3.2.2. Addition of hydrochloric acid

Laboratory tests, with addition of hydrochloric acid to co-digestion reactors operated under mesophilic conditions were performed in 1999-2000 [20]. Positive effects on volumetric gas production and VFA levels were noted in the digesters where pH was lowered with hydrochloric acid and full-scale acid addition was started at the plant in March 2002. On comparison of the operation performance of the plant in 2000-2001 with 2002-2003, the following direct and indirect effects were observed [19]: digester loading rate could be increased with 70 % on VS basis, gas production increased, acetate concentration decreased by 43 % and partial alkalinity concentration increased from 11 000 mg/L to 17 000 mg/L. On average, between the two periods, the amount of material increased by 20 % (fig 2B) whereas the dry substance of the material increased with 26 % during the same period (fig. 2A). Since also the percentage of slaughter house waste increased from 59 to 72 % in the material, the VS percentage of TS increased. Thus, the gas production increase was a result of the increased loading rate since the specific methane yield per kg VS was unchanged.

3.2.3. Addition of process additive KMB1

To further enhance process stability, and to increase the efficiency of the plant, a process additive known as KMB1 was developed at TVAB [21]. The main effects of the additive were: (1) more stable production, enabling (2) higher organic loading rate without process disturbances and heavy foaming [19], leading to (3) higher methane production. Also, the additive enabled the decrease and final removal of manure in the substrate mixture, and has been added to the plant since November 2003.

3.3. Plant performance after process improvements

After implementation of the three process improving additives mentioned above, a closer study of the process reveal the positive effects (data from 2004-2005). In the heated buffer tank the VFA levels fluctuate to a great extent and can occasionally get very high (up to 16 000 mg/L) while the pH is low (Fig. 3A). However, even though the buffer tank substrate display a low pH and high, fluctuating VFA concentrations (average 8400 mg/L, pH 5.5), the concentration of VFA in the digester is low and stable (average 1600 mg/L, max 2800 mg/L) and the digester fluid has a stable pH of 8.0 (7.9-8.1).

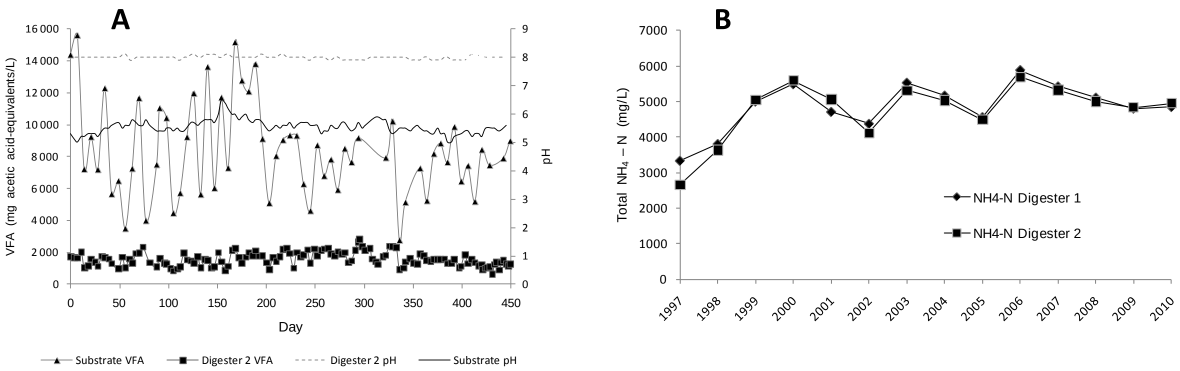

Figure 3 A). Volatile fatty acid (VFA) concentrations (mg acetic acid-equivalents/L) and pH levels in the heated buffer tank (denoted: substrate) and in the biogas digester (data from 2004-2005). B) Total ammonium (NH_4^+-N (aq) + NH_3-N (aq)) concentrations (mg/L) in the digesters during 1997-2010.

