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Lab and Pilot research with non-conventional anaerobic sequencing batch reactor

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Report

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Lab and Pilot research with non-conventional anaerobic sequencing batch reactor

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Summary

Slaughterhouse wastewater is a challenging waste stream to handle. The wastewater is characterized by high loads of biodegradable organic compounds, oil and grease, nitrogen, and phosphorus due to the presence of high suspended solids and colloidal compounds such as blood, protein, fat, and cellulose. A treatment train conventionally consist of pretreatment to decrease high suspended solids (SS) concentration, chemical addition (polymers), and biological treatment system are commonly implemented. The proposed self-regulated anaerobic sequencing batch reactor (AnSBR) technology for this TKI project could be applied to meat processing plants that produce large amounts of high-strength slaughterhouse wastewater, including its potential for on-site resource recovery of energy.

This TKI project aimed to test, using lab and pilot trials, a new anaerobic technology, a non-conventional, self-regulated anaerobic sequencing batch reactor (AnSBR) system, for the treatment of high-strength slaughterhouse wastewater. The novel system operates in sequences but does not work as a conventional AnSBR. This technology has been designed to enable reaction and settling phases to happen simultaneously within each cycle, which results in much longer settling periods without compromising the duration of the reaction phase. Even though other anaerobic technologies can reach high organic removal efficiencies, a robust, anaerobic single-stage (train simplification) and low-cost solution for the slaughterhouse industry is not currently available. Reactor stability/robustness for the high-strength slaughterhouse wastewater (extreme organic loading rate fluctuations and fat, oil, and grease (FOG) concentrations) is of highly important for the partners of the research proposal based on industrial experiences and for the direction of scaling up the reactor technology.

This TKI project successfully tested the novel AnSBR system technology for treating protein and lipid-rich wastewater from a slaughterhouse and determined the key operational parameters through continuous pilot/lab trials and batch tests. The experiments were carried out to understand the degradation of proteins/lipids compounds on slaughterhouse streams and assessed the overall performance of this reactor configuration.

The analysis of slaughterhouse wastewater revealed average protein and lipid (fat, oil and grease; i.e. FOG) concentrations of approximately 700 mg/L and 350 mg/L, respectively, with palmitic acid, stearic acid, and oleic acid being the main long-chain fatty acids present. The anaerobic biodegradability of the wastewater was found to be high, with an average biomethane potential of 307 Nml CH_4. $gCODsubstrate^{-1}$, indicating a degradation efficiency of 87%. Additionally, protein degradation was shown to be comparable across different FOG:Protein ratios, with similar anaerobic biodegradabilities in the range of 75-82% and no signs of VFA accumulation or inhibition within the first 48 hours of digestion. These findings demonstrate the potential for effective anaerobic treatment of the slaughterhouse wastewater.

The pilot anaerobic sequencing batch reactor system showed high tolerance to FOG, suspended solids, and fluctuations in the raw wastewater, and exhibited high treatment performance, good solids separation, and operational flexibility. The AnSBR system was tested on high-protein and FOG slaughterhouse wastewater and achieved a maximum of 90% TCOD (total chemical oxygen demand) removal and over 70% degradation efficiency without pre-treatment at an OLR (organic loading rate) below 6.2 kgCOD·L-1·d-1.
After a quick start-up, stable performance was reached at a VLR (volatile loading rate) of 3 gCOD/L, with a total COD removal efficiency of ~75% at pilot scale. The AnSBR pilot system proved to be efficient, achieving targets for total COD (80% total COD conversion to biogas) and digestion efficiency after about 50 days of trial for this type of wastewater.

The pilot AnSBR microbial structure showed high similarities in the buffer tank with the lab-scale AnSBR, while the reactor showed high abundance in Halobactereota phylum, which is mainly methanogens (i.e., Methanosaeta). Ammonification was correlated with the microbial community, confirming that protein degradation to ammonium is the key process governing the whole AnSBR system. Correspondingly, *Clostridium sensu stricto 1*, a bacteria involved in both protein and lipids degradation, was dominant in the microbial community composition in the reactor and buffer tank core microbiome.

Based on the results of the pilot trial and full-scale plant, the AnSBR presents several advantages over the conventional DAF+UASB system. These benefits include improved effluent quality, increased biogas and energy production, reduced operational costs, and a smaller footprint. The AnSBR's batch system is particularly well-suited for the slaughterhouse industry's batch operations, providing greater flexibility in handling load fluctuations while eliminating the need for DAF pretreatment and off-site transportation of FOG sludge. The use of Sparthane™ technology in the AnSBR ensures most biomass is converted into biogas, leading to a running cost of 0.60 euros per m^3 of wastewater, which is significantly lower than the DAF+UASB system's running cost of 1.33 euros per m^3, mainly due to sludge treatment and chemical usage.

Furthermore, the AnSBR's ability to achieve lower concentrations of N and P in the effluent makes downstream treatment more cost-effective. While anammox appears to be the best approach for N-removal, concentrating technologies like electrodialysis or ion exchange, which are less sensitive to feed concentration, can be used to recover N. Struvite precipitation, which is of high interest due to the value of phosphorous, is not currently feasible with the current P-concentrations achieved in the effluent, but rising prices may change this soon.

To further optimize the AnSBR cycle settings, research should be conducted in relation to the specific properties of the wastewater quality. Additionally, expansion of the existing biogas system, including the desulphurization system, piping, and boiler, may be necessary to accommodate the increased volume of biogas produced by the AnSBR. This would lead to less natural gas consumption when producing steam for the slaughterhouse.

As the nitrogen load in the anaerobic effluent increases, expansion of the aerobic system or incorporation of a nutrient recovery treatment step should be considered. Moreover, the presence of (dead) anaerobic bacteria in the anaerobic effluent may be contributing to the high Legionella levels observed in the following aerobic system. The use of an AnSBR, with its robust settling phase, may help to retain more (dead) anaerobic bacteria inside the anaerobic treatment step and could potentially be beneficial in mitigating high Legionella levels. Further investigation is required to confirm this hypothesis.

Contents

1 Introduction

1.1 General introduction

The meat processing industry consumes 29% of the total freshwater used in the agricultural and livestock industry worldwide. Meat processing plants produce large amounts of high-strength slaughterhouse wastewater because of the slaughtering process and cleaning of facilities (Figure 1). Slaughterhouse wastewater is a challenging waste stream to handle, because of high loads of biodegradable organic compounds, oil and grease, nitrogen, and phosphorus due to the presence of high suspended solids and colloidal compounds such as blood, protein, fat, and cellulose. Therefore, it needs multiple steps treatment for a safe discharge to the environment due to the high content of organics and nutrients, which leads to opportunities for recovery.

Figure 1 An example of slaughterhouse wastewater.

Although physical, chemical, and biological treatment can be used for the treatment, each of the processes has different advantages and drawbacks depending on the wastewater characteristics and regulations. Worldwide guidelines on water and energy management recommend the use of anaerobic reactors as a core technology for treating food industry wastewater. Anaerobic treatment is the preferred biological treatment because of its effectiveness in treating high-strength waste streams such as slaughterhouse wastewater. Anaerobically treated effluents typically require post-treatment to comply with required discharge limits and increased the removal of organic matter, nitrogen, and phosphorus. High ammonia and low organic matter concentrations are the principal characteristics of the slaughterhouse anaerobic effluents that will also provide opportunities for the recovery of nitrogen. On-site treatment is the preferred option for water reuse and energy recovery.

1.2 Conventional treatment of slaughterhouse wastewater

Since the slaughter industry is highly water-consuming and wastewater generator, new technologies towards a circular economy are needed, and treatment technologies need to move to the water resource recovery facility concept to take a step toward achieving the sustainability of wastewater reclamation and reuse schemes.

A treatment train for slaughterhouse wastewater consisting of pretreatment to decrease high SS concentration, chemical addition (polymers), and a biological treatment system are commonly implemented (Figure 2). Most of the available systems are based on “floating-sludge” based principles (either upstream, inside, or downstream of the anaerobic reactor). These systems, in the long term, cannot be efficient as they promote biomass floating; which happens naturally when a system is overloaded as all TSS and fats, oil and grease (FOG) are accumulated in the system (in the float) and not degraded; thus, COD removal efficiency could be high, but COD conversion to biogas is not high as all this fraction of substrate is not converted. Furthermore, sequencing batch reactor (SBR) systems work very well aerobically due to fast settling velocities of aerobic sludge (which do not take a big part of the sequence for settling). However, for anaerobic application time available for settling in a cycle compromises the time available for reaction. Moreover, it is subject to the effects of biogas up-flow that hinders sludge settling all this resulting in low rate systems (1-2 g COD/L day) and consequently in very high footprint systems.

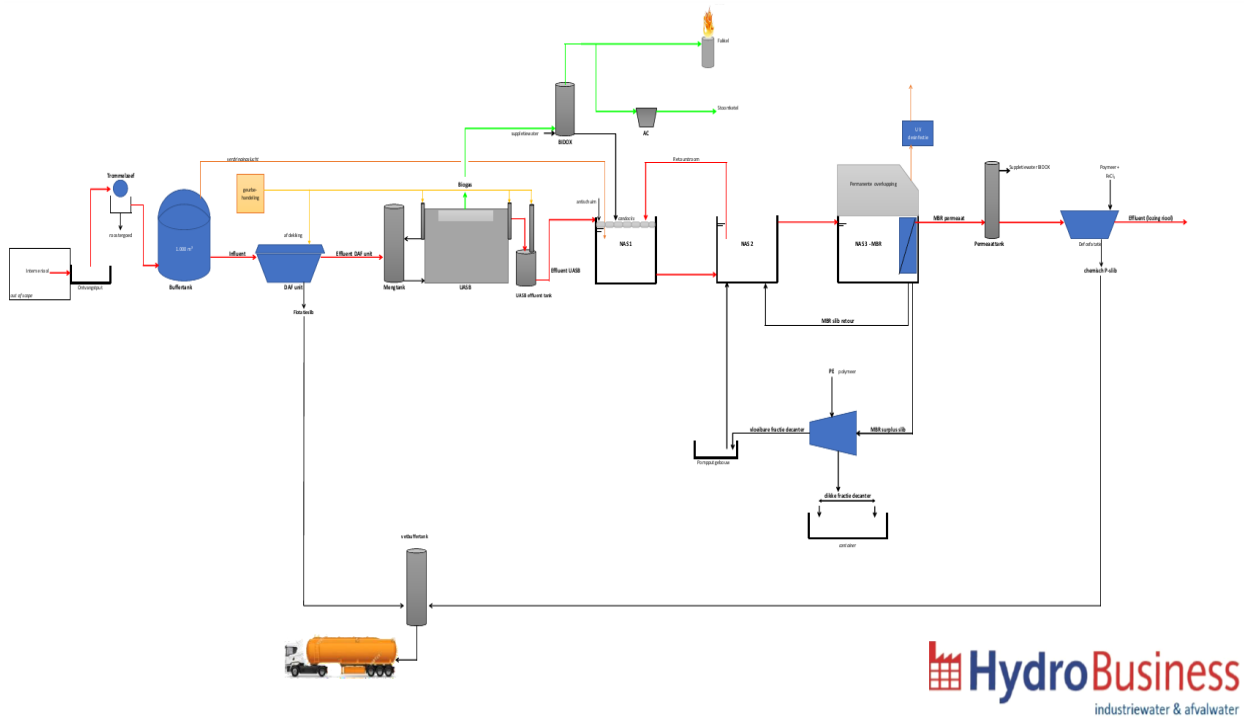


Figure 2 Slaughterhouse wastewater treatment train (courtesy HydroBusiness)

In the slaughterhouse, the slaughtered pigs first pass a hot bath to make the skins weaker. This simplifies the removal of hairs. The hot bath is constantly refreshed with small amounts of water during the day, but most of the solids will settle in the hot bath tank (in Dutch “Broeibak”) and will be drained to the waste water treatment plant when the hot bath is cleaned after the second shift ends. The draining of the hot bath forms a hydraulic peak to the waste water treatment plant. With this peak also, a significant part of the daily organic load is transported to the WWTP.

In the treatment train of the WWTP, as depicted in Figure 2, all waste water originating from the slaughterhouse is drained from the factory to the WWTP by an internal sewer system. The waste water is collected in an influent pit, from where the waste water is transported to an internally fed drum sieve (mesh wide 0,75 µm), in which solids are removed from the water. The solids are collected and transported off-site. The waste water is collected in a buffering tank. From the buffering tank, the waste water is transported in an equal flow to a Dissolved Air Flotation (DAF) unit. On weekdays, a grab sample and a 24-hour sample of the influent from the buffering tank, which is transported to the DAF unit, are analysed to monitor and characterize the loads to the WWTP.
In the DAF unit, fats are separated from the waste water. No coagulants or flocculants are dosed. The separated floatation sludge is collected in a tank and transported off-site. The effluent of the DAF unit is transported to the

current anaerobic reactor. The anaerobic reactor is an Upflow Anaerobic Sludge Blanket (UASB)-type reactor, in which the organic compounds are converted into biogas. Besides methane (CH4) and carbon dioxide (CO2), the produced biogas contains some H_2S, which is removed from the biogas by biological desulphurization followed by an activated carbon filter. The treated biogas is transported to the boiler, where it is mixed with natural gas and converted into steam. Excess gas can be transported to the biogas torch.

The anaerobic effluent is collected into a pump tank and transported to the aerobic system. The aerobic system uses Anammox bacteria to remove nitrogen and consists of three different zones: the first zone is the so-called partial nitritation zone, in which ammonia is partially converted into nitrite (NO_2) by aerating the waste water. In the second zone, which is mixed but not aerated (anoxic), Anammox bacteria convert leftover ammonia with nitrite to nitrogen gas. In the third zone, the leftover nitrogen is converted into nitrate (NO3) by aeration. Via a return flow, the produced nitrate is transported to the second, where nitrogen gas is formed by conventional denitrification. The aerobic sludge is separated from the waste water via membrane filtration, also in the third zone of the aerobic system. The membrane permeate is collected in a buffering tank and from there transported to a second DAF unit. Coagulant and flocculant are dosed, and phosphorus is removed from the permeate. The effluent of the second DAF unit is discharged to the communal sewer system. The chemical sludge which is formed in the second DAF unit is collected in a tank, together with the flotation sludge of the first DAF unit, and transported off-site.

The surplus sludge of the aerobic system is dewatered using a decanter centrifuge. PE is added to the surplus sludge. The dewatered sludge is transported off-site to an external sludge processor. In order to prevent aerosol formation in the aerated parts of the aerobic sludge system, the first zone is covered with floating blocks, and the third zone (MBR) is covered with a roof. The air from under this roof is collected and treated in a UV system.

1.3 Non-conventional anaerobic SBR technology (Sparthane™) to treat slaughterhouse wastewater

A robust, high-rate anaerobic single-stage (train simplification) and low-cost solution that is not currently available is being designed and proposed. This novel system operates in sequences but does not work as a conventional AnSBR. This technology has been designed to enable reaction and settling phases to happen simultaneously within each cycle, which results in much longer settling periods (up to 3 times longer than in a conventional AnSBR) without compromising the duration of the reaction phase.
This means that the technology can achieve total reaction times of 100% of the cycle time and in parallel settling times up to 70% of the cycle time. This is very important as the reaction cycle should be long enough to promote complete degradation of the entire batch fed, and the settling phase needs to be as long as possible to allow for good sludge separation but not too long to result in huge batches.
Biothane novel AnSBR system has incorporated an automatic operation control system based on each cycle performance that enables real-time monitoring of the system capacity in each cycle, something impossible in a continuous operation. The patented simultaneous phases operational regime and tank arrangement enable complete cycle time use for reaction (100% of cycle time is reaction phase) while providing up to 70% of the cycle time for completely undisturbed settling without compromising the duration of the reaction phase; this is something impossible to achieve in a conventional AnSBR. This real-time cycle follow-up also enables an overload control that does not allow the system to overload by any means (despite any stream variability), ensuring robust operation at all times. All this, relies on follow-up of the biogas profile of every batch cycle. Thus this technology is considered as a self-regulated system and safely operated.

Reactor stability/robustness for the high-strength slaughterhouse wastewater (extreme organic loading rate fluctuations and fat, oil, and grease (FOG) concentrations) is of extreme importance for the research and for the direction of scaling up the reactor technology.
The technology is designed to treat high-strength slaughterhouse wastewaters with a wide range of COD (5-50 g/L), high SS (1-20 g/L), and/or FOG (0-4 g/L) in a cost-efficient way (without pretreatment /or addition of chemicals).

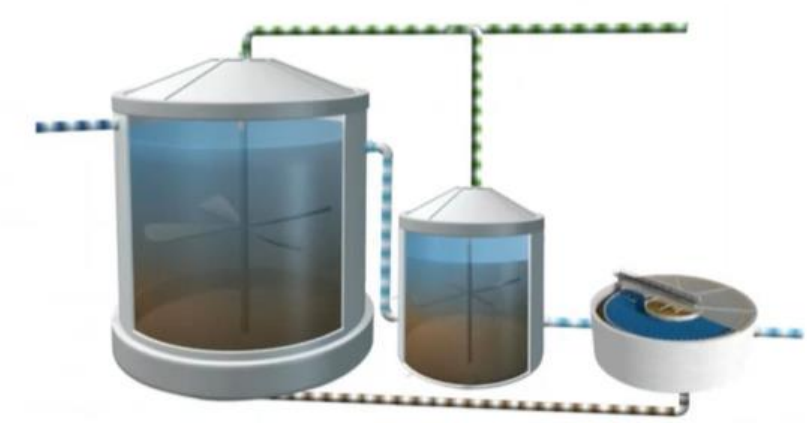


Figure 3 Schematic representation of AnSBR (Sparthane™ courtesy Biothane)

This research mainly focuses on how the implementation of the technology can contribute to the sustainability of the meat industry and the circular economy. With this project, an important step to the practical application of slaughterhouse wastewater treatment will be taken by testing and optimizing an innovative self-regulated anaerobic SBR technology.

1.4 Project aim and description

This project aims to test by means of a lab and pilot plant the performance of a self-regulated AnSBR system for the treatment of high strength (high SS, high FOG and protein) slaughterhouse wastewater. The goal is to achieve a robust and stable, treatment system for slaughterhouse wastewater treatment sites.

The specific objectives of the project are:

1. Implement an anaerobic self-regulated SBR bench-scale and pilot installation for the treatment of high-strength (high suspended solids, high FOG (fat, oil, and grease)) slaughterhouse wastewaters. Targeting protein/FOG degradation to guarantee a high conversion of particulate COD to energy (biogas).

2. Test and optimize the AnSBR system and process:
   a. By carrying out detailed slaughterhouse wastewater characterization, anaerobic assays on protein/FOG conversion, and monitoring of the targeted removal of water quality parameters (e.g., COD, , protein, volatile fatty acids (VFAs), total Kjeldahl nitrogen (TKN)).

   b. Monitoring and on-site sampling, testing, and tuning to determine the performance of the reactor system (Figure 4), the long-term stability, and the possible improvements to the AnSBR cycle sequence. .

3. Evaluate the technical and operational performance in time of the slaughterhouse treatment process and resource recovery potential by calculations that can lead to further optimization.

Implementing a pilot in an industrial installation provides a deeper insight into which operational parameters might be optimized and determine the feasibility of resource/energy recovery. By monitoring and process optimization, we can properly determine the discharges (quantity and quality) of the wastewater and quantify the potential of energy/resource recovery for future developments. By combining scientific background with the practical application of the anaerobic slaughterhouse wastewater system and testing its effectiveness with measurements in practice, insights for scalability and applicability in slaughterhouse wastewater of the technology can be obtained.

By means of supportive laboratory tests and pilots, research experience is gained in practice, and testing and validation of the robustness of the technology are achieved. This leads, on the one hand, to identify potentials and drawbacks for the scale-up of the process to make it suitable for slaughterhouse wastewaters, and on the other hand, opens possibilities for recovering resources (energy, nutrients) from the wastewater treatment process.

Figure 4 AnSBR containerized Pilot plant

2 Research design

This chapter presents the different work packages activities, monitoring plan overview and research questions formulated.

2.1 Overview of activities

Work package 1: Lab-tests preliminary research

Wastewater characterization and biodegradations tests were carried out to get insight into the strength of two slaughterhouse wastewaters, water quality parameters concentrations, and into conversion rates, achievable COD removal and biogas production to foresee adjustments to the process, and sub-research questions to address.

Activities:

1. Wastewater characterization from slaughterhouse location.
2. Current wastewater layout review to determine the best pilot plant location and sort out practical challenges.
3. Analysis of wastewater characterization historical data and treatment performance to already identify challenges and variability.
4. Anaerobic biodegradability tests of the main wastewater streams (average and high concentrations)

Work package 2: Monitoring plan

The performance of the novel AnSBR lab and pilot installation needs to be monitored, and a monitoring plan was discussed among the project partners. An overview of the parameters that need to be measured for reliable monitoring of the process is provided. Regular wastewater water quality performance indicators will be followed such as COD, TSS; also process stability parameters such as biogas production, and sludge settling performance such as zone settling velocity, among others (see Table 1) . Also laboratory tests, e.g., specific methanogenic activity, protein, and FOG degradation are carried out. The monitoring plan included:

a. Monitoring of the wastewater treatment performance at both lab and pilot scale by means of total and soluble COD removal,. Other variables such as ammonification and acidification levels in the buffer tank and AnSBR reactor are reported by analyzing Total Kjeldahl Nitrogen, Total Nitrogen, N-NH_4, and VFAs.
The operational values of the reactor such as organic loading rate (volumetric and specific), hydraulic retention time, and sludge retention time, among others, are registered regularly. The parameters that were measured for reliable monitoring of the process are summarized in Table 1. These process performance indicators were followed regularly on a daily and weekly basis.

b. Measuring and monitoring solids separation and sludge settleability through analysis of total suspended solids, volatile suspended solids, zone settling velocity (ZSV), and capillary suction time (CST).

c. Carry out supportive, detailed wastewater characterization tests and batch-test experiments on the degradation of protein/FOG to methane. They were implemented by carrying out: LCFAs/protein/amino acids/FOG characterization of wastewater, specific methanogenic activity (SMA), anaerobic batch tests of Protein/FOG acidification (VFAs), and overall degradation using a Biochemical Methane Potential (BMP) setup.

d. Evaluate the operational performance of the pilot and estimate the potential of nutrient and energy recovery for further AnSBR optimization.

Table 1. Analysis and measurements of the streams involved in the process. Daily measurements are from Monday to Friday at lab and pilot scale.

Analyses/measurements \ Stream			Process Wastewater	Buffer Tank	Sparthane Reactor Biomass	Sparthane Effluent	Biogas
Flow		L/day	Daily weight of "Feed bucket"		Daily weight of WAnS sampling	Daily weight of "Effluent bucket"	Daily Reading of Ritter Meter
Liq. Volume		L	Daily	Daily	Daily		
pH	from PC	-	Daily	Daily	Daily	Daily	
pH	from sample	-	Daily	Daily	Daily	Daily	
Temperature	from PC	°C	Daily	Daily	Daily	Daily	
Sludge settling	in the Process			Daily - EBT measurement			
Sludge settling	ex-situ method				1x / Week		
Biomass Morphology	CST	s			1x / Week		
Biomass Morphology	Viscosity	mPa.s			1x / Week		
Methanogenic Activity	SMA	gCOD/(gVSS.d)			1 x / Month		
TCOD	Total COD	mg/l	1 x / Week	3 x / Week	3 x / Week	3 x / Week	
CCOD	Colloidal COD	mg/l	1 x / Week	3 x / Week	3 x / Week	3 x / Week	
SCOD	Soluble COD	mg/l	1 x / Week	3 x / Week	3 x / Week	3 x / Week	
TSS	Total Suspended Solids	mg/l or g/l	1 x / Week	2 x / Week	2 x / Week	2 x / Week	
VSS	Volatile Suspended Solids	mg/l or g/l	1 x / Week	2 x / Week	2 x / Week	2 x / Week	
FOG	Fat, oil and grease	mg/l or g/l	1 x / Month	1 x / Month	1 x / Month	1 x / Month	
VFA	Volatile Fatty Acids (total)	meq/l	1 x / Week	3 x / Week		3 x / Week	
Alk	Alkalinity	meq/l	1 x / Week	1 x / Week		1 x / Week	
TKN or TN	Total Kjeldahl Nitrogen	mg N/l	1 x / Week	1 x / Week	1 x / Week	1 x / Week	
NH_4-N	Ammonia Nitrogen	mg N/l	1 x / Week	1 x / Week		1 x / Week	
NO_2-N	Nitrate	mg N/l	1 x / Month	1 x / Month		1 x / Month	
NO_3-N	Nitrite	mg N/l	1 x / Month	1 x / Month		1 x / Month	
Proteins	Lowry's Method	mg N/l	1 x / Week	1 x / Week		1 x / Week	
Total-P	Total Phosphate	mg P/l	1 x / Week	1 x / Week		1 x / Week	
PO_4-P	(ortho) Phosphate	mg P/l	1 x / Week	1 x / Week		1 x / Week	
Ca	Calcium	mg/l	1 x / Week	1 x / Week		1 x / Week	
Mg	Magnesium	mg/l	1 x / Week	1 x / Week		1 x / Week	
SO_4	Sulphate	mg SO_4/l	1 x / Week	1 x / Week		1 x / Week	
CH_4	Biogas 5000 / NaOH captu	%					Daily
CO_2	Biogas 5000 / NaOH captu	%					Daily

Work package 3: Laboratory scale test, supportive batch experiments/desk study, and Pilot preparation

The lab-scale and pilot experimental setups are adequate for the proper operation during the tests. On-line controlling, process operation, as well as the hydraulics and instrumentation, were checked, and sensors/software for online monitoring and control were improved and implemented. Batch experiments and desk study work were carried out to support the research targeting more fundamental questions (see section 2.2.1).

