

Article

Biogas Production from Thin Stillage on an Industrial Scale—Experience and Optimisation

Jan Moestedt ^{1,2,*}, Sören Nilsson Påledal ¹, Anna Schnürer ² and Erik Nordell ¹

¹ Department of Biogas R&D, Tekniska verken i Linköping AB (public), Box 1500, Linköping SE-581 15, Sweden; E-Mails: soren.nilsson-paledal@tekniskaverken.se (S.N.P.); erik.nordell@tekniskaverken.se (E.N.)

² Department of Microbiology, BioCenter, Swedish University of Agricultural Sciences, Box 7025, Uppsala SE-750 07, Sweden; E-Mail: anna.schnurer@slu.se

* Author to whom correspondence should be addressed; E-Mail: jan.moestedt@tekniskaverken.se; Tel.: +46-13-20-80-00; Fax: +46-13-20-80-06.

Received: 5 August 2013; in revised form: 27 September 2013 / Accepted: 15 October 2013 /

Published: 29 October 2013

Abstract: With the increasing demand for renewable energy and sustainable waste treatment, biogas production is expanding. Approximately four billion litres of bio-ethanol are produced annually for vehicle fuel in Europe, resulting in the production of large amounts of stillage residues. This stillage is energy-rich and can be used for biogas production, but is a challenging substrate due to its high levels of nitrogen and sulphate. At the full-scale biogas production plant in Norrköping, Sweden (Svensk Biogas i Linköping AB), thin grain stillage is used as a biogas substrate. This paper describes the plant operation and strategies that have been implemented to digest thin stillage successfully. High ammonia concentrations in the digester have resulted in syntrophic acetate oxidation (SAO) becoming the major pathway for acetate degradation. Therefore, a long hydraulic retention time (HRT) (40–60 days) is used to allow the syntrophic acetate-oxidising bacteria time to grow. The high sulphate levels in thin stillage result in high levels of hydrogen sulphide following degradation of protein and the activity of sulphate-reducing bacteria (SRB), the presence of which has been confirmed by quantitative polymerase chain reaction (qPCR) analysis. To optimise biogas production and maintain a stable process, the substrate is diluted with tap water and co-digested with grain residues and glycerine to keep the ammonium nitrogen (NH₄-N) concentration below 6 g L⁻¹. Combined addition of iron, hydrochloric acid and cobalt successfully precipitates sulphides, reduces ammonia toxicity and supplies microorganisms with trace element. Mesophilic temperature (38 °C) is employed to further

avoid ammonia toxicity. Together, these measures and doubling the digester volume have made it possible to increase annual biogas production from 27.7 TJ to 69.1 TJ.

Keywords: anaerobic digestion; thin stillage; full-scale; ammonia; sulphate; trace elements

Nomenclature:

HRT	hydraulic retention time
COD	chemical oxygen demand
OLR	organic loading rate
TS	total solids
VS	volatile solids
VFA	volatile fatty acids
DNA	deoxyribonucleic acid
qPCR	quantitative polymerase chain reaction
SAO	syntrophic acetate oxidation
SAOB	syntrophic acetate-oxidising bacteria
SRB	sulphate-reducing bacteria

1. Introduction

Anaerobic digestion is the microbiological degradation of organic material in the absence of oxygen. The product from this degradation is biogas, which mainly consists of bio-methane and carbon dioxide. Organic wastes from industry, wastewater treatment plants or households can be treated in biogas plants to produce energy-rich bio-methane, which may be used to produce vehicle fuel or electricity and heat. A liquid residue rich in nitrogen and phosphates formed during anaerobic digestion is suitable for use as organic fertiliser [1]. Therefore, anaerobic digestion is a feasible technique to treat organic wastes, produce renewable energy and recover nutrients for re-use in agriculture. As a consequence of the increased demand for renewable energy and sustainable waste treatment, the number of full-scale biogas production plants in Europe is increasing [2,3] and these require a supply of substrate, e.g., energy-rich industrial residues. Stillage is one such potential substrate. Approximately four billion litres of bio-ethanol for vehicle fuel are produced annually in Europe [4]. Stillage is separated from bio-ethanol after the fermentation process, with 0.8 kg total solids (TS) stillage (5–10 L) being produced per litre of bio-ethanol [5,6]. Stillage consists of materials which are not degraded during hydrolysis and bio-ethanol fermentation, such as high concentrations of proteins, amino acids, smaller amounts of sugars and yeast cells [7,8]. Moreover, stillage is energy-rich ($13.6 \text{ MJ kg}^{-1} \text{ TS}$) and has been shown to be appropriate as feedstock for anaerobic digestion [8]. Thin stillage used as feedstock for biogas production has a methane potential of 10.4 MJ kg^{-1} chemical oxygen demand (COD) [8]. Combining bio-ethanol production with biogas production also brings economic and environmental benefits, especially at sites where the respective plants are in close proximity to each other [6,9,10]. However, thin stillage presents some challenges in biogas production.

Degradation of the nitrogen-rich stillage results in high levels of total ammonium nitrogen ($\text{NH}_4\text{-N}$), which can result in process disturbance. The ammonia ($\text{NH}_3\text{-N}$) fraction of $\text{NH}_4\text{-N}$ is positively correlated with increased pH and temperature, is a toxic component and has been shown to be inhibitory to most microorganisms involved in the biogas process, but particularly aceticlastic methanogens [11,12]. Inhibition of aceticlastic methanogens has been suggested to result in selection of syntrophic acetate oxidation (SAO) (at approximately $3 \text{ g NH}_4^+\text{-N L}^{-1}$) [13,14].

SAO is performed in a sensitive syntrophic cooperation between syntrophic acetate-oxidising bacteria (SAOB) and hydrogenotrophic methanogens [13,14]. Co-cultures of SAOB and methanogens typically have lower growth rates than aceticlastic methanogens and introduction of the SAO pathway is thus correlated with lower gas yield [13,15]. In addition to high nitrogen levels, sulphuric acid is commonly used for pH regulation in bio-ethanol fermentation processes, contributing to high sulphate concentrations in stillage [16]. Sulphate is converted to sulphides by the activity of sulphate-reducing bacteria (SRB), which proliferate in different types of anaerobic digestion process [17,18]. The high protein content in thin stillage also results in the release of organic sulphur as sulphides, which have various negative effects on the industrial biogas production process. These include corrosion, precipitation of trace elements, inhibition of microorganisms and consumption of organic material [mainly alcohols, volatile fatty acids (VFA) and hydrogen] that would otherwise be used for methane production [11,18–20].