Since the plant's early years of operation, the total NH_4-N concentration has been high. The average for both digesters during 1999-2010 has been 5060 mg/L, with a maximum yearly average of 5880 mg/L (digester 1, 2006) and a minimum yearly average of 4120 (digester 2, 2002) (Fig. 3B). Labeling experiments were performed in 2008 to establish what type of methane formation pathway is prevalent – methane formation by acetate utilizing methanogens or via syntrophic acetate oxidation and hydrogenotrophic methanogens?

The labeling analysis showed production of high levels of labeled carbon dioxide in relation to labeled methane. The $^{14}CO_2/^{14}CH_4$ quota was determined to be 16; clearly showing that methane production in the digester occurred mainly through syntrophic acetate oxidation and hydrogenotrophic methanogenesis. Since the digester is operated at high ammonium levels (5300 mg NH_4^+-N/L at the time of sampling) this is a result that was expected and in accordance with the previous studies that have shown development of SAO in response to increasing ammonia levels[13]. The development of this prevailing metabolic pathway is likely the explanation to the stable operation of the process even at high ammonia levels. Given that methanogenesis via syntrophic acetate oxidation involves a hydrogenotrophic methanogen, that tolerates higher levels of ammonia than acetoclastic methanogens, methane production from acetate can still proceed even though the acetoclastic methanogens are inhibited. Furthermore, isolation and characterization of several ammonia tolerant hydrogen utilizing methanogens, as well as ammonia tolerant syntrophic acetate oxidizing bacteria, support this suggested mechanism for ammonia adaptation in biogas processes [22-24]. However, the generation time of a SAO culture was calculated to be approx. 28 days [13] which can be compared with the times of around 2 - 12 day for acetate utilizing methanogens [25]. Thus, the long retention time would seem to be a prerequisite to allow SAO to establish in the digester.

3.4. Practical experiences

The general plant operation experiences of anaerobic digestion of slaughterhouse waste concern two main themes: 1) logistics and transportation and 2) process and technology. At the slaughterhouse, the waste is grinded to ≤12 mm and treated with formic acid. The grinding at the slaughterhouse allows for transportation of the substrate in slurry form, and thereby a closed-system handling at the biogas plant, which prevents odor problems. Treatment with formic acid prevents foaming which would otherwise cause significant problems during transport and storage at the biogas plant. The thermal disintegration of the substrate in the heated buffer tank, and the fact that the substrate temperature is over 70 °C in large parts of the system, reduces potential problems with clogging and eases pumping of the substrate due to reduced viscosity. Furthermore, to achieve a stable process the type of material co-digested with the slaughterhouse waste is important, and the complimentary substrates should work well in the plant, both from a practical and a process point of view, which will lead to an even substrate mixture over time and thus an even organic loading rate and a stable biogas process.

4. Conclusions

From the long time experiences the following conclusions are established:

- It is possible to operate CSTR co-digestion of slaughterhouse waste, at substrate TS levels significantly over the original design level.
- The plant is operating well at high levels of ammonium, and the long HRT (45-55 day) enables establishment of a mesophilic syntrophic acetate oxidizing culture.
- With optimization of process parameters, substrate composition and through the addition of process additives, it has for 15 years been possible to achieve a continued increase of the biogas production, with basically the original plant capacity.

References

[1] Edström M., Nordberg Å., Thyselius L. Anaerobic treatment of animal byproducts from slaughterhouses at laboratory and pilot scale. Applied Biochemistry and Biotechnology, 109 (13), 2003, pp. 127 - 138.

[2] European Community. Regulation (EC) No. 1774/2002 of the European Parliament and of the Council laying down health rules concerning animal by-products not intended for human consumption. Official Journal, L 273, 2002, pp. 1 - 95.

[3] Hejnfelt A. and Angelidaki, I. Anaerobic digestion of slaughterhouse by-products. Biomass and Bioenergy 33 (8), 2009, pp. 1046 - 1054.

[4] Salminen E., Einola J., Rintala J. Characterisation and anaerobic batch degradation of materials accumulating in anaerobic digesters treating poultry slaughterhouse waste. Environ. Technol. 22 (5), 2001, pp. 577 - 585.

[5] Salminen E. and Rintala J. Semi-continuous anaerobic digestion of solid poultry slaughterhouse waste: effect of hydraulic retention time and loading. Water Research 36 (13), 2002, pp. 3175 - 3182.

