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BIOGAS PRODUCTION FROM CORN BIOETHANOL WHOLE STILLAGE: EVALUATION OF TWO DIFFERENT INOCULA

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Abstract

According to EU Strategy for the Baltic Sea Region, Lithuania obligates to ensure sustainable growth, gain and maintain good condition of marine environment until 2020. In accordance with the sustainability approach, every potential cost and energy cutting as well as social sustainability measure for wastewater treatment should be explored. Nonetheless, Lithuania wastewater treatment plants (WWTP) in the sustainability context have never been evaluated before. A comprehensive set of 30 sustainable development indicators (SDI) (9 functional, 11 environmental, 5 economical and 6 socio-cultural) in connection with functional unit were applied to medium-sized Jurbarkas WWTP (with a capacity of 2,540 m^3/d). Sustainability evaluation involved life cycle of WWTP maintenance phase as well as water inlet, outlet and manufacturing. Results revealed that in the general context of sustainability the stability of plant varied greatly. Nine SDI haven't reached the sustainability approach. Graphically systemized results in the four sustainability categories have shown that relatively highest environmental impact regarding the maximum covered plot is caused due to an economical unsustainability. Operational and maintenance costs per volume of wastewater treated were approximately 2.23 higher than the cost to consumers per one cubic meter of wastewater treated, therefore depreciation, repairs, material costs and wastewater treatment costs accounted to 87%. Methodology by using SDI for estimating sustainability of WWTP is adaptable to different capacity or technology of WWTP, comparable, simple to develop and improve.

Key words: sustainable development, sustainability indicators, wastewater treatment plant

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1. Introduction

The energy policies of the last decade, characterized by the goal of reducing oil-dependency and carbon-dioxide emissions by promoting renewable energy sources, have led to a considerable growth of the biofuel industries. Amongst them the volume of corn-based bioethanol production has steeply increased in the last decade (Abayomi et al., 2011; Moza and Mironescu, 2017; RFA, 2013), resulting in huge quantities of distillery by-products. The volume of bioethanol processing residues exceeds approximately 10 times the volume of produced ethanol, and their utilization raises serious problems (Krzywonos et al., 2009). Distillery by-products contain significant amounts of organic residues and are currently valorized as animal feedstock due to their nutrient content.

In the process of ethanol making whole stillage is produced in the distillation step that follows the alcoholic fermentation, where the produced ethanol is separated from the fermented mass. This fermentation residual is the „distillery wastewater" or „whole stillage", and it has a high content of soluted and

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particulate organic matter, made up of non-fermented sugars, residual starch, degraded yeast cells, proteins, fats and cellulolytic material. Whole stillage is usually further processed in products with longer shelf-life: in a centrifugation step its soluble part (thin stillage) is separated from the non-soluble fraction (distiller's wet grain or DWG). Thin stillage can be partly recycled into the ethanol making process; water is then evaporated from the remaining thin stillage to obtain the so called syrup or condensed distiller's solubles (CDS). The two fractions, syrup and DWG, are eventually mixed to form the so-called dried distillers grains with solubles (DDGS). Water is eliminated through evaporation so that the final product becomes stable and can be valorized as animal feedstock. The amount of DDGS produced normally exceeds the volume of bioethanol obtained (Rosentrater et al., 2012).

The above stillage processing and valorization method has several problematic aspects, such as the high phosphorus content of the CDS, limiting its use as DDGS component (Rosentrater et al., 2012), or the build-up of solids, lactic acid and sodium, caused by the recycling of the thin stillage (Shojaosadati et al., 1996; Wilkie et al., 2000). The most important drawback of stillage handling, however is that the evaporation of water from the whole stillage is very energy-consuming, accounting for approx. 47% of total energy consumption of a bioethanol plant (Eskicioglu et al., 2011b; Vasic et al., 2016). Hence the net energy balance ratio of the ethanol fuel is very modest, with a value of approx. 1.26 for corn ethanol, meaning that 1 unit of energy input is needed to produce 1.26 energy units of corn ethanol energy (Agler et al., 2008).

