

METHANE PRODUCTION FROM FAT-RICH MATERIALS

Małgorzata WORWAĞ*, Ewa NECZAJ, Anna GROSSER,
Dorota KRZEMIŃSKA
Czestochowa University of Technology
Faculty of Engineering and Environmental Protection
Institute of Environmental Engineering
Brzeźnicka st. 60A, 42 – 200 Czestochowa, Poland

Waste materials containing a lot of fats seem to be an attractive substrate for production of methane through the fermentation process. Yet, due to a changing content of reagents and the high concentration of higher fatty acids, they must be stabilized along with other biodegradable wastes in the process of co-fermentation. This process results in a higher fermentation-grade and a greater volume of produced biogas. However, the methane fermentation of sewage sludges or sewage containing higher fatty acids may be problematical, and requires widespread studies in order to get a better understanding of this process.

Keywords: fat-rich waste, co-fermentation, production of biogas

1. INTRODUCTION

During the last few decades, anaerobic digestion of organic matter has been presented as a suitable technology used for treatment of organic wastes and production of energy in the form of methane [18,31]. Digestion of lipids is certainly one of the most important and less known topics in this area, and research is necessary to enhance the application of anaerobic digestion to a range of difficult effluents, such as slaughterhouse wastewaters, fish waste, ice-cream waste, edible oil, dairy wastewaters or olive oil etc. [38,42].

The article presents the recent results of researches into fat-rich lipid materials anaerobic digestion and shows current knowledge of how this type of wastes affect processes. Additionally, we show different kinds of pre-treatment methods that have been used to enhance anaerobic digestion of lipids.

* Corresponding author. E-mail: mworwag@is.pcz.czest.pl

Lipids, natural oils, and fats are abundantly present in nature. Lipids are constituents of membranes of bacteria, archaea, and eukaryotes, while oils and fats are storage compounds for carbon and energy in all kinds of living organisms. Lipids are mostly esters of glycerol (animal fat –triglycerides) and long-chain fatty acids (LCFAs) or, in the case archaea, ethers of glycerol and long-chain alcohols [57]. LCFAs are monocarboxylic acids usually with aliphatic tails of 12 or more carbon atoms (Table 1). These can either be saturated, monounsaturated or polyunsaturated depending on the number of double bonds [57]. Quantity and kind the LCFA are dependent on raw materials. Table 2 presents the LCFA composition of lipid in different materials. Evidently, oleic acid and palmitic acid, respectively, is the most abundant unsaturated and saturated LCFA [4].

Table 1. Name and chemical description of some common long chain fatty acids [57]

Systematic name	Common name	Structure	Cn:d
Saturated Fatty Acids			
Dodecanoic acid	Lauric acid	$\text{CH}_3(\text{CH}_2)_{10}\text{COOH}$	C12:0
Tetradecanoic acid	Myristic acid	$\text{CH}_3(\text{CH}_2)_{12}\text{COOH}$	C14:0
Pentadecanoic acid	Valeric acid	$\text{CH}_3(\text{CH}_2)_{13}\text{COOH}$	C15:0
Hexadecanoic acid	Palmitic acid	$\text{CH}_3(\text{CH}_2)_{14}\text{COOH}$	C16:0
Heptadecanoic acid	Margaric acid	$\text{CH}_3(\text{CH}_2)_{15}\text{COOH}$	C17:0
Octadecenoic acid	Stearic acid	$\text{CH}_3(\text{CH}_2)_{16}\text{COOH}$	C18:0
Eicosanoic acid	Arachidic acid	$\text{CH}_3(\text{CH}_2)_{18}\text{COOH}$	C20:0
Docosanoic acid	Behenic acid	$\text{CH}_3(\text{CH}_2)_{20}\text{COOH}$	C22:0
Unsaturated Fatty Acids			
cis-9-hexadecanoic	Palmitoleic acid	$\text{CH}_3(\text{CH}_2)_5\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	C16:1
cis-9-octadecenoic	Oleic acid	$\text{CH}_3(\text{CH}_2)_7\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	C18:1
cis-9,12-octadecadienoic	Linoleic acid	$\text{CH}_3(\text{CH}_2)_4\text{CH}=\text{CHCH}_2\text{CH}=\text{CH}(\text{CH}_2)_7\text{COOH}$	C18:2