This paper describes results and experiences from a full-scale operation using thin grain stillage (hereafter called thin stillage) as the dominant substrate at an industrial biogas plant in Norrköping, Sweden run by Svensk Biogas i Linköping AB, a subsidiary company of the public municipal company Tekniska verken i Linköping AB (Linköping, Sweden). Major challenges and the solutions devised to cope with these are discussed and on-going work to optimise gas production efficiency is described.

2. Results and Discussion

Despite the challenges involved in using thin stillage as a substrate for biogas production, experiences show that large-scale gas production from this material is possible. Annual biogas production at the Norrköping plant has increased year on year, from 1.4 million m^3 (27.7 TJ) in 2008 to 3.5 million m^3 (69.1 TJ) in 2011 (Figure 1a). This increase in biogas production has been achieved by doubling the digester volume, increasing the amount of substrate treated annually from 1750 to almost 4000 t volatile solids (VS) in 2011 and improving specific biogas production (VS basis) from $680 \text{ m}^3 \text{ t}^{-1} \text{ d}^{-1}$ during 2008 to $860 \text{ m}^3 \text{ t}^{-1} \text{ d}^{-1}$ during 2011 (Figure 1b). The methane content was relatively low, $55\% \pm 1\%$, and thus the specific methane production (VS basis) has increased from 2008 to 2011 (370 to $466 \text{ m}^3 \text{ t}^{-1} \text{ d}^{-1}$), which is generally higher than previously reported for stillage fractions [8,10,21–23]. The high and increasing specific gas production during the period 2008–2011 can be explained by increased process efficiency and by the use of glycerine during 2011. Glycerine has high energy content and thus should theoretically result in higher biogas yield. From the second half of 2009, the organic loading rate (OLR) has stabilised at $2.4\text{--}3.2 \text{ kg VS m}^{-3} \text{ d}^{-1}$ (Figure 1c), determined by continuous measurements of TS and VS in the substrate. There is generally

a low concentration of VFA and high alkalinity, illustrating that the process is clearly stable (Figures 1d and 2).

Figure 1. Mean monthly values of different process parameters at the Norrköping biogas plant, 2008–2011: (a) total biogas production (●); (b) specific biogas production (□); (c) organic loading rate (OLR) (○); (d) alkalinity (Δ); (e) ammonium-nitrogen (▲); (f) ammonia-nitrogen (◇); and (g) pH (◆).

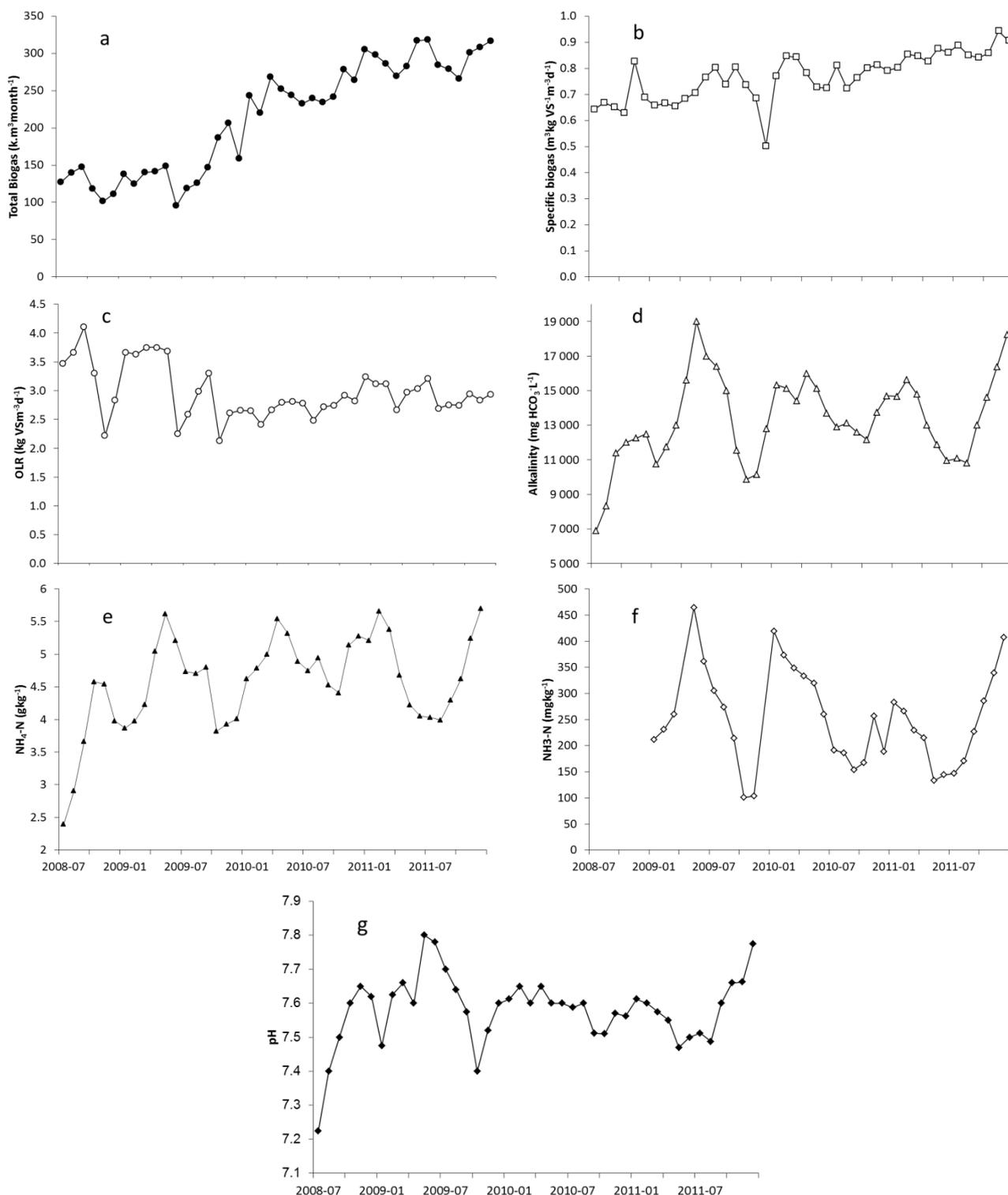
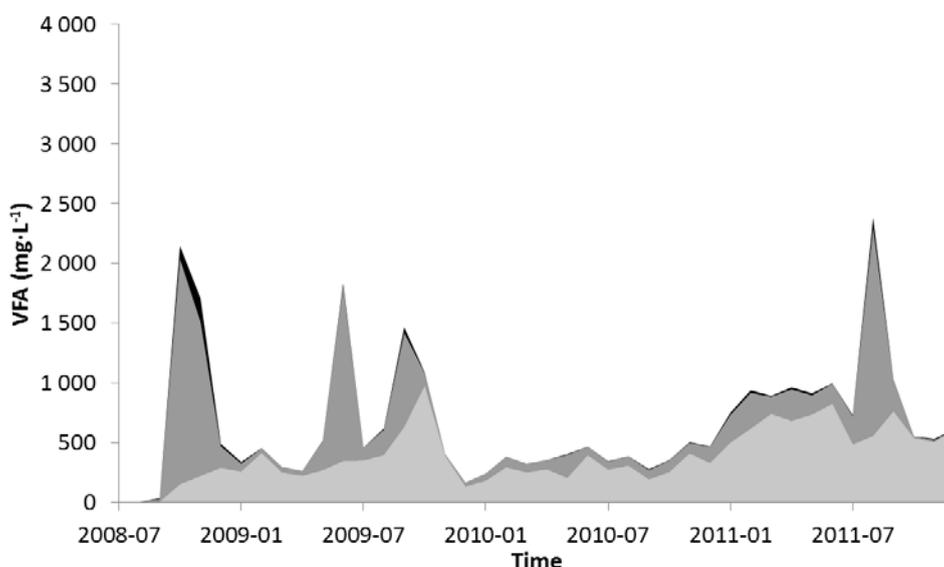


