Effect of Inoculum to Substrate Ratio on the Methane Potential of Microcrystalline Cellulose Production Wastewater

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The methane potential and influence of the inoculum to substrate ratio of wastewater originating from the production of microcrystalline cellulose (MCC) were studied. Laboratory experiments were carried out in a continuously stirred batch multi-reactor at mesophilic temperature (37 °C). Inoculum to substrate ratios (ISRs) of 2.0, 1.0, 0.8, and 0.5 based on volatile solids (VS) were evaluated. The results demonstrate the suitability of MCC wastewater at ISRs of 2.0, 1.0, and 0.8 with ultimate methane potentials of 333, 297, and 325 mL CH_4 per gram of volatile solids added, respectively, which correspond to anaerobic degradabilities of 91.4, 81.7, and 89.3%, respectively, compared to the theoretical potential. The inoculum to substrate ratio of 2.0 provided a faster methane production rate and a kinetic constant of 0.24 d^{-1}, reaching its ultimate yield at day 8 of incubation. The lowest ISR of 0.5 showed the occurrence of process inhibition due to accumulation of acids. Energy estimation suggests that considering the volume and VS of wastewater produced in a MCC mill, a total energy amount of 44,105 GJ/year can be produced, which can be used to replace 29.4% of the natural gas demand.

Keywords: Anaerobic digestion; Biogas; Biochemical methane potential; Inoculum to substrate ratio; Microcrystalline cellulose; Wastewater

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INTRODUCTION

The current global environmental concern focused on the increase in the emission of greenhouse gases has outlined the importance of studying alternative energies. As a result of the negative impacts of production and use of fossil fuels as well as the dependence of the global economy towards them, biogas technology has been one of the proposed energy solutions. Biogas is produced through anaerobic digestion (AD), which is a biological process in which organic material of a substrate is degraded by microorganisms in the absence of oxygen (Angelidaki *et al*. 2003). The result of this degradation is a mixture of methane, carbon dioxide, and some small quantities of H_2S, H_2, and NH_3. The composition of the biogas is dependent on the type of digested material and the operating parameters of the process (Rodriguez 2012).

The pulp and paper industry is one of the largest water consuming industries. It generates relatively large volumes and many types of wastewaters with different pollutants and characteristics depending on the process parameters and end product desired in each mill. Although technological advances have made the pulping process less water consuming, on average a pulp and paper mill will use 13 to 30 m^3 of water per ton of produced paper (Kamali and Khodaparast 2014). Anaerobic digestion of pulping

wastewater and sludge has been evolving to analyze the potential recovery of energy in the form of biogas. This process presents an effective wastewater treatment that produces biogas and moreover minimizes the volume of sludge generated by 30 to 70% (Ekstrand *et al*. 2013). Such an approach can decrease additional problems associated with exponential generation of waste.

Anaerobic digestion helps break down biodegradable organic fraction present in wastewater by turning it into biogas with high methane content, thus having a high energy use and a stabilized final effluent. Hence, anaerobic digestion presents a positive energetic balance, allowing both pollution prevention and recovery of sustainable energy (De Baere 2000). This not only offers the potential use of biogas for heat and electricity, but also allows the possibility of upgrading biogas to biomethane and using it as vehicle fuel.

Biochemical methane potential (BMP) tests are the most used tool to provide a measure of the anaerobic degradability of a given substrate; this is a key parameter for assessing design, economics, and managing issues for the full scale implementation of anaerobic digestion processes (Angelidaki *et al*. 2009). This method is a simple and fast way to determine the suitability of a substrate for anaerobic digestion and the potential methane yield therein resulting in a quantitative measurement of methane production. Different inoculum to substrate ratios can be tested in order to achieve the maximum methane production within the substrate.

Many studies have reported on the methane potential of different substrates such as municipal sludge, food waste, energy crops, and animal slurry (González-Fernández and García-Encina 2009; Rodriguez 2012; Browne and Murphy 2013). However, fewer studies have reported on the methane potential of pulp and paper mill effluents and have concluded on a positive yield of methane ranging from 40 to 60% of the theoretical potential (Bayr and Rintala 2012; Ekstrand *et al*. 2013; Hagelqvist 2013). However, a setback in using AD for pulp and paper mill wastewaters is the fact that these effluents are highly loaded with different toxic compounds that can inhibit the AD process and also have a high amount of lignin and extractives that have low degradability. Therefore, there is a need to select and test different independent effluent streams that have a higher potential or easily degradable organic content that can be utilized to produce high methane volumes.

