitoring of anaerobic digestion processes: A review perspective. *RenewSust Energy Rev* 15:3141-3155
- McInerney M, Bryant M, Hespell R, Costerton J (1981) *Syntrophomonas wolfei* gen. nov. sp. nov. an anaerobic syntrophic fattyacid-oxidizing bacterium. *Appl Environ Microbiol* 41: 1029-1039
- McKendry P (2002) Energy production from biomass (part 1): Overview of biomass. *Bioresour Technol* 83:37-46

- McMahon K, Stroot P, Mackie R, Raskin L (2001) Anaerobic co-digestion of municipal solid waste and biosolids under various mixing conditions – II: Microbial population dynamics. *Water Res* 35: 1817-1827
- Mehdizadeh SN, Eskicioglu C, Bobowski J, Johnson T (2013) Conductive heating and microwave hydrolysis under identical heating profiles for advanced anaerobic digestion of municipal sludge. *Water Res* 47:5040-5051
- Menéndez M, Paredes B, Iglesias O, Rendueles M, Diaz M (2006) Rheological behavior and organoleptic effects of ovalbumin addition in yogurt mousse production. *J Dairy Sci* 89(3): 951-962
- Menon V, Rao M (2012) Trends in bioconversion of lignocellulose: Biofuels, platform chemicals & biorefinery concept. *Prog in Energ and Combust* 38:522-550
- Miron J, Ben-Ghedalia D, Morrison M (2001) Invited review: Adhesion mechanisms of rumen cellulolytic bacteria. *J Dairy Sci* 84:1294-1309
- Moen G (2003) Anaerobic digester foaming: causes and solutions. *Water Environ Technol* 15(8): 70-73
- Mohsenzadeh A, Jeihanipour A, Karimi K, Taherzadeh MJ (2012) Alkali pretreatment of softwood spruce and hardwood birch by NaOH/thiourea, NaOH/urea, NaOH/urea/thiourea, and NaOH/PEG to improve ethanol and biogas production. *J Chem Technol Biot* 87:1209-1214
- Molina F, Ruiz-Filippi G, Garcia C, Lema JM, Roca E (2009) Pilot-scale validation of a new sensor for on-line analysis of volatile fatty acids and alkalinity in anaerobic wastewater treatment plants. *Environ Eng Sci* 26:641-649
- Monlau F, Sambusiti C, Barakat A, Guo XM, Latrille E, Trably E, Steyer J-, Carrere H (2012) Predictive models of biohydrogen and biomethane production based on the compositional and structural features of lignocellulosic materials. *Environ Sci Technol* 46:12217-12225
- Moosbrugger R E, Wentzel, M C, Ekama M W, Marais, G R (1993) A 5-point titration method to determine the carbonate and SCFA waek acid/bases in anaerobic systems. *Water Sci Technol* 28:237-248
- Morel E, Santamaria K, Perrier M, Guiot SR, Tartakovsky B (2004) Application of multi-wavelength fluorometry for on-line monitoring of an anaerobic digestion process. *Water Res* 38:3287-3296
- Mudhoo A, Kumar S (2013) Effects of heavy metals as stress factors on anaerobic digestion processes and biogas production from biomass. *Int J Environ Sci Technol* 10:1383-1398
- Murray WD, Van den Berg L (1981) Effect of support material on the development of microbial fixed films converting acetic acid to methane. *J Appl Bacteriol* 51:257-265
- Nges I, Björn A, Björnsson L (2012) Stable operation during pilot-scale anaerobic digestion of nutrient-supplemented maize/sugar beet silage. *Biores Technol* 118: 445-454
- Nges IA, Mbatia B, Björnsson L (2012) Improved utilization of fish waste by anaerobic digestion following omega-3 fatty acids extraction. *J Environ Manage* 110:159-165
- Nielsen HB, Ahring BK (2006) Responses of the biogas process to pulses of oleate in reactors treating mixtures of cattle and pig manure. *Biotechnol Bioeng* 95:96-105
- Nielsen HB, Uellendahl H, Ahring BK (2007) Regulation and optimization of the biogas process: Propionate as a key parameter. *Biomass Bioenerg* 31:820-830
- Nizami AS, Thamsiroj T, Singh A, Murphy JD (2010) Role of leaching and hydrolysis in a two-phase grass digestion system. *Energ Fuels* 24:4549-4559