Figure 2. Mean monthly concentration of volatile fatty acids (VFA) at the Norrköping biogas plant, 2008–2011. Acetic acid (■) and propionic acid (■) are presented separately, while the total concentration of residual acids (C4–C7) are presented together (■).



2.1. Nitrogen

One of the challenges with thin stillage is the high nitrogen level, giving rise to high levels of $\text{NH}_4\text{-N}$ during degradation. Different actions were taken at the plant to handle the high nitrogen concentration in thin stillage (Table 1). First, mesophilic temperature was chosen for the operation. It is a well-known fact that thermophilic temperature results in a relatively higher level of $\text{NH}_3\text{-N}$, and thus a higher degree of microbial inhibition [12]. As expected, $\text{NH}_4\text{-N}$ concentration in the process is high, $4000\text{--}5700\text{ mg L}^{-1}$ (Figure 1e). The average ammonia-nitrogen ($\text{NH}_3\text{-N}$) level during the period 2008–2011 was $252 \pm 34\text{ mg kg}^{-1}$ (Figure 1f), as a combined result of the $\text{NH}_4\text{-N}$ concentration, pH (7.6 ± 0.0) (Figure 1g) and temperature ($37.7 \pm 0.1\text{ }^\circ\text{C}$). Having an operating temperature of $55\text{ }^\circ\text{C}$ instead of $38\text{ }^\circ\text{C}$ would hypothetically have increased the $\text{NH}_3\text{-N}$ concentration from 252 mg kg^{-1} to 620 mg kg^{-1} , clearly exceeding the level of $\text{NH}_3\text{-N}$ previously reported to cause severe inhibition [11,12]. It has been shown that a temperature increase from $25\text{ }^\circ\text{C}$ to $35\text{ }^\circ\text{C}$ can result in higher methanogenic reaction rates despite increased ammonia concentration according to Garcia and Angenent [24], but this effect is unlikely to occur in the Norrköping plant since it involves higher pH and a larger temperature change. Higher temperature often results in higher pH too; therefore, a shift from mesophilic to thermophilic temperature would most likely further increase the ammonia concentration [25].

A second strategy to handle the high levels of nitrogen in the Norrköping biogas plant is to use a long hydraulic retention time (HRT) (45–60 days) (Table 1). Previous studies have shown that high levels of ammonia are selective for methane production via SAO, and batch degradation experiments with $[2\text{-}^{14}\text{C}]\text{-acetate}$ have shown that this is also true for the Norrköping plant [14,26]. Analysis of the degradation products showed $^{14}\text{CH}_4$ and $^{14}\text{CO}_2$ but with the latter dominating, resulting in a $^{14}\text{CO}_2\text{:}^{14}\text{CH}_4$ ratio of 34.0 ± 3.5 . Using a long HRT allows the environment to adjust and maintain the dominating slow-growing SAOB microorganisms in the process. Therefore dilution with water is kept to a minimum,

leading to high $\text{NH}_4\text{-N}$ concentrations in the digestate (Table 1). The presence of high levels of different known SAOB, *i.e.*, *Tepidanaerobacter acetatoxydans* (6.51 ± 0.16 log gene copies mL^{-1}), *Syntroacetivus schinkii* (10.23 ± 0.06 log gene copies mL^{-1}) and *Clostridium ultunese* (6.89 ± 0.11 log gene copies $\cdot\text{mL}^{-1}$) was confirmed by quantitative polymerase chain reaction (qPCR) analysis in a recent study by Sun, *et al.* [27].

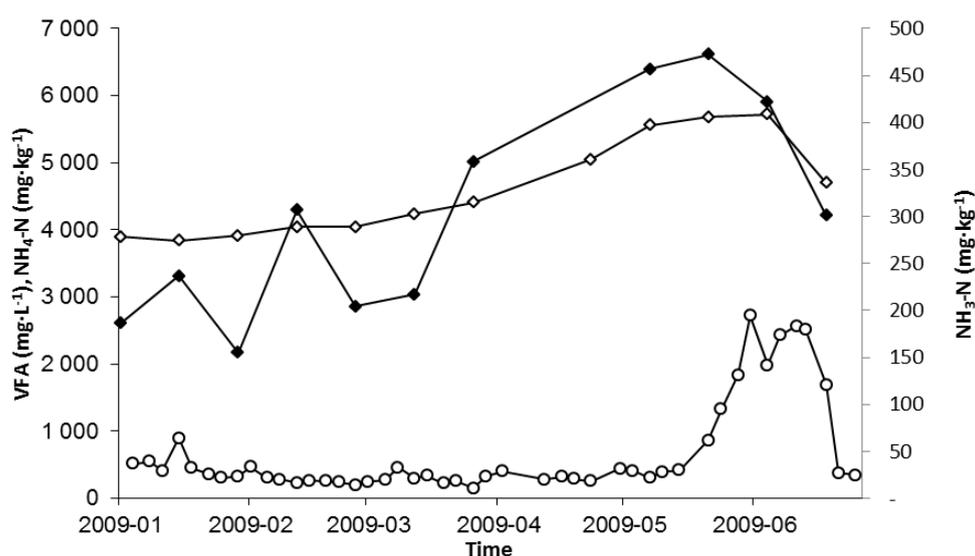
Table 1. Summary of the measures introduced to optimise anaerobic digestion of thin stillage at the Norrköping biogas plant, the underlying cause and the mode of action.