With animal feed markets getting saturated and ethanol co-product volumes rapidly increasing, the energetic valorization of these co-products through biogas production is attracting more and more attention. Recent studies suggest that the anaerobic digestion (AD) of the stillage would be more preferable than stillage drying, for more reasons: 1) it would eliminate the problems related to stillage handling; 2) it would save the energy used for stillage drying and 3) by transforming stillage into biogas, which can be used on-site, it could better the energy balance (and reduce the cost) of the ethanol production (Agler et al., 2008; Eskicioglu et al., 2011a; Mateescu and Constantinescu, 2010). Few studies can be found on anaerobic digestion of bioethanol by-products, and among these only three reports on the digestion of whole stillage: Eskicioglu et al. (2011b) described the mesophilic and thermophilic digestion of the whole stillage, while Eskicioglu et al. (2011a) showed the importance of inoculum-to-substrate ratio (ISR) on the mesophilic digestion of whole stillage. No research has been done, however, regarding the effect of the inoculum type on the digestion on any of the bioethanol by-products (including whole stillage), although it is known, that the type and quality of the inoculum must be considered in order to reach high biogas yields (Mateescu and Constantinescu, 2011). Both above mentioned studies assessed only one type of inoculum, collected from anaerobic digesters treating the primary sludge and the thickened waste activated sludge of municipal wastewater treatment plants. Since an AD process involves four major phases, hydrolysis, acidogenesis, acetogenesis, and methanogenesis, mediated by unique functional groups of microbes, the composition of the microbial community of the inoculum might be crucial for the whole AD process. Pandey et al. (2011) showed that the inoculum quality is critical on the startup of the AD process by balancing the populations of *Syntrophobacter* and methanogens, making syntrophic metabolism thermodynamically feasible. Research on the effect of inoculum source on the methane production efficiency has been done on several other substrates, showing that significant differences can be observed when using different inocula on the same substrate, in terms of maximum methane production, length of lag phase and resistance to toxicity (Elbeshbishy et al., 2012; Neves et al., 2010; Pereira et al., 2002). Such studies clearly show that besides the microbial load of the inoculum also its microbial composition affects the biogas production and the substrate degradation efficiencies. Inoculum quality is even more decisive on the performance of AD in the case of practically sterile substrates, such as the whole stillage, which leaves the bottom of the distillation column at 85-88°C.

The aim of this work was to analyze anaerobic digestion under mesophilic conditions of corn ethanol whole stillage using two different inoculum sources in order to quantify in what extent the inoculum choice may affect the biomethanation process of this substrate, in terms of biodegradability and specific methane yields. By the microbial characterization of the used inocula we tried to have a deeper insight into the AD process conducted on whole stillage substrate, and to find possible explanations for the observed differences.

2. Experimental

2.1. Substrate characterization

The whole stillage used as substrate has been procured from the SC Bio Fuel Energy SRL bioethanol plant (Zimnicea, Romania), where bioethanol is produced using a dry-grind process and continuous fermentation scheme (Gamureac, 2010). Total solids (TS) and volatile solids (VS) content determinations of the substrate were performed according to Standard Methods (Clesceri et al., 1999).

The total Kjeldahl nitrogen (TKN) content of the substrate were determined chemically (Horwitz, 2000) using an UDK 159 VELP Automatic Kjeldahl Distillation & Titration System apparatus. Digestion of samples (5 g) was made with concentrated H2SO4 and cupric catalyst in DK6 Heating Digester Unit (Velp Scientific).

Chemical oxygen demand (COD) determinations were accomplished using the Open

Reflux Method (Clesceri et al., 1999). Given the high solid fraction of the substrate, prior to the hydrolysis, all samples have been thoroughly blended using a high-rpm kitchen blender for better homogenization. For the soluble COD (SCOD) determinations first the particulate matter has been eliminated by filtration on a 0.45 μm glass-fiber filter disks; following this a regular COD measurement has been performed on the filtrate.

All reagents used for the chemical analyses were of analytical grade. Each measurement has been repeated three times to yield more reliable data, and mean values were calculated.