Microcrystalline cellulose (MCC) is a powder-like cellulose product which has a global market of more than 100,000 tons, with an annual increase of 5% (Ciechanska *et al*. 2010). Typically, MCC mills are equipped to handle relatively small throughputs (less than 10,000 tons/year) using a high amount of acid and low concentrations for cellulose cleavage. Due to these process characteristics, it is not economically viable to utilize released sugars from the process. Therefore, the generated wastewaters are normally led to the municipal wastewater treatment plant after pH neutralization in order to reduce organic load to the river and lake systems. However, Aalto University has developed a new method to produce MCC (Vanhatalo and Dahl 2014) by means of high volume, low acid dose, and high concentration; such an approach can lead to a high sugar content (30 to 80 g/L) in the process wastewater. These process conditions mean that the wastewater originating from the production of AaltoCell™ will have specific qualities that make it a suitable substrate for rapid biogas production.

The aim of this study is to analyze the stream of wastewater generated from the simplified production of MCC by the AaltoCell™ process invented by Aalto University, and also to test the most efficient methane production at different inoculum to substrate ratios. Additionally, the importance of produced methane as a source of energy for the AaltoCell™ process is estimated.

EXPERIMENTAL

Materials

The substrate used for this study was the hydrolyzed filtrate wastewater originating from the production of MCC by mild acid hydrolysis following the AaltoCell™ process, as detailed in earlier studies (Vanhatalo and Dahl 2014; Vanhatalo *et al*. 2014). Substrate sample was stored at –20 °C prior to its use. The original carbohydrate composition of the filtrate wastewater was as follows: arabinose 1.63 g/L, rhamnose 0.004 g/L, galactose 0.86 g/L, glucose 26.79 g/L, xylose 14.93 g/L, and mannose 11.61 g/L. The fresh substrate was analyzed prior to the start of the experiment using average values of triplicates, resulting in total solids (TS %) of 9.31, volatile solids (VS %) of 7.82, moisture content (%) of 90.69, and an initial pH value of 1.75. According to Angelidaki *et al*. (2009), the VS for acidic substrates can be underestimated due to volatile fatty acids (VFA) loss during the analysis of total solids. Therefore before TS and VS analysis, the pH of the substrate was adjusted to 7 using a NaOH 20% solution to decrease volatility of VFA during measurements. The elemental composition of the substrate was C (44.00%), H (4.45%), N (0.15%), and S (2.20%), from which the following empirical formula was determined: $C_{367}H_{445}O_{307}NS_7$.

The inoculum used for the batch tests originated from Suomenoja municipal wastewater treatment plant located in Espoo, Finland. It was taken fresh from their mesophilic anaerobic digester and degassed for 7 d at the same operating temperature (37.0 °C) prior to the start of the experiment. Using average values of triplicates, analysis resulted in total solids (TS%) of 1.7, volatile solids (VS%) of 0.9, and moisture content (%) of 98.3. A pH value of 7.6 was measured, with a total alkalinity (TA) of 6.8 g $CaCO_3$/L.

Methods

Experimental design

Experiments were carried out in an automatic methane potential test system (AMPTS), which is a laboratory scale batch methane potential analyzer developed for automatic real-time logging and measuring of methane production (Rodriguez 2012; Badshah *et al.* 2012; Browne and Murphy 2013; Browne *et al.* 2013). Measurements are expressed using the same unit for conventional BMP test found in literature, normalized mL of methane per gram of volatile solids added (N mL CH_4/gVS). It has a capacity for incubating 15 reactors of 500 mL each with an individual mixing motor and a defined carbon dioxide removal step in order to provide only methane yield.