Measure	Cause	Mode of action
Mesophilic temperature	Nitrogen toxicity	Keep the fraction of $\text{NH}_3\text{-N}$ as low as possible
Addition of hydrochloric acid (HCl) and Fe^{3+}	i) Nitrogen toxicity ii) pH optimum of microorganisms	i) Decrease the fraction of $\text{NH}_3\text{-N}$ with lower pH ii) Obtain beneficial pH for microbial growth
Optimised dilution of feedstock	i) Dominance of slow-growing SAOB	i) Long hydraulic retention time (HRT) to avoid wash-out of SAOB
-	ii) Improved value of digestate for agriculture	ii) As high nitrogen content in the digestate as possible
-	iii) Nitrogen toxicity	iii) Avoid too high $\text{NH}_4\text{-N}$ (keep below 6 g L^{-1})
Addition of iron ($\text{Fe}^{2+}/\text{Fe}^{3+}$)	High levels of H_2S	i) Decrease sulphide inhibition ii) Decrease precipitation of trace elements iii) Meet Swedish legislation for vehicle fuel regarding sulphide concentration
Addition of cobalt (Co^{2+})	i) Low concentration in feedstock	Obtain high process stability by supplying microorganisms with this critical trace element

A third strategy used for managing the high $\text{NH}_4\text{-N}$ levels in the process is to down-regulate the pH by addition of HCl and acidic iron (Fe^{3+}) ions to the substrate (Table 1). A decrease of 0.2 pH units can enhance biogas production significantly when degrading protein-rich substrates [28], by lowering the free $\text{NH}_3\text{-N}$ concentration and also creating a more optimum pH for methanogens [12,28,29]. Theoretically, without process additive addition to the process in the Norrköping biogas plant, the pH would be 0.2 pH units higher and the resulting $\text{NH}_3\text{-N}$ concentration would increase from the present level of 252 mg kg^{-1} to 360 mg kg^{-1} , a 48% increase. This would considerably increase the risk of the upper inhibitory limit being exceeded during high $\text{NH}_4\text{-N}$ periods (Figure 1f). Using the strategies described above allowed stable operation to be achieved during the majority of the period 2008–2011. Except for a few peaks, the VFA concentration was generally low in the period ($0.79 \pm 0.09 \text{ g L}^{-1}$) (Figure 2), even though the $\text{NH}_4\text{-N}$ and $\text{NH}_3\text{-N}$ concentration was far above the value reported to cause inhibition of anaerobic digestion [11]. However, during spring 2009 increasing $\text{NH}_4\text{-N}$ and $\text{NH}_3\text{-N}$ concentrations correlated with increased process instability (Figure 3). The $\text{NH}_4\text{-N}$ concentration increased from approximately 4000 mg kg^{-1} to 5700 mg kg^{-1} and the $\text{NH}_3\text{-N}$ level simultaneously increased to approximately 450 mg kg^{-1} . This unexpected increase in $\text{NH}_4\text{-N}$ may have been the result of several different factors, such as variations in the degree of degradation (organic nitrogen mineralisation), in the OLR or, most likely, in the substrate mixture due to fluctuating urea addition in the bio-ethanol production process. The increase in $\text{NH}_4\text{-N}$ resulted in accumulation of total VFA

to a total peak of 2.3 g L^{-1} , consisting of 93% propionic acid, which has previously been identified as a common product during $\text{NH}_3\text{-N}$ inhibition [30,31]. The level at which $\text{NH}_3\text{-N}$ caused inhibition in the process, 450 mg kg^{-1} , is similar to inhibitory levels reported previously [11,30,32]. This process disturbance was dealt with by significantly decreasing the OLR from $3.75 \text{ g L}^{-1} \text{ d}^{-1}$ to $2.25 \text{ g L}^{-1} \text{ d}^{-1}$, which decreased both the total VFA and the $\text{NH}_4\text{-N}$ and $\text{NH}_3\text{-N}$ concentration (Figure 3). After breakdown of the VFA, the OLR was then raised to the original OLR without any resulting process problems (Figures 1c and 2).

Figure 3. Total VFA concentration (C2–C7) (\circ) at the Norrköping biogas plant during a problem period of increasing ammonia-nitrogen (\blacklozenge) and ammonium-nitrogen (\diamond) concentrations.



