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Characterization of Lactose Consumption during the Biogas Production from Acid Whey by FT-IR Spectroscopy

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***Abstract*—**The consumption of lactose in acid cheese whey anaerobic fermentation process under fed-batch conditions was studied. During fermentation for 100 hours the biogas production (CO_2 and CH_4) was analyzed online. Among the standard analyses FT-IR spectroscopy was used to follow the consumption of lactose by bacteria. The absorption bands at 990, 894 and 787 cm^{-1} in the 2nd derivative spectra were shown to be characteristic for lactose and were used to follow the lactose conversion. It was shown that acid cheese whey lactose was converted by bacteria in first 7 hours. In the spectra of 17, 18 and 95 hour fermentation samples lactose was not identified and these results correlated with the HPLC data.

***Keywords*—**Acid whey, anaerobic digestion, biogas, FT-IR spectroscopy, lactose consumption.

I. Introduction

PRODUCTION of biogas from waste has become a topic of interest nowadays [1]. There is research in the field of utilizing different type of waste for producing biogas done [2]. In some cheese processing enterprises and dairy farms cheese whey is considered as waste water and is not being processed in an effective way.

More than 40% of the European Union (EU) milk is processed into cheese [1]. There are a lot of varieties of cheese resulting in different cheese making technologies, but on average the final volume of whey is about 85-90 % of the volume of the processed milk. Whey is used directly as animal feed or as field fertilizer, but usage of whey for energy production is not widespread. Cheese whey has sufficient biogas potential; however, it is a complicated substrate for biomethane production due to the unstability of the process. While whey may have further uses, many small and medium-size dairy industries do not have the technical know-how nor the economic incentive to do so, making it necessary to consider its treatment as a waste stream [3]. Due to the low bicarbonate alkalinity, pH control is required to avoid a failure of the anaerobic process [3] – [5]. The main components of cheese whey are: lactose (44.0-52.0 g/L), protein (6.1-6.6 g/L), fat (0.2-0.3 g/L) and minerals (5.0-7.9 g/L) [6]. Cheese whey has a very high content of organics (60 to 80 g COD/L) [3]. Dairy waste water contains complex organic substances, such as polysaccharides, proteins and lipids, which after hydrolysis form sugars, amino acids, and fatty acids [7]. In subsequent acidogenic reaction these intermediate products are converted to volatile fatty acids, which are further degraded by acetogens, forming acetate, CO_2, and H_2. Lastly, both acetate and H_2 or CO_2, are converted by methanogens to CH_4 [8]. The acid accumulation is due to the slower growth rate of the microbes that utilize the acetic and propionic acids than the rest of the microbial population in the reactor; therefore, if the microbes produce acids faster than is the capacity of acid utilization, there will be an accumulation of acids, and consequently, a drop in pH, unless the system is well buffered [9]. Infrared spectroscopy methods are widely used techniques for monitoring of various components during fermentation process [10]. Due to the complex composition of the cheese whey fermentation products the evaluation of lactose conversion by FT-IR (Fourier transform infrared) spectroscopy becomes complicated. In this study we investigated the biogas production from acid whey and the potential of FT-IR spectroscopy for the control of lactose consumption in biogas producing process from acid cheese whey.

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II. Materials and Methods

A. Cheese Whey

Acid cheese whey was supplied by a dairy product manufacturer „Smiltenes piens", Smiltene, Latvia. Filtration process or any other pretreatment was not used. The whey samples were provided from the manufacturer, collected in 5 L containers and stored at 4°C no longer than 1 week to avoid changes of the chemical composition.

B. Experimental Set Up

Cultivations were performed in 6.2 L glass bioreactor (EDF-5.3_1, Riga, Latvia) with a working volume of 4 L and

a height to diameter ratio of 3:1. The bioreactor was equipped with novel magnetic drive placed in the upper lid. Temperature (Pt-100), pH (Ingold, Toledo 405-DPAS SC K8S/325), CH_4 and CO_2 concentrations in exhaust gases (Bluesens, Germany) were measured online.

Once inoculated, the bioreactor was run at a stirrer speed of 40 rpm. Automatic control of pH at 7.2 ± 0.2 using 12 % sodium hydroxide solution was applied. NaOH solution was added in bioreactor using peristaltic pump (LongerPump, BT100-2J) working in on-off pumping rate mode. Temperature set point was 37.0 ± 0.2 ˚C.