Activities:

1. Lab-sale set-up preparation and start-up
2. Lab-scale experiments

- Reactor operation with pre-acidification/ammonification in the buffer tank.
- Analysis of samples and the anaerobic reactor performance based on all parameters
- Monitoring, data analysis, and reporting

3. Batch experiments
   - Detailed characterization of wastewater (FOG, Protein, FA/LCFA profile, amino acids profile, sterols profile)
   - Tests of methane potential at different Inoculum/Substrate ratios.
   - Test of protein degradation at different FOG concentrations.
   - Microbial analysis of samples taken from lab-scale and pilot systems with high throughput sequencing using the MiSeq Illumina platform (16S rRNA gene (V3-V4 region)

   Desk study
   - Potential of resource recovery.

4. Pilot preparation:
   - Software engineering implementation to improve and have better control and monitoring of the pilot plant
   - Implementation of new pipes, tubing, pumps, calibration, and hydraulic checkups.
   - Pilot installation and operation

Work package 4: Pilot test

This work package was mainly focused on finding the operational conditions that allowed to achieve the maximum conversion of the COD to biogas without compromising the quality of the solid's separation/sludge settleability.

Activities:
1. Commissioning, installation of the pilot plant on site
2. Operating the system, data logging, sampling/analysis on site
3. Monitoring, off-site (lab) analysis, data analysis, and reporting

Work Package 5: Dissemination

Dissemination of the project results will be carried out throughout the project with the help of regular progress updates on the TKI website, magazines, presentations in relevant national/international events/conferences, journal articles, and a final public TKI report in which the results of the lab-scale, batch experiments and pilot are summarized.

2.2 Overview of research questions

The main research questions to be answered have been divided into the two main phases, laboratory scale experiments and pilot tests:

Laboratory scale tests

For the laboratory scale tests, the following four research questions are formulated:

1. What is the biodegradability of slaughterhouse wastewater?
2. How is the acclimation process of biomass to slaughterhouse/protein-rich wastewater?
3. How does the increase of volumetric loading rate affect reactor performance?
4. How do the operational parameters (HRT, SRT, VLR/SLR) and processes (ammonification, acidification) impact the reactor performance (TCOD/SCOD removal) and the sludge settleability?

Support laboratory scale experiments

These research questions were formulated to identify any possible anaerobic conversion inhibition and the core microbiome involved in the conversion:

5. What are the amino acids, FAs/LCFA, and sterols composition profiles of the slaughterhouse wastewater?
6. What is the methane production achieved with the slaughterhouse wastewater at the different substrate to inoculum ratios?
7. What is the influence of lipids on protein conversion in the anaerobic reactor?
8. What is the core microbial community structure involved in the buffer tank and in the AnSBR reactor?

Desk study

9. What is the resource (methane/energy, nutrients) recovery potential from the slaughterhouse wastewater and effluent stream by applying the AnSBR technology?
10. What are the potential technologies for nutrient recovery?

Pilot tests

For the pilot plant tests, the following operational main research questions are formulated:

11. What is the impact of volumetric load and wastewater composition fluctuations on the performance of the system?
12. What are the specific loading rates that maximize digestion efficiency?

3 Results preliminary research: wastewater analysis and biodegradability tests

This chapter compile the materials and methods and preliminary results obtained from the slaughterhouse sampling campaign, wastewater characterization and anaerobic treatability tests.

3.1 Materials and Methods

To gain insight into the strength of two slaughterhouse wastewaters, characterization in terms of bulk parameters and specific compounds was carried out. The two types of samples were: regular wastewater and concentrated wastewater (After the hot bath tank, wastewater is released, called “Na Broeibak” in this report). The regular wastewater samples were collected directly from the wastewater treatment plant at the level of the 24h sampler in the influent line between the Buffer Tank and the DAF unit (see WWTP scheme in Figure 2). Composite and daily grab samples were collected. For “Na Broeibak” samples, daily grab samples were collected shortly after draining the Broeibak .

3.1.1 Wastewater analyses

Five sets of wastewater samples analyzed to characterize them in terms of bulk parameters and specific compounds. Daily samples were taken between 8/06/20 and 15/06/20, both grab samples and from the 24h sampler. The individual weekly grab samples were mixed together, making up the “Weekly Grab Sample”, respectively the 24h samples made up the “Weekly 24h sample”. During three days (16,18 and 19/06/20), grab samples of the highest COD load wastewater flows, after the hot bath “Broeibak” (from cleaning) process were taken and mixed, “Na Broeibak”. Also, high COD and low COD samples were taken on the same day were characterized. From these five sets, the first three were also selected to proceed with the biodegradability tests.
Solids and volatile fractions (TS, TSS, VS, and VSS) of wastewater, nitrogen concentrations (total Kjeldahl nitrogen (TKN), soluble Kjeldahl nitrogen (SKN), ammonium (NH4+)) were analyzed following standard methods (APHA, 1998).
HACH kits (HACH, Berlin, Germany) were used to quantify COD (LCK 400, 514, 014, and 914), calcium–magnesium (LCK327), and total organic carbon (LCK386) following the manufacturer’s protocol. Alkalinity in mol/L was quantified using TitraLab AT 1000 series (HACH, Berlin, Germany).
The volatile fatty acids composition (C2 to C6) was determined using gas chromatography (GC, 7820 A, Agilent Technologies, Amstelveen, Netherlands) equipped with a flame ionization detector using a CP 7614 column (WCOT fused Silica 25 m × 0.55 mm, CP-wax 58 FFAP capillary, Agilent Technologies).
Major inorganic anions: chloride, sulfate, phosphate, nitrate, and nitrite, were analyzed conform NEN-ISO 15923-1.
Major inorganic cations: sodium, magnesium; potassium; calcium, were analyzed in accordance with NEN 6953 (digestion NEN6961, measurement NEN-EN-ISO 17294-2 (2004)).

3.1.2 Anaerobic biodegradability tests

The batch biodegradability tests were carried out using AMPTS-II equipment. In glass bottles, known amounts of pH-neutralized wastewater (diluted to different predetermined COD concentrations with a $NaHCO_3$ solution) were

inoculated with a known quantity of seed biomass. The headspace of the bottles was made oxygen-free by flushing with an N_2:CO_2 (70%/30%) mixture, thereby providing a near-neutral pH buffered by HCO_3^-/CO_2. Test bottles for biogas production monitoring were prepared, along with equally prepared parallel bottles for liquid sampling and blank bottles (= substrate-free controls) to correct for the endogenous methanogenic activity of the sludge. The bottles were placed in a water bath or in a temperature-controlled cabinet at 36 ± 1°C. Gentle stirring assured good contact between the wastewater and the anaerobic biomass. In the AMPT-II system, the produced biogas is led over a caustic wash bottle (to remove CO_2 and H_2S) to gas counters that register the production of CH4. At selected time intervals, samples withdrawn from the parallel test bottles were analyzed on SCOD and VFA.

3.2 Results

The results are shown in the Table 2. Samples were very similar, and as expected, High COD and “Na Broeibak” were more concentrated. Overall, the samples were neutral, medium-strength, and high in solids, with about 50% of particulate COD. The FOG concentration ranged from 350 to 420 mg/L, thus accounting for 20 to 30% of samples' COD. Natural pre-acidification of the samples can also be observed by the presence of high GC-analyzed volatile fatty acids concentrations. These VFAs make up around 30% of the COD of the mixed samples. Nitrogen and Phosphorous contents followed the trend of the COD. TKN ranged between 298 to 597 mg N/L, and Total Phosphorous between 42 and 57 mg P/L. the concentrations of major anions (chloride, nitrate, nitrite, and sulfate) are also very low: 25 mg/L of Sulphate and Chloride while no Nitrate and Nitrite were detected. The presence of chloride was detected up to 260 mg/L. The presence of the remaining major anions (nitrate, nitrite, and sulfate) was not detected, with the exception of Sulphate at low levels in sample “Weekly 24h sample”. The samples contained low to moderate concentrations of Calcium, Magnesium, Sodium, and Potassium.
As mentioned above, three sets of samples were further subjected to the biodegradability tests. Results confirm overall high biodegradability (>80%) of the raw stream (before dissolve air flotation DAF). It was possible to observe a fast conversion of the COD. Within four days, the first two samples - “Weekly Grab Sample” and “Weekly 24h sample” - showed 80% SCOD conversion to CH4 and almost 30% particulate COD conversion. Within 15 days, maximum production was achieved with 80% TCOD conversion to CH4 and SCOD removal of 90%. The last sample - “Na Broeibak,” resulted in similar SCOD removal efficiencies; however, maximum COD conversion was around 10% lower.

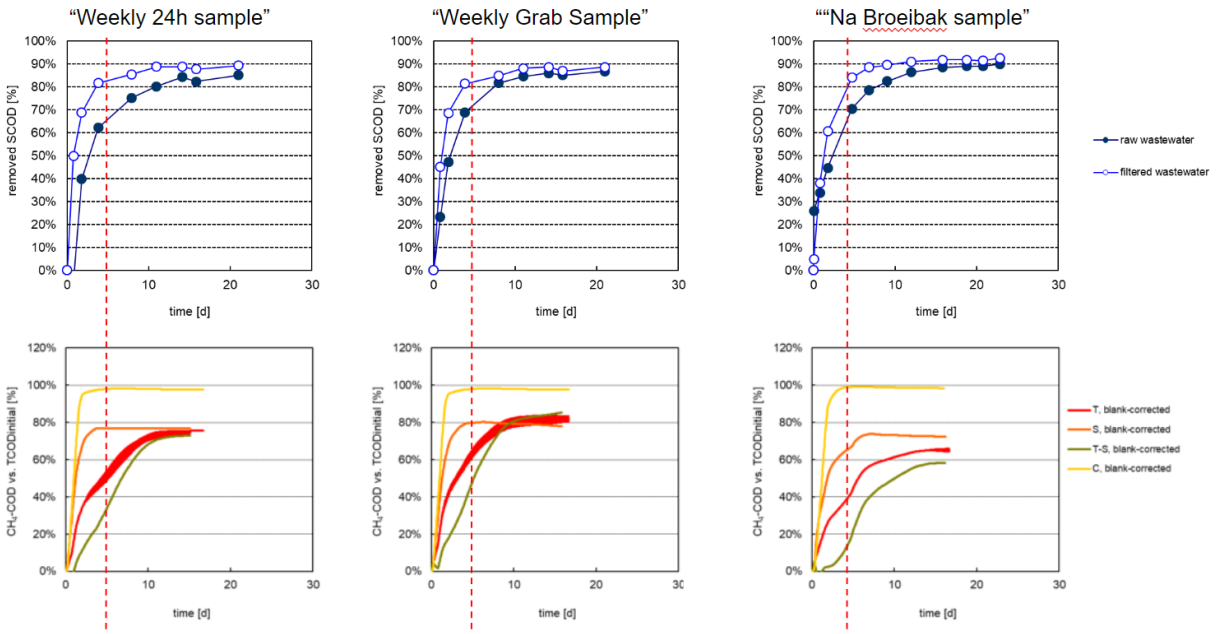


Figure 5 Biodegradability tests total and soluble COD removal efficiency [%].

Table 2 Wastewater characterization from sampling campaigns.

Parameter		Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
		"Weekly Grab Sample"	**"Weekly 24h sample"**	**"Na Broeibak"**	**High COD grab**	**Low COD grab**
Chemical Oxygen Demand		**mgCOD/L**	**mgCOD/L**	**mgCOD/L**	**mgCOD/L**	**mgCOD/L**
TCOD		4169	5255	7129	5773	3594
CCOD (supernatant after centrifugation)		2000	2143	3502	2571	738
SCOD (filtered <0.45µm after centrifugation)		1832	1980	3186	2357	616
Solids		g/kg	g/kg	g/kg	g/kg	g/kg
TS, Total Solids		2.747	3.664	4.471	3.886	2.857
VS, Volatile Solids		1.979	2.748	3.376	2.646	2.057
IS, Inorganic Solids		0.768	0.916	1.095	1.240	0.800
TSS, Total Suspended Solids		1.309	2.572	2.098	2.494	1.651
VSS, Volatile Suspended Solids		1.288	2.353	1.944	2.219	1.350
ISS, Inorganic Suspended Solids		0.021	0.219	0.154	0.275	0.301
Nitrogen		mgN/L	mgN/L	mgN/L	mgN/L	mgN/L
TKN, Total Kjeldahl Nitrogen		298	336	597	370	318
SKN, Soluble Kjeldahl Nitrogen		192	213	427	245	207
NH4-N, Ammoniacal Nitrogen		142	181	215	173	174
Phosphorous		mgP/L	mgP/L	mgP/L	mgP/L	mgP/L
P-tot, Phosphorous Total		43.6	56.3	56.9	47.8	41.9
ortho-P, ortho-Phosphate (H3O4 + H2PO4- + HPO42- + PO43-)		34.7	44.9	44.2	35.8	27.1
Volatile Fatty Acids		mg/L	mg/L	mg/L	mg/L	mg/L
C2, acetic acid	C2H4O2	390	515	655	335	24
C3, propionic acid	C3H6O2	260	360	410	210	17
iC4, isobutyric acid	C4H8O2	0	83	83	34	0
C4, butyric acid	C4H8O2	105	145	155	78	0
iC5, isovaleric acid	C5H10O2	140	195	150	0	0
C5, valeric acid	C5H10O2	31	37	28	18	0
C6, hexanoic acid	C6H12O2	0	0	0	0	0
Major Anions		mg/L	mg/L	mg/L	mg/L	mg/L
Cl-, chloride	as Cl	200	230	230	260	200
NO3-, nitrate	as N	0	0	0	0	0
NO2-, nitrite	as N	0	0	0	0	0
SO42-, sulphate	as SO4	0	6	0	0	15
Major Cations		mg/L	mg/L	mg/L	mg/L	mg/L
Ca2+, calcium	as Ca	41	87	76	90	75
K+, potassium	as K	12	25	25	24	22
Mg2+, magnesium	as Mg	60	110	140	120	100
Na+, sodium	as Na	130	220	220	230	200
Other analyses						
Conductivity	mS/cm	2.3	2.57	2.90	2.63	2.56
TOC	mg/L	1,196	1,410	1,926	1,607	848
Alkalinity Total (pH-4)	meq/L	18	22	20	27	21
pH		6.87	7.01	6.84	6.84	7.23
FOG	mg/L	350	420	420	/	/

3.3 Conclusions

- Wastewater characterization of the samples led to conclude that this slaughterhouse wastewater fits the characteristics of anaerobic treatment with the AnSBR (Sparthane™ technology).
- Namely, its high solids fraction, with particulate COD accounting for more than 50% of the COD and high TSS and FOG above 350 mg/L.
- Slaughterhouse wastewater samples were well and quickly degradable: 10 days of incubation with non-adapted anaerobic granular biomass led to 90% COD removal. No sign of toxicity was observed during the incubation of the three sets of samples tested.

4 Results of lab-scale testing of AnSBR

This chapter showed the materials and methods, and main results obtained from the lab-scale testing of the AnSBR system treating slaughterhouse wastewater.

4.1 Materials and Methods

4.1.1 Inoculum and wastewater characteristics

The inoculum sludge was taken from an anaerobic high-rate granular sludge bed reactor from a biochemical company in the Netherlands. The sludge had a solids content of 9210 mgTSS·L^{-1} and 8400 mgVSS·L^{-1}. The sludge was blended, and in total, 214 mL sludge was inoculated into the 30 L reactor.

The protein-rich feed of the process was raw wastewater collected from the inlet of a slaughterhouse wastewater treatment plant in the Netherlands. The main characteristics of the slaughterhouse wastewater and inoculum are shown in Table 3.

Table 3 Characteristics of protein-rich SWW and inoculum

	Units	SWW	Inoculum
pH	-	6.8-7.8	-
TSS	mg·L^{-1}	1,140 – 3,650	9,210
VSS	mg·L^{-1}	1,080 – 3,540	8,400
TCOD	mg·L^{-1}	4,737 – 6,488	-
SCOD	mg·L^{-1}	1,472 – 3,803	-
NH_4^+-N	mg·L^{-1}	175 – 424	-
Proteins	mg·L^{-1}	500 – 700	-
Carbohydrates	mg·L^{-1}	100 – 250	-
FOG	mg·L^{-1}	0 – 4,000	-

4.1.2 Reactor setup and operational conditions

The reactor setup consisted of a 10 L buffer tank (BT), a 30 L reactor, and a 12 L settling tank (Figure 6). The biogas was collected, and the volume was recorded by a gas meter (Ritter TG05/5 , Germany), whereas the treated effluent was collected in an effluent tank.

The anaerobic reactor (Figure 7) was operated as a sequencing batch reactor (AnSBR), with four cycles per day. During each cycle, the effluent of the buffer tank was fed to the reactor, and then raw slaughterhouse wastewater

was fed to the buffer tank. About 10 L of the reactor content was transferred to the settling tank for degassing and settling. At the end of the cycle, the liquid in the settling tank was discharged to the effluent tank, whereas the settled sludge was returned to the reactor.

The organic loading rate (OLR) in the reactor was gradually increased from 2 g COD $\cdot L^{-1} \cdot day^{-1}$ to the design threshold of 6.2 g COD $\cdot L^{-1} \cdot day^{-1}$ within three different phases of operation. During the startup phase (phase I, 0 – 55 days), the biomass was acclimated to the slaughterhouse wastewater. In phase II (Days 56 – 196), the reactor was operated with an average OLR of 2.0 ± 0.6 gTCOD$\cdot L^{-1} \cdot d^{-1}$ and an average specific loading rate (SLR) of 0.2 ± 0.1 gTCOD$\cdot gVSS^{-1} \cdot d^{-1}$. In phase III (Days 197 – 260), the OLR was gradually increased to around 5.0 gTCOD$\cdot L^{-1} \cdot d^{-1}$, to investigate the treatment capability of the reactor. The pH was maintained at 7.0 in the buffer tank and in the reactor. The temperature of the AnSBR was controlled at 36 ± 0.5 °C with a water bath (VWR International MX06S135, USA), whereas the buffer tank and the settling reactor were operated at ambient temperature. The operational parameters of the reactor are summarized in Table 4. The sludge retention time (SRT) was calculated by the total solids in the reactor divided by the wasted solids of the day. Correspondingly, the HRT was calculated standardly as effective working volume divided by the flow rate of the day.

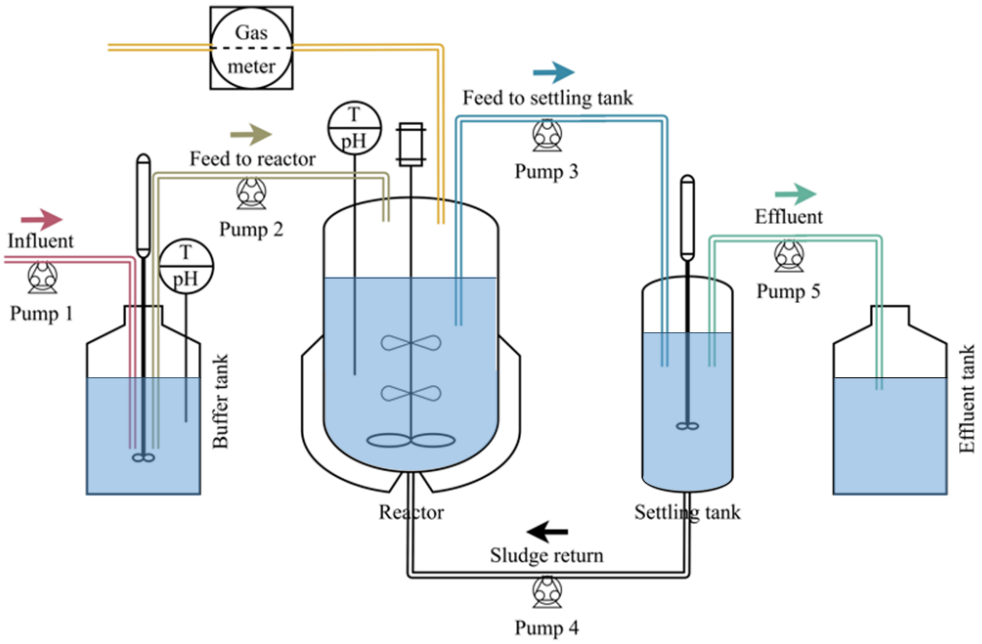


Figure 6. AnSBR (Sparthane™) scheme of the laboratory experimental setup at Biothane.

Figure 7. Laboratory scale AnSBR (Sparthane™) set-up.

Table 4 Operational parameters of the reactor

	OLR	SLR	HRT	SRT	TSS	VSS	Cycle length	pH	T*
	$gTCOD \cdot L^{-1} \cdot d^{-1}$	$gTCOD \cdot gVSS^{-1} \cdot d^{-1}$	d	d	$g \cdot L^{-1}$	$g \cdot L^{-1}$	h	-	°C
Buffer Tank	2 - 6	-	18 ± 12	-			6 (4 cycles/ day)	7.0	-
AnSBR	I: 1.8 ± 1.0	0.25 ± 0.13	2.9 ±1.6	79 ± 67	7.0 ± 1.0	6.0 ± 1.0	4	7.0	36
	II: 2.0 ± 0.6	0.17 ± 0.07	2.4 ± 1.3	124 ± 67	8.8 ± 1.1	7.9 ± 1.1	4	7.0	36
	III: 3.2 ± 1.0	0.23 ± 0.06	1.5 ±0.7	64 ± 19	10.6 ± 2.0	9.9 ± 2.0	4	7.0	36
Settling Tank	-						2	7.0	-

T*: temperature.

4.1.3 Sampling and Analysis

Analytical methods

Total (TCOD) and soluble COD (SCOD) were analyzed as indicated in the monitoring plan . The TCOD and SCOD (filtered through 0.45 µm Whatman filters) measurements were carried out using HACH-Lange kits (LCK014).
Total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), soluble Kjeldahl nitrogen (SKN), ammonium (NH4+), and volatile fatty acids (VFAs) were analyzed once per week. The solid's content and nitrogen concentrations were analyzed following standard methods (APHA, 1998). All samples were taken from the raw feed, buffer tank, reactor, and effluent tank.
Samples for VFAs analysis were first centrifuged at 13,500 x g for 5 min and filtered through 0.45 µm membrane filters (Whatman, Sigma Aldrich). The composition of VFAs was analyzed as described previously by Tan et al. (2021).
Proteins and carbohydrates concentrations from the buffer tank and the reactor were also analyzed once per week. Samples were centrifuged at 6,500 xg for 10 min. The protein and carbohydrate content in the supernatant and supernatant after filtration with 1 µm or 0.45 µm was measured. Protein concentrations were assessed following the manufacturer protocol of the Bicinchoninic acid kit (BCA protein assay, BCA1-1KT, Sigma Aldrich), measured by a spectrometer at 562 nm, with BSA as standard. Carbohydrates were analyzed following the Dubois method (Dubois et al., 1956).
Particle size distribution (PSD) of the sludge was measured between 0.01 to 2000 µm using Blue wave light scattering Micrortac (Retsch Technology GmbH, Germany) with Microtrac FLEX 11.1.0.2 software at a flowrate of 25%.