Cn:d where n is the number of carbon atoms and d the number of double bonds

Lipids are a group of organic pollutants whose conversion into biogas has been considered very difficult [11]. Anaerobic treatment of wastes with high lipid content cause few operational problems, such as: clogging, sedimentation hindrance, scum formation and flotation of biomass [14,27,9]. Moreover, intermediate compounds (LCFAs) may inhibit anaerobic microbial activity [18,32], and can also reduce the efficacy of anaerobic treatment processes by reducing the transport of soluble substrates to the bacterial biomass [8]. Inhibition depend on several factors, such as: type of bacteria present, specific

surface area of sludge, LCFA: biomass ratio, the sludge origin, activity of the sludge and the carbon chain length and saturation of LCFAs [54,15,9]. Moreover, research carries out by Cramer and Koster [31] showed that LCFAs have a synergistic effect. Nevertheless, fat-rich materials are attractive substrates for methane production, since theoretically their degradation produces more biogas with higher methane content, when compared with proteins or carbohydrates (Table 3) [4]. For example, about 1.01 dm³ methane at STP (standard temperature and pressure) can be produced from 1 g oleate acid, while only 0.37dm³ can be produced from 1 g of glucose [29,28].

Table 2. Percentage of the main LCFAs found in the different materials

Material	Fatty acid							Reference
	Lauric acid C12:0	Myristic acid C14:0	Palmitic acid C16:0	Stearic acid C18:0	Oleic Acid C18:1	Linoleic acid C18:2	Other LCFA	
OFMSW	0.2	2.2	32.9	14.9	33.8	5.6	10.4	Fernandez et al., 2005 ¹
Animal fat		3.0	3.0	17.0	38.0	6.0	6.0	
Vegetable fat	45.5	18.5	10.4	3.3	8.7	2.2	11.4	
SW			35.0	15.0	50.0	0	0	Hwu et al., 1998 ²
WM			21.0	6.0	39.0	13.0	21.0	Komatsu et al., 1991 ²
WM	7.0	6.0	21.0	6.0	39.0	13.0	8	Hanaki et al. 1981 ¹
Beef tallow	1.0	2.6	28.1	20.0	37.6	2.9	7,8	Alves et al. 2009 ¹
Domestic sewage		2.2	16.4	8.1	30.5	29.2	13,6	
Soybean oil		1.0	11.0	4.8	39.0	10.0	34,2	
STP-FOGW		7.77	59.65	15.04	17.54		0	Martín-González L. et al.2010 ¹
SC-OFMSW		3.27	41.28	33.27	22.18		0	
Sewage sludge	21.29	10.98	7.72	8.32	46.49		5.21	Casado et al. 1998 ¹
Dairy wastewater			27	7	37	13	16	Kim et al. 2004 ¹
Milk fat	3.6	10.5	23.5	10.0	21.0	1.8	29.6	Petry & Lettinga 1997 ¹

OFMSW – organic fraction of municipal solid wastes, SW – slaughterhouse wastewater, WM- whole milk, STP-FOGW- grease waste from sewage treatment plants, SC-OFMSW- source collected organic fraction of municipal solid wastes; 1 - % of total LCFA 2 - COD percentage

Table 3. Potential biogas production from different classes of substrates [9]