The reactor was inoculated with 1000 ml digestate originated from lab-scale working reactor (HRT - 50 days, substrate – granules of lucerna) with volume 50 L. The anaerobic environment was ensured by flushing with N_2 until the dissolved oxygen was zero.

At the beginning 1 L of undiluted acid whey was added. To avoid pH drop, 3 M NaOH was added manually till the pH reached 7.2.

Fed-batch mode was used, and 0.2 l of acid whey was added after 42, 72 and 90 hours.

C. Analyses

Standard method was applied for pH measurement (LUTRON PH-208). Total solids (TS) were determined by drying at 105 °C for eight hours. Volatile total solids and ash were analyzed using muffle furnace (Snol 60/300 LFN) at 550 ℃ for 90 minutes.

Total non-organic carbon (TC) and total volatile acid (TVA) measurements were done with titration method. As reagent 0.1M H_2SO_4 was used. The effluent was diluted three times with deionized water.

The chemical oxygen demand (COD) was analyzed with Hatch Lange cuvettes method (Hach Calorimeter DR 890).

For FT-IR spectroscopy analyses cell free fermentation liquid supernatant samples (2-10 µL) were poured out by drops on a 384 place silicon plate and dried at T<50 ºC. FT-IR spectra were recorded on a Vertex 70 with HTS-XT extension - microplate reader (Bruker, Germany) over the range 4000 - 600 cm^{-1}, with a resolution of 4 cm^{-1}, accumulating 64 scans. Baseline was corrected by rubber band method, CO_2 and H_2O bands excluded. Data were processed with OPUS 6.5 (Bruker, Germany).

The HPLC analyses was performed on a Waters separation module (model 2414), using Phenomenex NH_2 4,6´150 mm column. The mobile phase consisted of acetonitrile and water (75:25 v:v) used at a flow rate of 1.3 mL/min.

III. Results and Discussion

The chemical composition of acid cheese whey and inoculum data are shown in Table I.

TABLE I
INOCULUM AND WHEY ANALYSES

Parameter	Inoculum	Whey
pH (20 °C)	7.83	4.65
Total solids (% w/w)	1.84	6.00
Volatile solids (% w/w)	1.20	11.92
$COD_{unfiltred}$ (mg O_2/L)	-	73.30
$COD_{filtred}$ (mg O_2/L)	-	29.50
Ash (% w/w)	0.59	0.62
Protein (% w/w)	-	0.30
NPN (% w/w)	-	0.20
Lactose (% w/w)	-	4.85
Fat (% w/w)	-	0.05

Whey contained high amounts of total solids (6.0%) and volatile solids (11.92%), but inoculum - much lower TS and VS content (1.84% and 1.2%). Whey contained 4,85% lactose. After water (93 to 94 % of whey), the main component of whey is lactose, accounting for about 70-72 % of whey solids [11, 12]. It is known that the composition of whey depends on the method of cheese manufacturing as well as the location [13].

TABLE II
THE RESULTS OF COD MEASUREMENTS

Time, h	COD, mg/L
0	20,24
17	13,36
65	10,46

The average chemical oxygen demand data obtained from three time points are shown in Table II. The reduction of COD after the first day was 34 %.

Fig. 1 shows the daily methane production rate. The methane content in biogas increased very rapidly in the first 24 hours. After whey addition on 42th hour methane content increased rapidly for 15 hours. In anaerobic digestion of cheese whey the main organic compounds are broken down to gaseous products. Using Buswell's Equation, the amount of biogas produced can be estimated [14]:

$$C_aH_bO_cN_d + \left(\frac{4a-b-2c+3d}{4}\right)H_2O \rightarrow \left(\frac{4a+b-2c-3d}{8}\right) \cdot CH_4 + \left(\frac{4a-b+2c+3d}{8}\right)CO_2 + dNH_3 \quad (1)$$

For lactose ($C_{11}H_{22}O_{11}$) the proportion of methane and carbon dioxide is calculated as:

$$C_{11}H_{22}O_{11} + H_2O \rightarrow 6CH_4 + 6CO_2 \quad (2)$$

Thus, 1 mole of lactose produces 6 moles of methane and 6 moles of carbon dioxide. After recalculation – 0.786 L of gas is produced when 1g of lactose is consumed. In this study, the first whey feed at 41 hours was 1L (48.5 g lactose), or 27 g lactose/day. The theoretical yield of methane is 38.12 l/d, but the experimental yield was 1.45 L/day which is 3.8 % of the theoretical in the first two days.