4.2 Results

4.2.1 AnSBR performance

The AnSBR was operated for 260 days, divided into three different phases as mentioned above. After the first 10 days of the startup phase (I), the TCOD removal efficiency gradually increased by applying an OLR of 1.8 ± 1.0 $gTCOD \cdot L^{-1} \cdot d^{-1}$, and achieved an average of 78 ± 10 % during this phase (Figure 8). In phase II, in which a stable volumetric loading rate of 2.0 ± 0.6 $gTCOD \cdot L^{-1} \cdot d^{-1}$ was aimed (Figure 9), an average of 81 ± 5% TCOD removal efficiency was obtained, whereas in the last phase (III), the TCOD removal reached an average of 83 ± 6% at increasing OLR up to 6.2 $gTCOD \cdot L^{-1} \cdot d^{-1}$. The SCOD removal was 87± 5% during phase I, and it decreased to 82 ± 8% - 83± 6% during the last two phases due to increasing OLR (Figure 8).
Throughout the trial, the sludge showed very good settling behavior. This can be observed by the high TCOD removal efficiencies, as well as the low solids concentration in the effluent. The sludge concentration inside the reactor ranged between 6 and 13 g TSS/L, and the effluent TSS was, on average, 200 mg/L (Figure 10).
The conversion of organic compounds to CH_4 was registered as digestion efficiency (%). Unlike the relatively stable COD removal, the degradation efficiency constantly decreased with the increase of OLR. During phase I, the digestion efficiency had an average of 79 ± 12%; it decreased to 72 ± 15% during phase II and further decreased to an average of 70 ± 14% in phase III with the higher loading rate applied (Figure 9).
During phases I and II, the SRT was 79 ± 67 and 124 ± 67 days to maintain the biomass within the reactor (Figure 10). During phase III, the SRT was gradually decreased to 64 ± 19 days to avoid excessive TSS content in the reactor. During phases I and II, the HRT was maintained at an average of 2.9 ± 1.7 days and 2.4 ± 1.3 days, respectively, and in phase III, the HRT was controlled at 1.5 ± 0.7 days. The increase in OLR led to the decrease of HRT and, concomitantly, a decreasing digestion efficiency.

The VFA production as a percentage of TCOD and SCOD was recorded to evaluate the effect of pre-acidification on the COD removal. Due to the high solids content, the VFA production as TCOD was maintained below 40%, whereas the fraction of VFA in SCOD was generally higher than 60% after phase I. During phase III, i.e., days 196 – 250, the degree of pre-acidification was varied by adjusting the HRT in the buffer tank to enlarge the range of VFA fraction in TCOD and SCOD.
Ammonification percentage was determined as the ratio between the measured ammonium concentration (mgN·L-1) divided by the measured total Kjeldahl nitrogen (TKN) concentration (mgN·L-1). During phase I, the average ammonification efficiency in the buffer tank was 57 ± 9%, and in the reactor was 71 ± 19%. In the reactor, the ammonification efficiency increased from 50% to 85% during phase I, indicating that the microbes were acclimated to the slaughterhouse wastewater. During phase II, the ammonification efficiency decreased with the increasing OLR in the buffer tank, with an average of 53 ± 18%, whereas in the reactor, the ammonification efficiency increased to an average of 82 ± 12%. Especially days 125 – 200, the ammonification efficiency was increased to 92 ± 12 %. During phase III, the ammonification efficiency in the buffer tank was further decreased to an average of 47 ± 10%. In the reactor, the ammonification efficiency decreased from above 90% to below 80% with the increase from 3.5 $gCOD \cdot L^{-1} \cdot d^{-1}$ to 6.2 $gCOD \cdot L^{-1} \cdot d^{-1}$ in OLR.; the average ammonification efficiency during phase III was 87 ± 20%. Based on the results, a moderate OLR between 2.0 $gCOD \cdot L^{-1} \cdot d^{-1}$ and 3.5 $gCOD \cdot L^{-1} \cdot d^{-1}$ is required to maintain a high ammonification percentage (≥85%).

To investigate the organic residuals in the reactor, protein and carbohydrate concentrations in the broth were determined. Both protein and carbohydrate concentrations decreased within phases I and II. The protein concentration decreased from 419 ± 128 $mg \cdot L^{-1}$ to 297 ± 108 $mg \cdot L^{-1}$, and the carbohydrate concentration decreased from 38 ± 17 $mg \cdot L^{-1}$ to 23 ± 5 $mg \cdot L^{-1}$ at a stable OLR of 2.0 ± 0.7 $gCOD \cdot L^{-1} \cdot d^{-1}$. This is attributed to the fact that the microbes were acclimated to the feed wastewater, which was also shown in the increase in ammonification

efficiency. In phase III, the protein concentration increased from 200 mg·L^{-1} to 550 mg·L^{-1} when the OLR was increased from 3.5 gCOD·L^{-1}·d^{-1} to 6.2 gCOD·L^{-1}·d^{-1}, and the carbohydrates concentration also had an increase from 20 mg·L^{-1} to 35 mg·L^{-1}. Under the applied operational conditions, it took about 150 days to achieve a relatively low organic residual concentration in the reactor.

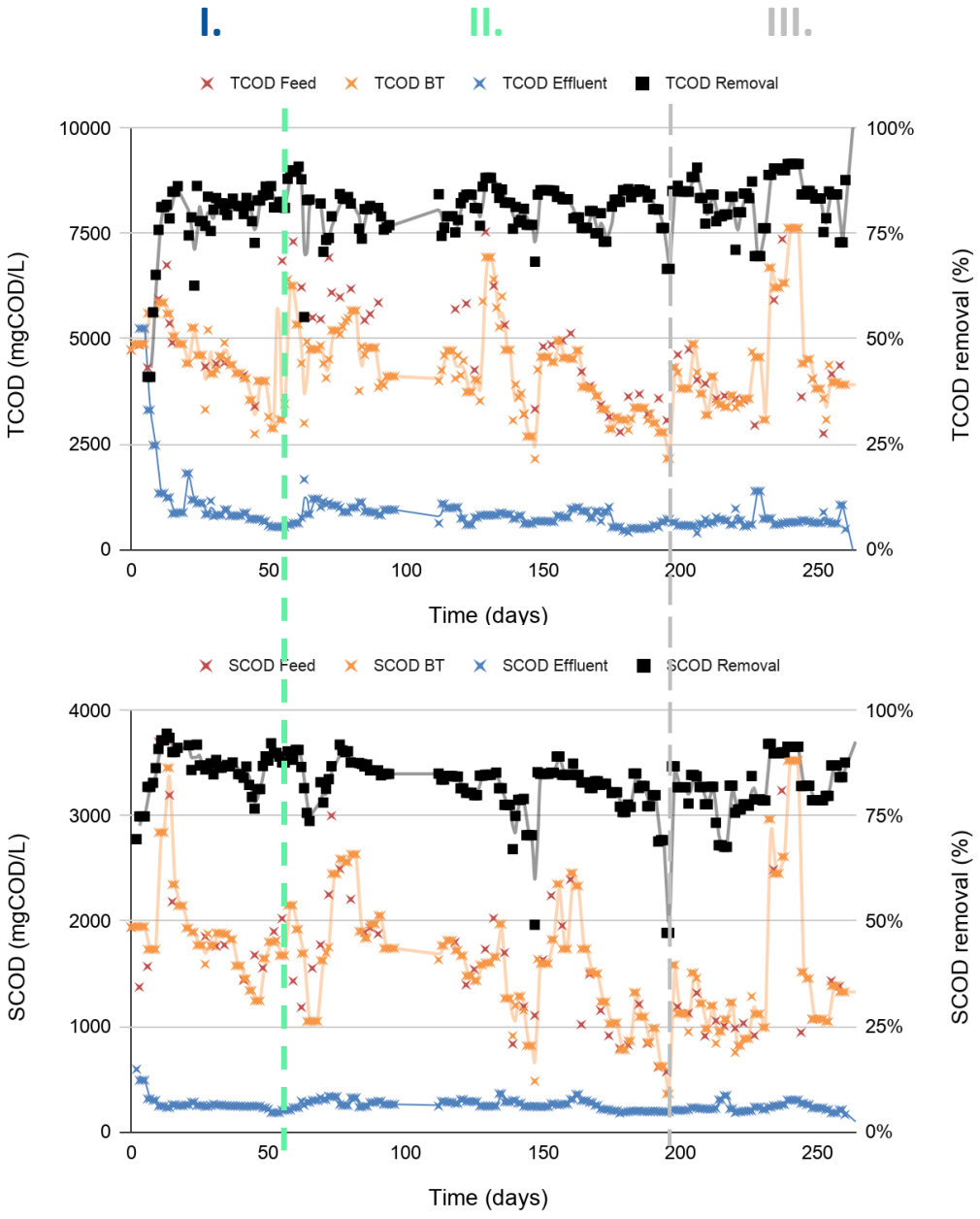


Figure 8 Total and soluble COD removal.

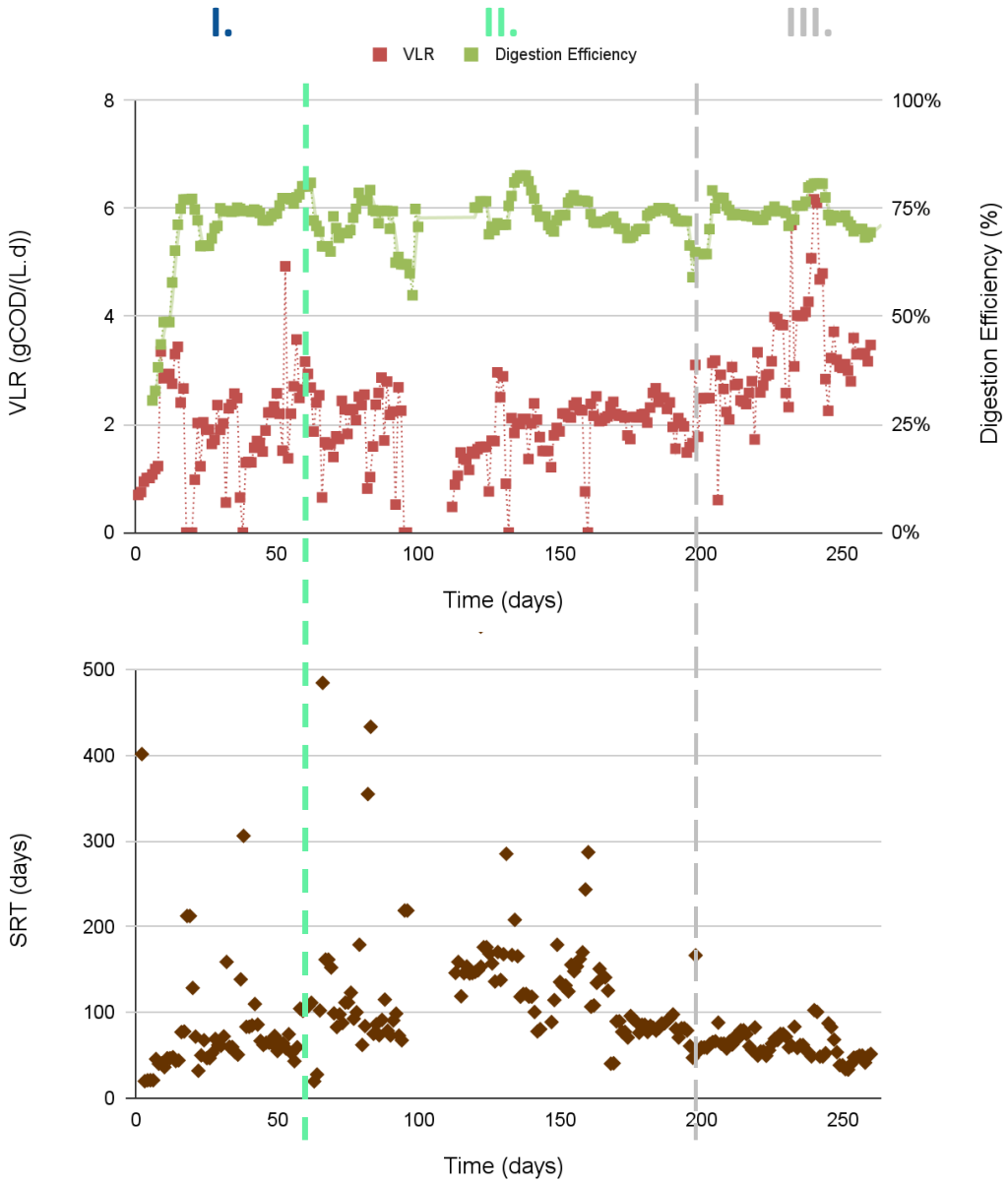


Figure 9 Volumetric loading rate, digestion efficiency and sludge retention time during lab trials.

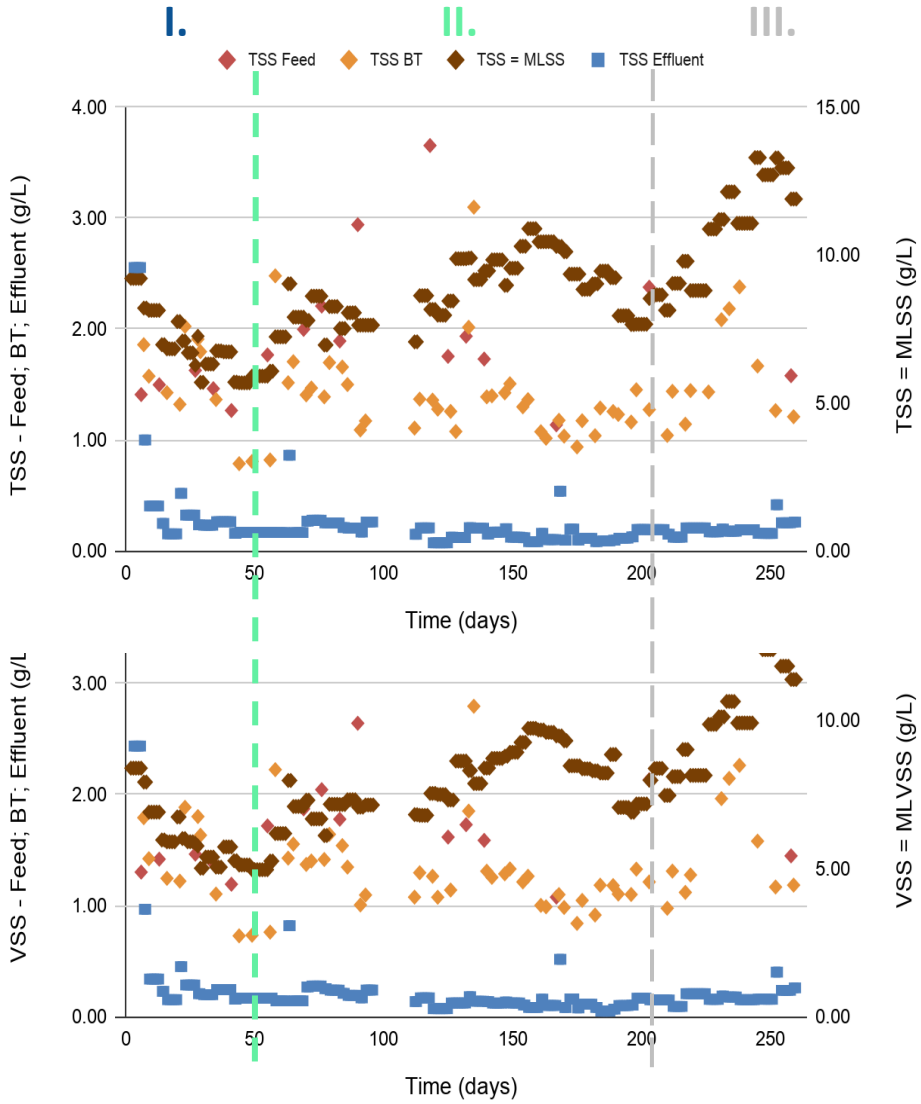


Figure 10 Solids concentration during the lab-scale trial.

4.3 Conclusions

The novel AnSBR system achieved a max of 90% TCOD removal and over 70% degradation efficiency without pre-treatment at an OLR below 6.2 $kgCOD \cdot L^{-1} \cdot d^{-1}$ treating a high-protein and FOG slaughterhouse wastewater.
Changes in OLR in the reactor and HRT in the buffer tank impacted the COD removal substantially, impacting the performance of the AnSBR, parallel with the SLR, HRT, and protein degradation efficiency.
The sludge settleability was affected by the HRT in the buffer tank and reactor, which regulated the carbohydrates concentrations and ammonification efficiencies.

5 Results laboratory batch tests and microbial community analysis

This chapter presents materials and methods, and results obtained from the batch tests carried out and microbial community analysis to support the research of the AnSBR system treating slaughterhouse wastewater.

5.1 Materials and Methods

5.1.1 Slaughterhouse wastewater characterization

Sampling

Two different sampling campaigns were carried out from the slaughterhouse wastewater. Samples were taken from the influent of the current wastewater treatment plant (after the buffer tank). One sample was a grab sample from the expected time to get the higher wastewater strength of the plant (sample A), and another as 24 h composed sample (Sample B).

Analytical methods

Measurements were made with the corresponding Hach-Lange test method. The chemical oxygen demand (COD), subdivided into total COD (CODt) and the dissolved fraction COD (CODs), were determined using the same type of Hach-Lange measurement. For soluble COD, the sample was filtered over a 0.45 µm filter. For ammonium (NH_4^+), total Kjeldahl nitrogen (TKN), total soluble nitrogen (TNs), and soluble phosphate, the samples were also filtered, and dilutions were made when necessary.

The FOG content was measured using the Soxhlet extraction method described in the NEN 6671:2013. In the method, FOG soluble in petroleum ether is extracted from the sample and determined gravimetrically. The minimum level of detection was 20 $mg.L^{-1}$. The total Kjeldahl nitrogen (TKN) was determined following the NEN-ISO 5663 method.

The total solids (TS), volatile solids (VS), total suspended solids (TSS), and volatile suspended solids (VS) were measured on weight base (gL^{-1}) according to the standard methods for the examination of water and wastewater (APHA, 1998).

The amino acid composition of the wastewater was determined by implementing the method REG CE 152/09 27/01/09 ALL III MET F PTO 5.3 for water samples, including relative density (specific gravity) determination. The lipid fraction of the sample was analysed for acidic composition and sterols composition using GC-FID following the methods UNI EN ISO 12966-2:2011 + 15282:1997 + UNI EN ISO 12966-1:2015+UNI EN ISO 12966-4:2015 and UNI EN 15282:1997 + REG CEE 2568/1991 ALL V REG UE 1604/2019, correspondingly.

5.1.2 Methane production potential of slaughterhouse wastewater

The slaughterhouse wastewater was loaded into 500 mL digesters with three (3) different inoculum (I) to substrate (S) ratios (I/S = 0.5, 2.0, and 4.0) to evaluate the wastewater methane conversion. The inoculum used was from an anaerobic high-rate granular sludge bed reactor from a biochemical company in the Netherlands. The anaerobic digestion tests were performed using an Automated Methane Potential Test System AMPTS II (Bioprocess Control,

Sweden) (see Figure 11), following the protocols suggested by Holliger et al. (2016); Loosdrecht et al. (2016). The AMPTS calculates and records the volume of gas under normal conditions (NmL, 0°C, 100 kPa). The experiments were carried out at 35 °C. CO_2 and H_2S gas were stripped from the biogas by a 3 M NaOH solution before entering the methane-flow cell meter. The tests were conducted in triplicate. Positive control experiments using cellulose were carried out.

Figure 11. Lab-set up for batch anaerobic batch tests (AMPTS).

Cumulative methane production (CMP)

The CMP is the net methane production per gram substrate COD added during the entire incubation period (subtracting the methane production from the blank) at standard temperature and pressure (T = 0 °C and P = 1 atm). It is expressed as NmL $CH_4.gCOD_{substrate}^{-1}$.

The CMP tests were stopped when the daily methane production was less than 5% of the CMP_t , i.e., when CMP_t-$CMP_{t-1}/CMP_t \leqslant 0.05$, where CMP_t is the average cumulative methane production at time *t* (NmL $CH_4 \cdot gCOD_{substrate}^{-1}$) and CMP_{t-1} is the average cumulative methane production one day before *t* (NmL $CH_4.gCOD_{substrate}^{-1}$) (Ghasimi et al., 2016).

Biochemical Methane Potential (BMP)

The BMP is an indication for the potential biogas that can be produced determined by the net methane production per gram substrate $COD_{substrate}$ added during the entire incubation period (subtracting the methane production from the blank) at standardized temperature and pressure (T = 0 °C and P = 1 bar) which is expressed as NmL $CH_4.gCOD_{substrate}^{-1}$.

Biodegradability

Anaerobic biodegradability was assessed as the experimental ultimate methane production (expressed in g COD) over the initial total COD (tCOD) of the substrate (Raposo et al., 2011). It is noted that the methane produced will

be lower than the theoretical value, as it does not take into account the COD needed for bacterial cell growth and their maintenance, which has been reported typically 5–10% of organic material degraded, depending on the type of substrate (Angelidaki & Sanders, 2004). Moreover, during bioconversion, non-mechanized biodegradable or non-biodegradable intermediates may occur, lowering the actual methane yield of the substrate.

5.1.3 Effect of fat, oil and grease (FOG) on protein degradation

The effect of lipids on anaerobic protein degradation in batch tests at different ratios of FOG and Protein concentrations (expected from slaughterhouse wastewater characteristics) were assessed. Bovine serum albumin (BSA, Sigma Aldrich) was used as the protein source, while Oleic acid was used as the main lipid (FOG) identified. The experiment was carried out using the AMPTS II as in 5.1.2 and sludge (inoculum) of the AnSBR to determine the biogas production, and in the liquid phase, the protein degradation and VFAS production during the first 48 h of digestion. An inoculum to substrate ratio (I/S) of 4 was used.

Protein concentrations in the batch reactors were assessed following the manufacturer protocol of the Bicinchoninic acid kit (BCA protein assay, BCA1-1KT, Sigma Aldrich). Protein was determined using the Lowry method assay measured by a spectrometer at 660 nm using BSA as standard.

Samples for VFAs analysis were first centrifuged at 13,500 x g for 5 min and filtered through 0.45 µm membrane filters (Whatman, Sigma Aldrich). The composition of VFAs was analyzed as described previously by Tan et al. (2021).

5.1.4 Microbial community analysis from Lab-scale AnSBR

Microbial community analysis

Microbial community analysis was performed to get insight into the core microbiome and find relations with the key processes of ammonification and COD conversion to biogas. 10 mL sludge samples were taken regularly from the buffer tank and reactor and centrifuged at 13,500 x g for 5 min. The supernatant was discarded, and the biomass pellets were stored at – 20 °C in Eppendorf tubes (Eppendorf, Germany). The sludge samples were then sent for DNA extraction and amplicon sequencing (Novogene, UK).
Before the DNA extraction, the biomass pellets were thawed, and biomass of duplicate samples were combined, weighted to 250 mg, and transferred to PowerBead Pro tubes. DNA was extracted with the DNeasy PowerSoil Pro Kit (Qiagen, Germany), and DNA quality and quantity was verified by Agarose Gel Electrophoresis and using a 5400 Fragment Analyzer System (Agilent, US). DNA (16S rRNA gene) amplification was carried out by Illumina Novaseq 6000 platform by Novogene, using the primers 341F [(5′–3′) CCTAYGGGRBGCASCAG] and 806R [(5′–3′) GGACTACNNGGGTATCTAAT] for bacteria/archaea in the V3–V4 regions.
Alpha and beta diversities were calculated with the phyloseq library (McMurdie & Holmes, 2013). A PCoA was plotted to visualize the beta diversity differences between samples using the unweighted UniFrac distance metric. Only the ASVs that prevailed in 95% of all resamples were considered as the core microbiota. Core members within the operational phases were analyzed with BLAST against the refseq RNA database to identify the closest related species. The sequences were deposited in the SRA (NCBI) database under the accession number PRJNA847614.
A Permutational Multivariate Analysis of Variance (PERMANOVA) was calculated with the vegan library (Oksanen et al., 2007) to correlate the changes in the microbial community composition (distances) in samples of the AnSBR system with the operational parameters and performance indicators. The data sets were considered statistically different when a p-value ≤ 0.05 was determined.