- Nkemka VN, Murto M (2013) Two-stage anaerobic dry digestion of blue mussel and reed. *Renew Energy* 50:359-364
- Noike T, Endo G, Chang J- (1985) Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic digestion. *Biotechnol Bioeng* 27:1482-1489
- Noike T, Endo G, Chang J- (1985) Characteristics of carbohydrate degradation and the rate-limiting step in anaerobic digestion. *Biotechnol Bioeng* 27:1482-1489
- Nordberg Å, Ekström M (2005) Co-digesting of energy crops and the source-sorted organic fraction of municipal solid waste. *Water Sci Technol* 52(1): 217-222
- Nordberg Å, Hansson M, Sundh I, Nordkvist E, Carlsson H, Mathisen B (2000) Monitoring of a biogas process using electronic gas sensors and near- infrared spectroscopy (NIR). *Water Sci Technol* 41:1-8
- Nordberg Å, Hansson M, Kanerot M, Krozer A, Löfving B and Sahlin E (2010) Robusta mättekniker on-line för optimerad biogasproduktion. Waste refinery report no 11. SP Sveriges tekniska forskningsinstitut.
- Nordell E, Hansson AB, Karlsson M (2013) Zeolites relieves inhibitory stress from high concentrations of long chain fatty acids. *Waste Manage* 33: 2659-2663.
- Novaes E, Kirst M, Chiang V, Winter-Sederoff H, Sederoff R (2010) Lignin and biomass: A negative correlation for wood formation and lignin content in trees. *Plant Physiol* 154:555-561
- Okeh OC, Onwosi CO, Odibo FJC (2014) Biogas production from rice husks generated from various rice mills in Ebonyi State, Nigeria. *Renew Energy* 62:204-208
- Olivet D, Valls J, Gordillo M, Freixo A, Sanchez A (2005) Application of residence time distribution technique to the study of the hydrodynamic behavior of a full-scale wastewater treatment plant plug-flow bioreactor. *JChem Technol Biotechnol* 80: 425-432
- Ong H.K, Greenfield P.F, Pullammanappallil P.C (2002) Effect of mixing on biomethanation of cattle-manure slurry. *Environ Technol* 23: 1081-1090
- O'Sullivan CA, Burrell PC, Clarke WP, Blackall LL (2005) Structure of a cellulose degrading bacterial community during anaerobic digestion. *Biotechnol Bioeng* 92:871-878
- Palatsi J, Lauren M, Andrés MV, Flotats X, Nielsen HB, Angelidaki I (2009) Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors. *Bioresour Technol* 100:4588-4596
- Palatsi J, Viñas M, Guivernau M, Fernandez B, Flotats X (2011) Anaerobic digestion of slaughterhouse waste: Main process limitations and microbial community interactions. *Bioresour Technol* 102:2219-2227
- Parawira W, Read JS, Mattiasson B, Björnsson L (2008) Energy production from agricultural residues: High methane yields in pilot-scale two-stage anaerobic digestion. *Biomass Bioenerg* 32:44-50
- Park J, Park S, Kim M (2014) Anaerobic degradation of amino acids generated from the hydrolysis of sewage sludge. *Environ Technol (United Kingdom)* 35:1133-1139
- Pavlostathis SG, Giraldo-Gomez E (1991) Kinetics of anaerobic treatment: A critical review. *Crit Rev Env Contr* 21:411-490