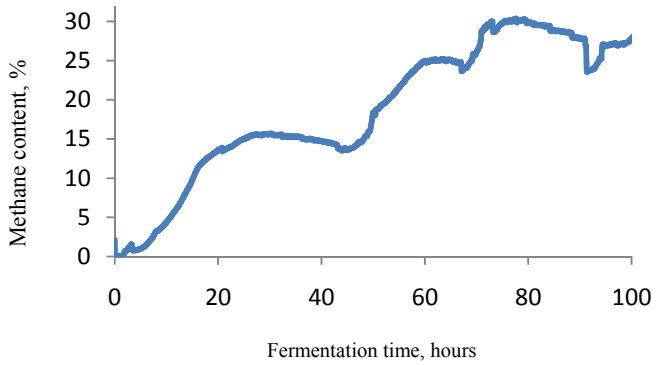

Fig. 1 Daily methane production

During all fermentation period the gas production yield was 12% from the theoretical. From these results it can be concluded that lactose hydrolysis process was very fast, because after feeding with whey the biogas production yield increased very rapidly in first three hours after which it decreased (Fig. 2).

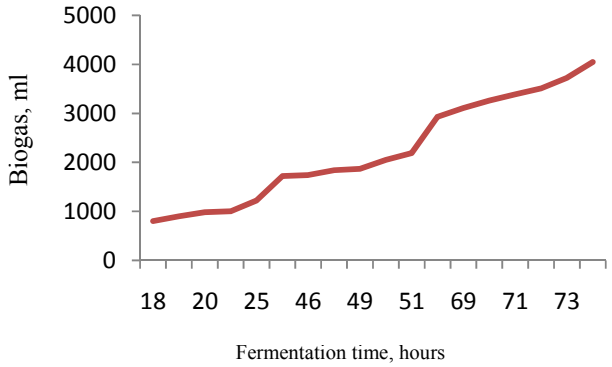

Fig. 2 Daily biogas production

In previous studies it was demonstrated that during hydrolysis of whey about 82 % of lactose is transformed into lactate. Rapid formation of the total volatile acids and acidification are characteristic by a drop in pH and reduction of alkalinity [15].

The total alkalinity of an anaerobic digester is parameter of its ability to neutralize organic acids and maintain constant pH. TVA increased up to 11.2 g/kg. During anaerobic conversion of the organic matter, the end products of acidogenesis are volatile fatty acids [12].

According to Cobb and Hill [16] low TVA concentration indicates stable digester conditions, but high TVA concentration is associated with digester failure. The TVA measurements showed that the TVA increased considerably in the first day (Fig. 3). After that the increase was slower but at the end the concentrations still became too high. The level of volatile fatty acids had the major effect on the pH.

The lactose content of whey is readily degraded by acidogenic microorganisms; this results in the occurrence of acid inhibition because of the differences in the rates of acidogenesis and methanogenesis. Consequently, in a single phase reactor often there is an acidic environment, which depresses the biogas productivity, the methane yield and the stability, thereby resulting in low treatment efficiency [17].

It has been shown earlier that when pre-fermented or untreated acid whey was used, a higher organic loading rate resulted in a lower pH, but with the pre-fermented whey the pH range remained within the optimum range of methanogenesis (pH 7.15–8.15), whereas the pH of the reactors fed with untreated acid whey varied in a lower range [17].

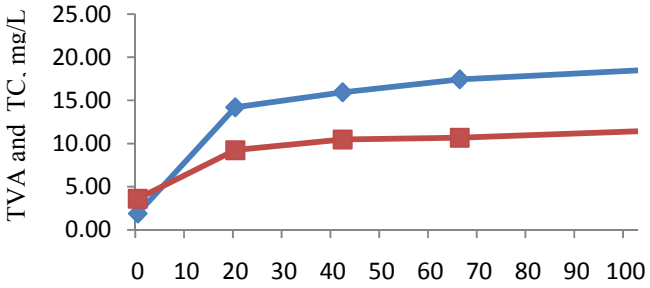

Fig. 3 Total volatile acids and total non-organic carbon

It must be noted that during the anaerobic digestion of acid cheese whey a base was used to control the pH which resulted in an increase in the total solids. The other problem is $CaCl_2$ salt, which was added in dairy manufacturing to produce cottage cheese.

The total organic acids and total organic carbon amount increased till 25th hour after which these amounts increased very slowly, even after whey addition (Fig. 4). TS measurements did not show a significant change.