Component	Methanogenic reaction	Biogas (lg-1)	CH ₄ (%)
Lipids	$C_{50}H_{90}O_6 + 24.5H_2O \rightarrow 34.75CH_4 + 15.25CO_2$	1.425	69.5
Carbohydrates	$C_6H_{10}O_5 + H_2O \rightarrow 3CH_4 + 3CO_2$	0.830	50.0
Proteins	$C_{16}H_{24}O_5N_4 + 14.5H_2O \rightarrow 8.25CH_4 + 3.75CO_2 + 4NH_4^+ + 4HCO_3^-$	0.921	68.8

2. THE BIODEGRADATION OF LCFA IN ANEAROBIC DIGESTION

The biodegradation of solid fatty residues is limited by their low bioavailability [9]. The anaerobic digestion of the tri-glyceride esters is achieved by a combination of hydrolytic, fermentative, syntrophic acetogenic (SAB) and methanogenic microorganisms [42]. In an anaerobic environment, the neutral fats are first hydrolysed (lipolized) into free long-chain fatty acids (LCFAs) and glycerol. Fig. 1 presents biodegradation of lipids.

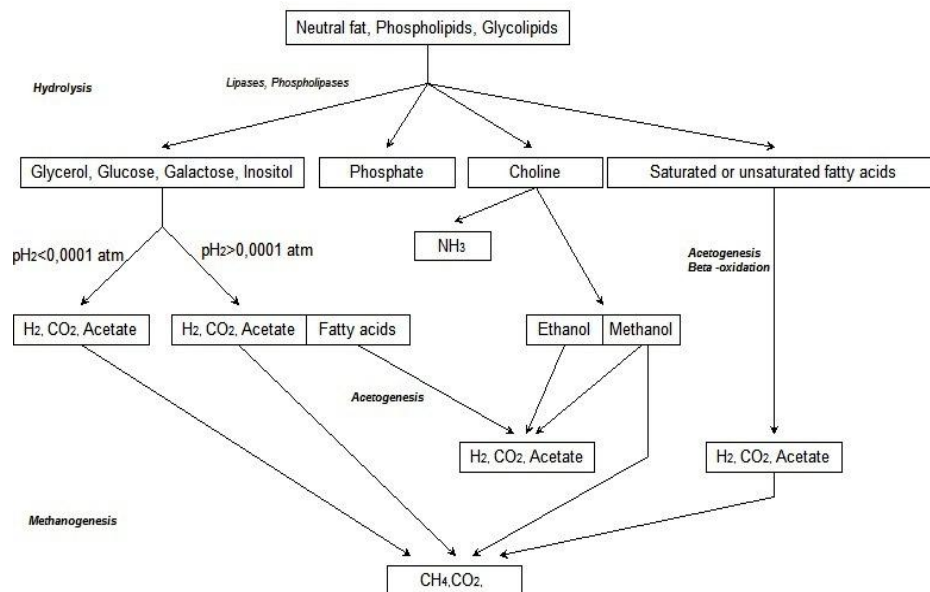


Fig. 1. Anaerobic degradation of fats (Gallert, C. and Winter, J. (2005). Bacterial Metabolism in Wastewater Treatment Systems. In: Jördening H.J. and Winter J. (eds), pp. 1-48, Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim)

The hydrolysis process is catalysed by extracellular lipase released by acidogenic bacteria. The free LCFAs are subsequently oxidized to shorter chain fatty acids by acetogenic bacteria. The oxidation process becomes thermodynamically unfavourable unless the hydrogen partial pressure is maintained at extremely low levels [21]. The acetate and hydrogen produced by hydrolytic bacteria and syntrophic acetogenic bacteria are finally converted to biogas by methanogenic bacteria [9]. The process thus relies on the ability of the hydrogenotrophic methanogenesis to utilize the molecular hydrogen produced during fatty acid oxidation [39].