- Pechan Z, Knappová O, Petrovicová B, Adamec O (1987) Anaerobic digestion of poultry manure at high ammonium nitrogen concentrations. *Biol Wastes* 20:117-131
- Peck MW, Chynoweth DP (1992) On-line fluorescence-monitoring of the methanogenic fermentation. *Biotechnol Bioeng* 39:1151-1160
- Pereira MA, Sousa DZ, Mota M, Alves MM (2004) Mineralization of LCFA associated with anaerobic sludge: Kinetics, enhancement of methanogenic activity, and effect of VFA. *Biotechnol Bioeng* 88:502-511
- Pereira MA, Pires OC, Mota M, Alves MM (2005) Anaerobic biodegradation of oleic and palmitic acids: Evidence of mass transfer limitations caused by long chain fatty acid accumulation onto the anaerobic sludge. *Biotechnol Bioeng* 92:15-23
- Pind PF, Angelidaki I, Ahring BK (2003) A new VFA sensor technique for anaerobic reactor systems. *Biotechnol Bioeng* 82:54-61
- Pind PF, Angelidaki I, Ahring BK (2003) A new VFA sensor technique for anaerobic reactor systems. *Biotechnol Bioeng* 82:54-61
- Pobeheim H, Munk B, Lindorfer H, Guebitz GM (2011) Impact of nickel and cobalt on biogas production and process stability during semi-continuous anaerobic fermentation of a model substrate for maize silage. *Water Res* 45:781-787
- Poggi-Varaldo H M, Fernández-Villagómez G, Esparza-García F (1996) Pre-treatment of black liquor spills effluents. In 51st Purdue University Industrial waste conference proceedings, page 651-661
- Poggi-Varaldo HM, Rodríguez-Vázquez R, Fernández-Villagómez G, Esparza-García F (1997) Inhibition of mesophilic solid-substrate anaerobic digestion by ammonia nitrogen. *Appl Microbiol Biotechnol* 47:284-291
- Prajapati SK, Kaushik P, Malik A, Vijay VK (2013) Phycoremediation coupled production of algal biomass, harvesting and anaerobic digestion: Possibilities and challenges. *Biotechnol Adv* 31:1408-1425
- Quemada D, Beril C (2007) On the flow curve of colloids presenting shear-induced phase transitions. Cornell University Library, <http://arxiv.org/abs/cond-mat/0703560>
- Ramsay IR, Pullammanappallil PC (2001) Protein degradation during anaerobic wastewater treatment: Derivation of stoichiometry. *Biodegradation* 12:247-257
- Rickard D, Luther III GW (2006) Metal sulfide complexes and clusters. *Rev Mineral Geochem* 61:421-504
- Rintala JA, Puhakka JA (1994) Anaerobic treatment in pulp- and paper-mill waste management: A review. *Bioresour Technol* 47:1-18
- Risberg K, Sun L, Levén L, Horn SJ, Schnürer A (2013) Biogas production from wheat straw and manure - Impact of pretreatment and process operating parameters. *Bioresour Technol* 149:232-237
- Risén E, Gregeby E, Tatarchenko O, Blidberg E, Malmström ME, Welander U, Gröndahl F (2013) Assessment of biomethane production from maritime common reed. *J Clean Prod* 53:186-194
- Rodrigue A, Effantin G, Mandrand-Berthelot M- (2005) Identification of rcnA (yohM), a nickel and cobalt resistance gene in *Escherichia coli*. *J Bacteriol* 187:2912-2916