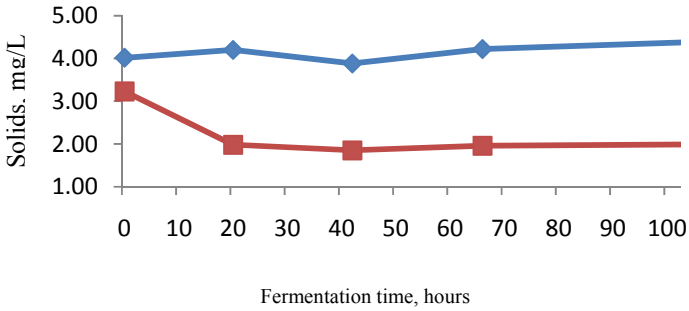

Fig. 4 Total solids and volatile organic solids

FT-IR spectra of cheese whey showed a strong absorption in carbohydrate region 900-1100 cm^{-1} (Fig. 5). As the spectra were recorded in the absorption range 0.25-1.25, the absorption band intensity was directly proportional to the concentration of a particular component. Thus comparison of the spectral profiles of cheese whey and cell free fermentation supernatants showed the consumption of cheese whey in time scale. Moreover it can be said that mostly cheese whey

carbohydrates were converted by bacteria in 7 hours of fermentation. The spectra of 18 and 95 hour samples did not show the cheese whey carbohydrates. As the HPLC analyses of 18 hour fermentation supernatant sample did not show lactose the attempt was made to identify lactose in fermentation supernatant sample IR spectra.

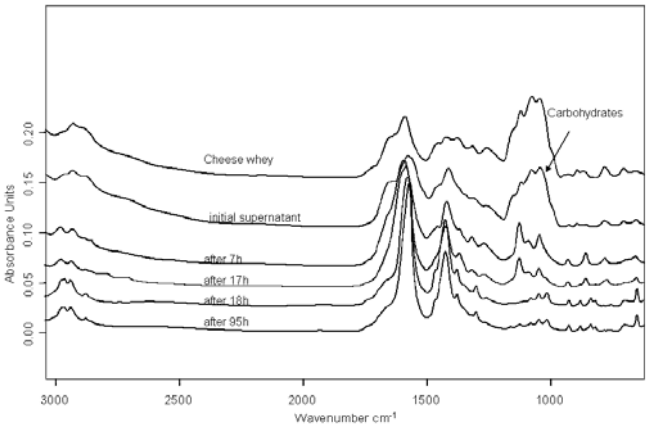

Fig. 5 FT-IR spectra of cheese whey and cell free fermentation supernatants

It is known that lactose is the main carbohydrate in cheese whey, yet in the raw spectra it was not clearly identified. Broad absorption bands (in our case carbohydrate band) in FT-IR spectra originate from overlapping of several components. These absorptions of particular components can be seen in the 2nd derivative spectra. Thus the spectra of lactose, cheese whey and supernatant were compared (Fig.6). Three absorption bands were identified as the characteristic for lactose – 990, 894 and 787 cm^{-1}. These three bands are clearly seen in all spectra except the supernatant sample after 7 hours.

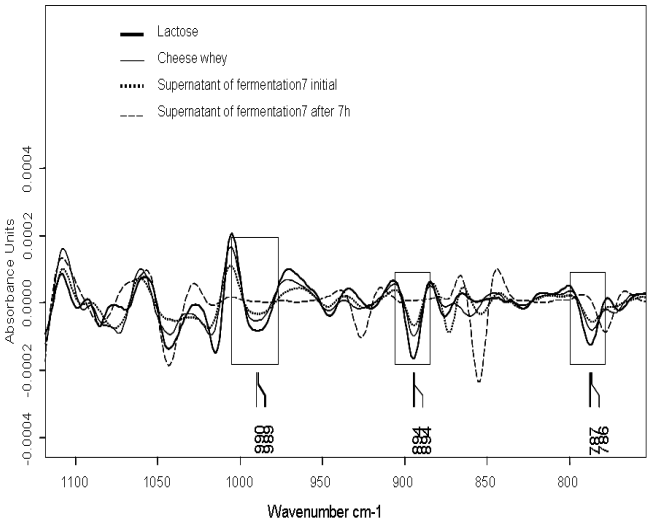

Fig. 6 Lactose, cheese whey and supernatant FT-IR spectra

The intensity of bands at 990 and 894 cm^{-1} decreased to zero in the spectra of cell free fermentation supernatant sample demonstrating the consumption of lactose by bacteria. The frequency shift of lactose band at 787 cm^{-1} in the spectra of supernatant samples after 7 hour fermentation indicates other extracellular components produced by bacteria or unconverted growth medium components.