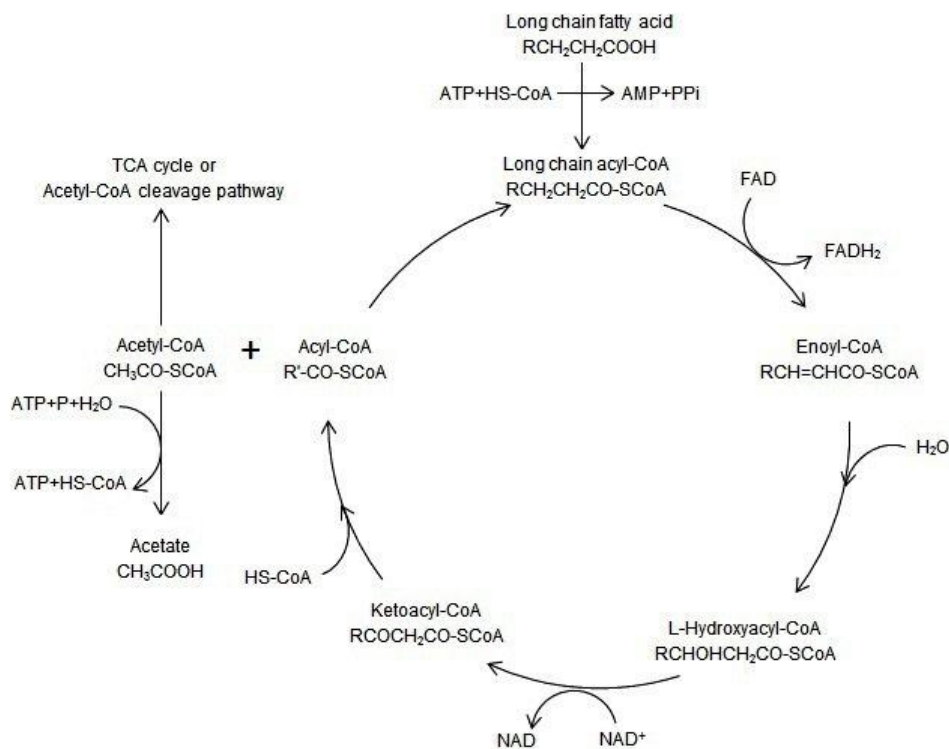


Fig. 2. The β -oxidation pathway involved in LCFA degradation in sulfate-reducing bacteria, and acetogenic bacteria that grown in syntrophy with methanogens

In recently years much attention has focused on LCFA degradation due to its perceived status as “limiting steps” of the anaerobic digestion process [42]. Hydrolysis was followed by a significant accumulation of free LCFAs in the reactor, suggesting that LCFA oxidation was the rate-controlling process of lipid anaerobic degradation [39]. The methane fermentation of lipids is achieved by the concerted action of three groups of bacteria: hydrolytic fermentative,

syntrophic acetogenic (obligate hydrogen-producing bacteria, OHPA) and methanogenic bacteria [9]. LCFA degradation pathways have been studied extensively in methanogenic and sulfatereducing communities at biochemical or genetic level. LCFA biodegradation occurs through sequential steps: (1) LCFA adsorption to the cell surface, (2) LCFA uptake, and (3) LCFA conversion to lower molecular weight components via β -oxidation. The end product in this cycle is acetylo-CoA [57]. The β -oxidation pathway LCFA presents in Fig. 2.

In sulphate-reducing bacteria that degrade LCFA completely to CO_2 , acetylo-CoA is further degraded via the acetylo-CoA cleavage pathway or a modified citric acid cycle. Acetylo-CoA can also be converted to acetate as is the case for LCFA-degrading bacteria in methanogenic environments and in several sulphate-reducing bacteria. Acetate is then a substrate for acetoclastic methanogens (*Methanosarcina* and *Methanosaeta*) or acetate-utilizing sulphate-reducing bacteria (*Desulfobacteria*, *Desulfobacterium*, *Desulforhabdus*, *Desulfobacca*) [57].

LCFA toxicity varies with the type of anaerobic sludges and is more correlated to their physical characteristic, specific surface area and size distribution, than to their biological ones [24].