- Rodríguez-Abalde A, Fernández B, Silvestre G, Flotats X (2011) Effects of thermal pre-treatments on solid slaughterhouse waste methane potential. *Waste Manage* 31:1488-1493
- Rudnitskaya A, Legin A (2008) Sensor systems, electronic tongues and electronic noses, for the monitoring of biotechnological processes. *J Industr Microbiol Biotechnol* 35:443-451
- Saha BC (2003) Hemicellulose bioconversion. *JIndust Microbiol Biotechnol* 30:279-291
- Saito MA, Moffett JW, Chisholm SW, Waterbury JB (2002) Cobalt limitation and uptake in *Prochlorococcus*. *Limnol Oceanogr* 47:1629-1636
- Salminen EA, Rintala JA (2002) Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: Effect of hydraulic retention time and loading. *Water Res* 36:3175-3182
- Salminen E, Einola J, Rintala J (2003) The methane production of poultry slaughtering residues and effects of pre-treatments on the methane production of poultry feather. *Environ Technol* 24:1079-1086
- Sanders WTM, Geerink M, Zeeman G, Lettinga G (2000) Anaerobic hydrolysis kinetics of particulate substrates. *Water Sci Technol* 41:17-24
- Savreux F, Jay P, Magnin A (2007) Viscoplastic fluid mixing in a rotaion tank. *Chem Eng Sci* 62: 2290-2301
- Schink B (1997) Energetics of syntrophic cooperation in methanogenic degradation. *Microbiol Mol Biol Rev* 61:262-280
- Schmidt T, Nelles M, Scholwin F, Pröter J (2014) Trace element supplementation in the biogas production from wheat stillage - Optimization of metal dosing. *Bioresour Technol*, in press.
- Schmidt T, Pröter J, Scholwin F, Nelles M (2013) Anaerobic digestion of grain stillage at high organic loading rates in three different reactor systems. *Biomass and Bioenerg* 55:285-290
- Schnürer A, Houwen FP, Svensson BH (1994) Mesophilic syntrophic acetate oxidation during methane formation by a triculture at high ammonium concentration. *Arch Microbiol* 162:70-74
- Schnürer A, Nordberg Å (2008) Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. *Water Sci Technol* 57:735-740
- Schwarz WH (2001) The cellulosome and cellulose degradation by anaerobic bacteria. *Appl Microbiol Biotechnol* 56:634-649
- Sessiecq I, Ferasse J.H., Roche N (2003) State-of-the-art: rheological characterization of wastewater treatment sludge. *Biochem Eng J* 16: 41-56
- Shakeri Yekta S, Gustavsson J, Svensson BH, Skyllberg U (2012a) Sulfur K-edge XANES and acid volatile sulfide analyses of changes in chemical speciation of S and Fe during sequential extraction of trace metals in anoxic sludge from biogas reactors. *Talanta* 89:470-477
- Shakeri Yekta S, Gonsior M, Schmitt-Kopplin P, Svensson BH (2012b) Characterization of dissolved organic matter in full scale continuous stirred tank biogas reactors using ultrahigh resolution mass spectrometry: A qualitative overview. *Environmental Science and Technology* 46:12711-12719
- Shakeri Yekta S, Svensson BH, Björn A, Skyllberg U (2014) Thermodynamic modeling of iron and trace metal solubility and speciation under sulfidic and ferruginous conditions in full scale continuous stirred tank biogas reactors. *Appl Geochem*, in press.

Shakeri Yekta S, Lindmark A, Skyllberg U, Danielsson A, Svensson BH (2014b) Importance of reduced sulfur for the equilibrium chemistry and kinetics of Fe(II), Co(II) and Ni(II) supplemented to semi-continuous stirred tank biogas reactors fed with stillage. *J Hazard Mater*

Sierra-Alvarez R, Field JA, Kortekaas S, Lettinga G (1994) Overview of the anaerobic toxicity caused by organic forest industry wastewater pollutants. *Water Sci Technol* 29:353-363

Smith DS, Bell RA, Kramer JR (2002) Metal speciation in natural waters with emphasis on reduced sulfur groups as strong metal binding sites. *Comp Biochem Phys - C* 133:65-74