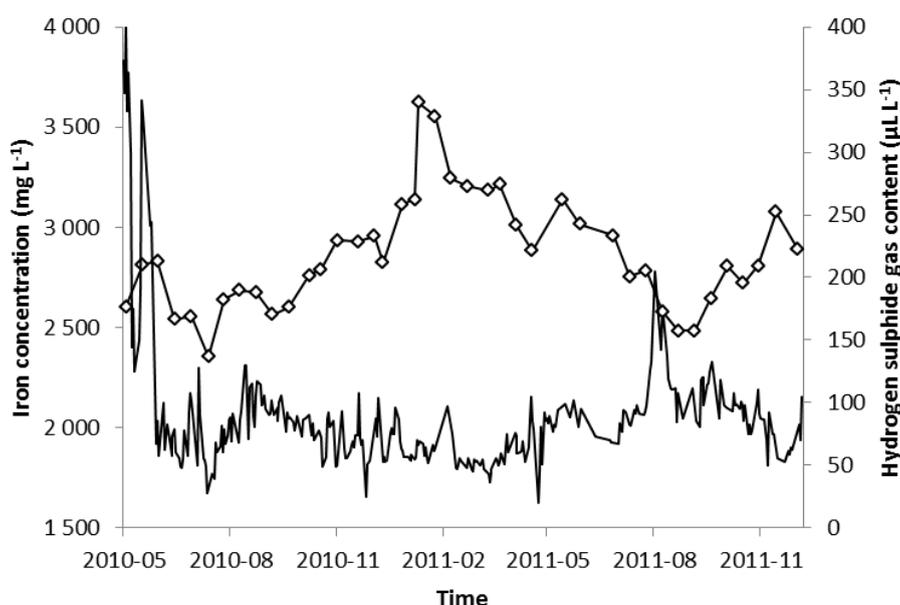
2.2. Hydrogen Sulphide

In addition to its high nitrogen concentration, thin stillage typically contains high levels of sulphur in amino acids but also in the form of sulphate, originating from the sulphuric acid used for pH regulation during bio-ethanol production. Irrespective of source, sulphur causes problems during anaerobic digestion as it is converted to hydrogen sulphide (H_2S), either during the degradation of amino acids or through the activity of SRB [11]. In addition to the malodorous and corrosive properties of H_2S gas, it is also toxic to microorganisms and can cause precipitation of trace elements required for microbial growth and activity [11,20,33]. Furthermore, the H_2S formed have to be removed to meet Swedish legal requirements on vehicle fuel and to reduce negative effects on anaerobic digestion. The thin stillage used at the Norrköping biogas plant contains approximately $44 \text{ kg sulphate t}^{-1} \text{ TS}$, resulting in high levels of H_2S due to the reduction of sulphate by SRB, leaving only trace amounts of sulphate in the digestate [17]. SRB have been shown to exist in most types of biogas processes and to increase in number in response to increasing sulphate levels [18]. qPCR analysis performed during a five-month period clearly revealed a stable population of SRB at the Norrköping plant between 1.8×10^5 and 7.3×10^5 gene copies mL^{-1} .

However, the levels were no higher than in biogas plants running with substrates containing lower levels of sulphate [18]. To cope with the issues related to high levels of H_2S , e.g., precipitation of

trace elements, toxicity to microorganisms and cost associated with upgrading of the biogas, the process was supplemented with continuous addition of iron (Table 1). This strategy resulted in a rather low sulphide concentration ($89 \pm 6 \mu\text{L L}^{-1}$) through precipitation of sulphides as iron sulphide. To achieve this low concentration of sulphide, iron needed to be added to the digester at an average concentration of $2814 \pm 72 \text{ mg kg}^{-1}$. Since there has been no period without addition of iron, the inherent concentration of H_2S is difficult to predict. However, judging from the iron demand to keep the H_2S concentration low, the inherent concentration in the gas would be 8,000–10,000 $\mu\text{L L}^{-1}$. There is a negative correlation ($R^2 = 0.34$) between the H_2S and iron concentrations in the digester during operation, showing that iron is indeed necessary (Figure 4).

Figure 4. Negative correlation between hydrogen sulphide (—) and iron concentration in the digester at the Norrköping biogas plant (\diamond).



2.3. Trace Elements and Co-Digestion

Trace element addition, in particular cobalt and nickel, or co-digestion with other substrates has previously been reported to be necessary for successful anaerobic digestion of stillage fractions [10,34,35]. In addition, trace element addition during anaerobic digestion has been proven to be important in supplying microorganisms with the necessary trace elements, particularly when digesting $\text{NH}_3\text{-N}$ -rich substrates [34,36–38]. Addition of a combination of trace elements and iron has also been shown to enhance the capacity for degradation of VFA, particularly propionic acid, in biogas processes with methanogenesis mainly performed via SAO [37,39]. A combined additive containing cobalt and iron has therefore been developed for use in the Norrköping biogas plant. This has most likely enhanced process performance considerably and may be the reason why the VFA concentration, in particular propionic acid, is relatively low despite the high nitrogen concentration. Inclusion of other substrates (grain residues and glycerine) is also used to provide a more complete nutrient composition of the feedstock. The C:N ratio of these substrates is lower than that of thin stillage, contributing to lower nitrogen in the feedstock and thus decreasing the nitrogen inhibitory effect. Glycerine also contributes to higher gas yields due to its high energy content.

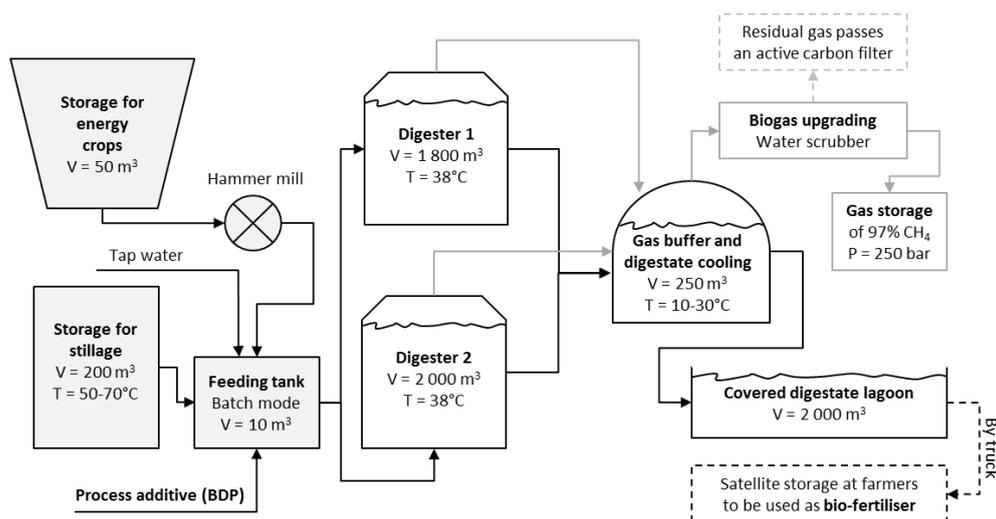
2.4. Further Measures

In summary, a number of measures have been introduced to optimise the anaerobic digestion process at the Norrköping biogas plant. These measures have been governed by the high nitrogen and sulphate concentrations in the feedstock (Table 1). Further measures are being planned to decrease the $\text{NH}_4\text{-N}$ concentration, reduce the concentrations of sulphides and supply a more complete set of nutrients to the process. This can be accomplished by mixing in other substrates, thus increasing the C:N and C:S ratio [35]. It has been shown in an experimental simulation of the Norrköping plant that the HRT could be reduced to 25 days while keeping OLR at $4.6 \text{ kg VS m}^{-3} \text{ d}^{-1}$ without any process disturbance, as a result of inclusion of household waste (up to 75% of total OLR) (unpublished data). Inclusion of household waste is planned at the Norrköping plant and most of the challenges coupled with nitrogen and sulphides are predicted to be reduced by this action. Furthermore, negative effects of temporary process disturbance owing to VFA accumulation (Figure 1c) can be better handed in the future, since a post-digester has now been installed (in 2012). With this post-digester, the outgoing VFA from the primary digesters will be degraded, increasing the efficiency of the plant during high VFA peaks. The post-digester is also predicted to increase methane production at the plant by ~5%, even in absence of VFA, as a result of increased degree of degradation. This would reduce methane emissions from digestate storage and increase the level of mineralised $\text{NH}_4\text{-N}$ in the residue used as fertiliser [40].