Four different ISRs based on VS% were evaluated: 2.0, 1.0, 0.8, and 0.5. All sample tests were prepared in triplicates for statistical significance. The BMP tests were carried out using a working volume of 400 mL. In order to achieve the desired ISRs based on VS content, the volumes of inoculum and substrate were calculated (Table 1). Triplicate blank samples with no substrate were run to determine the produced background methane originating from the inoculum alone. Triplicate control samples containing Avicel® PH-101 pure cellulose (Sigma-Aldrich, USA) were also run to verify inoculum activity. Finally, to increase buffering capacity throughout the experiment, 4 g/L of sodium hydrogen carbonate ($NaHCO_3$) was added to each sample reactor at the beginning of the experiment (zero time) as suggested by Abdulkarim and Evuti (2010) and Raposo *et al*. (2011). After filling each reactor, bottles where sealed with a hermetic rubber stopper connected to a mechanical agitator and placed in a water incubator at 37.0 ± 0.5 °C.

Table 1. Substrate and Inoculum Volume Used for Each Reactor

Inoculum to substrate ratio (ISR)	Inoculum amount (mL)	Inoculum VS load (gVS)	Substrate amount (mL)	Substrate VS load (gVS)	Total volume (mL)	Total load (gVS)
2.0	378	3.41	22	1.71	400	5.12
1.0	359	3.24	41	3.24	400	6.64
0.8	350	3.16	50	3.95	400	7.11
0.5	308	3.17	92	6.34	400	9.51

To chemically remove carbon dioxide (CO_2) and hydrogen sulfide (H_2S) formed during anaerobic digestion, each reactor was individually connected to another small bottle containing 80 mL of an alkali solution of 3 M NaOH. Thymolphthalein pH indicator was added to each bottle to determine when the solution has been spent and needed replacement. Each alkali solution bottle was then connected to the measuring device and finally all reactors were flushed with pure nitrogen gas (N_2) for 5 min, to ensure anaerobic conditions.

BMP calculation

Results from the BMP tests were used to evaluate the anaerobic digestion rate and profile of the substrate in respect to different ISR over time. The methane potential was calculated as the accumulated methane produced per gram of VS added to each reactor, as determined in Eq. (1) (Strömberg *et al*. 2014),

$$BMP = \frac{V_{sample} - V_{inoc}\frac{gVS\ is}{gVS\ ib}}{gVS_{substrate}} \quad (1)$$

where *BMP* is the normalized volume produced per gram VS of substrate added ($mLCH_4/gVS$), V_{sample} is the mean value of accumulated methane produced from the reactor with inoculum and substrate mixed, V_{inoc} is the mean value of the accumulated volume produced by the blanks, gVS_{is} is the mass of volatile solids of the inoculum added in the sample, gVS_{ib} is the mass of volatile solids of the inoculum added in the blanks, and $gVS_{substrate}$ is the mass of volatile solids added of the substrate in the reactor.

Anaerobic degradability

The anaerobic degradability of a given substrate can be expressed by product formation (methane production). The anaerobic degradability achieved for each ISR tested is defined as the ratio between the experimental methane yield (M_{exp}) and the maximum theoretical potential (M_{th}) expressed as a percentage (Eq. 2) (Raposo *et al*. 2011):

$$AD\ (\%) = (M_{exp} / M_{th}) * 100 \quad (2)$$

Given the determined empirical formula for the substrate ($C_{367}H_{445}O_{307}NS_7$), by application of the Buswell equation (Eq. 3), the maximum theoretical potential (M_{th}) of methane formation can be calculated (Symons and Buswell 1933; Buswell and Mueller 1952):

$$C_nH_aO_bN_c + \left(n - \frac{a}{4} - \frac{b}{2} + \frac{3c}{4}\right)H_2O \rightarrow \left(\frac{n}{2} + \frac{a}{8} - \frac{b}{4} - \frac{3c}{8}\right)CH_4 + \left(\frac{n}{2} - \frac{a}{8} + \frac{b}{4} + \frac{3c}{8}\right)CO_2 + cNH_3 \quad (3)$$

Analytical methods

Total solids (TS), volatile solids (VS), and moisture content in fresh samples of substrate and inoculum were determined gravimetrically following standard methods described in APHA (2005). Total alkalinity (TA) to pH 4.5 was measured by Standard Method 2320 B. The pH measurements were performed using a pH meter model Thermo Scientific Orion 2-star (Singapore).