Song H, Clarke WP, Blackall LL (2005) Concurrent microscopic observations and activity measurements of cellulose hydrolyzing and methanogenic populations during the batch anaerobic digestion of crystalline cellulose. *Biotechnol Bioeng* 91:369-378

Sousa DZ, Pereira MA, Smidt H, Stams AJM, Alves MM (2007) Molecular assessment of complex microbial communities degrading long chain fatty acids in methanogenic bioreactors. *FEMS Microbiol Ecol* 60:252-265

Sousa DZ, Smidt H, Alves MM, Stams AJM (2009) Ecophysiology of syntrophic communities that degrade saturated and unsaturated long-chain fatty acids. *FEMS Microbiol Ecol* 68:257-272

Steyer P, Bouvier JC, Conte T, Gras P, Harmand J, Delgenes JP (2002) On-line measurements of COD, TOC, VFA, total and partial alkalinity in anaerobic digestion processes using infra-red spectrometry. *Water Sci Technol* 45:133-138

Stroot P.G, McMahon K.D, Mackie R.I, Raskin L (2001) Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions – I. digester performance. *Water Res* 35(7): 1804-1816

Sun C-, Qin C, Wang X-, Su Z- (2013) Metal-organic frameworks as potential drug delivery systems. *Expert Opin Drug Deliv* 10:89-101

Sun Y, Cheng J (2002) Hydrolysis of lignocellulosic materials for ethanol production: A review. *Bioresour Technol* 83:1-11

Sundberg C, Al-Soud WA, Larsson M, Alm E, Yekta SS, Svensson BH, Sørensen SJ, Karlsson A (2013) 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters. *FEMS Microbiol Ecol* 85:612-626

Sutherland I.W (2011) Biofilm exopolysaccharides: a strong and sticky framework. *Microbiology* 147: 3-9

Svensk avfallshantering (2013) Report from Avfall Sverige

Taherzadeh MJ, Karimi K (2008) Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *Int J Mol Sci* 9:1621-1651

Takashima M, Shimada K, Speece RE (2011) Minimum requirements for trace metals (iron, nickel, cobalt, and zinc) in thermophilic and mesophilic methane fermentation from glucose. *Water Environ Res* 83:339-346

Takashima M, Speece RE (1989) Mineral nutrient requirements for high-rate methane fermentation of acetate at low SRT. *Res J Water Pollut Control Fed* 61:1645-1650

Tampio E, Ervasti S, Paavola T, Heaven S, Banks C, Rintala J (2014) Anaerobic digestion of autoclaved and untreated food waste. *Waste Manage* 34:370-377