Inhibition on methane production during anaerobic digestion of lipids can be attributed essentially to accumulation of LCFAs. LCFA can be toxic to the bacteria [21]. High LCFA concentrations can destabilize anaerobic digestion due to inhibition of methanogenic bacteria by possible damage to cellular membrane [18,5]. Thus, concentrations of inhibition are in the range of 30-300 mg/l for oleic acid [32,3,2,1], 100-300 mg/l for stearic acid [32], or 30 mg/l for linoleic acid [33].

The results obtained in this work evidence the important role of transport limitations imposed by LCFA in the anaerobic digestion process. Besides the potential toxic effect, LCFA accumulation onto the sludge can create a physical barrier and hinder the transfer of substrates and products (e.g., biogas release), inducing a delay on the initial methane production. Although metabolic inhibition of LCFA may also occur, the important feature is that the metabolic or physical effect that is behind a temporary decrease in the methanogenic activity is a reversible phenomenon, which is eliminated after the mineralization of the biomass-associated LCFA (Pereira et al., 2005).

Different kinds of pre-treatment have been applied to hydrolyse and dissolve lipids in order to improve their biological degradation (Carballa and Vestraete, 2010). Research carry out by Cirne [14] to prove, that addition enzymes hydrolytic such as: lipases enhances lipid hydrolysis up to 35%. Moreover different methods may improve the stability of lipids by lowering the size of fat globules are: addition NaOH [39]; ultrasounds [43], hydrothermal cracking processes (190°C), which increased the lipids degradation field from

67 to 84% [7]; electrochemical treatment increase the anaerobical biodegradation of lipids wastewaters [20]; adding calcium ions (CaCl_2) enhanced mineralization to methane linoleic acid inhibition [58]. Saponification is a promising processing technique for enhancing the emulsification and bioavailability of fatty residues. However hexane extractible matter (HEM) degradation depends on pH change, regardless of the alkali agent used to saponify greases, e.g. the HEM degradation performances were enhanced by 40% at pH 8.5 versus 10% at 6.5 [40]. The effect of bioaugmentation by an anaerobic lipolytic bacterium on anaerobic digestion of lipid-rich waste was also studied by Cirne et al. The addition of the bioaugmenting lipolytic strain led to an increase in the methane production rate and accordingly, a reduction in the digestion period required to obtain the same methane yield as the control [14].

3. ANAEROBIC WASTEWATER TREATMENT

The amount of lipid-rich wastewater increases every year due to urbanization and development of factories, and the treatment of such wastewater is still a challenge. Lipids, usually in the form of fats and oils, are common pollutants present in domestic sewage and industrial effluents, such as the ones from dairy industry [44], food processing industry, slaughterhouses [11], the edible oil processing industry and olive oil mills [19]. Different reactor technologies have been development for anaerobic treatment of lipid-rich wastewater. Among these technologies, the continuously stirred tank reactor (CSTR), upflow anaerobic sludge bed reactor (UASB) and expanded granular sludge bed reactor (EGSB) are the most widely used. EGSB reactor is a variant of the UASB concept with the larger ratio of reactor height/surface area to obtain higher upflow velocity and improve the contact between substrate and microorganisms. The various COD removals (50÷97%) are experienced during the treatment of wastewater containing LCFA in UASB reactors [22]. The UASB treatment failures mainly due to flotation of sludge granules and inhibitory effects of LCFA on anaerobic microorganism. Fang at al. (2010) studied anaerobic digestion of palm oil mill effluent (POME) in UASB and EGSB reactors. Authors showed high and similar COD removal efficiency (more than 90%) and methane yield (ca. 450 ml- CH_4 /gVS) reactors and in both reactors. However UASB reactor was found to be more stable than EGSB reactor under the same organic loading rate. On the contrary Renzema (1993) took advantage of the EGSB reactors and observed significant improvement of lauric acid as compared to UASB reactor. On the over hand, for the ice-cream wastewater, the sludge granulation was unsuccessful, and the most adequate method for that kind of wastewater was the fixed bed system [22]. Rinzema [50] concluded that the application of conventional UASB reactors LCFA containing wastewaters