3. Experimental Section

The full-scale biogas plant in Norrköping, Sweden, has been operating since 2007 using thin stillage from bio-ethanol production as the main substrate to produce biogas. Initially, the plant was operated with one top-stirred continuously stirred tank reactor (1800 m^3), but in 2009 the plant was expanded with another digester (2000 m^3) (Figure 5).

Figure 5. Schematic diagram of process flow at the Norrköping biogas plant. Black arrows represent organic material flows, while grey arrows represent gas flows. Grey boxes represent upflow processes, white boxes represent main production process and boxes with crosshatched lines represent downflow processes.



The plant is operated at mesophilic conditions (38 °C) and receives a mixture of thin stillage (TS ≈ 10%, VS ≈ 9%) and condensed thin stillage (TS ≈ 30%, VS ≈ 27%), occasionally combined with hammer-milled grain residues (TS ≈ 90%, VS ≈ 88%; corresponding to on average 17% of OLR) and glycerol (TS ≈ 80%, VS ≈ 70%; corresponding to 10% of OLR, 2011 only). The stillage originates from a grain-based bio-ethanol plant (Lantmännen Agroetanol AB, Norrköping, Sweden) in close proximity to the biogas facility and is kept in a storage tank (Figure 5). The different substrates are mixed with tap water in a feeder tank, which is operated in batch mode. The OLR (measured as VS) is on average $3.0 \pm 0.1 \text{ kg m}^{-3} \text{ d}^{-1}$, resulting in a HRT in the digesters of 45–60 days. The average sulphate content (measured as amount of TS) is 44 g kg^{-1} and the Kjeldahl nitrogen concentration is 36 g kg^{-1} . A process additive named BDP, developed at Tekniska verken i Linköping AB and sold by Kemira Oyj (Helsinki, Finland), has been used since 2008. It contains iron (11%, a mixture of $\text{Fe}^{2+}/\text{Fe}^{3+}$), cobalt and hydrochloric acid [41]. The iron dose is regulated to keep the hydrogen sulphide concentration low and is on average $2814 \pm 72 \text{ mg kg}^{-1}$. Cobalt is added to achieve a concentration of approx. 0.5 mg L^{-1} in accordance with other studies [34,38] and earlier laboratory simulations of the plant, the concentration was on average $0.49 \pm 0.01 \text{ mg L}^{-1}$. The liquid outflow from the plant (digestate) is collected in a covered digestate lagoon and distributed to local farmers via satellite storage. The digestate is certified according to SPCR120 [42] and is also accepted as an organic fertiliser by the Swedish association for organic farming (KRAV). The biogas produced is upgraded with a water scrubbing technique (Malmbergs, Åhus, Sweden) to $\geq 97\%$ methane (Figure 5). The upgraded gas is then compressed (200–250 bar), stored, distributed and sold as vehicle fuel for urban buses, taxis and private cars.

Analyses

Data on full-scale process parameters, amount of biogas produced, biogas composition, substrate mixtures and quantities was taken from the SCADA system (Cactus Uniview AB, Mölndal, Sweden). All data presented in this paper refer to the period 1 July 2008–31 December 2011. All volumetric gas data were converted to standard conditions at pressure 1.01325 bar and temperature 273.2 K. Confidence intervals were calculated for all means according to the students' T-test ($p < 0.05$) using values from the whole of study period.

For process monitoring purposes, TS and VS are analysed on each batch of incoming substrate to calculate the correct OLR. Representative sampling of the digesters was performed at dedicated sampling points in the biogas plant. pH, alkalinity and VFA were monitored and analysed in order to assess process stability and process efficiency. Nitrogen fractions were determined to predict nitrogen inhibition. Hydrogen sulphide was analysed to determine the necessary iron addition and metals in order to confirm correct metal addition. qPCR analysis was performed on frozen samples (stored at -20 °C), while chemical analyses were performed on fresh samples. VFA (C2–C7) were analysed with a Clarus 550 gas chromatograph (Perkin Elmer, Waltham, MA, USA) with a packed Elite-FFAP column (Perkin Elmer) for acidic compounds according to Jonsson and Borén [43]. Ammonium nitrogen ($\text{NH}_4\text{-N}$) was analysed as the sum of $\text{NH}_4\text{-N (aq)} + \text{NH}_3\text{-N (aq)}$ by distillation (Kjeltec 8200, FOSS in Scandinavia, Hillerød, Denmark) in an acidic solution (H_3BO_3). $\text{NH}_4\text{-N}$ was determined in a second step by titration with HCl (Titro 809, Metrohm, Herisau, Switzerland).

Kjeldahl nitrogen was analysed with the same procedure and equipment as for the $\text{NH}_4\text{-N}$ analysis, with the exception that the samples were pre-treated with H_2SO_4 and subsequently heated to $410\text{ }^\circ\text{C}$ for 1 h. The ammonia nitrogen ($\text{NH}_3\text{-N}$) concentration was calculated from the $\text{NH}_4\text{-N}$, pH and temperature according to Hansen, *et al.* [12]. Bicarbonate alkalinity was measured by titration with hydrochloric acid to pH 5.4 (Titro 809, Metrohm). pH was measured with a potentiometric pH meter with a Hamilton electrode (WTW Inolab GmbH, Weilheim, Germany) at $25\text{ }^\circ\text{C}$. TS was measured by oven-drying at $105\text{ }^\circ\text{C}$ for 20 h. VS was subsequently measured by combusting the TS sample at $550\text{ }^\circ\text{C}$ for 3 h. The content of hydrogen sulphide in the biogas produced was analysed with a micro IV sensor (GfG GmbH, Dortmund, Germany). For qPCR analysis, samples from the reactors were withdrawn and frozen. Deoxyribonucleic acid (DNA) was extracted from small aliquots ($300\text{ }\mu\text{L}$) using the FastDNA® SPIN kit for soil (MP Biomedicals, Solon, OH, USA) according to the manufacturer's instructions, with small adjustments according to Moestedt, *et al.* [18]. For quantification of SRB, the primers *DSRp2060F-GC* (5' CAA CAT CGT YCA YAC CCA GGG 3') and *DSR4R* (5' GTG TAG CAG TTA CCG CA 3') [44] were used according to the protocol described by Dar, *et al.* [45], with adjusted initial touchdown protocol using 10 cycles instead of 20 for the step-wise decrease of annealing temperature from $65\text{ }^\circ\text{C}$ to $55\text{ }^\circ\text{C}$. Quantification was performed in triplicate for each extraction and the optimal dilution (1:10, 1:50 and 1:100) was evaluated to observe and avoid inhibiting effects of the amplification reaction. To determine the pathway of acetate degradation, samples from the plant were collected and treated according to Schnürer and Nordberg [14]. In short, aliquots of digester liquid (20 mL) were incubated with [$1\text{-}^{14}\text{C}$]-acetate (final concentration 0.11 mCi mL^{-1} ; Larodan Fine Chemicals, Malmö, Sweden). The degradation of [$2\text{-}^{14}\text{C}$]-acetate was detected by scintillation counting in a xylene-based scintillation fluid. A $^{14}\text{CO}_2\text{:}^{14}\text{CH}_4$ ratio less than 1 indicates aceticlastic methanogenesis, while a ratio above 1 indicates dominance of SAO [14,26].