- Tielbaard M, Wilson T, Feldbaumer E, Driessen W (2002) Full-scale anaerobic treatment experiences with pulp mill evaporator condensates. *Int Environ Conf*:621-634
- Tommaso G, Ribeiro R, Varesche MBA, Zaiat M, Foresti E (2003) Influence of multiple substrates on anaerobic protein degradation in a packed-bed bioreactor. *Water Sci Technol* 48:23-31
- Vairavamurthy A, Mopper K (1987) Geochemical formation of organosulphur compounds (thiols) by addition of H₂S to sedimentary organic matter. *Nature* 329:623-625
- Vanrolleghem P A, Lee, D S (2003) On-line equipment for wastewater treatment processes: State of the art. *Water Sci Technol* 47:1-34
- Van Velsen AFM (1979) Adaptation of methanogenic sludge to high ammonia-nitrogen concentrations. *Water Res* 13:995-999
- Vavilin VA (2010) Anaerobic degradation of organic waste: An experience in mathematical modeling. *Microbiology* 79:334-341
- Vavilin VA, Rytov SV, Lokshina LY (1996) A description of hydrolysis kinetics in anaerobic degradation of particulate organic matter. *Bioresour Technol* 56:229-237
- Vidal G, Diez MC (2005) Methanogenic toxicity and continuous anaerobic treatment of wood processing effluents. *J Environ Manage* 74:317-325
- Vintiloiu A, Lemmer A, Oechsner H, Jungbluth T (2012) Mineral substances and macronutrients in the anaerobic conversion of biomass: An impact evaluation. *Eng Life Sci* 12:287-294
- Waldron KJ, Robinson NJ (2009) How do bacterial cells ensure that metalloproteins get the correct metal? *Nature Rev Microbiol* 7:25-35
- Wang J, Huang CP, Allen HE (2003) Modeling heavy metal uptake by sludge particulates in the presence of dissolved organic matter. *Water Res* 37:4835-4842
- Wang W, Zhuang X, Yuan Z, Yu Q, Qi W, Wang Q, Tan X (2012) High consistency enzymatic saccharification of sweet sorghum bagasse pretreated with liquid hot water. *Bioresour Technol* 108:252-257
- Ward A, Hobbs P, Holliman P.J, Jones D (2008) Optimisation of the anaerobic digestion of agricultural resources. *Biores Technol* 99: 7928-7940
- Weimer P.J (1992) Cellulose degradation by ruminal micro-organisms. *Critl Rev Biotechnol* 12: 189-223
- Weimer PJ (1998) Manipulating Ruminant Fermentation: A Microbial Ecological Perspective 1. *J Anim Sci* 76:3114-3122
- Wilson DB (2008) Three microbial strategies for plant cell wall degradation. *Ann oN Y Acad Sci* 1125:289-297
- Worm P, Feroso FG, Lens PNL, Plugge CM (2009) Decreased activity of a propionate degrading community in a UASB reactor fed with synthetic medium without molybdenum, tungsten and selenium. *Enzyme Microb Technol* 45:139-145
- Yao Y, He M, Ren Y, Ma L, Luo Y, Sheng H, Xiang Y, Zhang H, Li Q, An L (2013) Anaerobic digestion of poplar processing residues for methane production after alkaline treatment. *Bioresour Technol* 134:347-352
- Zaimes GG, Khanna V (2013) Microalgal biomass production pathways: Evaluation of life cycle environmental impacts. *Biotechnology for Biofuels* 6

Zamalloa C, Boon N, Verstraete W (2012) Anaerobic digestibility of *Scenedesmus obliquus* and *Phaeodactylum tricornutum* under mesophilic and thermophilic conditions. *Appl Energy* 92:733-738

Zamalloa C, Vulsteke E, Albrecht J, Verstraete W (2011) The techno-economic potential of renewable energy through the anaerobic digestion of microalgae. *Bioresour Technol* 102:1149-1158

Zandvoort MH, van Hullebusch ED, Feroso FG, Lens PNL (2006) Trace metals in anaerobic granular sludge reactors: Bioavailability and dosing strategies. *Eng Life Sci* 6:293-301

Zehnder AJB Ed (1988) *Biology of Anaerobic Microorganisms* John Wiley & Sons Inc. New York 875p

Zhang L, Jahng D (2010) Enhanced anaerobic digestion of piggery wastewater by ammonia stripping: Effects of alkali types. *J Hazard Mater* 182:536-543

Zhao X, Zhang L, Liu D (2012) Biomass recalcitrance. Part I: The chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. *Biofuel, Bioprodu Bior* 6:465-482

Zhao X, Liu D (2011) Fractionating pretreatment of sugarcane bagasse for increasing the enzymatic digestibility of cellulose. *Shengwu Gongcheng Xuebao/Chinese J Biotechnol* 27:384-392

Örlygsson J, Houwen FP, Svensson BH (1994) Influence of hydrogenotrophic methane formation on the thermophilic anaerobic degradation of protein and amino acids. *FEMS Microbiol Ecol* 13:327-334.

Örlygsson J, Houwen FP, Svensson BH (1995) Thermophilic anaerobic amino acid degradation: Deamination rates and end- product formation. *Appl Microbiol Biotechnol* 43:235-241