Methane production was measured using the AMPTS II from Bioprocess Control AB, Sweden (System Version 2.0 V1.08), which works by the principle of liquid displacement and buoyancy. Volumes of gas are corrected to standard temperature and pressure (STP) conditions at 273 K and 1013 mbar air pressure.

Elemental analysis of the substrate for C, H, N, and S was determined by duplicate samples using a Perkin Elmer Model 2400 Series II CHNS Elemental Analyzer (USA).

RESULTS AND DISCUSSION

Methane Potential

The ultimate methane production and flow rate from the substrate at ISRs of 2.0, 1.0, 0.8, and 0.5 were obtained. Methane production was monitored at a temperature of 37.0 ± 0.5 ºC for 21 d, which resembled time after which the methane production curves reached a plateau stage. The accumulated methane production of the different inoculum to substrate ratios tested is shown in Fig. 1. Daily methane production in each sample bottle was corrected by subtraction of the average background gas formed in the blank samples.

The values of cumulative methane production at STP, pH, and total alkalinity values after digestions are expressed in Table 2.

Table 2. Sample Characterization after Digestion

Inoculum to Substrate ratio (ISR)	Final VS (gVS)	Ultimate methane yield (mL CH_4/gVS)	Final pH	Final alkalinity (g $CaCO_3$/L)	Final conductivity (mS/cm)
2.0	2.00	332.9	7.6	7.9	11.5
1.0	2.69	297.4	7.8	8.7	12.0
0.8	2.60	325.0	7.8	8.9	12.1
0.5	5.19	59.4	5.6	3.1	16.7
All values are mean averages of triplicate samples					

For an ISR of 0.5, one can observe an accumulated methane yield of 59.4 mL/gVS, which is significantly lower than the other ISRs tested and implies a serious process imbalance and a severe inhibition of methanogenesis. Methane production ceased during day 5. This is mostly attributed to the excessive acid formation originating from the high initial load of easily available sugars in the substrate; this would in turn decrease the pH in

the reactor, which is validated with the final pH value of 5.6 taken from the ISR 0.5 samples after the digestion experiment. The same behavior was also observed with sugarcane bagasse (Badshah *et al*. 2012). The pH value of the reactor is usually controlled by a bicarbonate buffer, which is formed by the reaction of carbon dioxide from the biogas with the natural mineral alkalinity of the wastewater.

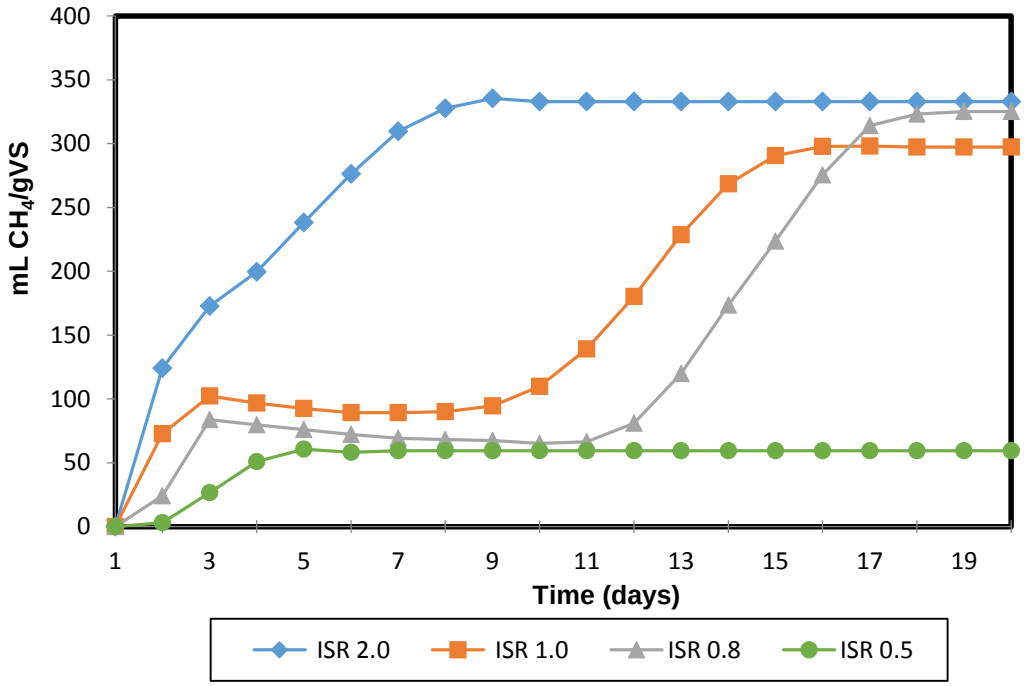

Fig. 1. Accumulated methane production at different inoculum to substrate ratios expressed as a function of time