4. Conclusions

Full-scale anaerobic digestion of thin stillage is associated with multiple problems, such as high $\text{NH}_4\text{-N}$ concentrations (low C:N ratio) and high sulphide concentrations (low C:S ratio). Combined addition of hydrochloric acid, iron and cobalt, together with long HRT, are necessary for successful biodigestion of thin stillage. Ammonia and sulphide toxicity is counteracted by lowering the pH, precipitation of hydrogen sulphides and addition of cobalt. In addition, mesophilic temperature is applied to avoid $\text{NH}_3\text{-N}$ inhibition, while the long HRT avoids the risk of wash-out of slow-growing syntrophic acetate-oxidising bacteria. However, operating problems can still arise, e.g., a temporary increase in the nitrogen content of thin stillage increased the $\text{NH}_3\text{-N}$ level to 450 mg L^{-1} , resulting in stress to the microorganisms.

Acknowledgments

Thanks to all personnel at Svensk Biogas i Linköping AB and Tekniska verken i Linköping AB involved in planning and operation of the Norrköping biogas plant. This project was funded by Tekniska verken i Linköping AB (publ.) and the Swedish Research Council Formas and formed part of the thematic research programme MicroDrivE at the Swedish University of Agricultural Sciences.

Conflicts of Interest

The authors declare no conflict of interest.

References

1. Abubaker, J.; Risberg, K.; Pell, M. Biogas residues as fertilisers—Effects on wheat growth and soil microbial activities. *Appl. Energy* **2012**, *99*, 126–134.
2. EurObserv'ER. Biogas barometers. *J. Énergies Renouv.* **2012**, *212*, 66–79.
3. Weiland, P. Biogas production: Current state and perspectives. *Appl. Microbiol. Biotechnol.* **2010**, *85*, 849–860.
4. EurObserv'ER. Biofuels barometer. *J. Énergies Renouv.* **2012**, *210*, 42–62.
5. Schmidt, T.; Pröter, J.; Scholwin, F.; Nelles, M. Anaerobic digestion of grain stillage at high organic loading rates in three different reactor systems. *Biomass Bioenergy* **2013**, *55*, 285–290.
6. Borjesson, P.; Tufvesson, L.M. Agricultural crop-based biofuels—Resource efficiency and environmental performance including direct land use changes. *J. Clean. Prod.* **2011**, *19*, 108–120.
7. Kim, Y.; Mosier, N.S.; Hendrickson, R.; Ezeji, T.; Blaschek, H.; Dien, B.; Cotta, M.; Dale, B.; Ladisch, M.R. Composition of dry-grind ethanol by-products: DDGS, wet cake and thin stillage. *Bioresour. Technol.* **2008**, *99*, 5165–5176.
8. Wilkie, A.C.; Riedesel, K.J.; Owens, J.M. Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstock. *Biomass Bioenergy* **2000**, *19*, 63–102.
9. Martin, M.; Eklund, M. Improving the environmental performance of biofuels with industrial symbiosis. *Biomass Bioenergy* **2011**, *35*, 1747–1755.
10. Agler, M.T.; Garcia, M.L.; Lee, E.S.; Schlicher, M.; Angenent, L.T. Thermophilic anaerobic digestion to increase the net energy balance of corn grain ethanol. *Environ. Sci. Technol.* **2008**, *42*, 6723–6729.
11. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.* **2008**, *99*, 4044–4064.
12. Hansen, K.H.; Angelidaki, I.; Ahring, B.K. Anaerobic digestion of swine manure: Inhibition by ammonia. *Water Res.* **1998**, *32*, 5–12.
13. Schnürer, A.; Zeller, G.; Svensson, B.H. Mesophilic syntrophic acetate oxidation during methane formation in biogas reactors. *FEMS Microbiol. Ecol.* **1999**, *29*, 249–261.
14. Schnürer, A.; Nordberg, A. Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. *Water Sci. Technol.* **2008**, *57*, 735–740.
15. Westerholm, M. Biogas Production through the Syntrophic Acetate-Oxidising Pathway. Ph.D. Thesis, Swedish University of Agricultural Sciences, Uppsala, Sweden, 2012.
16. Karlsson, R. *Personal Communication*; Lantmännen Agroetanol AB: Norrköping, Sweden, 2013.
17. Rabus, R.; Hansen, T.A.; Widdel, F. Dissimilatory Sulfate- and Sulfur-Reducing Prokaryotes. In *The prokaryotes*; Springer: Singapore, 2006; Volume 2, pp. 659–768.
18. Moestedt, J.; Nilsson Påledal, S.; Schnürer, A. The effect of substrate and operational parameters on the abundance of sulphate-reducing bacteria in industrial anaerobic biogas digesters. *Bioresour. Technol.* **2013**, *132*, 327–332.