2.2. Inoculum characterization

The two types of inocula used in this study were obtained from 1) the anaerobic digester of the municipal wastewater treatment plant of Sfantu Gheorghe, Romania (referred to as "suspended inoculum" in the following), and 2) from the up-flow anaerobic sludge blanket reactor (UASBR) of a wastewater treatment facility treating the brewery wastewater of Heineken S.A. Miercurea Ciuc (referred to as "granular inoculum" in the following). Both inocula have been collected in airtight containers and kept in dark for 72 hours before being used in the degradation experiments. Prior to the AD experiments inoculum samples were freeze-dried and kept in lyophilized form until being analyzed trough the EL-FAME method.

The physicochemical characterization of the inocula has been done using the same methods and instrumentation described in the Substrate Characterization subsection.

The microbial characterization of the inocula was done both with cultivation-dependent and –independent approaches. Traditional colony-forming unit (CFU) counts were carried out in order to evaluate different groups of cultivable bacteria. Different sample dilutions have been spread into four different media:

1) Thioglycolate medium for anaerobes (L^{-1}): pancreatic digest of casein 17.5 g, papaic digest of soybean meal 2.5 g, dextrose 10 g, NaCl 5 g, sodium thioglycolate 1 g, K_2HPO_4 2 g, methylene blue 0.002 g, agar 15 g.

2) Nutrient medium for aerobes and facultative aerobes (L^{-1}): peptone 5 g, meat extract 1 g, yeast extract 2 g, NaCl 5 g, Agar 15 g.

3) Acetogen medium for acetogenes (values are per 421.8 mL): $NaHCO_3$ 2.4 g, NH_4Cl 0.2 g, yeast extract 0.2 g, stock salt solution #1 40 mL, potassium phosphate buffer 20 mL, clarified rumen fluid 20 mL, stock salt solution #2 4 mL, trace mineral solution 4 mL, vitamin solution 4 mL, reducing agent 4 mL, tungstate solution 0.4 mL, resazurin (0.1% solution) 0.4 mL, agar-agar 6.33 g. For details please refer to Atlas (2004).

4) Standard growth medium for methanogens (L^{-1}): K_2HPO_4 0.3 g, KH_2PO_4 0.3 g, $(NH_4)_2SO_4$ 0.3 g, NaCl 0.6 g, $MgSO_4 \cdot 7H_2O$ 0.13 g, $CaCl_2 \cdot 2H_2O$ 0.008 g, $FeSO_4 \cdot 7H_2O$ 0.002 g, sodium acetate 0.5 g, yeast extract 0.5 g, trypticase 0.5 g, cysteine-HCl 0.5 g, Resazurin 0.001 g, trace mineral solution 10 mL (detailed description in Garcia et al., 2006).

Petri dishes were incubated at 37°C in dark, in Memmert 400 incubator. To assure anaerobic conditions GasPak EZ Anaerobe Container System was used, and BD BBL Dry Anaerobic Indicator Strips were used to verify the presence of oxygen.

As cultivation independent approach ester linked fatty acid methyl esters (EL-FAME) analysis has been used. The analysis was carried out on 1 g of lyophilized inoculum sample, using organic solvent extraction, as previously reported by Crognale et al. (2013). Methylated fatty acids (FAs) were identified according to their mass spectra and using BAME 24 (bacterial acid methyl ester) and 37 FAME (fatty acid methyl ester) Mix (47080-U and 47885-U respectively, Sigma-Aldrich) as qualitative standards.

FAs are described using the standard nomenclature, given by the total number of carbon atoms:number of double bonds, followed by the position of the double bond from the methyl (aliphatic) end (ω) of the molecule. Anteiso- and iso-branched FAs are marked by the "a" and "i" prefixes, while the prefix "cy" indicates cyclopropane FAs.

2.3. Anaerobic digestion set-up

The anaerobic degradation assays were conducted in 50 mL serum bottles. In each bottle 6 mL of substrate has been added, except in the control bottles, which contained starch as reference substrate, having a known biogas yield (Raposo et al., 2011). The inoculum quantity has been determined so that the inoculum-to-substrate ratio expressed in TS was 3:1. Alkalinity was added to the mixtures in order to adjust pH to 7. The serum bottles were than adjusted to 25 mL with distilled water, so that the remaining headspace in each bottle was equal. No trace metal solution has been added to the bottles, as the trace elements concentrations in the corn whole stillage are of a level comparable to the mineral solutions normally supplemented to anaerobic digestion media (Belyea et al., 2006). Probes were performed in triplicates, hence a total of 15 bottles have been used for the two types of inocula, the controls (with reference substrate) and the blanks (containing only inoculum). The controls – by giving an idea of the inocula response towards a standard substrate – served to ensure that the activity of the used inocula is not particularly low.