5.2 Results

5.2.1 Slaughterhouse wastewater characterization

The characterization results of the wastewater composition are summarized in Table 5. The two different samples exhibited very similar results in all parameters, indicating the wastewater strength of the samples was very similar. An average difference between samples of 5.0% was observed. The highest differences were found to be TNs, TKNs, and organic acid, with differences of 18%, 10%, and 8%, respectively, between the samples. The slaughterhouse wastewater (SWW) is mainly characterized by high organic contain (COD 5250±99 mg/L) from which 56% is particulate (CODs 2285±7 mg/L), high suspended solids (TSS 1855±7 mg/L, which is 52% of TS), high protein concentration (715±70 mg/L), considerable lipids concentration (FOG 345±7 mg/L), and nutrient content (Total Nitrogen 354±16 mg/L, and Total Phosphate 40±16 mg/L). These results were in agreement with those obtained during the preliminary sampling campaign (Chapter 3).
The short FA and LCFA content of the SWW lipid fraction indicated that short FA were not detected, while LCFA were mainly from C14-C20 present (see table 6). The major three LCFAs were determined as % from the lipid fraction where Palmitic acid (C16:0), Stearic acid (C18:0), and Oleic acid (C18:1) with a content fraction of 28,97±0,97%, 26,95±0,94%, and 35,26±1,09%, respectively, which counted as a total of 91% of the total lipid composition. Accordingly, the total monosaturated and saturated FA were 38±1% and 59±1%, respectively.
An insight into the amino acids composition of the wastewater samples indicated that only aspartic acid, glutamic acid, and leucine were observed with concentrations of 139±69, 117±68, and 134±69 mg/L, respectively (Table 7).
Additionally, since sterols have been identified as inhibitors for anaerobic digestion, the sterols characterization of the wastewater was carried out. The results showed only a concentration of 27±8 mg/L of total sterols, with cholesterol and beta-sitosterol as the main compounds (see Table 15, Appendix I)
Overall, the wastewater composition was confirmed to be suitable for anaerobic digestion and applying the AnSBR technology.

Table 5 Slaughterhouse wastewater characterization from two different sampling campaigns.

Parameter	Average Sampling A [mg/L]	Average Sampling B [mg/L]	Total Average	STD
COD	5320	5180	5250	99
CODs	2290	2280	2285	7
TN	365,6	342,8	353,75	16
TNs	272,8	222,8	247,8	35
TKN	325	315	320	7
TKNs	180	200	190	14
N-NH4	71,4	73,8	72,6	2
P-PO4t	40,8	39,2	40	1
P-PO4s	18	17,1	17,55	1
Organic acids	1015	936	975,5	56
Proteins	666	764	715	69
FOG	350	340	345	7
TS	3620	3450	3535	120
TSS	1850	1860	1855	7
VS	2420	2490	2455	49
VSS	1650	1710	1680	42

Table 6 Fatty acids (FA) and Long chain fatty acids (LCFA) characterization of the slaughterhouse wastewater lipid fraction.

FA/LCFA	[%]	FA/LCFA	[%]
CAPRONIC ACID (C 6:0)	N.D	PALMITOLEIC ACID (C 16:1)	2,74±0,20
ENANTIC ACID (C 7:0)	N.D	HEPTADECANOIC ACID (C 17:0)	0,55±0,06
CAPRILIC ACID (C 8:0)	N.D	HEPTADECENOIC ACID (C 17:1)	0,09±0,04
CAPRIC ACID (C 10:0)	N.D	STEARIC ACID (C 18:0)	26,95±0,94
CAPROLEIC ACID (C 10:1)	N.D	OLEIC ACID (C 18:1)	35,26±1,09
LAUROLEIC ACID (C 12:1)	N.D	LINOLEIC ACID (C 18:2)	3,13±0,23
LAURIC ACID (C 12:0)	N.D	LINOLENIC ACID (C 18:3)	N.D
TRIDECENOIC ACID (C 13:1)	N.D	ARACHIC ACID (C 20:0)	0,36±0,04
TRIDECANOIC ACID (C 13:0)	N.D	EICOSENOIC ACID (C 20:1)	0,27±0,04
MYRISTIC ACID (C 14:0)	1,5±0,11	BEENIC ACID (C 22:0)	N.D
MYRISTOLEIC ACID (C 14:1)	N.D	ERUCIC ACID (C 22:1)	N.D
PENTADECANOIC ACID (C 15:0)	0,75±0,07	LIGNOCERIC ACID (C 24:0)	N.D
PALMITIC ACID (C 16:0)	28,97±0,97		

FA/LCFA	[%]
Monounsaturated fatty acids	38±1
Polyunsaturated fatty acids > C20	ND
Polyunsaturated fatty acids	3±0
Saturated fatty acids	59±1

Table 7 Amino acids composition of the slaughterhouse wastewater samples after hydrolysis.

Parameter	[mg/L]
ASPARTIC ACID	139±69
GLUTAMIC ACID	117±68
ALANINE	<LOD
ARGININE	<LOD
PHENYLALANINE	<LOD

GLYCINE	<LOD
ISOLEUCINE	<LOD
HYSTIDINE	<LOD
LEUCINE	134±69
LYSINE	<LOD
PROLINE	<LOD
SERINE	<LOD
TYROSIN	<LOD
THREONINE	<LOD
VALINE	<LOD

LOD: Limit of detection

5.2.2 Methane production potential of slaughterhouse wastewater

Methane production potential of the slaughterhouse wastewater (SWW) was measured at different inoculum to substrate (I:S) COD ratios (0.5, 2, 4) to assess the biodegradability and potential inhibition. The results (see Table 8) showed that at an I:S ratio of 0.5, a relatively low average methane potential of 168 Nml CH_4. gCOD substrate^{-1} was obtained, with a biodegradability of 48%, indicating potential inhibition of methanogenesis under this ratio. On the contrary, at I:S ratio of 2 and 4 average biomethane potential of 211 and 307 Nml CH_4. $gCOD_{substrate}^{-1}$ were obtained. Substantial increase in anaerobic biodegradability of slaughterhouse wastewater to 60% and 87% were found at ratios of 2 and 4 correspondingly. The positive control with cellulose indicated a biomethane potential of 316 Nml CH_4. gCOD substrate^{-1}, equivalent to biodegradability of 90%. The latter is within the range of the expected crystalline cellulose methane production, i.e., 315 - 439 Nml $CH_4 \cdot gVS^{-1}$. It is expected that with a sludge acclimated to a high protein and lipid-rich wastewater, the methane production can be slightly higher and faster.

Table 8 Anaerobic biodegradability tests at different I:S ratios.

	Average Cumulative CH_4 [Nml]	Time [days]	BMP [Nml CH_4. $gCOD_{substrate}^{-1}$]	Biodegradability (g CH_4-COD. $gCOD_{substrate}^{-1}$) [%]
I:S= 0.5	338	13,3	168	48%
I:S= 2	363	13,5	211	60%
I:S= 4	433	25,5	307	87%
Cellulose (I:S= 2)	516	16,6	316	90%

5.2.3 Effect of FOG on protein degradation

The presence of lipids in protein degradation was assessed through anaerobic batch experiments.
The results showed that within the first 48 hours, protein degradation took place similarly independently of the lipid concentration. However, at higher FOG: Protein ratio, the degradation seemed to be slower at the beginning

but reached similar protein concentrations after 48 h, except for the experiment at a ratio of 1. This can be most likely due to mass transfer limitation caused by the presence of a higher lipids concentration.

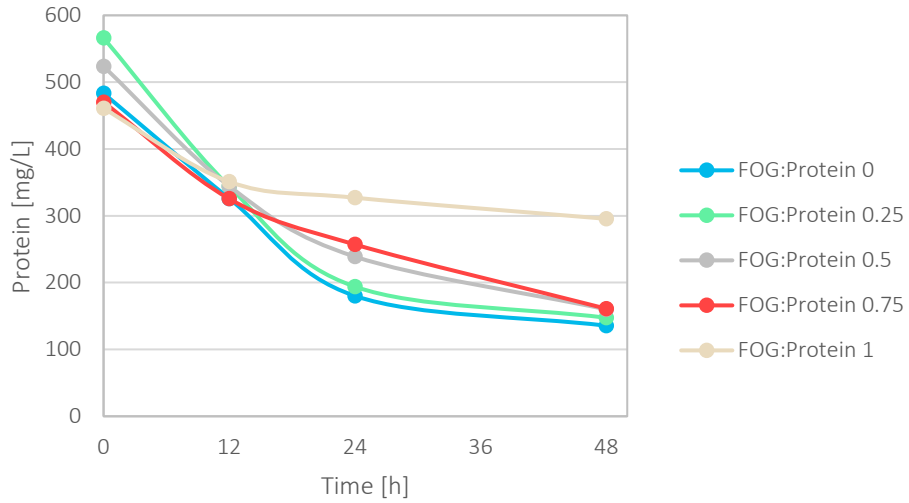


Figure 12 Protein degradation at different FOG:Protein ratios (0-1).

The concentrations of VFAs at 0, 12, 24, and 48 hours were also measured, indicating that after 12 hours, only in the sample from FOG:Protein of 0.25 still 6 mg/L of VFAs was present, while in the sample of 0.75 mg/L, 6 mg/L of VFAs was found at 24 h. From the rest, concentrations of VFAs of up to 20 mg/L were determined at time 0, which were completely converted to biogas at 48h, showing no accumulation of VFAs in the degradation of proteins due to the presence of lipids. Biogas production results showed that the biomethane potential of each of the different experiments slightly varied per gram of COD substrate added (FOG + Protein), reaching all of them anaerobic biodegradability between 75-82%. A longer digestion time for the experiments with higher FOG concentration was observed. These results confirm that protein degradation is not impacted by the FOG(lipids): Protein ratio under the concentrations expected in the real slaughterhouse wastewater, indicating that the AnSBR sludge was well acclimated to this type of wastewater matrix.

Table 9 Methane production and anaerobic biodegradability at different FOG:Protein ratios.

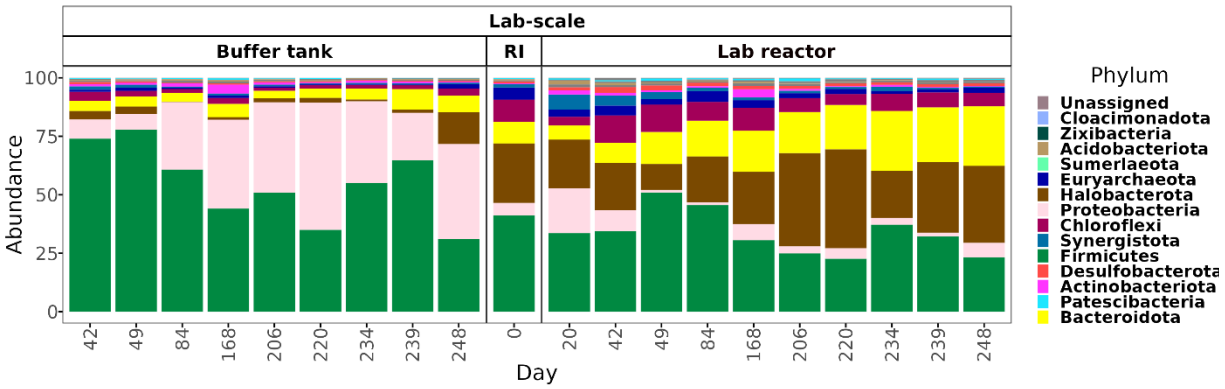
	Cumulative CH_4 Produced [Nml]	Time [days]	BMP [Nml CH4.g COD $_{substrate}^{-1}$]	Biodegradability (g CH_4-COD. g COD $_{substrate}^{-1}$) [%]
FOG:Protein 0	150,0	9,9	263,2	75%
FOG:Protein 0.25	203,7	11,6	279,0	79%
FOG:Protein 0.5	246,3	10,5	286,4	82%
FOG:Protein 0.75	257,3	10,6	265,3	76%
FOG:Protein 1	299,8	15,6	280,2	80%

5.2.4 Microbial community analysis from Lab-scale

Microbial community analysis from Lab-scale reactor

The microbial community structure and dynamics of the buffer tank and reactor biomass were determined (Figure 13). The dominant bacteria in the buffer tank belonged to the phylum Firmicutes, followed by Proteobacteria and Bacteroidota (Figure 13A). Firmicutes relative abundance decreased from 75.9±2.8% in phase I to 52.4±11.8% and 47.4±11.2% in phases II and III respectively, whereas Proteobacteria increased from 7.5±1.1% in phase I to 37.8±11.2% in phase III. On the contrary, Bacteroidota phylum relative abundance remained about 4.9±0.6% along the whole operation, all of them belonging to the class *Bacteroidia*. The most abundant genera were *Clostridium sensu stricto 1* (21.3±7%), *T34* (18.9±13.3%) especially in phase III, *Brachymonas* (8.5±6.7%), *Proteiniclasticum* (6.2±2.4%), *Terrisporobacter* (5.1±1.8%), *Lactobacillus* (4.0±3.3%), *Methanosaeta (*2.9±4.0%), *Streptococcus* (2.5±0.9%), *Bacteroidetes vadinHA17* (1.6±0.6%) and *Romboutsia* (1.6±0.6%) (Figure 13 B). *Clostridium sensu stricto 1* metabolizes various compounds such as proteins/amino acids, carbohydrates, short fatty acids (Wiegel et al., 2006) and is majorly involved in lipids/LCFAs degradation having a syntrophic relationship with methanogens.

A



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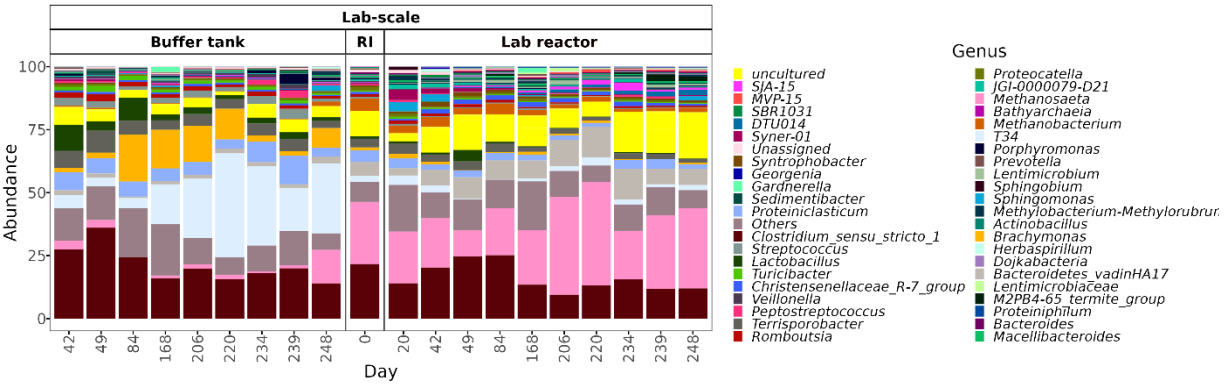

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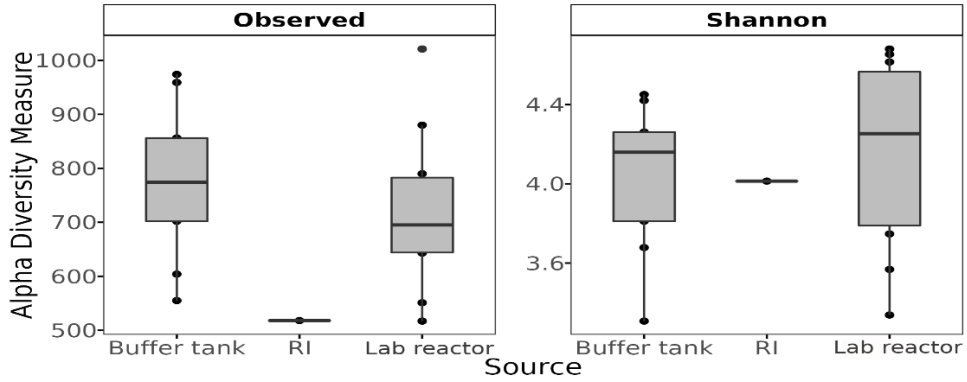

Figure 13. Microbial community dynamics in the buffer tank and reactor at the A. Phylum, B. Genus level. Phase I: up to day 55, Phase II: up to day 197, Phase III: up to day 248. C. Alpha diversity plots for the microbial community in the buffer tank, inoculum, and AnSBR Left: Observed OTU numbers, Right: Shannon's index

During the entire operation, the most dominant bacteria in the reactor belonged to phylum Firmicutes (34.2±9.1%), Bacteroidota (16.5±6.8%),) Chloroflexi (7.7±2.7%), and Proteobacteria (5.5±5.2%) while the dominant archaea to the Halobacterota (25.9±9.5%) and Euryarchaeota (2.9±1.3%) phylum (Figure 13A). Jabari et al. (2016) also reported that the most detected bacteria in an anaerobic reactor treating protein-rich slaughterhouse wastewater belonged to Firmicutes, specifically to the class Clostridia and Bacteroidota. Similarly that in the buffer tank, *Clostridium sensu stricto 1* was the prevalent bacteria genus in the AnSBR but decreased his relative abundance from 18.4±4.1% in phase I to 13.1±1.7% in phase III at higher OLR. On the contrary, the archaea genus *Methanosaeta* increased from 21.6±2.7% to 30.4±8.9% at phase III (Figure 13B), indicating enrichment of methanogens in the reactor. It is important to mention that the identified core microbiome contained mainly protein/blood/amino acids degraders such as *Turicibacter sp Turicibacter sanguinis strain MOL361, Romboustia sp. Romboutsia timonensis strain DR1, Proteiniclasticum, Clostridium sensu stricto 1, sp. Clostridium disporicum strain DS1* in the buffer tank and *Proteiniclasticum and Clostridium sensu stricto 1* in the AnSBR (Figure S1; Table 16 Supplementary material). Other protein and amino acids degraders genera, such as *Proteocatella* and *Proteiniphilum*, were present but in lower relative abundances (Figure 13B).

Alpha diversity indices (Lemos et al., 2011) were used to compare the evenness and richness of the microbial population of the buffer tank, inoculum, and AnSBR during the entire operation (Figure 13C). The median alpha diversity metrics from the observed ASVs was about 775 for the buffer tank compared to 696 and 525 for the AnSBR and inoculum, respectively. Even though the buffer tank had the highest microbial reachness, the variation range of the scores indicated no substantial difference among the samples. On the contrary, the Shannon index showed a higher score in the AnSBR of about 4.27 compared to 4.16 and 4.02 for the buffer tank and inoculum, respectively (Figure 13C). Because Shannon's index score considers the richness and evenness of the microbial population, the highest value observed in the reactor indicated a more even microbial population in the AnSBR than in the buffer tank, even though it was not the richest. The differences in the diversity among samples were attributed to the changes in organic loading rates during the 248 days of operation. These changes could promote higher diversity but could also lead to variable microbial community function (Santillan et al., 2019), i.e., a high alpha diversity maintains a relatively stable COD removal in the reactor, but a decreasing conversion to methane was observed even though enrichment of methanogens was observed. Moreover, the principal coordinates analysis of beta diversity indicated that the microbial community in the AnSBR clustered together with the inoculum sample and could be distinguished from the buffer tank (Figure S.2).

Microbial community analysis statistical analysis

PERMANOVA statistical test (Table 10) was carried out to analyse the correlation between the core microbial community structure, the AnSBR performance indicators and operational parameters. A statistically significant correlation ($p<0.05$) was only identified between the microbial community and the attained ammonification efficiency (R2= 0.14). The R2 of PERMANOVA represents the correlation of the distances matrix with a given variable, which is the variance or the differences in composition between samples explained by this variable. Even though the coefficient of determination is statistically low, it infers that protein and amino acids degradation to ammonium might be the key process governing the AnSBR treatment performance.

Table 10 Statistical significance for Ammonification in AnSBR by PERMANOVA analysis (using unweighted unifrac distance).

Factor	R^2	Pr(>F)
TCOD removal [%]	0.12	0.291
SCOD removal [%]	0.11	0.481
OLR [$gCOD \cdot L^{-1} \cdot d^{-1}$]	0.12	0.291
SLR [$gCOD \cdot gVSS^{-1} \cdot d^{-1}$]	0.10	0.757
HRT [d]	0.09	0.798
SRT [d]	0.14	0.050
Acidification (VFAs) TCOD [%]	0.102	0.747
Acidification (VFAs) SCOD [%]	0.11	0.442
Ammonification [%]	**0.14**	**0.012**

5.3 Conclusions

- Average protein and lipid (FOG) concentrations of about 700 mg/L and 350 mg/L were determined correspondingly in the slaughterhouse wastewater.
- Palmitic acid (C16:0), Stearic acid (C18:0), and Oleic acid (C18:1) acids were determined as the main long-chain fatty acids, which accounted for about 91% of the total lipid composition of the FOG, being the Oleic acid the most abundant.
- An average biomethane potential of 307 Nml CH_4. $gCOD_{substrate}^{-1}$ of the slaughterhouse wastewater was obtained at an inoculum to substrate ratio (I:S) of 4, which is equivalent to an anaerobic biodegradability of 87% when using a seed sludge that is not yet adapted to this wastewater matrix.
- The degradation of protein at different FOG (lipid): Protein ratios (characteristic of SWW) showed that within the first 48 hours of digestion, protein degradation was comparable within the different tests independently of the lipid concentration. Furthermore, similar anaerobic biodegradabilities were obtained for all FOG:Protein ratios in the range of 75-82%, with no signals of VFA accumulation or inhibition.
- Ammonification was correlated with the microbial community, confirming that protein degradation to ammonium is the key process that is governing in the whole AnSBR system. *Clostridium sensu stricto 1* was dominant in the core microbiome, which is involved in both protein and lipids degradation.

6 Results of pilot-scale testing of AnSBR

This chapter summarized the materials and methods and results obtained from the AnSBR pilot trial with slaughterhouse wastewater and the corresponding microbial community analysis.

6.1 Materials and Methods

6.1.1 Inoculum and wastewater characteristics

The inoculum sludge was taken from a slaughterhouse wastewater treatment plant in the Netherlands, with a conventional system of DAF followed by UASB. 300 Liters of UASB granular biomass were blended and inoculated into the 800 L reactor.

The raw wastewater collected from the inlet of a slaughterhouse wastewater treatment plant was sent by truck in IBC containers to the pilot site, 3 times per week, and stored in a refrigerated container, kept at 4^{o}C.

6.1.2 Pilot set-up and operational conditions

The study was performed using a containerized AnSBR (Sparthane™) pilot plant. The container consisted of two spaces - a pilot room and an operation/laboratory room- to generate reference data for the full-scale process design. The Sparthane™ pilot unit was fully automated. The process is controlled and protected by means of a Programmable Logic Control (PLC) unit. A PC and a laboratory table were equipped in the operation room to facilitate system control (via PLC), sample preparation, and analysis.

Sparthane™ is an anaerobic sequential batch reactor technology (AnSBR) that comprises the buffer tank (BT), a sequentially operated flocculent biomass reactor, and an external batch settling tank equipped with tilted plates. In this Sparthane™ configuration, the patented operational regime of Sparthane™ consists of simultaneous phases; feed and reaction phases occur in the reactor while degassing, settling, and effluent discharge phases take place in the external batch tank (EBT).

Prior to the BT, a feed IBC of 1 m^{3} containing the wastewater was placed inside a separate refrigerated container, kept at 4^{o}C. New shipments of fresh wastewater were sent to Biothane directly from the slaughterhouse facility for testing. The BT had a liquid volume of 400 L. It was equipped with a pH meter and dosing facilities controlled via the pH probe and the PLC program. The pH control pump, controlled by the pH probe and PLC, was connected to a caustic bottle filled with NaOH (2M). The pH was controlled in the BT at a setpoint of 5.3.

At the start of the batch cycle, the wastewater from the BT is pumped into the Sparthane™ reactor. The Reactor was equipped with a heater, and the temperature of the process was controlled and maintained at 36.0 ± 0.5 °C. Sludge discharge is carried out with the WAnS pump.

A batch of reactor content transferred to the EBT is degassed, settled, and effluent is discharged using the effluent pump. At the end of the cycle, the settled sludge is returned in concentrated form to the reactor; and a new cycle resumes. The EBT is fully enclosed, and the headspace is connected to the reactor headspace. Hence, when the batch transfer to (or sludge return from) takes place, an equal amount of biogas is displaced, thus eliminating large pressure variations. In addition to this, a gas bag is also present to buffer changes during the feed and the effluent discharge.

Biogas produced was collected at the gas headspace dome of the settler and flowed through a wet (bio)gas flow meter to monitor the biogas production. A gas bag connected to the reactor headspace was placed outside the container.