resulted in local overloading of LCFA and severe washout caused by flotation. Pereira also observed EGSB treatment failures during an oleic-based synthetic wastewater [45]. The author showed that after oleic acid addition, that methane yield decreased from 280 to 27 l CH₄/kg COD-removed in EGSB reactor, and 362 to 91 l CH₄/kg COD-removed in anaerobic filter. Pereira et al. [45] also studied the anaerobic degradation of LCFA in EGSB reactor continuously fed with oleic acid (50% COD) and co-substrate, skimmed milk (50% COD). The authors showed that methane production decreased to 20-30 % of the value obtained when 50% COD fed was a co-substrate. It was found that adsorption of palmitic acid onto the sludge occurs before biodegradation and presence of oleic acid inhibits further β -oxidation of palmitic acid.

On the other hand, UASB reactors have been successfully employed for dairy wastewater in full scale applications for almost two decades. COD removal rate varied between 60-99% and was strongly dependent on organic loading rate and hydraulic retention time [48].

4. CO-DIGESTION

Sewage sludge comprises lipids, carbohydrates, and proteins. Among them lipids are the most significant substances in the anaerobic digestion, since a larger amount of methane can be produced from lipids than from other components [21]. Co-digestion with other organic wastes is an attractive strategy to increase the biogas yield [56]. The energy value of lipids makes them an ideal co-substrate to increase the economic feasibility of any anaerobic digestion plant based on co-digestion concepts.

The investigation carried out by Lansing et al. proved that adding small amounts of grease to the influent is a simple way to double energy production without affecting other digester benefits. A small volume of grease (2.5%), which corresponded to a 113% increase in organic matter, increased methane production by 124% [34].

Research carried out by Hejnfelt and Angelidaki to prove, co-digestion of 5% pork by-products mixed with pig manure at 37°C showed 40% higher methane production compared to digestion of manure alone. The animal waste constitutes a good substrate for biogas production with a methane potential of mixed animal waste of 619 dm³/kg, which is much higher than the methane potential of manures (20-30 dm³/kg). Dilution of the by-products had a positive effect on the specific methane yield with the highest dilutions giving the best results. High concentrations of LCFA and ammonia in the by-products were found to inhibit the biogas process at concentrations higher than 5 g lipids/dm³ and 7g N/dm³ respectively [23].

Lustarinen et al. have found feasible up to grease trap sludge addition with mixture co-digestion, which make 46% of feed VS with HRT of 16 d and OLR of up to 3.46 kgVS/m³d at 35°C. The high methane production potential of grease trap sludge (918 m³/tVS_{added}) resulted in significantly increased specific methane production in reactor experiments (maximum 463 m³/tVS_{added}) compared to digestion of sewage sludge alone (278 m³/tVS_{added}). Grease trap sludge additions of 55% and 71% of feed VS resulted in increased VS and COD_{sol} in digested material and decreased methane production indicating overloading and LCFA inhibition (Lustarinen et al. 2009).

Co-digestion of by-products from meat-processing industry and sewage sludge (respective feed ratios 1:7 and 1:3), gave the highest methane yield and the steadiest digestate quality at 20-days-HRT. At 14-days-HRT, methane production decreased indicating too high OLR. Hygienization pre-treatment of the feed ratio 1:7 was found efficient at improving degradation and thus increasing methane production, the latter being higher than with the digester of feed ratio 1:3. Hygienization caused an estimated 0.55– 0.66 GWh/a more energy despite the energy consumed by the pre-treatment itself (Luste and Luostarinen, 2010).