19. McCartney, D.M.; Oleszkiewicz, J.A. Sulfide inhibition of anaerobic degradation of lactate and acetate. *Water Res.* **1991**, *25*, 203–209.
20. Van der Veen, A.; Feroso, F.G.; Lens, P.N.L. Bonding form of metals and sulfur fractionation in methanol-grown anaerobic granular sludge. *Eng. Life Sci.* **2007**, *7*, 480–489.
21. Dererie, D.Y.; Tobro, S.; Momeni, M.H.; Hansson, H.; Blomqvist, J.; Passoth, V.; Schnurer, A.; Sandgren, M.; Ståhlberg, J. Improved bio-energy yields via sequential ethanol fermentation and biogas digestion of steam exploded oat straw. *Bioresour. Technol.* **2011**, *102*, 4449–4455.
22. Nasr, N.; Elbeshbishy, E.; Hafez, H.; Nakhla, G.; El Naggar, M.H. Comparative assessment of single-stage and two-stage anaerobic digestion for the treatment of thin stillage. *Bioresour. Technol.* **2012**, *111*, 122–126.
23. Alkan-Ozkaynak, A.; Karthikeyan, K.G. Anaerobic digestion of thin stillage for energy recovery and water reuse in corn-ethanol plants. *Bioresour. Technol.* **2011**, *102*, 9891–9896.
24. Garcia, M.L.; Angenent, L.T. Interaction between temperature and ammonia in mesophilic digesters for animal waste treatment. *Water Res.* **2009**, *43*, 2373–2382.
25. Eskicioglu, C.; Kennedy, K.J.; Marin, J.; Srehler, B. Anaerobic digestion of whole stillage from dry-grind corn ethanol plant under mesophilic and thermophilic conditions. *Bioresour. Technol.* **2011**, *102*, 1079–1086.
26. Schnürer, A.; Houwen, F.P.; Svensson, B.H. Mesophilic syntrophic acetate oxidation during methane formation by a triculture at high ammonium concentration. *Arch. Microbiol.* **1994**, *162*, 70–74.
27. Sun, L.; Müller, B.; Westerholm, M.; Schnürer, A. Syntrophic Acetate Oxidation in Industrial CSTR Biogas Digesters. Department of Microbiology, BioCenter, Swedish University of Agricultural Sciences, Uppsala, Sweden. Unpublished work, 2013.
28. Karlsson, A.; Ejlertsson, J. Addition of HCl as a means to improve biogas production from protein-rich food industry waste. *Biochem. Eng. J.* **2012**, *61*, 43–48.
29. Strik, D.; Domnanovich, A.M.; Holubar, P. A pH-based control of ammonia in biogas during anaerobic digestion of artificial pig manure and maize silage. *Process Biochem.* **2006**, *41*, 1235–1238.
30. Calli, B.; Mertoglu, B.; Inanc, B.; Yenigun, O. Effects of high free ammonia concentrations on the performances of anaerobic bioreactors. *Process Biochem.* **2005**, *40*, 1285–1292.
31. Poggi-Varaldo, H.M.; Rodríguez-Vázquez, R.; Fernandez-Villagómez, G.; Esparza-García, F. Inhibition of mesophilic solid-substrate anaerobic digestion by ammonia nitrogen. *Appl. Microbiol. Biotechnol.* **1997**, *47*, 284–291.
32. Siles, J.A.; Brekelmans, J.; Martin, M.A.; Chica, A.F.; Martin, A. Impact of ammonia and sulphate concentration on thermophilic anaerobic digestion. *Bioresour. Technol.* **2010**, *101*, 9040–9048.
33. Lopes, S.I.C.; Capela, M.I.; Lens, P. Sulfate reduction during the acidification of sucrose at pH 5 under thermophilic (55 °C) conditions. I: Effect of trace metals. *Bioresour. Technol.* **2010**, *101*, 4269–4277.
34. Gustavsson, J.; Svensson, B.H.; Karlsson, A. The feasibility of trace element supplementation for stable operation of wheat stillage-fed biogas tank reactors. *Water Sci. Technol.* **2011**, *64*, 320–325.

35. Westerholm, M.; Hansson, M.; Schnürer, A. Improved biogas production from whole stillage by co-digestion with cattle manure. *Bioresour. Technol.* **2012**, *114*, 314–319.
36. Banks, C.J.; Zhang, Y.; Jiang, Y.; Heaven, S. Trace element requirements for stable food waste digestion at elevated ammonia concentrations. *Bioresour. Technol.* **2012**, *104*, 127–135.
37. Karlsson, A.; Einarsson, P.; Schnürer, A.; Sundberg, C.; Ejlertsson, J.; Svensson, B.H. Impact of trace element addition on degradation efficiency of volatile fatty acids, oleic acid and phenyl acetate and on microbial populations in a biogas digester. *J. Biosci. Bioeng.* **2012**, *114*, 446–452.
38. Nordell, E.; Moestedt, J.; Wiberg, L.; Karlsson, M. A Trace Metal Formulation that Reinforces the Effects of a Cobalt Additive. In Proceedings of the Fourth International Symposium on Energy from Biomass and Waste, Venice, Italy, 12–15 November 2012; Volume 4.
39. Ek, A.; Hallin, S.; Vallin, L.; Schnürer, A.; Karlsson, M. Slaughterhouse Waste Co-Digestion—Experiences from 15 Years of Full-Scale Operation. In Proceedings of the World Renewable Energy Congress, Linköping, Sweden, 8–13 May 2011; pp. 64–71.
40. Nordell, E.; Karlsson, M. Post Digestion of Biogas Production Residues at Mid-Range Mesophilic Temperature. In Proceedings of the International Symposium on Anaerobic Digestion of Solid Waste and Energy Crops, Vienna, Austria, 28 August–1 September 2011.
41. Ejlertsson, J. Method, a Device, and an Additive for Digesting Organic Matter. U.S. Patent 20070184542 A1, 9 August 2007.
42. Petersson, A. *English Summary of SPCR 120—Certification Rules for Digestate from Biowaste by the Quality Assurance System of Swedish Waste Management*; Swedish Gas Centre: Borås, Sweden, 2013.
43. Jonsson, S.; Borén, H. Analysis of mono- and diesters of *o*-phthalic acid by solid-phase extractions with polystyrene—Divinylbenzene-based polymers. *J. Chromatogr. A* **2002**, *963*, 393–400.
44. Geets, J.; Borremans, B.; Diels, L.; Spingael, D.; Vangronsveld, J.; van der Leilie, D.; Vanbroekhoven, K. *DsrB* gene-based dgge for community and diversity surveys of sulfate-reducing bacteria. *J. Microbiol. Methods* **2006**, *66*, 194–205.
45. Dar, S.A.; Yao, L.; van Dongen, U.; Kuenen, J.G.; Muyzer, G. Analysis of diversity and activity of sulfate-reducing bacterial communities in sulfidogenic bioreactors using 16S rRNA and *dsrB* genes as molecular markers. *Appl. Environ. Microbiol.* **2007**, *73*, 594–604.