Accumulations of acetic or other volatile acids tend to neutralize this mineral alkalinity, causing the pH value to fall from about 7.5 to about 6.5 which releases dissolved carbon dioxide (bicarbonate) back into the biogas. In severe cases, where the concentration of organic acids exceeds the concentration of mineral alkali, the pH value will fall to around 5.5 and be controlled by an acetate (or mixed volatile acid) buffer (McCarty and Mosey 1991); this is the case for the samples of the ISR of 0.5, which have a lower alkalinity and pH measurement than the rest of samples. Similar results were reported by Eskicioglu and Ghorbani (2011), where at a high organic load rate (0.46 ISR), reactors experienced volatile fatty acid accumulation and pH decrease.

The highest value of methane yield corresponds to the ISR of 2.0, with an ultimate yield of 332.9 mL/gVS, followed closely by ISRs 0.8 and 1.0, with values of 325.0 and 297.4 mL/gVS, respectively. From the ISR 2.0 curve, one can see the rapid methane production in a continuous semi-linear manner until day 8, after which it reaches a plateau stage corresponding to its ultimate yield. Both ISRs 1.0 and 0.8 curves have a sigmoid behavior where a lag phase is observed from day 4 to day 10 before reaching a plateau; this may be due to the accumulation of VFA during the first days. However, within this lag phase, the bicarbonate buffering formed in each reactor is sufficient to neutralize the acid formation and reestablish a neutral pH environment. After day 10 both curves showed a rapid methane production until they reached their ultimate yield at day 18. This lag phase can be called an acclimation period and is observed in substrates with inhibiting or toxic

compounds and substrates that create a stress situation such as high organic loadings (Meyer and Edwards 2014). Before reaching a certain threshold, the microbial community can become tolerant to the chemical or physical stressor and adapt to metabolize the substrates. Similar acclimation periods were observed in Wiegant and Lettinga (1985), where easily degradable substrates such as glucose, sucrose, and volatile fatty acids experienced a lag phase during the first 8 to 9 days of digestion and after this period produced a significant amount of methane.

Cellulose control samples reached their ultimate yield of 315.0 mL/gVS on day 10, which can be compared to the maximum theoretical methane value of 414.0 mL/gVS calculated using the Buswell equation (Buswell and Mueller 1952); this ensures that the inoculum used to seed the reactors has the sufficient bacteria consortium to efficiently perform the anaerobic digestion process.

Methane Production Rate (MPR)

The methane production rate was calculated using the daily values of methane volume produced and applying the same formula from Eq. 1. Results (Fig. 2) show that for ISRs of 1.0 and 0.8, a similar pattern can be seen where there is an increased MPR over the first 4 days followed by a significant drop in MPR through days 4 to 10, and again another increase in MPR over days 11 to 17 followed by a gradual decrease towards a basal level. The reduced methane production from day 4 to 10 corresponds to the acclimation period described in the previous section. This means an estimated hydraulic retention time (HRT) of 18 to 19 days for ISRs of 1.0 and 0.8 when applied in a continuously fed system.