5. CONCLUSIONS

The preceding sections show the challenges posed by the presence of rich -lipids material in wastewater. Hence new approaches and methods (both biological and physicochemical) are still required to full understand the behavior of lipids in biological waste and wastewater treatment processes and to enhance their removal [12]. Provided the appropriate technology is utilized and the right feeding strategy is followed, lipids can be effectively converted to methane (by syntrophic consortia of acetogenic bacteria and methanogenic archaea. Driving the methane production from lipids/LCFA at industrial scale, without risk of inhibition, is still a challenge that has the potential for filling a gap in the existing processes and the existing processes and technologies for biomethane production associated to waste and wastewater treatment [4]. Table 4 presents results of treatment of fat rich materials in different reactors.

Table 4. Results of treatment of fat rich materials in different reactors

Kind of substrate	Type of reactor	Temperature (°C)	HRT (days)	OLR (g COD/ l)	COD removal (%)	Reduction of VS (%)	CH ₄ yield (l/gCOD)	Methane content [%]	Reference
GS & SS		35	16	1.67-4.4 ¹	ND	52-72	0.315-0.463 ²	61-66	Luostarinen et al. 2009
GS & SS		35	13	2.4-2.5 ¹	ND	55-58	0.295-0.344 ²	66-69	Davidsson et al. 2008
LCFA mixture ³	CSTR+UASB	35	2.9	0.2-2.7	60-95	ND	ND	ND	Kim et al. 2004
Ice cream wastewater	UASB	35	1.62	2.19	49	ND	0.19	69.6	Hawkes et al. 1995
	AF	35	0.93	6.38	66.9	ND	0.36	75.3	
	Fluidized bed reactor	35	1.47	4.2	55.7	ND	0.37	70.1	
	Contact process	35	5.51	1.05	81.8	ND	0.39	76.9	
HSC:SS	CSTR	37	17	2.9 ⁴	ND	68 ⁵	0.23 ⁶	52	Rosenwinkel and Meyer 1999
FT:SS	CSTR	37	15	1.5 ⁴	ND	58 ⁵	0.32 ⁶	66	
TWAS&PS: &FOG	laboratoryscale anaerobic digesters	35	12	4.35	47.8	45	0.449 ⁷	ND	Kabouris et al. 2009
		52	12	4.35	54.6	51.2	0.512 ²	ND	
Palm oil mill wastewater	UASFF	38	1.5-3.0	2.63-23.25	89.5-97.5	ND	0.310-0.346	62-84	Najafpour et al. 2006
ABP:SS		35	14-25	1.8-4.0 ¹	ND	ND	0.340-0.430 ²	56-67	Luste and Luostarinen 2010
Dairy wastewater	UASB	35	6-40	2.0-4.5	79-99	ND	ND	68-74	Gavala et al. 1999
	draw and fill digester	35	26-40	1.5-2.3	83-94	ND	ND	ND	
Poultry slaughterhouse wastewater	SGBR	22	1.25-2.5	0.64-4.97	85.0-97.8	ND	0.25	ND	Debig and Coskun 2009
	SASBR	22	1.25-2.5	0.64-4.97	72.2-98.6	ND	0.27	ND	
Slaughterhouse wastewater	UASB	37	1.6-7.2	1.7-3.0	approx. 89	ND	0.343-0.349	ND	Rodriguez Martinez et al. 2002
Slaughterhouse wastewater	UASB	30	10	3.5	70	ND	0.280	ND	Manjunath et al. 2000
PSW:FPP	CSTR	35	18	4.6 ¹	ND	68	0.33 ²	ND	Salminen and Rintala 1999
Slaughterhouse wastewater	ASBR	20	2.3	0.9-2.75	93	86 ⁷	ND	78.4	Masse D.I. and Masse L. 2001
		25	2.3-3.5	1.93-2.94	92-95	80-93 ⁷	ND	76.9	
		30	2	4.39-4.93	93	91 ⁷	ND	74.7	
Slaughterhouse wastewater	combination sludge blanket and filter arrangement in a single reactor	35	1.5	2.49-6.94	90.2-96.2	83.6-91.4	0.287-0.349	65-74	Borja et al. 1998
FVW:SCSM:SCSSW	laboratoryscale anaerobic digesters	35	30	1.1-1.3 ¹	ND	51.7-67.4	0.04-0.35 ²	25-57	Alvarez and Lidén
Slaughterhouse wastewater	ESGB	35	0.2	15	ND	67	ND	ND	Núñez and Martínez 1999
Slaughterhouse wastewater	UASB	37	1.2-6.5	1.03-6.58	44.9-91.5	ND	ND	52.9-70.6	Ruiz et al. 1997
	AF	37	0.5-7.1	0.88-11.21	59-93	ND	ND	18.1-51.1	