All bottles were degassed with nitrogen for 2 min to ensure anaerobic conditions were attained and were placed in incubator at 37°C. The degradation tests were conducted for 45 days, and mixing was assured by inverting the bottles each day three times. The volume and the methane concentration of the produced biogas were measured each day at the beginning of the experiment, and less frequently later on, as the intensity of biogas production decreased. COD measurements were performed at initial and

final time of the experiment. The results of the blank probes (the biogas production of the inocula due to endogenous respiration (Ferrer et al., 2008)), were substracted from the observed biogas volumes. Finally, the gas volumes have been adjusted to normal conditions (101325 Pa, 0°C).

2.4. Biogas analytical assay

The volume of the produced biogas has been measured by displacement of acidulated water (pH=2) in an upside-down graduated cylinder (Walker et al., 2009); the observed values were then corrected for temperature. At each volume measurement the biogas has been let out of the serum bottles through a syringe needle inserted in the septum (and conducted into the upside-down cylinder) until atmospheric pressure inside the bottle was reached.

The methane content of the produced biogas was determined by gas chromatography analysis, using a HP 5890 Series II gas chromatograph equipped with a Thermal Conductivity Detector (TCD) and a Mol Sieve 5A PLOT Capillary GC Column (Supelco). Nitrogen has been used as carrier gas, and the injector, detector and oven temperatures were 120°C, 120°C and 80°C respectively. Prior to each measurement session, four-point calibration of the gas chromatograph has been performed with analytical grade methane (Merck). Biogas sampling has been done using a Hamilton GasTight 250 µL syringe.

3. Results and discussion

3.1. Chemical characterization of the substrate and of the inocula

The results of the substrate characterization are shown in Table 1. The pH and the TS content of the whole stillage were rather low, but within the range of values reported in the literature (Doušková et al., 2010; Eskicioglu et al., 2011b). The same holds for the TS and VS content of the substrate.

The COD of the substrate was very high, but in line with values reported in other studies on whole stillage. About 24% of the substrate's COD was found to be in dissolved form. Note that the accuracy of COD determinations was rather low (standard deviation of almost 10%) due to the inhomogeneous nature of the substrate.

The TKN content of the whole stillage was of 3460 mg N/L, which gives a COD:TKN ratio of 100:2.9, somewhat higher than the most commonly recommended 100:2.5 ratio for anaerobic digestion (Mara and Horan, 2003). Given the nitrogen content of the substrate in excess as compared to the required, it should not limit the AD process.

The pH of the two inocula was close to neutral. This was expected, since both bioprocesses the inoculants came from use pH correction. The TS and VS content of the inocula correspond to values practiced/observed in low-solid anaerobic digesters.

The COD determination accuracy for the inocula was considerably better than in the case of the substrate. For both inocula the soluble COD fraction was found to be very low, accounting for 1-2% of the total COD. This means that only insignificant quantities of residual soluble substrate were present in the inocula mass.

3.2. Microbial inoculum characterization

Table 2 shows the results of the cultural counts performed on four different selective media. For the ease of comparison, the values are expressed on a dry matter basis, eliminating this way the biases due to the different TS content of the two inocula. As can be seen, the observed bacterial loads are of the order of 108-109 cells·g^{-1}, which corresponds well to data reported on granular and suspended anaerobic sludges (Lozano et al., 2009; Shuangjiang et al., 1993).