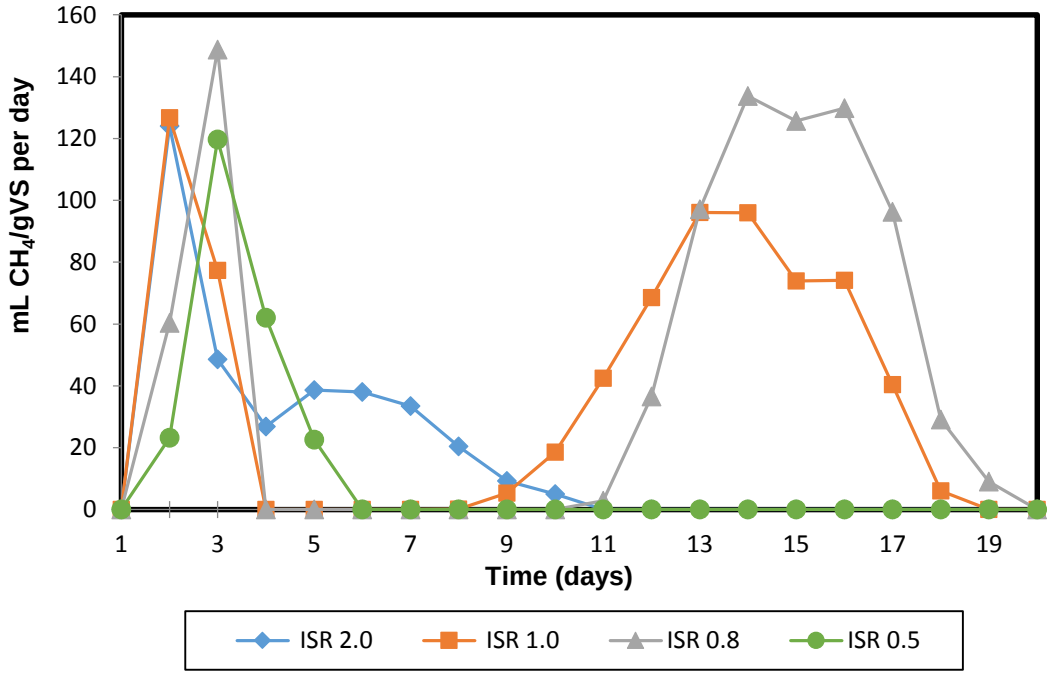

Fig. 2. Evolution of daily methane production rates for each inoculum to substrate ratio tested

The curve for ISR 2.0 shows an initial high MPR from the beginning peaking at day 3, which later slowly decreased and which finally ceased at day 11. This indicates a HRT of 8 to 11 days. Studies in pulp and paper mill residues (Elliott and Mahmood 2007; Bayr *et al.* 2013) state how these residues have long HRT of 20 to 30 days, making them

less popular for anaerobic digestion. However several pretreatment methods which include microwave, ultrasonic, and hydrothermal were shown to reduce the HRT by 50% (10 to 15 days). Results in the present study show the efficiency of the digestion system at a shorter HRT of 8 to 11 days without the need of pretreatment, which provides higher techno-economic advantages such as reduced costs in pretreatments and smaller digester volumes.

The ISR 0.5 curve, which presented severe inhibition from day 5, exhibited the lowest peak in MPR. A tendency observed was that the lower the ISR, the longer time span over which methane was being produced. Also, the higher the ISR, the earlier the MPR peaks were observed. This may be due to the higher microbial activity present in the larger amount of inoculum used for higher ISRs. Increasing the ISR increases the amount of active methanogens in the inoculum, which moreover decreases the time required for the growth of sufficient methanogenic population to initiate methanogenic activity.

Alkalinity measurement was taken after digestion (Table 2) for all samples, with the exception of ISR 0.5, which ranged from 7.9 to 8.9 expressed as g $CaCO_3$/L. This exceeds the average buffering capacity needed to provide resistance to significant and rapid changes in pH (Raposo *et al.* 2011). However, the lag phase observed in curves of ISRs 1.0 and 0.8 can also be attributed to a slight drop in the pH due to VFA accumulation, and converting carbon dioxide to bicarbonate will require a time lag for gas equilibrium to occur.

Many authors (Chudoba *et al.* 1991; Neves *et al.* 2004; Raposo *et al*. 2006) clearly state that the ISR used is one of the most important parameters in batch tests. Previous research shows that the use of high ISR (2 to 4) is recommended because it ensures microbial activity, and there is low risk of overloading and inhibition because the substrate is more dilute. Similar results are found in Raposo *et al*. (2006) which reported that at different ISRs, the ultimate methane yields had slight variations; however a ISR of 1 resulted in higher maximum specific methane production rate.