The organic loading rate (OLR) in the reactor was gradually increased up to 6.2 g COD ·L-1·Day-1 within three different phases of operation. The biomass was acclimated to the slaughterhouse wastewater during the startup

phase (phase I, 0 – 10 days). In phase II (Days 11 – 39), the reactor was operated with an average OLR of 2.7 ± 0.4 gTCOD·L^{-1}·d^{-1} and an average specific loading rate (SLR) of 0.3 ± 0.1 gTCOD·gVSS^{-1}·d^{-1}. In phase III (Days 40 – 52), the OLR was increased up to 6.2 gTCOD·L^{-1}·d^{-1}, to investigate the treatment capability of the reactor. The pH was maintained at 7.0 in the buffer tank and in the reactor. The temperature of the AnSBR was controlled at 35 ± 0.5 °C, whereas the buffer tank and the settling reactor were operated at ambient temperature. The operational parameters of the reactor are summarized in Table 2.

Table 11 Operational parameters of the reactor

	OLR	SLR	HRT	SRT	TSS	VSS	Cycle length	pH	T*
	gTCOD·L^{-1}·d^{-1}	gTCOD·gVSS^{-1}·d^{-1}	d	d	g·L^{-1}	g·L^{-1}	h	-	°C
AnSBR	I: 1.6 ± 0.7	0.14 ± 0.1	3.0 ± 2.7	34 ± 17	22.4 ± 2.1	11.6 ± 1.1	4	7.1	35
	II: 2.7 ± 0.4	0.33 ± 0.1	1.1 ± 0.2	65 ± 37	16.5 ± 4.2	8.7 ± 2.2	4	7.0	35
	III: 4.5 ± 1.1	0.61 ± 0.1	1.0 ±0.3	54 ± 24	11.7 ± 0.8	7.3 ± 0.6	4	7.0	35

T*: temperature.

Figure 14 Pilot plant setup.

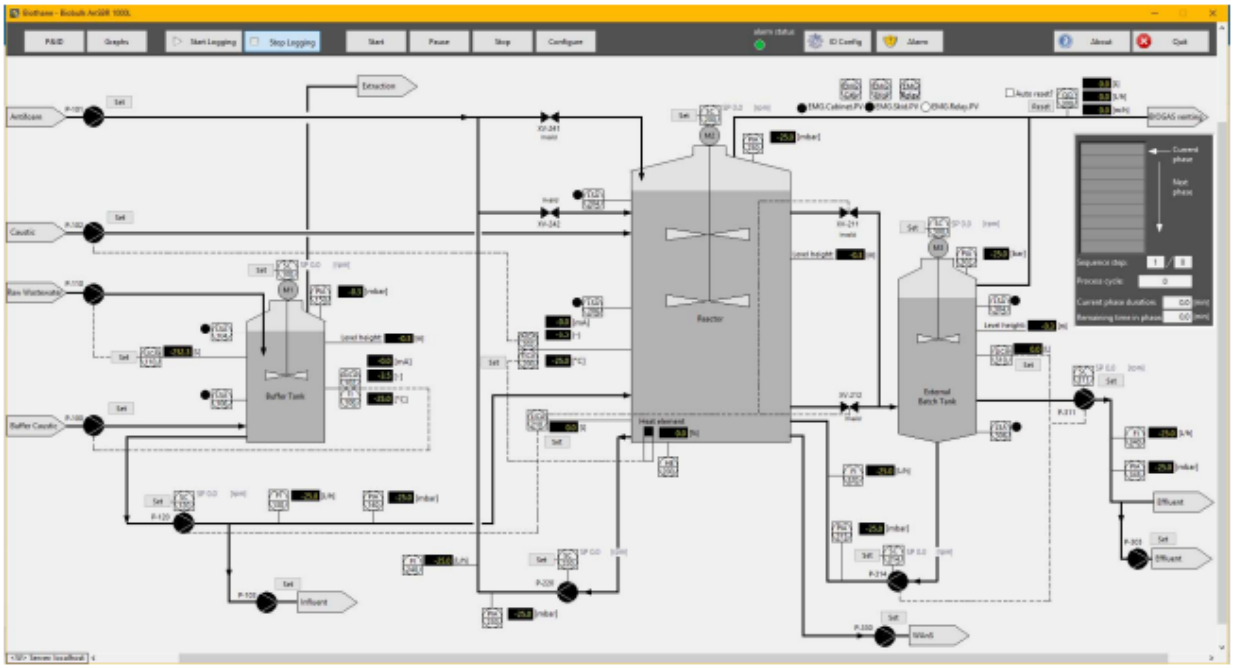

Figure 15 AnSBR (Sparthane™) process control interface.

6.1.3 Sampling and analysis

Total (TCOD) and soluble COD (SCOD) were analysed between 3 to 5 times a week. The TCOD and SCOD (filtered through 0.45 µm Whatman filters) measurements were carried out using HACH-Lange kits (LCK 400, 514, 014, and 914). Total suspended solids (TSS), volatile suspended solids (VSS), total Kjeldahl nitrogen (TKN), soluble Kjeldahl nitrogen (SKN), ammonium (NH4+), and volatile fatty acids (VFAs) were analyzed once to thrice per week. Solids and volatile fractions (TS, TSS, VS, and VSS) of wastewater, nitrogen concentrations (total Kjeldahl nitrogen (TKN), soluble Kjeldahl nitrogen (SKN), ammonium (NH4+)) were analyzed following standard methods (APHA, 1998). The volatile fatty acids composition (C2 to C6) was determined using gas chromatography (GC, 7820 A, Agilent Technologies, Amstelveen, Netherlands) equipped with a flame ionization detector using a CP 7614 column (WCOT Fused Silica 25 m × 0.55 mm, CP-wax 58 FFAP capillary, Agilent Technologies).

6.1.4 Microbial community analysis from AnSBR Pilot

Microbial community analysis

Microbial community analysis of a few samples from the buffer and reactor of the pilot was carried out similar to the ones performed in 5.1.4.

6.2 Results

The AnSBR (Sparthane™) reactor was inoculated with blended granular biomass from a current UASB system treating slaughterhouse wastewater. This biomass was collected from the UASB reactor treating the wastewater after a DAF system. Thus the biomass was already partly acclimatized to this type of wastewater. This allowed a quicker start-up phase of the pilot. After seeding the system, the reactor was filled with wastewater and placed in batch mode to increase temperature and acclimate the biomass. Once the reactor reached an operational temperature above 35°C, the entire system and its sequence was started up. The load applied to the system was

increased daily. Within 10 days, a load of OLR of 3 gTCOD·L^{-1}·d^{-1} was reached. During that period, the system showed gradual improved TCOD removal efficiency, up to 70%, and SCOD removal efficiency up to 90%.
During phase II. the target was to increase the load applied to the system to determine its operational and biological limits. However, over the trial period, the wastewater was very diluted, on average 2.9 gCOD/L. Therefore, it was not possible to apply a load above 3 gTCOD·L-1·d-1. Therefore, on operation day 21, biomass was extracted from the system to quickly increase the sludge loading rate (SLR) applied to the reactor. The TSS and VSS concentrations dropped from 21 gTSS/L and 11 gVSS/L, to 14 gTSS/L and 7 gVSS/L. Nevertheless, the system showed a very good stable operation at ORL 2.7 ± 0.4 gTCOD·L-1·d-1. TCOD and SCOD removal efficiencies were very stable at respectively 79% and 89%. Digestion efficiency also showed continued improvement compared to Phase I., stabilizing at around 80%.
In the final phase of the trial, the robustness of the AnSBR was tested. To increase the COD concentration of the influent, collected concentrated stream “Broeibak water” was mixed together with the raw wastewater and fed to the system. This caused significant increases and variations in the daily load applied. ORL up to 6.2 gTCOD·L^{-1}·d^{-1} was applied, and the system was able to cope with it and performed in a very stable manner. TCOD and SCOD removal efficiencies remained at an average of 83% and 90%, respectively, and digestion efficiency at 83%.

Table 12 Overall results of pilot plant operation

Parameter (Average)	TCOD removal	SCOD removal	Digestion Efficiency	Effl. VFA	pH Reactor
Units	%	%	%	meq/L	-
Target	80	80	70	<5	7
Phase I.	45 ± 12	83 ± 6	46 ± 14	0	7.1
Phase II.	79 ± 4	89± 1	79 ± 4	0	7.0
Phase III.	83 ± 4	90 ± 1	83 ± 4	0	7.0

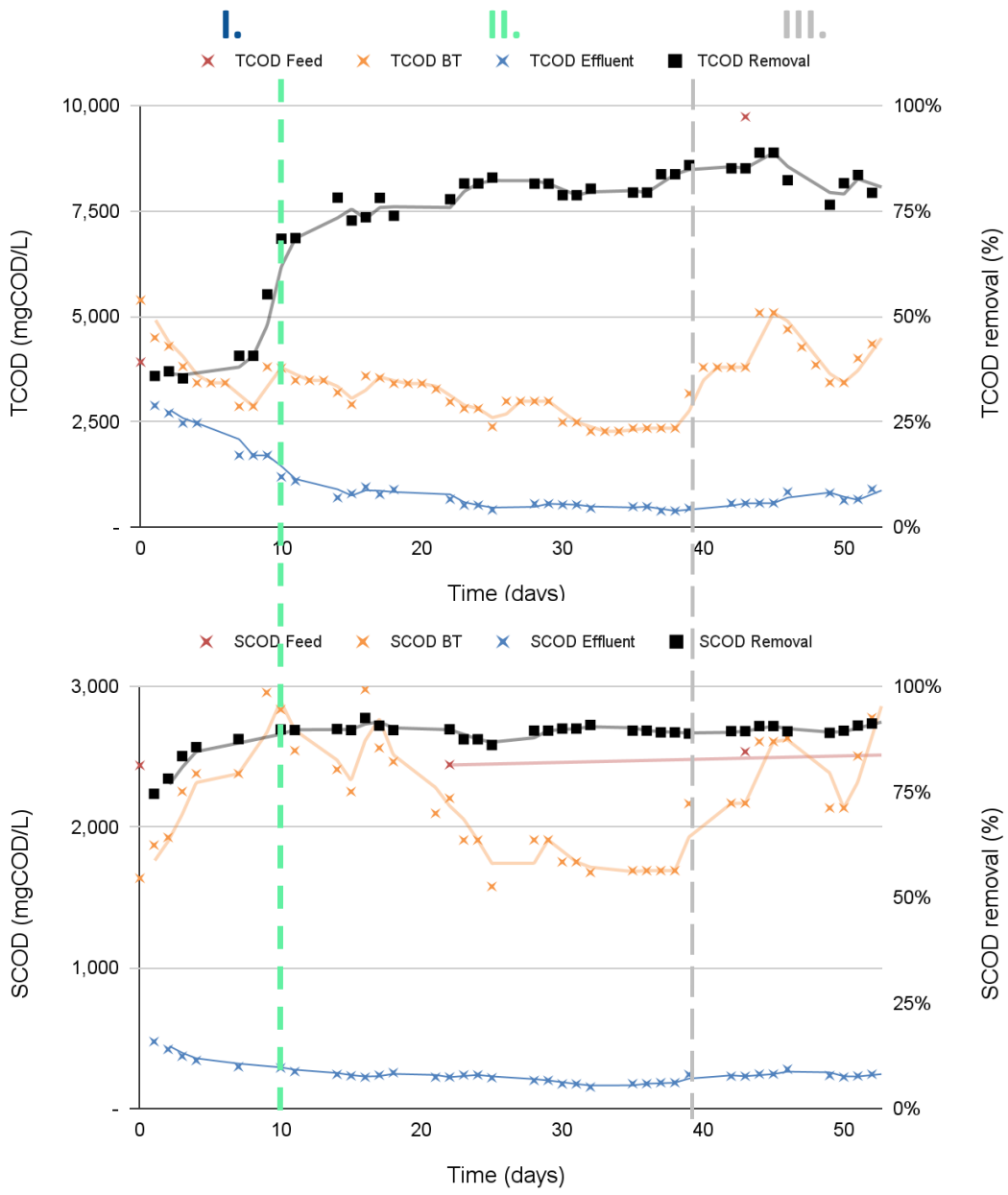

Figure 16 Sparthane™ process control interface.

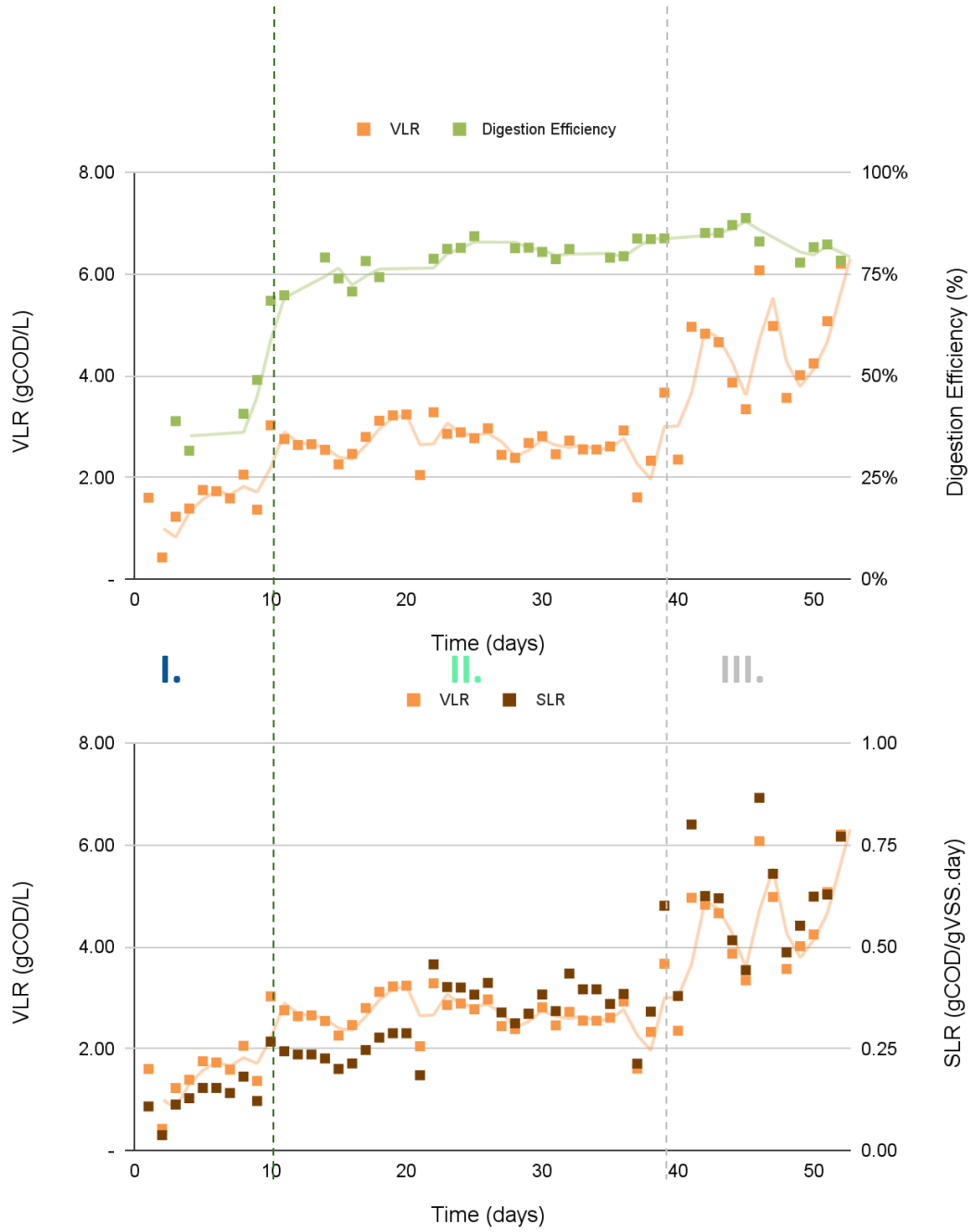

Figure 17 Volumetric loading rate vs Digestion Efficiency [%] and volumetric loading rate vs sludge loading rate.

6.2.1 Microbial community analysis from Pilot-scale reactor

The microbial community structure and dynamics of the buffer tank and reactor biomass were also determined at the pilot scale during its operation (Figure 18). Similar to in the lab-scale AnSBR, the dominant bacteria in the buffer tank belonged to the phylum Firmicutes, followed by Bacteroidota and Proteobacteria. Firmicutes relative abundance increased from 44.0 to 48.5%, whereas Bacteroidota decreased from 32.1 to 21.2% at the end of the operation. On the contrary, Proteobacteria relative abundance increased from 7.9 to 15.2 %.
The most dominant bacteria in the reactor belonged to phylum Bacteroidota (15.5±0.6%),) Firmicutes (10.9±2.3%), Chloroflexi (5.3±2.3%), while the dominant archaea to the Halobacterota (53.4±11.8%) and Euryarchaeota (2.0±1.5%) phylum. Interestingly, the pilot reactor had an almost double relative abundance of the lab-scale

reactor, indicating a clear difference in the sludge seed; and a similar relative abundance for the bacteria. This can be confirmed by the comparison PCoA analysis (Figure 19), in which the samples from the buffer tanks in both scales clustered together, and the samples from the pilot reactor clustered together with the lab-scale, but clearly from a seed sludge which is very different to that the lab-reactor case. Based on this analysis, we can hypothesize that with a longer operational time of the pilot, the reactor samples will become more similar to the lab-scale

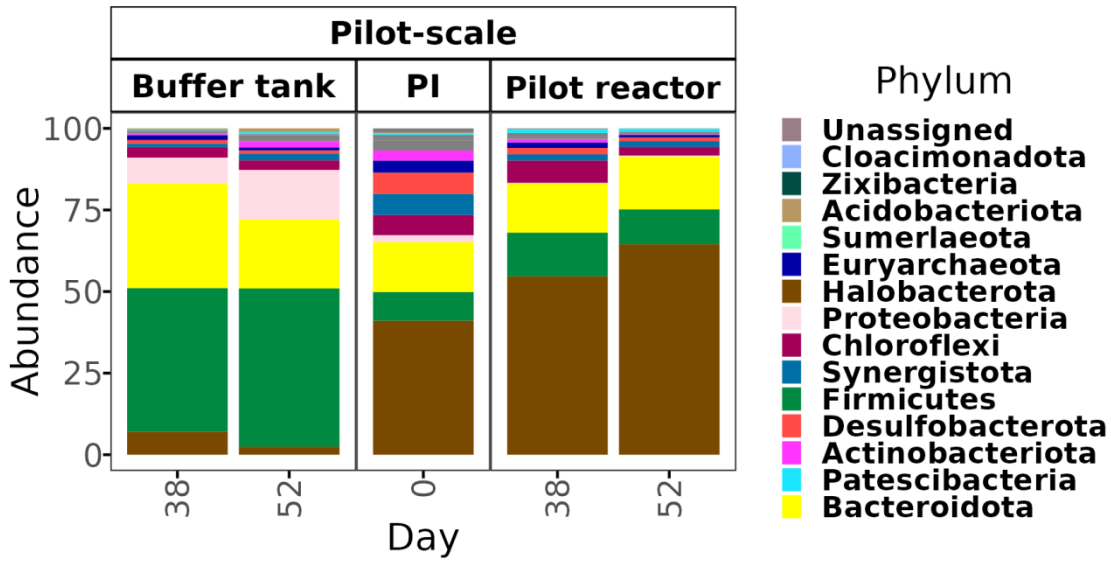

Figure 18. Microbial community dynamics in the buffer tank and pilot reactor at the Phylum level.

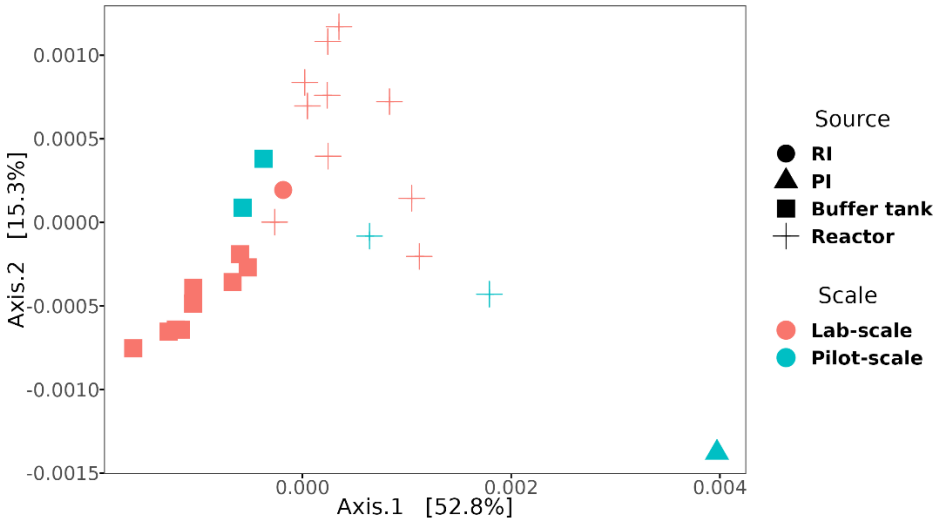

Figure 19 Beta diversity PCoA analysis among buffer tank and AnSBR samples at lab and pilot scale. RI: Reactor (lab) inoculum, PI: Pilot inoculum.

6.3 Conclusions

- The AnSBR system proved to be efficient, i.e., targets of total COD and digestion efficiency were achieved after the about 50 days of trial for this type of wastewater.
- After a quick start-up (10 days) and increase of the VLR, a stable performance was reached at VLR 3 gCOD/L with TCOD removal efficiency of ~75%.

- In the last phase of the trial, Broeibakwater (concentrated wastewater flow) was added to the wastewater to increase the COD concentration of the feed to the system. VLR up to 6.2 gCOD/(L.d) was applied to the system simultaneously to significant daily variations in load. The system coped with this increase and performed in a very stable manner.
- Pilot AnSBR microbial structure showed high similarities in the buffer tank with the lab-scale AnSBR, while the reactor showed to be highly abundant in Halobactereota phylum, which is mainly methanogens (i.e., *Methanosaeta*).

7 Results from desk research on resource recovery potential from slaughterhouse wastewater

This chapter presents the a comparison desk study of treating slaughterhouse wastewater with a conventional DAF-UASB system and the tested AnSBR from a point of view of resource recovery (energy and nutrients) potential.

7.1 Materials and Methods

7.1.1 Potential of resource recovery

Nitrogen (N), phosphorus (P), and energy balance of the slaughterhouse wastewater plant were made using excel sheets to analyze the potential differences in resource recovery by implementing an AnSBR system instead of a conventional DAF+UASB treatment train. Calculations were carried out taking into account historical data from the full-scale WWTP and the results obtained during the pilot operation.

The following standard calculation for biogas and energy equivalent (low heat value) was used:

Q (m^3/d)*C (kg COD/m^3)*0.35 m^3 CH_4/kg COD = m^3 CH_4/d

m^3 CH_4/d * 9,87 kWh/m^3 = kWh/d ; m^3 CH_4/d * 35,43 MJ/m^3 = MJ/d

7.2 Results

7.2.1 Potential of resource recovery

Resources (N, P, biogas) balance

In the current wastewater treatment plant installation (see Figure 2), wastewater enters first to a drum filter, then goes to a buffer tank, and the FOG present in the wastewater makes it necessary to pretreat the wastewater in a DAF installation before it can be treated in an anaerobic granular sludge reactor (UASB). The FOG sludge is sent to an external processor. The UASB produces biogas, which is desulphurized before it can be on the steam boiler fired, in addition to natural gas. The slaughterhouse process demands a high amount of steam, and biogas is aimed to be utilized on-site for its production. Therefore, the maximization of biogas production is desired for more sustainable wastewater treatment and resource recovery in the form of biogas converted to energy.
The long-term data analysis of the influent wastewater indicated a similar composition to the early discussed. By combining it to the DAF pretreatment average results, the baseline scenario of the wastewater treatment plant is as follows (Figure 20)

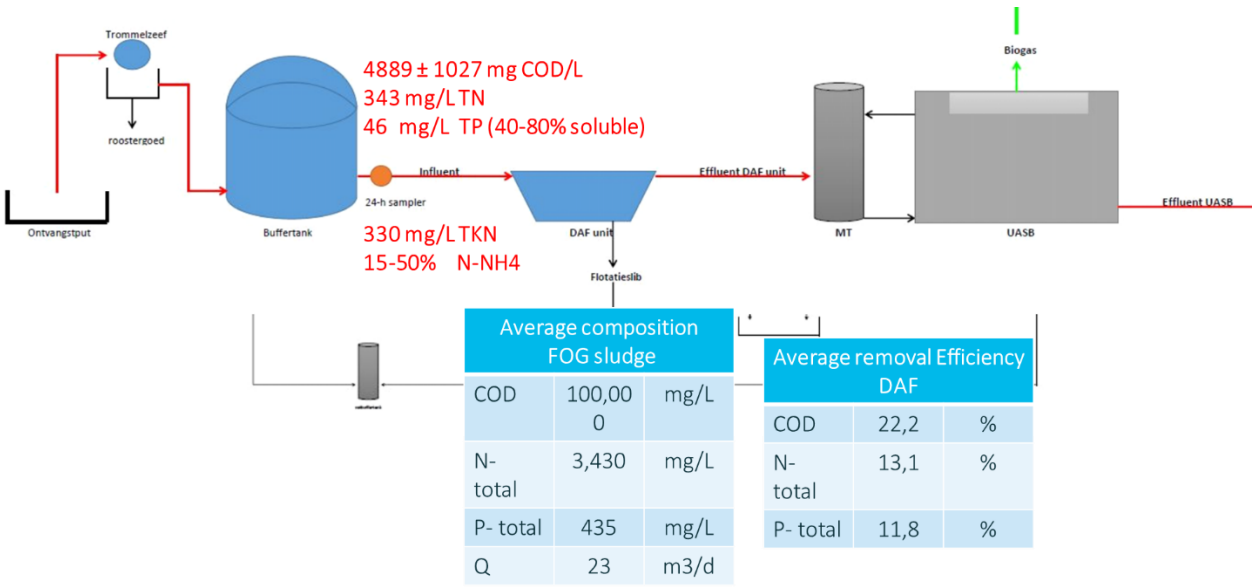

Figure 20 Baseline scenario of current slaughterhouse WWTP.