Table 1. Chemical characterization of substrate and inocula (Gyenge et al., 2014)
(Data represent arithmetic mean of replicates, standard deviations and number of measurements are shown in parenthesis)

Parameters / Material	***Whole stillage***	***Granular inoculum***	***Suspended inoculum***
pH	3.53	7.29	7.24
TS [%, w/w]	7.04 (0.1; 3)	8.01 (0.0; 3)	10.44 (0.1; 3)
VS [%, w/w]	6.74 (0.026; 2)	3.85 (0.043; 3)	4.87 (0.071; 3)
TS/VS [%]	95.7 (0.3; 2)	48.1 (0.6; 3)	46.2 (0.4; 3)
COD [mg/L]	117609 (9359; 2)	70834 (3058; 2)	57698 (361; 2)
SCOD [mg/L]	28232 (2177; 2)	879 (29; 2)	3450 (411; 2)
TKN [mg N/L]	3460 (13.8; 3)	-	-

Table 2. Microbial characterization of the two inocula by cultural counts on selective media

Microbial species	***Bacterial count [CFU·g^{-1} dry matter]***	
	Granular inoculum	***Suspended inoculum***
Aerobic & facultative aerobic bacteria	$3.11 \cdot 10^9$	$1.66 \cdot 10^9$
Anaerobic bacteria	$2.81 \cdot 10^8$	$4.35 \cdot 10^9$
Acetogens	$4.82 \cdot 10^9$	$7.51 \cdot 10^9$
Methanogens	$4.84 \cdot 10^8$	$3.21 \cdot 10^9$

Methanogens in the granular sludge were found to be one order of magnitude less numerous than other bacteria. This confirms the observation reported in other studies that methanogens in most of the cases make up less than 10% of the microbial community of anaerobic digesters (Wirth et al., 2012). In the case of the suspended sludge, however, this phenomenon was less evident. In general, the suspended inoculum showed higher microbial load than the granular one, only the aerobic/facultative aerobic population was slightly higher in this latter.

Microbial characterization of the substrate has not been performed, because the ethanol co-products result from the distillation and evaporation processes at high temperatures, and thus are practically sterile. To simulate this condition, the substrate has been autoclaved prior to the batch degradation experiments.

3.3. EL-FAME analysis of the inocula

Considering that cultivable counts are substrate-dependent methods, and over- or underestimations of several order of magnitudes of the microbial groups can easily occur (Elferink et al., 1998), a complementary, culture-independent method has been used to better depict the microbial community of the inocula. FAs are useful biomarkers that give an instant profile of the bacterial community and can be extracted from the samples without cultivation. The determination of the FA profile is a well-established method to study viable biomass and microbial community structure in environmental samples, such as soils and sediments, but still not common for anaerobic systems (Schwarzenauer and Illmer, 2012). As the El-FAME analysis uses FAs of intact cell membrane as target molecules, while FAs released by lysed membranes are not revealed, the method can be considered an indirect measure of the active/vital fraction of the sample. For this reason in some cases it can be more informative than DNA-based methods, which does not permit such differentiation.

A total of 16 different FA biomarkers have been identified in the two inocula in this study (Fig. 1).

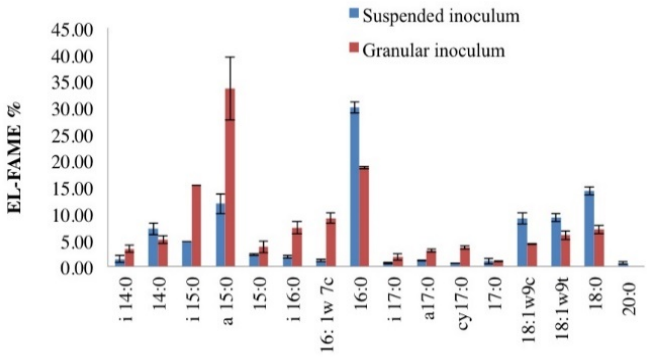

Fig. 1. EL-FAME analysis of the two inoculum types

Almost all of the detected FA biomarkers were present in both samples, the only exception being the eicosanoate (20:0), a marker of protozoa, found only in the suspended sludge. Considering that the suspended inoculum came from a digester with 10 days of retention time, while the granulated inoculum originated from an UASBR with a solids retention time of 70-80 days, one may assume that the long residence time of protozoa in the UASBR negatively affects their vitality. This hypothesis seems to be confirmed also by the dynamics of protozoa growth in anaerobic digesters reported by Priya et al. (2008).