IV. Conclusion

FT-IR spectroscopy was used for the monitoring of the lactose consumption in biogas production process from whey. This study showed that FT-IR spectroscopy was, fast, non-destructive and useful tool to follow the conversion of substrate during fermentation. FT-IR spectra analyses showed that lactose was consumed already in the first 7 hours. Lactose was consumed very quickly in the mode of fed-batch fermentation, but volatile organic acid degradation into methane and carbon dioxide were slower. Other fermentation modes, adaptation or bioaugmentation could provide a better biogas yield.

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References

[1] „Milk and milk products in the European Union", ISBN 92-79-02199, 2006.
[2] M. Balat, and H. Balat, "Biogas as a renewable energy source - A review", *Energy Sources, Part A: Recovery, Utilization and Environmental Effects*, Vol. 31, 2009, pp. 1280–1293.
[3] G. Mockaitis, S.M. Ratusznei, J.A.D. Rodrigues, M. Zaiat, E. Foresti, „Anaerobic whey treatment by a stirred sequencing batch reactor (ASBR) effects of organic loading and supplemented alkalinity", *J. Environ. Manage.*, vol.79, 2006, pp. 198-206.
[4] K.V. Lo and P.H. Liao, „Digestion of cheese whey with anaerobic rotating biological contact reactor", *Biomass*, vol. 10, 1986, pp. 243-252.
[5] B. Kavacik and B. Topaloglu, "Biogas production from co-digestion of a mixture of cheese whey and dairy manure", *Biomass and Bioenergy* , vol. 34, no. 9, 2010, pp. 1321-1329.
[6] P.F. Fox, T.P. Guinee, T.M. Cogan and P.L.H. McSweeney, "Fundamentals of Cheese Science", *Aspen Publishers*, Inc., Gaithersburg, 2000.
[7] F.R. Hawkes, R. Dinsdale, D.L. Hawkes, and I. Hussy, "Sustainable fermentative hydrogen production: challenges for process optimization", *Int. J. Hydrogen Energy,* vol. 27, 2002, pp. 1339–1347.
[8] R.L. Irvine and W.M Moe, "Periodic biofilter operation for enhance performance during unsteady state loading condition", *Water Sci. Technol.,* vol. 45, no. 3, 2001, pp. 231–239.
[9] G.L. Lybertos and I.V. Skidas, „Modeling of Anaerobic Digestion - A review", *Global Net. Intern. J.*, vol. 1, no. 2, 1999, pp. 63-76.
[10] S. Sivakesava, J. Irudayaraj and A. Demirci, „Monitoring a bioprocess for ethanol production using FT-MIR and FT-Raman spectroscopy, *J. Ind. Microbiol. Biotechnol.*vol. 26, 2001, pp. 185-190.
[11] A.E. Ghaly and D.R. Ramkumar, „Controlling the pH of Acid Cheese Whey in a Two-Stage Anaerobic Digester with Sodium Hydroxide", *Energy Sources,* vol. 21, 1999. pp. 475-502.
[12] A.E. Ghaly, D.R Ramkumar, S.S. Sadaks, and J.D. Rochon, „Effect of reseeding and pH control on the performance of a two stage mesophilic anaerobic digester operating on acid cheese whey", *Can. Agric. Eng.,* vol. 42, 2000, pp. 173-183.
[13] A.J. Mawson, „Bioconversions for whey utilization and waste abatement", *Biores. Technol., vol.* 47, 1994, pp. 195-203.
[14] G. Tchobanoglous, H. Theisen, and S. A. Vigil, "Integrated solid waste management: engineering principles and management issues", 2 edition, *McGraw-Hill*, Science/Engineering/Math; 1993.

[15] M. Chartrain, and J. G. Zeikus, "Microbial ecophysiology of whey biomethanation: characterization of bacterial trophic populations and prevalent species in continuous culture", *Appl. Environ. Microbiol*., vol. 51, no. 1, 1986, pp. 188–196.
[16] S.A. Cobb and D.T. Hill, „Volatile fatty acid relationships in attached growth anaerobic fermenters", *Transactions of the ASAE ,* vol. 36, no 6, 1991, pp. 2564-2572.
[17] S. Göblös, P. Portöro, D. Bordás, M. Kálmán, and I. Kiss, "Comparison of the effectivities of two-phase and single-phase anaerobic sequencing batch reactors during dairy wastewater treatment," *Renewable Energy*, vol. 33, no. 5, 2008, pp. 960–965.