[6] Zinder S. H. Microbiology of Anaerobic Conversion of Organic Wastes to Methane: Recent Developments. ASM news, 50 (7), 1984.

[7] Ochieng' Otieno F. A. Anaerobic digestion of wastewaters with high strength sulphates. Discovery and Innovation 8 (2), 1996, pp. 143 - 150.

[8] Chen Y., Cheng J. J., Creamer K. S. Inhibition of anaerobic digestion process: A review. Bioresource Technology 99 (10), 2008, pp. 4044 - 4064.

[9] Jiunn-Jyi L., Yu-You L., Noike, T. Influences of pH and moisture content on the methane production in high-solids sludge digestion. Water Research 31 (6), 1997, pp. 1518 - 1524.

[10] Tada C., Yang Y., Hanaoka T., Sonoda A., Ooi K., Sawayama S. Effect of natural zeolite on methane production for anaerobic digestion of ammonium rich organic sludge. Bioresource Technology 96 (4), 2005, pp. 459 - 464.

[11] Nordell E., Hallin S., Johansson M., Karlsson M. The diverse response on degradation rate of different substrates upon addition of zeolites. Third International Symposium on Energy from Biomass and Waste, Venice, Italy, 2010. ISBN 978-88-6265 -008-3.

[12] Siegrist H., Vogt D., Garcia-Heras J.L., Gujer W. Mathematical model for meso- and thermophilic anaerobic sewage sludge digestion. Environmental Science and Technology, 36 (5), 2002, pp. 1113 - 1123.

[13] Schnürer A. and Nordberg Å. Ammonia, a selective agent for methane production by syntrophic acetate oxidation at mesophilic temperature. Water Science and Technology 57 (5), 2008, pp. 735 - 740.

[14] Hellman J., Ek A. E. W., Sundberg C., Johansson M, Svensson B. H. and Karlsson M. Mechanisms of increased methane production through re-circulation of magnetic biomass carriers in an experimental continuously stirred tank reactor. 12th World Congress on anaerobic digestion, Guadalajara, Mexico, 2010.

[15] URL: http://microdrive.phosdev.se/index.php?page=Companies

[16] Tafdrup S. Centralized biogas plants combine agricultural and environmental benefits with energy production. Water Science and Technology 30 (12), 1994, pp. 133 - 141.

[17] Alvarez R. and Lidén G. Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. Renewable Energy 33 (4), 2008, pp. 726 - 734.

[18] Deublein D. and Steinhauser A. Biogas from waste and renewable sources. Weinheim, Germany. Wiley-VCH, 2008.

[19] Vallin L, Christiansson A., Arnell M., Undén P. D2.2 Operational experiences of cost effective production in Linköping, Sweden. Biogasmax Integrated Project No. 019795, 2007.

[20] Ejlertsson J. (2005). Swedish Patent No SE 525 313.

[21] Holm S., Ejlertsson J., Carlson B. (2005). Swedish Patent No SE 526 875.

[22] Schnürer A., Schink B., Svensson B.H. *Clostridium ultunense* sp. nov., a mesophilic bacterium oxidizing acetate in syntrophic relationship with a hydrogenotrophic methanogenic bacterium. International Journal of Systematic Bacteriology 46 (4), 1996, pp. 1145 - 1152.

[23] Schnürer A., Zellner G., Svensson B.H. Mesophilic syntrophic acetate oxidation during methane formation in different biogas reactors. FEMS Microbiology Ecology 29, 1999, pp. 249 - 261

[24] Karlsson M., Roos S., Schnürer A. Description of '*Candidatus Syntrophicus schinkii*' an anaerobic, syntrophic acetate-oxidizing bacterium isolated from mesophilic digester operating at high concentration of ammonia. FEMS Microbiology Letters, 309 (1), 2010, pp. 100 – 104.

[25] Jetten M. S. M., Stams A. J. M., Zehnder A. J. B. Methanogenesis from acetate: A comparison of the acetate metabolism in *Methanothrix soehngenii* and *Methanosarcina* spp. FEMS Microbiology Letters, 88 (3-4), 1992, pp. 181 - 197.