Due to the fact that the current DAF pretreatment removed about 22.2 %, 13.1%, and 11.8% of COD, N-total, and P-total, respectively it resulted in an average flow of approximately 23 m3/d of FOG sludge. On the contrary, by implementing an AnSBR system without DAF-pretreatment, the 22% COD (removed by DAF) can be converted to biogas/energy and nutrients can be solubilized (Figure 21).

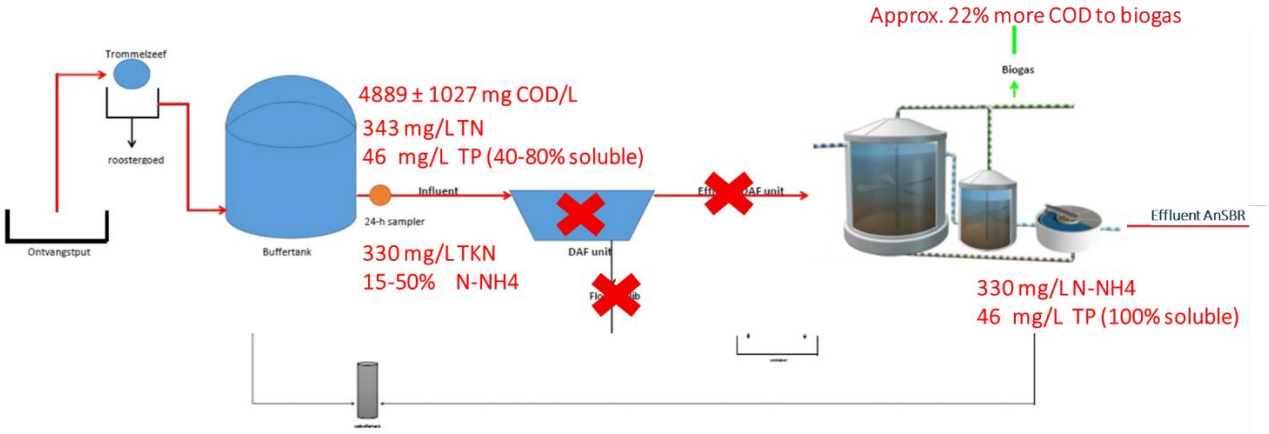

Figure 21 Future baseline scenario if AnSBR is implemented.

Based on the average data, it can be assumed that in the best-case scenario, an effluent with N-NH_4 and P-PO_4 concentrations of about 330 mg.L^{-1} and 46 mg.L^{-1} will potentially be the feeding to any nutrient recovery technology.

The analysis of historical data within the same period in which the pilot plant was operated resulted in the following scenario of the full-scale WWTP regarding COD, nitrogen, and phosphorus, as presented in Figure 22:

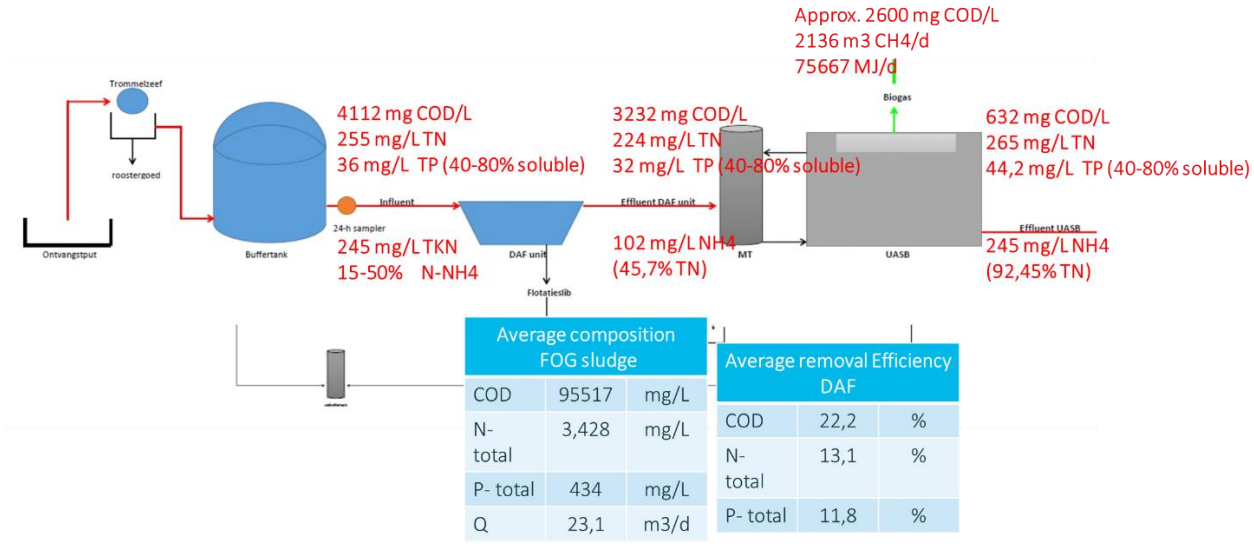

Figure 22 Current scenario of full scale slaughterhouse wastewater treatment plant. COD, N, and P balance during same period than pilot plant was operated.

An influent wastewater stream of about 4112 mg COD/L, 255 mg TN/L, and 36 mg TP/L goes to the DAF pretreatment. As effluent of the DAF, an influent to the UASB containing about 3232 mg COD/L, 224 mg TN/L, and 32 mg TP/L (40-80% soluble), enters the anaerobic system. From this stream, approximately 2600 mg COD/L are converted to energy as biogas of about 2136 m3 CH4/d, which is equivalent to 75667 MJ/d or 21080 kWh/d. The resulting UASB effluent contains 632 mg COD/L, 245 mg N-NH4/L (about 92.45% of the TN), and 44.2 mg TP/L.

For comparing with the operation of the pilot plant (assuming the same inflow of 2370 m3/d) and taking into account the concentrations and results observed (see Figure 23), with an influent concentration of 3264 mg COD/L, about 2250 m3 CH4/d equivalent to 79729 MJ/d or 22212 kWh/d would be expected as biogas/energy recovery of the AnSBR, asssuming an 83% average digestion efficiency. Furthermore, an effluent with nutrient concentrations of about 275 mg N-NH4/L (82.2% of TN) and 42 mg TP/L was observed. This indicates a slightly lower ammonification than in the UASB and a similar P solubilization.

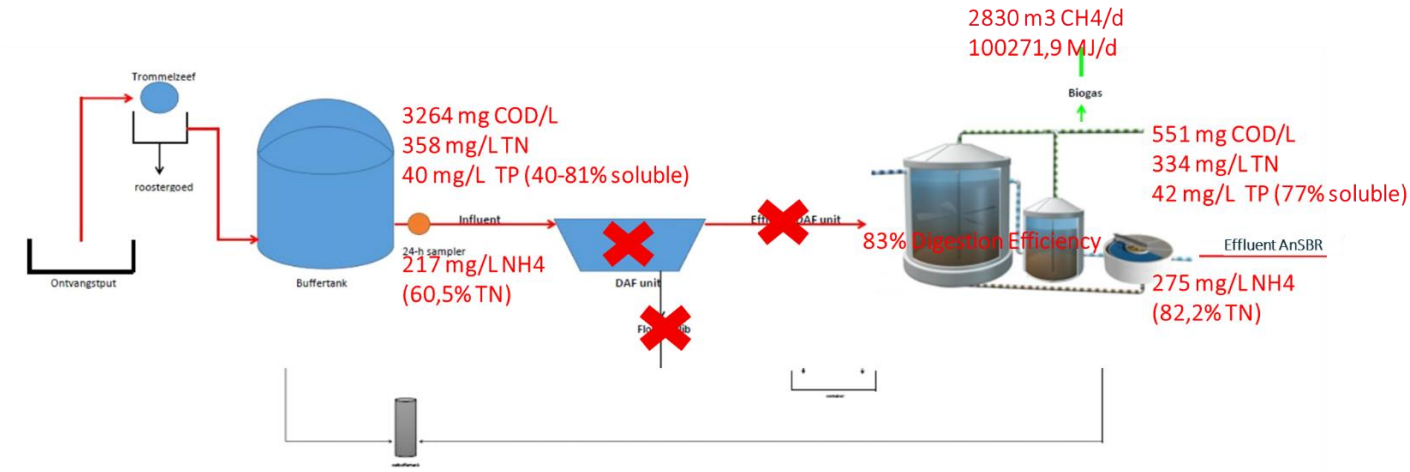

Figure 23 Current scenario of pilot slaughterhouse wastewater treatment plant. COD, N, and P balance.

However, to be able to compare the current and future scenarios by implementing an AnSBR, the same influent concentrations are assumed, and the obtained ammonification, P solubilization, and digestion efficiency of the pilot are used for the mass balances (Table 13):

Table 13 COD, TN, and TP mass balance in the pilot-scale wastewater treatment plant assuming the same inflow.

	Current scenario with DAF + UASB				Future scenario AnSBR	
	Influent	DAF sludge	Influent UASB	Effluent UASB	Influent	Effluent AnSBR
COD [kg/d]	9745	2159	7585	1483	9745	1657
TN [kg/d]	604	79,2	525	623	604	563
TP [kg/d]	85,1	10	75,1	104	85,1	89,4
N-NH4 [kg/d]	N.D	N.D	240	576	366	463
Q [m3/d]	2370	23,1	2347	2347	2370	2370

Under these assumptions, the biogas production and energy recovery of the AnSBR is 2831 m^3 CH_4/d and 100300 MJ/d or 27942 kWh/d, which is about 25% higher compared to the UASB. A slightly higher COD concentration in the effluent of the AnSBR is expected, but in terms of nutrients, lower concentrations of N and P will be obtained, which will make the further anammox and aerobic treatment of the stream more cost-effective.

Perspectives of nutrients recovery: N and P

The removal of nutrients has largely focused on taking advantage of nutrient cycling reactions whereby reactive forms of nutrients are converted to unreactive forms (e.g., ammonia to nitrogen gas). Ammonium and phosphate ions are the main present forms of N and P, respectively. Ammonia is normally biologically removed by nitrification-denitrification, while the removal of phosphate is mainly achieved by chemical precipitation (Liu et al., 2017; Ye et al., 2017). Recovery of nutrients through different technologies (rather than destruction or emission) is important not only for sustainability reasons but also due to economic drivers around the demand for fertilizers for agricultural purposes. However, the market penetration of specific nutrient recovery technologies depend on: the capital and operating costs; the engineering feasibility, maturity, and reliability; the credibility and completeness of available information on the technologies; the safety profile; and the environmental concerns and benefits associated (Mehta et al., 2015).

An overview of the most suitable nutrient recovery technologies for a stream after anaerobic digestion of slaughterhouse wastewater is summarized based on the current state-of-the-art literature in Appendix IV. Some of the technologies described have not yet reached very high-efficiency levels (Ward et al., 2018), but significant progress has been made during the last years. For instance, phosphorous recovery has reached recovery rates ranging from 25 to 90%, depending on the process, but also at different costs (Egle et al., 2016). However, from the AnSBR or UASB systems, expected concentrations of about 45 mg/L Total P are considered rather low to think directly on precipitation as struvite. Since Struvite precipitation/crystallization is known to be more feasible at P-concentrations higher than 100 $mg.L^{-1}$, therefore does not seem to be an option with the current concentrations achieved in the effluent of the AnSBR. However, P recovery is critical, and prices have recently risen significantly, so this can change soon. With struvite precipitation, only about 10-20% of the ammonium will be converted, leaving the other 80-90% for a concentrating technology to favor the further recovery or removal by anammox.

The first step in the recovery of nitrogen in the ammonia form from wastewater involves its preconcentration. From both the UASB and the AnSBR, expected effluent concentrations on the range of 300 mg NH4-N/L (or less) are

expected, which is considered rather low. Direct stripping, for instance, will not be economically feasible with the observed concentration of N-NH4 ammonium of 330 mg/L in the effluent of the AnSBR (< 1.0 g $NH4.L^{-1}$). Ion exchange and electrodialysis are both emerging technologies but are less sensitive to feed concentration. Therefore, it is likely that the eventual industrial wastewater process will be struvite precipitation followed by concentration through electrodialysis or ion exchange, depending on the final product desired. However, at the actual concentrations of $N\text{-}NH_4$ of 330 mg/L, anammox seems more adequate for N-removal than concentrating for N-recovery. Therefore an AnSBR + anammox process configuration can still be expected on the location. Struvite precipitation is still a question mark but of high interest due to the value of phosphorous.

Potential of the AnSBR as treatment technology for slaughterhouse wastewater

A treatment train that normally consists of pretreatment to decrease high SS concentration, chemical addition (polymers), and a biological treatment system is commonly implemented. Most of the available systems are "floating-sludge" based principles (either upstream, inside, or downstream of the anaerobic reactor). In the long term, these systems cannot be efficient as they promote biomass floating, which happens naturally when a system is overloaded as all TSS and FOG are accumulated in the system (in the float) and not degraded. Thus, COD removal efficiency could be high, but COD conversion to biogas is not increased as all this fraction of substrate is not converted. Furthermore, while a sequencing batch reactor (SBR) works very well aerobically due to fast settling velocities of aerobic sludge (which do not take a big part of the sequence for settling), for the anaerobic application, the time available for settling in a cycle compromises the time available for reaction. Therefore, it is subject to the effects of biogas up-flow that hinders sludge settling resulting in low rate systems (1-2 g COD/L day) and consequently in very high footprint systems. Hence, the AnSBR can become an up-and-coming alternative to be implemented in the slaughterhouse sector. Since the slaughter industry consumes a lot of water and produces wastewater, new technologies towards a circular economy are needed. Treatment technologies need to move to the water resource recovery facility concept to take a step toward achieving the sustainability of wastewater reclamation and reuse schemes. In this regard, the results confirm that AnSBR provides clear advantages since overall effluent quality is better, especially in terms of nutrients, which will benefit the following biological/physicochemical nutrient removal and aerobic treatment. A biogas and energy production higher than 20%, without the need for a DAF as pretreatment, will benefit the slaughterhouse because it is a highly demanding industry, especially for steam, from which biogas can be efficiently utilized for a boiler and produce a part of the required steam for operations. Besides, the FOG sludge must no longer be transported off-site to the external sludge processor. This saves transport (CO_2 emission) and costs. Moreover, the nature of a batch system of the AnSBR complies very well with this industry's batch operation and maintenance, and the technology facilitates dealing much better with fluctuations in load.
Additionally, since it is one single-stage treatment without pretreatment, the footprint of the total treatment system will be lower. Some disadvantage, when compared with the conventional systems, is that the operation by batch may bring some challenges or complexity to the operation of the reactor system, even though it is self-regulated. Moreover, the quality of the sludge produced by a flocculent sludge-based technology as the AnSBR is different compared to a granular sludge bed reactor and, therefore, not valuable to be sold as seed material.

When comparing the DAF+UASB with the AnSBR in terms of operational costs under the same flow and load basis, the Sparthane™ shows clear advantages (Figure 24). The most significant impact can be found in the sludge treatment of the two different systems (840k€ DAF+UASB compared to 154.6k€ AnSBR), and the impact of coagulant use, as well as dewatering, is noticeable in the chemical usage and sludge disposal. Sparthane™ ensures that most of the biomass coming into the system will be converted into biogas, as shown in the biogas generation. In short, we estimate that the running cost per m^3 of wastewater amounts to 1.33 euros for the DAF+UASB configuration and 0.60 euros for the Sparthane™ operation.

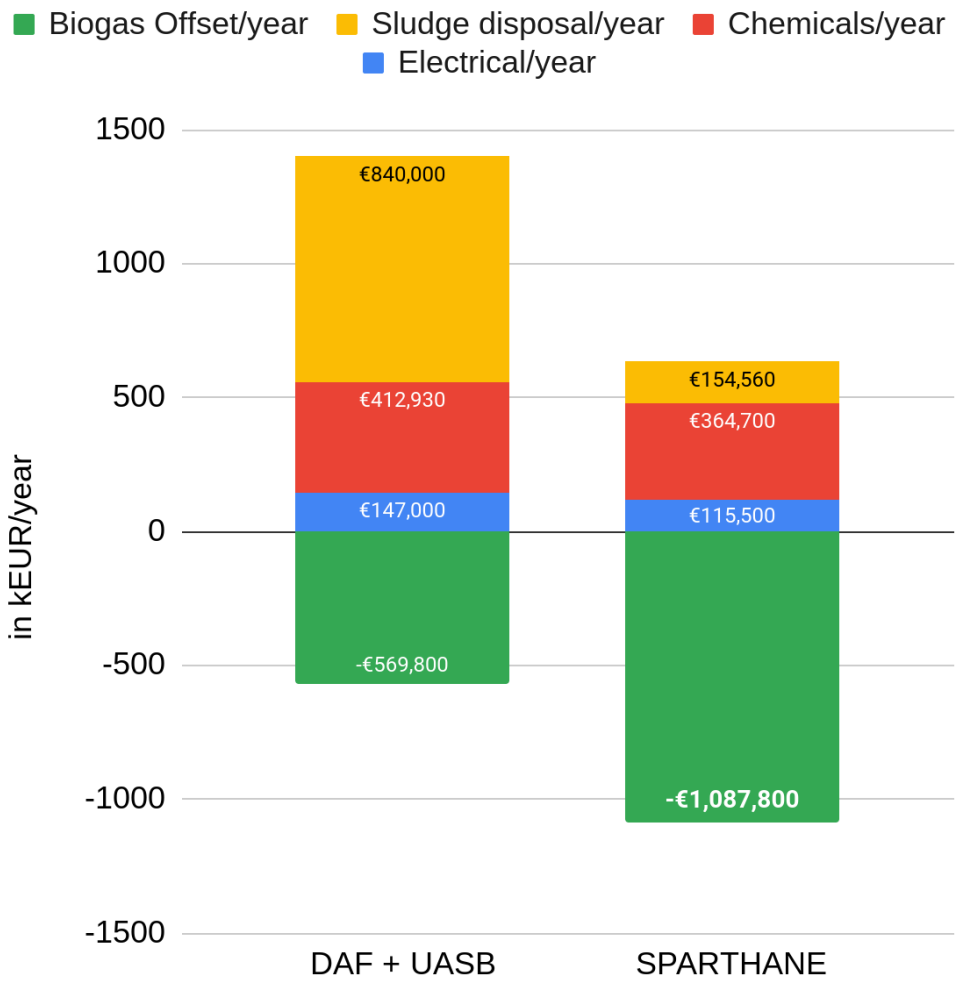

Figure 24 Operation costs comparison between a DAF+UASB system and Sparthane™

7.3 Conclusions

- Assuming similar conditions than achieved in the pilot trial and current full scale plant, the AnSBR is capable of producing 2831 m3 CH4/d and recovering 100300 MJ/d or 27942 kWh/d, which is approximately 25% higher than the UASB. While a slightly higher COD concentration is expected in the effluent, lower concentrations of N and P can be achieved, making the downstream anammox and aerobic treatment of the stream more cost-effective.
- Direct stripping is not consider a viable economic option for N-NH4 ammonium concentration of 330 mg/L found in the effluent of the AnSBR. Struvite precipitation/crystallization is not feasible with current P-concentrations achieved in the effluent, but P recovery remains crucial, and rising prices may change this soon. Although struvite precipitation can only convert 10-20% of ammonium, it can be combined with concentrating technologies such as electrodialysis or ion exchange for further recovery or removal by anammox. These technologies are less sensitive to feed concentration, making them ideal for an industrial wastewater process. However, at the current concentrations of N-NH4, anammox seems more appropriate for N-removal than concentrating for N-recovery, suggesting that an AnSBR + anammox process configuration is still expected on-site. Struvite precipitation remains a question mark but is of high interest due to the value of phosphorous.

- The AnSBR offers clear advantages over the DAF+UASB system in terms of effluent quality, biogas and energy production, lower operational costs, and a smaller footprint. The AnSBR's batch system is well-suited to the slaughterhouse industry's batch operation and maintenance and can handle load fluctuations. Additionally, it eliminates the need for DAF pre-treatment and the transportation of FOG sludge off-site. However, the AnSBR's flocculent sludge-based technology produces sludge of lower value than granular sludge bed reactors. While the AnSBR's batch operation may present challenges, it is self-regulated. The Sparthane™ technology used in the AnSBR ensures most biomass is converted into biogas, resulting in a running cost of 0.60 euros per m3 of wastewater, compared to 1.33 euros for the DAF+UASB system, mainly due to sludge treatment and chemical usage.

8 Conclusions and recommendations

The wastewater characterization indicated that slaughterhouse wastewater has particulate COD accounting for more than 50% of the COD, high TSS, Protein, and FOG, about 700 and 350 mg/L, correspondingly. Palmitic acid (C16:0), Stearic acid (C18:0), and Oleic acid (C18:1) acids were determined as the main long-chain fatty acids, which accounted for about 91% of the total lipid composition in the wastewater, with Oleic acid being the most abundant. The amino acid composition of the wastewater indicated that aspartic acid, glutamic acid, and leucine were present with concentrations of 139±69, 117±68, and 134±69 mg/L, respectively.

The anaerobic biodegradability tests achieved with non-adapted anaerobic granular biomass about 90% COD removal within 10 days of incubation and no sign of toxicity. Therefore, the slaughterhouse wastewater fits the characteristics of anaerobic treatment with the Sparthane™ technology. An average biomethane potential of 307 Nml CH_4. $gCOD_{substrate}^{-1}$ of the slaughterhouse wastewater was obtained at an inoculum to substrate ratio (I:S) of 4, which was equivalent to an anaerobic biodegradability of 87%. Further analysis with simulated wastewater with similar protein and FOG characteristics that the slaughterhouse wastewater showed that protein degradation was not significantly affected by the high lipid concentration. Furthermore, similar anaerobic biodegradabilities were obtained for all FOG:Protein ratios (0, 0.25, 0.5, 0.75, 1) in the range of 75-82%, with no signals of VFAs accumulation or inhibition.

The blended granular sludge treating chemical wastewater used as the seed of the AnSBR showed very good acclimation to the slaughterhouse wastewater matrix. The start-up was carried out slowly, increasing the volumetric loading rate, achieving total COD removal higher than 75% and soluble COD removal of about 85%. The VLR impacted the performance of the AnSBR substantially, together with the SLR, HRT, and protein degradation efficiency. The AnSBR system achieved a max of 90% TCOD removal and over 70% degradation efficiency without pre-treatment at a VLR below 6.2 $kgCOD \cdot L^{-1} \cdot d^{-1}$. The sludge settleability was only affected when high carbohydrate concentrations in the reactor broth were observed and was highly correlated with the ammonification. Total suspended solids in the effluent were less than 200 mg/L. Ammonification was also correlated with the microbial community dynamics of the reactor, confirming that protein degradation to ammonium is the key process that is governing in the whole AnSBR system when treating such protein and lipid-rich wastewater streams. The dominant bacteria in the buffer tank belonged to the phylum Firmicutes, followed by Proteobacteria and Bacteroidota. *Clostridium sensu stricto 1* was the dominant species in the core microbiome of the AnSBR, which is involved in both protein and lipids degradation.