Some of the differences were found in the case of universal markers (for example 16:0), which are not attributable to any specific microbial group, and thus alone do not provide useful information for this analysis. From the point of view of the AD process four significant differences have been identified between the two inocula, when assigning the biomarkers to microbial groups:

1) protozoa were not present in the granular inoculum. Priya et al. (2008) suggested that the absence of protozoa affects the COD removal in the anaerobic system substantially.

2) the saprophytic fungi group (18:1ω9c, 18:1ω9t (Hanif et al., 2012)) accounts for almost 20% of the identified FAs in the case of the suspended inoculum, while it makes up only 10% in the granular one. Fungi in general contribute to the hydrolytic degradation of the organic matter, thus their presence might influence the initial phase of the AD process.

3) the group of sulphate reducing bacteria (SRB) and other anaerobic bacteria (16:0, a17:0, i17:0, 17:0, 18:0), calculated according to Pratt et al., (2012) accounts for 47.3% of the viable biomass for the suspended inoculum and only 30.6% in the granular one. The high level of SRB in the suspended sludge might decrease the overall methane yields through two types of inhibition: first because SRB might out compete methanogens for hydrogen and acetate, second because of the inhibitory effect of the resulting sulphide (Chen et al., 2007).

4) the cy17:0/16:1ω7c ratio (cyclopropyl/precursor ratio), was 0.17 for the granular inoculum, indicating a low level of stress, vs. 0.47 found for the suspended inoculum. Cyclopropyl accumulation is normally a response of bacteria to starvation, therefore the cy17:0/16:1ω7c ratio is often used as a starvation or stress indicator of the microbial biomass (Kaur et al., 2005).

3.4. Biogas production efficiency

Cumulative methane productions using the two inoculum types are shown on (Fig. 2). The results show significantly higher methane yields when using granular inoculum: at the final time of the experiment the difference in the ultimate methane production was about 25% in its favor. The dynamics of the digestion process are also different for the two inocula.

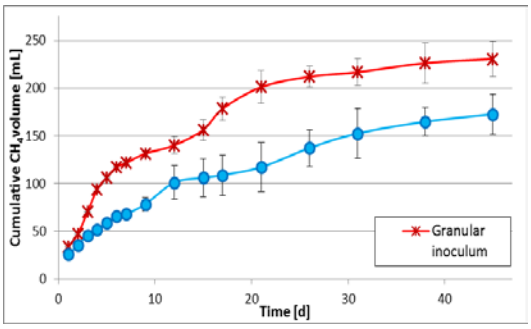

Fig. 2. Cumulative methane productions using the two inoculum types. Final values are 230±22 mL and 173±25 mL of CH_4 for the granular and the suspended inoculum, respectively

The granular inoculum produced large quantities of CH_4 at the beginning, and very little after the 21st day (the methane production curve is almost flat after day 21). In numbers, about 90% of the total CH_4 production took place in the first 21 days of the experiment, and on day 45 (when the experiment has been interrupted) the daily biogas production was less than 1 mL/day. The suspended inoculum showed less intense CH_4 production at the beginning, but its methane production curve does not become flat so fast, arriving to the 90% of the total CH_4 production on day 31. In this case the final daily methane production was about 1 mL/day at the end of the experiment. Noteworthy that the total biogas production, the average methane concentrations of the biogas and the maximum daily methane production rates observed in the probes with the granular inoculum were all higher than in the other case (Table 3).

These data are in concordance with the measured COD consumptions and give specific methane yields of 0.19 and 0.20 L/g $COD_{consumed}$ for the two inocula. Expressed relative to the VS added in form of whole stillage, the specific methane yields are 0.57 L/g VS_{added} (granular) and 0.43 L/g VS_{added} (suspended), comparable to data reported on anaerobic digestion of stillage. Eskicioglu et al. (2011a) reported specific methane yields of 0.4-0.5 L/g VS_{added} for mesophilic, and 0.6 L/g VS_{added} for the thermophilic digestion of this substrate. In this perspective the yields obtained with the granular inoculum are very promising, while those obtained with the suspended inoculum are modest. Note also, that the revealed 25% difference in the methane production translates to a daily quantity of about 40000 m^3 methane for a bioethanol facility of 130 million L/year.