HRT- hydraulic retention time, OLR – organic loading rate, COD – chemical oxygen demand, VS – volatile solids, ND-not determined, GS – grease trap sludge, SS – sewage sludge, HSC-hog stomach contents, FT-flotation tailings, PS-primary sludge, TWAS- thickened waste activated sludge, FOG- fat, oil, and grease, ABP – animal by-product, PSW- poultry slaughterhouse wastes, FPP- food packing plant wastes, FVW- fruit and vegetable waste, SCSM - Solid cattle and swine manure, SCSSW - solid cattle–swine slaughterhouse waste, CSTR – continuously stirred tank reactor, UASB - up-flow anaerobic sludge bed reactor, AF – anaerobic filter, UASFF - up-flow anaerobic sludge-fixed film reactor, SGBR- Static Granular Bed Reactor SASBR - Static Anaerobic Sludge Bed Reactor, ASBR - anaerobic sequencing batch reactor, ESGB – expanded granular sludge bed reactor;

1 – g VS/ l·d; 2 – l/gVSadd; 3 - the synthetic wastewater comprised a glucose and LCFA mixture; 4 – g TS/ l·d; 5- removal TS; 6 – l/gTS, 7 – suspended solid removal.

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PRODUKCJA METANU Z SUBSTRATÓW BOGATYCH W TŁUSZCZE

Streszczenie

Odpady zawierające wysoką zawartością tłuszczów wydają się najbardziej atrakcyjnym substratem do produkcji metanu w procesie fermentacji. Z uwagi na zmienny skład reagentów oraz znaczne stężenia wyższych kwasów tłuszczowych muszą być one stabilizowane z innymi biodegradowalnymi odpadami w procesie ko-fermentacji. W procesie ko-fermentacji dochodzi do rozcieńczenia substancji toksycznych oraz poprawy równowagi nutrientowej. Ponadto obserwuje się wyższy stopień przefermentowania osadów i większą produkcję biogazu. Podczas stabilizacji beztlenowej, tłuszcze w pierwszym etapie są hydrolizowane do wyższych kwasów tłuszczowych oraz glicerolu. W kolejnych fazach wyższe kwasy tłuszczowe oraz glicerol rozkładane są do kwasów lotnych, octanu i wodoru. Mimo, że hydroliza uważana jest za fazę limitującą jeden z etapów konwersji tłuszczy, niektórzy autorzy wskazują iż proces ten zależy od czasu zatrzymania osadu (SRT). Przy SRT poniżej 8 dni dochodzi do akumulacji wyższych kwasów tłuszczowych i inhibicji całego procesu fermentacji. Jednakże fermentacja metanowa osadów ściekowych lub ścieków zawierających tłuszcze na wysokim poziomie może być problematyczna. Główne problemy spowodowane przez tłuszcze podczas stabilizacji beztlenowej to pienienie, flotacja osadów, zapychanie się instalacji oraz nieprzyjemne odory. Tak więc kofermentacja odpadów z dużą zawartością tłuszczy może być problematyczna i wymaga dalszych badań mających na celu wyjaśnienie tego procesu.