The pilot AnSBR system proved to be efficient for treating slaughterhouse wastewater. Stable performance was reached at VLR 3 $gCOD.L^{-1}.d^{-1}$, achieving a total COD removal efficiency of about 75%. Furthermore, it also showed to be robust to concentration fluctuations when concentrated wastewater was added to the system. Volumetric loading rates of up to 6.2 gCOD/(L.d) were applied to the system, with significant daily variations in load. The system coped with the increase in load and performed in a very stable manner despite the variations.
By comparing the energy and nutrients balances of the conventional DAF+UASB with the new AnSBR based on the pilot trials results and the full-scale data, the biogas production and energy recovery of the AnSBR is about 25% higher (2831 m3 CH4/d , 100300 MJ/d , 27942 kWh/d) when compared to the DAF+UASB case. However, a slightly higher COD concentration in the effluent of the AnSBR and total suspended solids is expected. Regarding nutrients, lower concentrations of N and P will be obtained with the AnSBR, which will make the stream's further anammox and aerobic treatment more cost-effective.

Overall, Sparthane™ is a suitable technology for direct treatment (without any pretreatment) of slaughterhouse wastewater. The non-conventional, self-regulated AnSBR demonstrated to be a robust, high-rate anaerobic single-stage solution that is not currently available in the market for this application.

For further research, the cycle settings of the AnSBR can be further optimized in relation to the specific properties of the wastewater quality.
The existing biogas system (biogas desulphurization system, piping, boiler) should possibly be expanded in order to be able to process the higher volume of produced biogas when retrofitting the existing WWTP with an AnSBR instead of the DAF-unit followed by a UASB reactor. As the biogas is mixed with a higher volume of natural gas, higher biogas production will result in less natural gas needed to produce the required amount of steam for the slaughterhouse.

The aerobic system treating the anaerobic effluent should be expanded if the nitrogen load in the anaerobic effluent increases. Alternatively, a nutrient recovery treatment step for nitrogen would have to be incorporated between the anaerobic and aerobic treatment steps, such as a struvite reactor (in which ammonia and phosphorus are removed from the anaerobic effluent).
Furthermore, in the current treatment train of the slaughterhouse wastewater, the effluent quality of the anaerobic UASB reactor seems to be a perfect food source for *Legionella* bacteria in the following aerobic system, possibly due to the presence of (dead) anaerobic bacteria in the anaerobic effluent. Although this hypothesis for the sometimes extremely high *Legionella* levels in the aerobic system is still under investigation, the use of an AnSBR might be beneficial in this respect as possibly more (dead) anaerobic bacteria remain inside the anaerobic treatment step as the AnSBR cycle has a robust settling phase.

9 Overview of dissemination activities

Public Media

TKI Water Technology Website
Project description: Innovatieve technologie voor de behandeling van afvalwater uit slachterijen (tkiwatertechnologie.nl) (in Dutch)
News item: Start pilot innovatieve technologie afvalwaterbehandeling slachthuizen (tkiwatertechnologie.nl); July 2021

KWR Website
Project description: TKI Innovative slaughterhouse Wastewater Treatment Technology - KWR (kwrwater.nl): May 2019
News item: Pilot starts with innovative technology for treating slaughterhouse wastewater - KWR (kwrwater.nl); July 2021

H2O Waternetwerk
News item: Nieuwe techniek moet afvalwater van slachthuizen beter zuiveren (h2owaternetwerk.nl); 16 July 2021

Professional Journals:

Waterforum Magazine, nummer 6, jaargang 17 – november 2021: "Nieuwe zuivering eet meer vet" door Marga van Zundert (in Dutch).

Conference presentations:

Muñoz Sierra J.D. and Smet D. Innovative Technology for Treating Slaughterhouse Wastewater. Aquatech Amsterdam. Industry Hub: Organics in Water. 2021, 3rd November.

Scientific Publications:

1. Impact of operational parameters on the performance of an anaerobic SBR treating protein-rich wastewater. Environmental Science and Ecotechnology. *Under review may 2023.*
2. Biogas production enhancement by non-conventional AnSBR treating slaughterhouse wastewater. Renewable Energy. *In preparation may 2023.*

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I Wastewater characterization

I.I Wastewater characterization

Table 14 Wastewater characterization from initial sampling campaigns.

Parameter					Sample 1			Sample 2			Sample 3			Sample 4			Sample 5		
					"Weekly Grab Sample"			"Weekly 24h sample"			"Na Broeibak"			High COD grab			Low COD grab		
Chemical Oxygen Demand					**mgCOD/L**	% of TCOD		**mgCOD/L**	% of TCOD		**mgCOD/L**	% of TCOD		**mgCOD/L**	% of TCOD		**mgCOD/L**	% of TCOD	
TCOD					**4.169**			**5.255**			**7.129**			**5.773**			**3.594**		
CCOD (supernatant after centrifugation)					**2.000**			**2.143**			**3.502**			**2.571**			**738**		
SCOD (filtered <0.45µm after centrifugation)					**1.832**	43,9%		**1.980**	37,7%		**3.186**	44,7%		**2.357**	40,8%		**616**	17,1%	
SCOD centrifuged																			
ΔCOD, non-soluble					2.337	56,1%		3.275	62,3%		3.943	55,3%		3.416	59,2%		2.978	82,9%	
Solids					**g/kg**	% of TS		**g/kg**	% of TS		**g/kg**	% of TS		**g/kg**	% of TS		**g/kg**	% of TS	
TS, Total Solids					**2,747**			**3,664**			**4,471**			**3,886**			**2,857**		
VS, Volatile Solids					**1,979**	72,0%		**2,748**	75,0%		**3,376**	75,5%		**2,646**	68,1%		**2,057**	72,0%	
IS, Inorganic Solids					0,768	28,0%		0,916	25,0%		1,095	24,5%		1,240	31,9%		0,800	28,0%	
					g/kg	% of TSS		**g/kg**	% of TSS		**g/kg**	% of TSS		**g/kg**	% of TSS		**g/kg**	% of TSS	
TSS, Total Suspended Solids					**1,309**			**2,572**			**2,098**			**2,494**			**1,651**		
VSS, Volatile Suspended Solids					**1,288**	98,4%		**2,353**	91,5%		**1,944**	92,7%		**2,219**	89,0%		**1,350**	81,8%	
ISS, Inorganic Suspended Solids					0,021	1,6%		0,219	8,5%		0,154	7,3%		0,275	11,0%		0,301	18,2%	
Nitrogen					**mgN/L**	% fo TKN		**mgN/L**	% fo TKN		**mgN/L**	% fo TKN		**mgN/L**	% fo TKN		**mgN/L**	% fo TKN	
TKN, Total Kjeldahl Nitrogen					298			336			597			370			318		
SKN, Soluble Kjeldahl Nitrogen					192	64,4%		213	63,4%		427	71,5%		245	66,2%		207	65,1%	
NH4-N, Ammoniacal Nitrogen					142	47,7%		181	53,9%		215	36,0%		173	46,8%		174	54,7%	
Phosphorous					**mgP/L**	% of P-tot		**mgP/L**	% of P-tot		**mgP/L**	% of P-tot		**mgP/L**	% of P-tot		**mgP/L**	% of P-tot	
P-tot, Phosphorous Total					43,6			56,3			56,9			47,8			41,9		
ortho **-P, ortho-Phosphate** (H_3O_4 + $H_2PO_4^-$ + HPO_4^{2-} + PO_4^{3-})					34,7	79,6%		44,9	79,8%		44,2	77,7%		35,8	74,9%		27,1	64,7%	
Volatile Fatty Acids					mg/L	mgCOD/L	meq/L	mg/L	mgCOD/L	meq/L	mg/L	mgCOD/L	meq/L	mg/L	mgCOD/L	meq/L	mg/L	mgCOD/L	meq/L
		g/mol	*gCOD/mol*	*gCOD/g*															
C2, acetic acid	C2H4O2	60,1	64	1,07	390	416	6	515	549	9	655	698	11	335	357	6	24	26	0
C3, propionic acid	C3H6O2	74,1	112	1,51	260	393	4	360	544	5	410	620	6	210	318	3	17	26	0
iC4, isobutyric acid	C4H8O2	88,1	160	1,82	0	0	0	83	151	1	83	151	1	34	62	0	0	0	0
C4, butyric acid	C4H8O2	88,1	160	1,82	105	191	1	145	263	2	155	281	2	78	142	1	0	0	0
iC5, isovaleric acid	C5H10O2	102,1	208	2,04	140	285	1	195	397	2	150	305	1	0	0	0	0	0	0
C5, valeric acid	C5H10O2	102,1	208	2,04	31	63	0	37	75	0	28	57	0	18	37	0	0	0	0
C6, hexanoic acid	C6H12O2	116,2	256	2,20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total VFA						1.348	13		1.980	18		2.113	21		915	10		51	1
Major Anions					mg/L	mM	meq/L	mg/L	mM	meq/L	mg/L	mM	meq/L	mg/L	mM	meq/L	mg/L	mM	meq/L
		g/mol																	
Cl^-, chloride	as Cl	35,5			200	6	6	230	6	6	230	6	6	260	7	7	200	6	6
NO_3^-, nitrate	as N	14			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
NO_2^-, nitrite	as N	14			0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
SO_4^{2-}, sulphate	as SO4	96,1			0	0	0	6	0	0	0	0	0	0	0	0	15	0	0
SO_4-S, sulphur	as S	32,1			0			2			0			0			5		
Total inorganic anions							6			7			6			7			6
Major Cations					mg/L	mM	meq/L	mg/L	mM	meq/L	mg/L	mM	meq/L	mg/L	mM	meq/L	mg/L	mM	meq/L
		g/mol																	
Ca^{2+}, calcium	as Ca	40,1			41	1	2	87	2	4	76	2	4	90	2	4	75	2	4
K^+, potassium	as K	39,1			12	0	0	25	1	1	25	1	1	24	1	1	22	1	1
Mg^{2+}, magnesium	as Mg	24,3			60	2	5	110	5	9	140	6	12	120	5	10	100	4	8
Na^+, sodium	as Na	23,0			130	6	6	220	10	10	220	10	10	230	10	10	200	9	9
Total inorganic cations							13			24			26			25			21
Elements					mg/L	mM		mg/L	mM		mg/L	mM		mg/L	mM		mg/L	mM	
Other analyses																			
Conductivity					2,3	mS/cm		2,57	mS/cm		2,90	mS/cm		2,63	mS/cm		2,56	mS/cm	
TOC					1.196	mg/L		1.410	mg/L		1.926	mg/L		1.607	mg/L		848	mg/L	
Alkalinity Total (pH-4)					18	meq/L		22	meq/L		20	meq/L		27	meq/L		21	meq/L	
Wateringen HCO3 (pH-3)					13	meq/L		13	meq/L		0	meq/L		18	meq/L		16	meq/L	
pH					6,87			7,01			6,84			6,84			7,23		
FOG					350	mg/l		420	mg/l		420	mg/l		#N/A	mg/l		#N/A	mg/l	

II Wastewater characterization

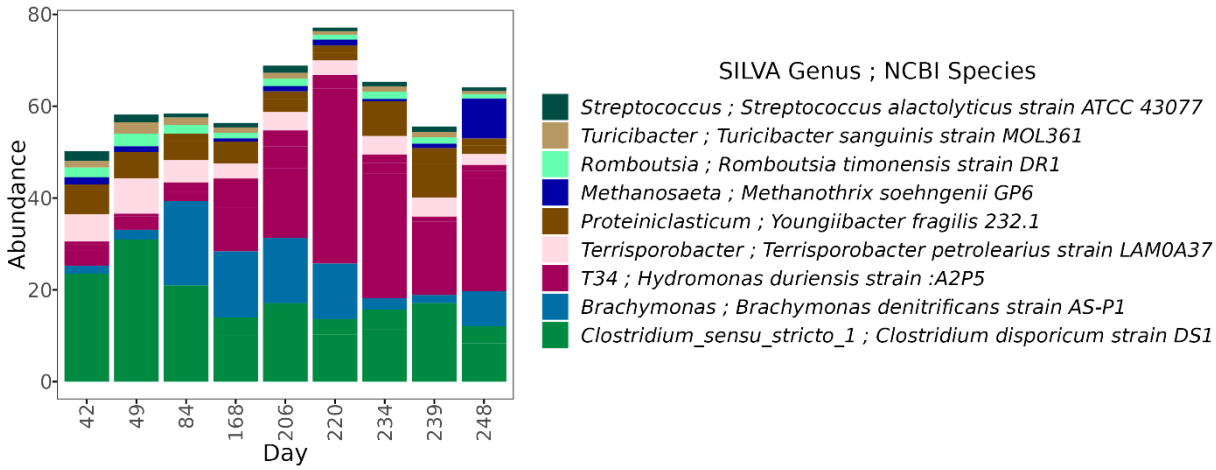
II.I Sterolic composition of the slaughterhouse wastewater

Table 15 Sterolic composition of the slaughterhouse wastewater.

Sterols	mg/L	STDEV
Cholesterol	14,20	0,8
Brassicasterol	N.D	N.D
24-methylencholesterol	0,39	0,18
Campesterol	3,2	0,41
Campestanol	0,2	0,08
Stigamasterol	2,31	0,42
delta-7-campesterol	N.D	N.D
delta-5,23-stigmastadienol	N.D	N.D
clerosterol	N.D	N.D
beta-sitosterol	6,64	0,55
sitostanol	1,49	0,34
delta-5-avenasterol	0,76	0,22
delta-7,9(11)-stigmastadienol	N.D	N.D
delta-5,24-stigmastadienol	N.D	N.D
delta-7-stigmastenol	N.D	N.D
delta-7-avenasterol	N.D	N.D
7-dehydrocholesterol	N.D	N.D
Total sterols	27,07	7,81

III Microbial community analysis Supplementary Material

A.



B.

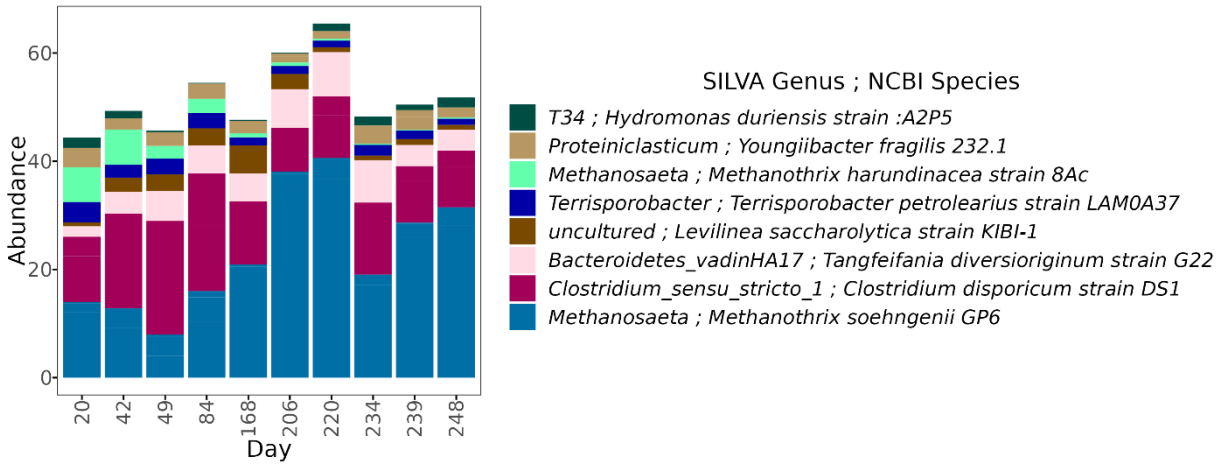


Figure S1 Core microbiome or predominant ASVs with average relative abundance > 1% in A. Buffer tank. B. Reactor at species level.

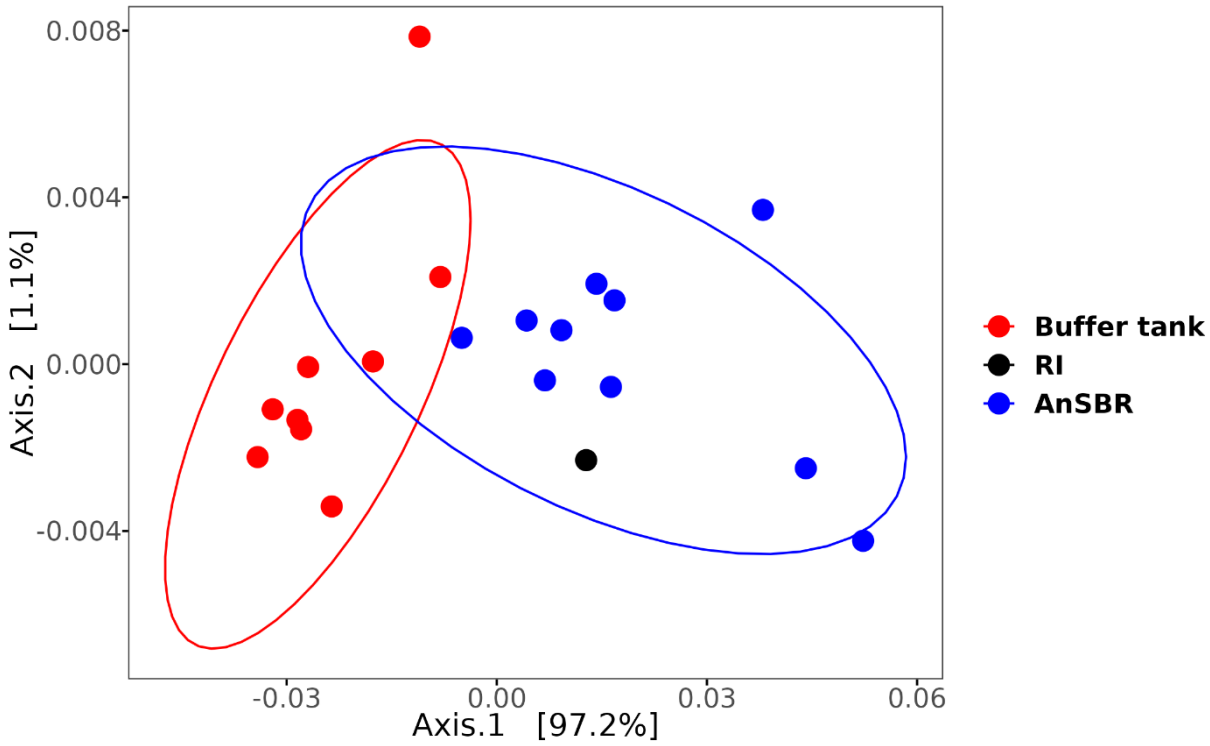


Figure S2. Beta diversity PCoA analysis among buffer tank and reactor samples.

Table 16 Most prevalent genera in AnSBR and buffer tank and their best BLAST similarity hits with species reported in the NCBI taxonomy.

Accesion	Similarity E-value	SILVA Genus	NCBI taxonomy	Source
NR_040972.1	88.9	*uncultured*	*Levilinea saccharolytica strain KIBI-1*	AnSBR
NR_118108.1	96.4	*Proteiniclasticum*	*Youngiibacter fragilis 232.1*	AnSBR
NR_026491.1	**100.0**	*Clostridium_sensu_stricto_1*	*Clostridium disporicum strain DS1*	AnSBR
NR_137408.1	**99.3**	*Terrisporobacter*	*Terrisporobacter petrolearius strain LAM0A37*	AnSBR
NR_043203.1	**99.7**	*Methanosaeta*	*Methanothrix harundinacea strain 8Ac*	AnSBR
NR_102903.1	**100.0**	*Methanosaeta*	*Methanothrix soehngenii GP6*	AnSBR
NR_102903.1	96.9	*Methanosaeta*	*Methanothrix soehngenii GP6*	AnSBR
NR_102903.1	97.5	*Methanosaeta*	*Methanothrix soehngenii GP6*	AnSBR
NR_145650.1	96.1	*T34*	*Hydromonas duriensis strain :A2P5*	AnSBR
NR_118108.1	94.9	*Proteiniclasticum*	*Youngiibacter fragilis 232.1*	AnSBR
NR_134211.1	88.2	*Bacteroidetes_vadinHA17*	*Tangfeifania diversioriginum strain G22*	AnSBR
NR_134211.1	86.9	*Bacteroidetes_vadinHA17*	*Tangfeifania diversioriginum strain G22*	AnSBR
NR_118108.1	96.4	*Proteiniclasticum*	*Youngiibacter fragilis 232.1*	Buffer tank
NR_026491.1	98.3	*Clostridium_sensu_stricto_1*	*Clostridium disporicum strain DS1*	Buffer tank
NR_026491.1	**100.0**	*Clostridium_sensu_stricto_1*	*Clostridium disporicum strain DS1*	Buffer tank
NR_041781.1	**100.0**	*Streptococcus*	*Streptococcus alactolyticus strain ATCC 43077*	Buffer tank
NR_028816.1	**99.1**	*Turicibacter*	*Turicibacter sanguinis strain MOL361*	Buffer tank
NR_137408.1	**99.3**	*Terrisporobacter*	*Terrisporobacter petrolearius strain LAM0A37*	Buffer tank
NR_144740.1	**100.0**	*Romboutsia*	*Romboutsia timonensis strain DR1*	Buffer tank
NR_102903.1	96.9	*Methanosaeta*	*Methanothrix soehngenii GP6*	Buffer tank
NR_145650.1	96.1	*T34*	*Hydromonas duriensis strain :A2P5*	Buffer tank
NR_145650.1	94.9	*T34*	*Hydromonas duriensis strain :A2P5*	Buffer tank
NR_145650.1	95.7	*T34*	*Hydromonas duriensis strain :A2P5*	Buffer tank
NR_118108.1	94.9	*Proteiniclasticum*	*Youngiibacter fragilis 232.1*	Buffer tank
NR_025834.1	**100.0**	*Brachymonas*	*Brachymonas denitrificans strain AS-P1*	Buffer tank

IV Nutrient Recovery

A literature overview of potential technologies to be applied in a slaughterhouse wastewater treatment train is presented:

Ammonia Stripping

Ammonia stripping is developed at full-scale and sometimes implemented for wastewater treatment. Commercially available stripping technologies are (*AMFER*, Colsen (NL), *ANAStrip*, GNS (DE), and the stripping processes developed by the manufacturers: Anaergia (Canada, CA), Branch Environmental Corp (USA), Europe Environnement (France, FR), and RVT Process Equipment (DE), AECO-NAR Nijhuis (NL, Menkveld and Broeders (2018)). Theoretically, these systems may achieve NH_3-removal efficiencies up to 98 %, but they are generally operated to reach 80–90 % removal to reduce the operating costs. The ammonium sulfate-fertilizer content in the recovered solution ranges from ±25 % ammonium sulfate (*ANAStrip*, GNS) and 30 % ammonium sulfateS (Branch Environmental Corp) to ±38 % ammonium sulfate (Anaergia; RVT Process Equipment), 40 % ammonium sulfate (*AMFER*, Colsen; Europe Environnement)(Vaneeckhaute et al., 2017), 25-40% ammonium sulfate AECO-NAR(Nijhuis)(Menkveld & Broeders, 2018).

Based on results and estimations with an influent stream of 2.5 g $N\text{-}NH_3 \cdot L^{-1}$, Menkveld and Broeders (2018) inferred that the cost per kg N decreases significantly at an increasing influent concentration of NH_3-N, varying within a range of 1.0 – 3.0 euros/Kg N. The cost drivers such as heating, caustic dosage, process stability, and CAPEX remain similar while increasing the NH_3-N concentration. The costs for sulphuric acid dosage cancel out the market value of ammonium sulfate. Based on a 200 $m^3 \cdot d^{-1}$ plant, the consumption of caustic, sulphuric acid, and heat represent about 19%, 20%, and 20% of the total cost of the process, respectively (Menkveld & Broeders, 2018). Thereby, the use of residual heat available from biogas conversion is crucial to make the process feasible.
Looking at conventional technologies, the economic feasibility of N-only recovery is still low, largely due to the chemical cost of adjusting pH to increase the free ammonia concentration (NH_4^+ to NH_3), due to the heat required to decrease ammonia gas solubility and drive ammonia stripping, or due to the relatively low cost of competing for ammonia products from the Haber–Bosch process.