Two important observations can be made on the basis of the above results:

1. The inoculum choice seems to affect not only the start-up of the AD process, but also the ultimate biodegradability of the whole stillage, since differing specific methane yields and COD consumptions were observed at the end of the experiment. This is in agreement with the findings of Pereira et al. (2002), where the studied granular inoculum exhibited higher specific methanogenic activity than the suspended one on a number of different substrates. A possible explanation of this phenomenon in our case might be that the granular inoculum came from a brewery, thus it was supposedly adapted to substrates with composition similar to that of the corn ethanol whole stillage. Long-term digestion tests are required to determine whether the adaptation to the substrate of the suspended inoculum could compensate for the revealed differences. The microbial loads of the inocula (determined by the bacterial counts) and the observed methane yields are not proportional. Despite its lower microbial load (in particular regarding the number of methanogens), the granular inoculum proved to be more efficient in terms of methane production. Possible explanation to this might be given by the limitations of the culture-dependent quantification method. However, also the "quality" of the microbial population (ratios between different microbial groups) may have significant effect on the biomethanation performance.
2. The AD process is a complex chain of biochemical transformations mediated by syntrophic associations of microorganisms, where the balance between microbial groups is very delicate and might easily cause bottlenecking or inhibition of the whole process. As the results of the EL-FAME analysis showed, high levels of SRB have been revealed in the suspended inoculum, and this is a potential reason for low methane yields. Another factor heavily affecting the biogas production is the methanogens-to-acetogens ratio (M/A). Amani et al. (2011) reported critically reduced biogas production at overly high levels of M/A. In our case this would mean, that the M/A of 1:2.3 revealed for the suspended inoculum is too high, while M/A=1:9.9 measured for the granular inoculum is closer to the optimum.

Table 3. Process parameters of whole stillage anaerobic degradation (results represent the mean values of 3 replicates)

Parameter	***Granular inoculum***	***Suspended inoculum***
Final biogas volume [mL]	377	320
Average CH_4 concentration [%]	61	54
Final CH_4 concentration [%]	67	58
Maximum CH_4 production rate [mL/day]	37	28.5
COD reduction [%]	67	61
Specific CH_4 yield [L/g $COD_{consumed}$]	0.19	0.20
Specific CH_4 yield [L/g VS_{added}]	0.57	0.43
Specific CH_4 yield [L/L whole stillage]	38	29

Considering that the bacterial counts and the EL-FAME analysis – besides their indisputable strengths – present limitations in terms of specificity, making possible the classification of the microbial community only into overlapping general groups, a further method of investigation (for example denaturing gradient gel electrophoresis, DGGE) could represent a complementary approach to better describe the microbial community, allowing for a deeper insight into the cause-effect relationships and underlying phenomenology of the AD process of corn whole stillage.

4. Conclusions

Our experiments demonstrate that the choice of inoculum is very important for the anaerobic digestion of the corn ethanol whole stillage. It affects the biogas production rate and seemingly also the biodegradation efficiency of the whole stillage.

In this work the granular inoculum gave considerably higher specific methane yields than the suspended inoculum. The observed 25% difference in the specific methane yields using different inocula justifies the importance of inoculum characterization prior to the start-up of the AD process. Inoculum characterization, however, should not limit to simple bacterial counts.

Our results highlight that the "quality" of the inoculum, the balance between different microbial groups (such as the M/A ratio or SRB level) is just as important as the microbial load of the inoculum. In this sense, additional methods would be needed for a more complete microbial characterization of the inoculum.

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Abbreviations

AD – anaerobic digestion
BAME – bacterial acid methyl ester
CDS – condensed distiller's solubles
COD – chemical oxygen demand
DDGS – dried distillers grains with solubles
DGGE – denaturing gradient gel electrophoresis
DWG – distiller's wet grain
EL-FAME – ester-linked fatty acid methyl esters
FAME – fatty acid methyl ester
FAs – fatty acids
ISR – inoculum-to-substrate ratio
M/A – methanogens-to-acetogens ratio
SCOD – soluble chemical oxygen demand
SRB – sulphate reducing bacteria
TCD – thermal conductivity detector
TKN – total Kjeldahl nitrogen
TS – total solids
UASBR – up-flow anaerobic sludge blanket reactor
VS – volatile solids

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