Electrodialysis
Electrodialysis (ED) is more applicable specifically for N and K recovery, as P can be effectively removed using other lower-cost methods. Electrodialysis is appropriate at low nutrient concentrations (below 2000 mg L^{-1}) due to a lower potential for membrane fouling or scale formation. The N and K recovery by ED from anaerobic reactors is a relatively new application, and the limits of nutrient concentration for this wastewater stream are still under evaluation (Mehta et al., 2015; Thompson Brewster et al., 2017).
It has been applied to recover ammonia from pig manure and also source-separated urine. A maximum ammonium concentration of 14.25 g L^{-1} was achieved in the concentrate, which was 10 times that in the manure. Waste streams with an acidic to slightly alkaline pH (<8.0) are preferred due to improved nutrient solubility and ion transfer through membranes. The process required about 3.25–3.60 kWh to remove 1 kg of $N\text{–}NH_3$. (Ippersiel et al., 2012; Mondor et al., 2009; Mondor et al., 2008).
In a pilot plant, 7.1 ± 0.3 g $N\text{-}NH_4$/L and 2.49 ± 0.04 g K/L were achieved by concentrating nutrient ions from the centrate wastewater dilute feed stream using electrodialysis. The average total current efficiency was 76 ± 2% (NH_4-N transport 40%, K transport 14%). The electrode power consumption was 4.9 ± 1.5 kWh/kgN on average.

This value is lower than competing technologies for NH_4-N removal and production and far lower than previous electrodialysis laboratory trials (Ward et al., 2018). This makes the process economically competitive for nitrogen removal by advanced reactive technologies such as anammox. The anammox treatment process consumes about 1.50-5.02 kWh/kg NH_4-N treated (Schaubroeck et al., 2015). Electrodialysis has a lower footprint (HRT 0.3 h), simpler in operation (single-stage process), and recovers the nitrogen rather than removing it (Ward et al., 2018).

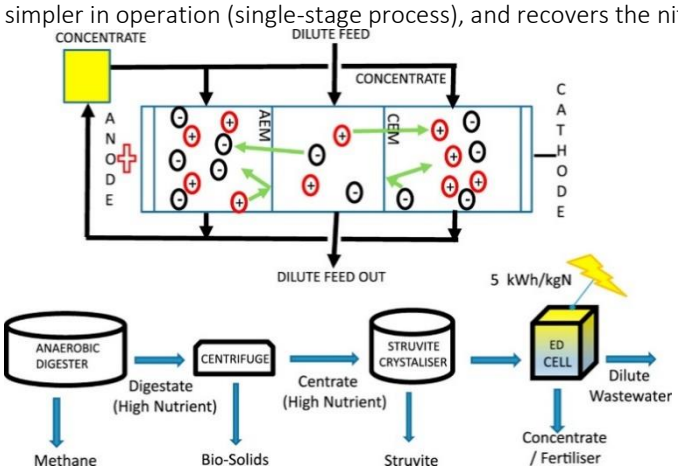


Figure 25 Nutrient recovery through pilot-scale Electrodialysis (Ward et al., 2018).

Wang et al. (2015) also used electrodialysis to simultaneously recover NH_4-N and PO_4-P from synthetic wastewater, coupled with a struvite crystallization reactor. Removal ratios of 96-100% for NH_4-N and 86 -94% for PO_4-P were obtained with this integrated process. Membrane scaling is reduced by more than 50% by coupling these two processes (Thompson Brewster et al., 2017), having struvite crystallization as a pre-treatment to the centrate.

Ion Exchange by zeolites

Both natural and synthetic zeolites have been widely evaluated for ammonium removal due to their high cation exchange capacity (Thornton et al., 2007). They have been identified as suitable sorbents for wastewaters taking benefit of their properties (e.g., mechanical and thermal, cation-exchange capacity, easy operation and maintenance, low treatment costs, high selectivity, and the release of non-toxic exchangeable cations (K^+, Na^+, Ca^{2+} , and Mg^{2+})(Sancho et al., 2017).

Adsorption and ion-exchange technology are suitable for waste streams with a range of nutrient concentrations (0.1–2000 mg L^{-1}) but relatively low solids concentrations (<2000 mg L^{-1}). Hence, anaerobic membrane bioreactors effluent is very suitable for applying ion-exchange-based nutrient recovery technologies. Waste streams with an acidic pH (<8.0) are favored to increase nutrient solubility and maximize the adsorption of the resin. For concentrated waste streams (>2000 mg L^{-1}), modified zeolite and clinoptilolite are usually used for nitrogen and potassium recovery. Maximum loading capacities have been reported to be 21.5 g N kg^{-1} for clinoptilolite (Sprynskyy et al., 2005).

Removal rates for N-NH4 between 61.5 and 84.6% were achieved at N-NH4 concentrations typical for sludge liquor (0.9 to 2.3 g.L^{-1}). Zeolite loadings ranged from 5 to 8 mg N-NH4.g^{-1} after 90 min of loading. Regeneration rates were between 42.9 and 49.7% but increased to 64.8% with simultaneous air stripping. As a result of matrix effects in sludge liquor (e.g., flocculants, competing ions), a minimal decrease in the ammonium removal rate was observed. Substantial phosphate reduction in the sludge liquor occurred after ion exchange due to potential struvite or apatite precipitation (Ellersdorfer, 2018). Regeneration of loaded ammonium zeolites generates rich ammonium/ammonia concentrates (2–6 g NH_3/L) in NaCl, NaOH, or NaOH/NaCl solutions (Sancho et al., 2017). Chemicals required for the regeneration of the sorbents, biofouling, large amounts of resin required for complete removal, limited resin life, and competitive foreign ion adsorption are some of the challenges at full-scale implementation. To reduce regeneration costs, some studies have tried to use media biological regeneration rather than chemicals (Jung et al., 2004; Wei et al., 2011)

Natural zeolites, especially those containing clinoptilolite, are very promising for N-NH4 rich streams due to their high treatment capacity (theoretical cation exchange capacity of 202 mEq/100 g), selectivity, and removal efficiency, low costs, and fast kinetics (Pabalan & Bertetti, 2001). Nonetheless, ion exchange may be hampered economically as a result of the large amounts of salts (e.g., NaCl) needed for regeneration (Deng et al., 2014). Deng et al. (2016) tested the simultaneous regeneration of exhausted zeolite for ammonium recovery at alkaline pH by air stripping. The results emphasize that the ammonium exchange capacity of regenerated zeolites was significantly reduced with increasing regeneration frequency. It decreased by 50% after ten times reuse, 70% after 20 times reuse, and 80% after 24 times reuse of exhausted zeolite. By applying regeneration with high pH and air stripping, the chemical cost was 20 times less than the conventional regeneration (using NaCl) and was at least half the cost of alkaline regeneration. Regeneration turned out to be the limiting step for ammonium recovery. An overview of removal rates of ammonium and phosphorus in several studies is presented in Table 17.

Ion Exchange by synthetic resins

The demo plant from SMART Horizon 2020 also provides an insight into the feasibility of accomplishing sequential N and P recovery by using ion exchange (see *Figure 26*). Guida; et al. (2019) studied, on this demonstration scale, the feasibility of P and N recovery through an ion exchange process. For this, they used a synthetic zeolite and a synthetic hybrid anion exchanger (HAIX) for the recovery of N and P, respectively, achieving 90% NH_4-N recovery and 95% PO_4-P removal. The regenerant solutions were made of 10% KCl and 2% NaOH to regenerate the synthetic zeolite and the HAIX.

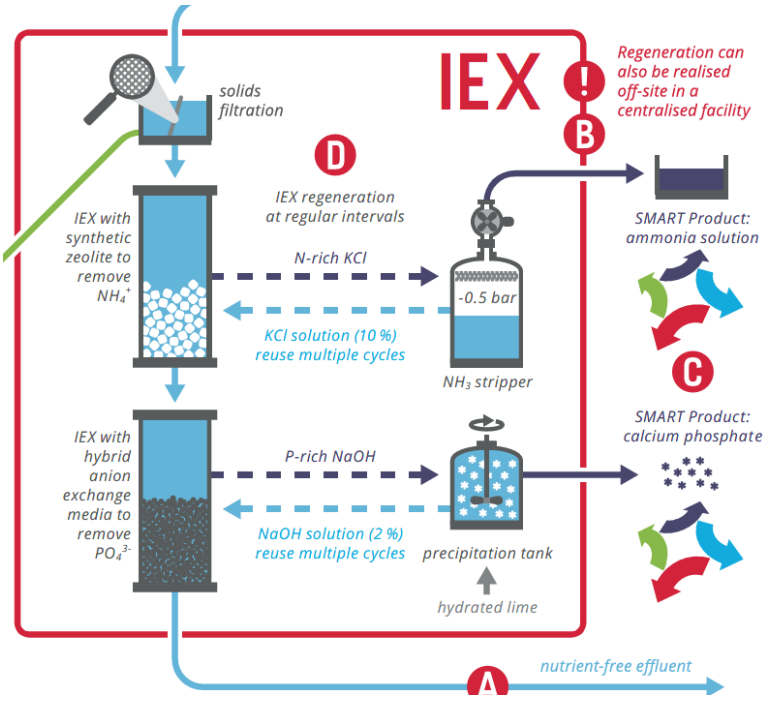


Figure 26 Ion Exchange with synthetic zeolite and hybrid anion exchange for Ammonia and Phosphorus Recovery from Wastewater (Guida; et al., 2019)

Competing anions in the wastewater may reduce the ion-exchange potential application. Pilot-scale studies should address different challenges for full-scale implementation, such as reversible fouling of the adsorbent and irrecoverable fouling of the adsorbent (i.e., capacity reduction after several adsorption regeneration cycles), and cost-benefit analyses.

Precipitation/Crystallization (P- recovery)

Different processes for P recovery from wastewater treatment plants have been tested on different scales, including phosphate salts precipitation/crystallization, which currently is one of the most promising technologies for recovering P and N in wastewater treatment plants (Li et al., 2019), achieving a technology readiness level (TRL) of 7 or above. Anaerobic digestion as a solubilization technique is a primary step in facilitating the precipitation of struvite in many commercial P recovery processes such as Crystalactor®, NuReSys®, Pearl®, Phosnix® , and PHOSPAQ™ (Schoumans et al., 2015). An overview of different technologies with different sources of streams within the wastewater treatment plant is given in appendix IV.

Struvite

Struvite precipitation as a recovery route for ammonia and phosphate has gained a lot of interest in the last decades, and it is applicable on a large scale. It can remove between 80 and 90% of PO_4^{3-} and 20–30% of $NH4^+$ (Le Corre et al., 2009). Struvite, which is magnesium ammonium phosphate ($MgNH4PO_4{\cdot}6H_2O$) is formed in WWTPs through spontaneous precipitation if the Mg concentration is high enough. Usually, Mg salt is added to fully remove soluble P as struvite from these streams (Münch & Barr, 2001).

The N and P fractions in struvite are slowly soluble, which makes struvite usable as a slow-release commercial fertilizer suitable for soils with a low pH value (Xie et al., 2016). The crystallization of struvite and other P-rich precipitates during precipitation results in a very low degree of impurities. Struvite contains 6% nitrogen by weight and 12% phosphate, only 10% of the available nitrogen will be removed through struvite precipitation.

Effective struvite precipitation can only be achieved if P concentrations are above 100 $mg.L^{-1}$ and depend on the ammonium concentration and pH value. At lower concentrations, struvite precipitation is probably not feasible since lower P concentrations lead to low recovery rates, longer precipitation reaction times, and higher pH values (Xie et al., 2016). The formation and growth of struvite crystals in WWTPs are affected by various parameters, such as pH, temperature, mixing energy and turbulences, and the presence of other ions like calcium or carbonates. Struvite precipitation can be promoted by stripping carbon dioxide and raising the pH, followed by adding magnesium chloride and sodium hydroxide, magnesium hydroxide, or magnesium oxide.

The cost of recovering struvite after sludge digestion by adding magnesium salt could be as high as €2 per m^3 of raw wastewater. However, struvite recovery can significantly reduce the sludge volume due to its subsequent improved dewaterability, and thereby this technique may decrease sludge handling and disposal costs (Cornel & Schaum, 2009). The chemical cost has been identified as a major contributor to the global P recovery process via struvite crystallization (van Alderen, 2019). The costs, without savings and revenues, of struvite crystallization P recovery systems are approximately €6 to €10 per kg of P recovered, or 0.8 to €1.7 PE per year (Egle et al., 2016). Struvite is currently marketed at values between €0–100 per ton but also at higher prices of €350 per ton (Phoshorgreen) to €1000 (Pearl) per ton. This implies that in about 80–87% of the cases, struvite is sold at lower prices than the estimated market value of its macronutrients, namely €250–412 per ton (Muys et al., 2021). Struvite was first successfully recovered in the Netherlands in 2006, but its implementation seemed not to have a breakthrough (Kehrein et al., 2020). The number of industrial-scale applications is small due to shortcomings such as limited product quality, limited economic feasibility, or restrictions in use due to the legal framework (Yetilmezsoy et al., 2017).

The commercial Airprex® process (see Table 19) precipitates struvite directly in the sludge stream and takes economic benefits concerning the scaling of pipes and sludge dewatering equipment. Moreover, the struvite product is sold for €50–100/t for fertilizer production (Melia et al., 2017).

Vivianite

Significant iron (Fe) enters the WWTP due to iron dosing, including for the use to remove P chemically. This results in iron phosphate precipitates in their sludge lines. About 40–50% of the total influent P precipitate is in the form of vivianite ($Fe_3^{2+}(PO_4)_2{\cdot}8H_2O$) and is the most abundant form of phosphate in digested sludge. The extraction of pure vivianite in crystal form still requires more knowledge about its formation (Wilfert et al., 2016). An innovative pilot system (‘ViviMag’) using magnetic separation to recover vivianite from digested sewage sludge is currently under

construction in the Netherlands (Prot et al., 2019). Vivianite for P recovery from wastewater offers potential economic opportunities due to high market prices for vivianite (approximately €10,000 per ton) compared to struvite (€100-500 per ton) (Wu et al., 2019). Vivianite is a source for the manufacture of lithium iron phosphate (LiFePO4), which is increasingly being exploited as a precursor when fabricating Li-ion secondary batteries (Priambodo et al., 2017).

Some of the disadvantages of recovering P as vivianite are:

- Vivianite is formed in the sludge phase as crystals or aggregates with sizes ranging between 10 and 150 µm, which are difficult to separate from sludge.
- The presence of impurities (i.e. magnesium or calcium). Impure vivianite is oxidized within 48 h, while pure vivianite can remain stable for several weeks (Wilfert et al., 2018).
- Vivianite separation and purification methods such as crystallization, magnetic separation, and centrifugation based on the different densities of sludge and vivianite (1 and 2.68 g/cm3 respectively) are not yet well developed.

Table 17 P recovery technologies at pilot and industrial scales (adapted from Robles et al. (2020)).

Source	Source	PO_4-P (mg/L)	NH4-N (mg/L)	Mg source	pH adjustment	Reactor type	Scale	Technology	Recovery efficiency (%)	Crystal shape and size	References
Thickener supernatant	Elutriated sludge	96 ± 14	102 ± 9	$MgCl_2$	NaOH	CSRT	Pilot	PHORWATER®	87	Coarse crystals (>200 µm)	(Bouzas et al., 2019)
	Elutriated sludge	NA	NA	$MgCl_2$	NaOH	UFBR	Industrial	PEARL®/WASSTRIP ®	83–90	Round pellets (0.9–3 mm)	(Cullen et al., 2013)
Anaerobic sludge	Digested sludge	387–400	NA	$MgCl_2$	Aeration	Airlift	Industrial	AirPrex®	14–21	Coarse crystals (>200 µm)	(Zhou et al., 2019)
		220	NA	$MgCl_2$	Aeration	CSTR	Industrial	NuReSys®	86[b]	Round pellets (1–3 mm)	nuresys.be
Digester supernatant	Dewatering centrate	NA	NA	$MgCl_2$	NaOH	UFBR	Industrial	PEARL®	83–90	Round pellets (0.9–3 mm)	(Egle et al., 2015), (Li et al., 2019)
		200–260	2100–3600	$MgCl_2$	NaOH	CSRT	Pilot	STRUVIA™	80–95	Coarse crystals (100–500 µm)	cordis.europa.eu/project/rcn/105528
		NA	NA	$MgCl_2$	NaOH, aeration	UFBR	Industrial	DHV Crystalactor®	70	NA	(van Houwelingen & Piekema, 2018)
		60–407	150–800	MgO	Aeration	CSRT	Industrial	PHOSPAQ™	75–95	Coffin shaped crystals (~0.7 mm)	(Abma et al., 2010), (Driessen et al., 2018)
		450	NA	$MgCl_2$	NaOH	CSTR	Industrial	NuReSys®	95[b]	Round pellets (1–3 mm)	nuresys.be

a: Dilution 1:4; b: Precipitation recovery; NA: not available

Table 18 Removal rates achieved in studies using IEX by Zeolites (adapted from Kotoulas et al. (2019))

Wastewater Type	Zeolite Type	Pollutant	Removal efficiency percentage and specific removal rate	Reference
Olive mill wastewater	Natural (normal zeolite particles)	Nitrate Phosphorus Potassium	78% 48.3% 66.6%	(Aly et al., 2014)
Olive mill wastewater	Natural (nanoparticles)	Nitrate Phosphorus Potassium	92.79% 92.64% 99.96%	(Aly et al., 2018)
Simulated swine wastewater	Chemical modification with NaCl	Ammonium	40%–95%	(Huang et al., 2015)
Dairy farm wastewater treated in constructed wetlands	Natural	Phosphorus Ammonium	86%–99% 88%–99%	(Schierano et al., 2017)
Municipal wastewater	Natural	Ammonium	5.03 mg/g 75.6%	(Beebe et al., 2013)
Aqueous solution	Modified with fly ash	Ammonium	41.73%–45.25%	(Das et al., 2017)
Aqueous solution	Natural	Ammonium	22.90 mg/g	(Ding & Sartaj, 2015)
Secondary wastewater effluents	Natural Modified (Z-Al)	Phosphate (single) Ammonium(single) Phosphate (single) Ammonium (single)	0.6 mg/g 33 mg/g 7.0 mg/g 30 mg/g	(Guaya et al., 2015)
Municipal wastewater	Natural Modified (Z-Fe)	Phosphate (single) Ammonium (single) Phosphate (single) Ammonium (single)	0.6 mg/g 33 mg/g 3.4 mg/g 27 mg/g	(Guaya et al., 2016)
Aqueous solution	Natural Modified with potassium permanganate	Ammonium	5.85 mg/g 3.68 mg/g	(Guo et al., 2016)
Aqueous solution	Modified with lanthanum oxide	Phosphorus Ammonium	8.96 mg/g 21.2 mg/g	(He et al., 2017)
Aqueous solution	Natural	Ammonium	67.4%–81.1%	(Huang et al., 2010)

Simulated reclaimed wastewater	Modified with NaCl	Ammonia, nitrogen Phosphorus	98.46% 99.8%	(Huo et al., 2012)
Aqueous solution	Natural	Ammonium	75%–95.3%	(Khosravi et al., 2014)
Aqueous solution	Natural (eight different types)	Ammonium	15.7%–32.4%	(Langwaldt, 2008)
Municipal wastewater	Natural	Phosphorus Ammonium	46%–100% 70%	(Lin et al., 2014)
Aqueous solution	Natural	Phosphate	Up to 26.48 mg/g	(Oliveira et al., 2015)
Post-treated municipal wastewater	Natural	Ammonium	23 ± 0.8 mg/g	(Sancho et al., 2017)
Fermentation liquids	Natural	Ammonium Phosphate	94.06% 98.28%	(Wan et al., 2017)
Aqueous solution	Modified with coal fly ash (Ze–Na) and potassium (Ze–K)	Ammonium	109 ± 4 mg/g 33 ± 1 mg/g	(You et al., 2017)

Table 19 Commercial processes for P recovery and final products (adapted from Melia et al. (2017); Muys et al. (2021))

Process	Information and process description	Final product
AirPrex® process	Crystallization of struvite applied directly in the digested sludge stream. CO_2 is stripped to increase pH. $MgCl_2$ is added. AirPrex® systems are currently operational at several WWTPs in Germany and The Netherlands. The world's largest AirPrex® system is being constructed at the WWTP of Amsterdam. Developed by Berliner Wasserbetriebe (Germany).	Struvite
DHV Crystalactor®	The sludge side stream is fed into the reactor and recirculated. Quartz sand is initially added as seed material to accelerate precipitation. Pellets settle to the bottom. Developed by DHV (NL).	Struvite, Mg-P or Ca-P
NuReSys® process	Air is initially added and CO_2 is stripped from the side stream followed by $MgCl_2$ addition in the stirred crystallizer tank where struvite forms pellets. NaOH is added to maintain pH in the range 8.1–8.3. Pellet size can be controlled by stirring speed. Developed by Akwadok/NuReSys (Belgium).	Struvite
Ostara Pearl® process	Struvite crystallization is achieved through treatment of sludge side stream in a fluidized bed crystallizer. Effluent is recirculated and $MgCl_2$ and NaOH are added as the Mg source and for pH maintenance respectively. Developed at the University of British Columbia and introduced at full-scale by Ostara Nutrients Recovery Technologies Inc. (USA).	Struvite (Crystal Green®)

Phosnix® process	A cylindrical reaction zone with a conical bottom section is applied. $Mg(OH)_2$ and NaOH added as a source of Mg and for the control of pH respectively, and aerated to strip CO_2. Struvite settles to the bottom where it is removed with the effluent recirculated. Developed by Unitika Ltd (Japan).	Struvite
PHOSPAQ™ process	A side stream process consisting within an aerated zone. Air lift is designed to provide mixing, strip CO_2 and increase pH, and provide DO for biological treatment. MgO is used as the Mg source for the precipitation of struvite. Developed by Paques (The Netherlands).	Struvite
FIX-Phos	Calcium silicate hydrate (CSH) particles are added into the anaerobic digester. The CSH adsorbs P as Ca-P and controls struvite formation by reducing the P concentration in the digestate. The Ca-P on CSH can be separated and recovered from the digested sludge.	Ca-P on CSH
P-RoC®	P recovery from waste water similar to the Crystalactor® process however complex pre-treatment steps such as pH adjustment or CO_2 stripping can reportedly be avoided. Crystallization products showed a P content of 11%–13% which was comparable to phosphate rock.	Ca-P on CSH
PHOXNAN	The process combines low pressure wet oxidation with two membrane filtration steps. High temperature and pressure at acidic conditions (sulphuric acid added to adjust pH to 1.5) are used for sludge oxidation with pure oxygen. Organic components are decreased and organic pollutants are oxidised. Due to the low pH, P exists in solution mainly as H_3PO_4 and H_2PO_4. The first membrane uses ultrafiltration to separate solids, the second membrane uses nanofiltration to eliminate metal ions.	H_3PO_4
Aqua Reci	Commercially, the process makes use of supercritical water oxidation. Leaching is accomplished with a base, which selectively dissolves P. By addition of calcium, P can be precipitated.	Ca-P
EcoPhos®	HCl or H_2SO_4 is used for the digestion of any phosphate raw material including P-rock or SSA. The EcoPhos® process involves the treatment of the obtained slurry to remove dissolved impurities and solid residues and produces a phosphate product such as dicalcium phosphate or H_3PO_4.	DCP or H_3PO_4
Mephrec	The process utilizes temperatures of up to 2000 °C where the sewage sludge melts under the addition of oxygen, with all organic pollutants destroyed. The metals obtained can be recycled, the slag is a form of fertilizer with high plant availability, free of heavy metals/metalloids and organic pollutants – similar to Thomas phosphate fertilizer (a P-rich slag produced in the steel industry).	Detoxified mineral P

Suez Phosphogreen	Phosphogreen uses mainly airlift for CO_2 stripping and $MgCl_2$ for pH control and Mg source respectively. It uses upflow and aerated FBR reactor achieving P removal efficiencies of 99%.	Struvite
AshDec	Ash and natural earth alkali salts are exposed to a temperature of 1000–1050 °C. The heavy metals/metalloids react with the salts, become gaseous and evaporate. The phosphate compounds are transformed into plant available species.	Detoxified mineral P
Colsen ANPHOS	Anphos uses mainly air stripping and $Mg(OH)_2$ for pH control and Mg source respectively. It uses aerated mixed and FBR reactors achieving P removal efficiencies of 60-98 %, depending of the source (UASB effluent or centrate).	Struvite