Anaerobic Degradability

Knowing the empirical formula of the substrate utilized throughout the experiments and applying the Buswell equation (Buswell and Mueller 1952), the maximum theoretical methane production (M_{th}) was calculated giving 364 mL of CH_4/gVS. By applying the formula previously indicated (Eq. 3), the anaerobic degradation of the samples for each of the ISR tested was determined.

The highest degradability rate was for ISR 2.0, reaching 91.5%, followed closely by ISR 0.8, with 89.3%, and ISR 1.0, with 81.7% degradability. As previously stated, ISR 0.5 had an inhibition process and resulted in a low anaerobic degradability of 16.3%.

During anaerobic digestion, the organic fraction of the substrate is mostly converted to methane and carbon dioxide. However, other fractions are converted to sulfide by sulfate reducing bacteria, which compete with methanogens (Chynoweth *et al*. 2001), and it is estimated that about 5 to 15% of the organic fraction removed is used in the generation of new biomass (Owens and Chynoweth 1993). Therefore, an anaerobic degradability higher than 85% may be considered to be in the maximum range and implies a high degradable substrate/system for methane production. In this study, samples for ISRs of 2.0, 1.0, and 0.8 represent an efficient anaerobic degradation profile, and samples of ISR 0.5 are considered outliers.

The interlaboratory study performed by Raposo *et al.* (2011) describes the anaerobic degradability of substrates with high volatile solids content that are naturally biodegradable (starch, cellulose, gelatine and mung bean). Their results demonstrate the

high anaerobic degradability achieved that ranges from 85 to 88% and assumes that the substrates can be fully degraded and hence the average amount of organic matter used for cell metabolism and new cells calculated by subtraction was around 12 to 15%. A similar behavior was found with the substrate in this study. Due to the specific production parameters of the AaltoCell™ process, the resulting wastewater was partially hydrolyzed, had a low sulfur content, and had a high content of naturally biodegradable sugars ranging from 30 to 80 g/L. Such a mixture led to a high anaerobic degradability and methane production.

However, comparing these results with other effluents from the pulp and paper industry, one can observe a vast difference. Ekstrand *et al.* (2013) carried out research with 62 samples of wastewater from 10 different processes in pulp and paper mills, from which only 19% of samples reached a degradability ranging from 50 to 65%. The reduced degradability may be attributed to the high content of lignin and sulfur compounds that are dissolved during the typical pulping processes into the wastewater. The results found in the present study show the potential and suitability for enhanced and rapid methane production of this particular MCC wastewater over other studied pulping effluents.

Kinetic Evaluation

Specific methane production is often modelled using the first-order kinetic model, a simple and useful model that has been commonly applied to anaerobic digestion systems (Raposo *et al*. 2011) and follows Eq. 4,

$$Y = Ym\ (1 - e^{-kt}) \quad (4)$$

where Y is the cumulative methane yield at time *t*, mL CH_4/gVS added; Ym is the ultimate methane yield, mL CH_4/gVS added; and *k* is the first-order rate constant, d^{-1}. The parameters Ym and *k* may be estimated using a nonlinear regression fit to the experimental yield data of a triplicate set.

This model however is recommended when hydrolysis is the rate limiting step in the anaerobic digestion process and there is an assumption that there is no accumulation of intermediate products such as VFA in the system (Veeken and Hamelers 1999; Yu *et al.* 2013). In this study, samples of ISR 0.5 showed total inhibition and therefore were considered as an outlier and not taken into further consideration. Curves of ISR 1.0 and 0.8 showed a lag phase, which is assumed to be caused by the slight accumulation of VFA; hence, this model does not present the best fit for the experimental data of ISR 1.0 and 0.8, generating very low rate constants of 0.0015 and 0.0007 d^{-1}, respectively.

For ISR 2.0, the experimental data showed an adequate fit to the model (Fig. 3), and by nonlinear regression using the Microsoft Solver tool, a rate constant of 0.24 d^{-1} was calculated. This rate constant is consistent with other studies using the same ISR of 2.0 for glucose-based substrates that rapidly degrade, such as starch and cellulose (Raposo *et al*. 2011).

The same substrate used in this study has not been tested in previous literature. For the MCC wastewater substrate, the kinetic rate constant was clearly affected by the ISR. Lower ISR (1 to 0.5), meaning a higher substrate concentration, resulted in lower rate constants and did not follow the first-order kinetic model.

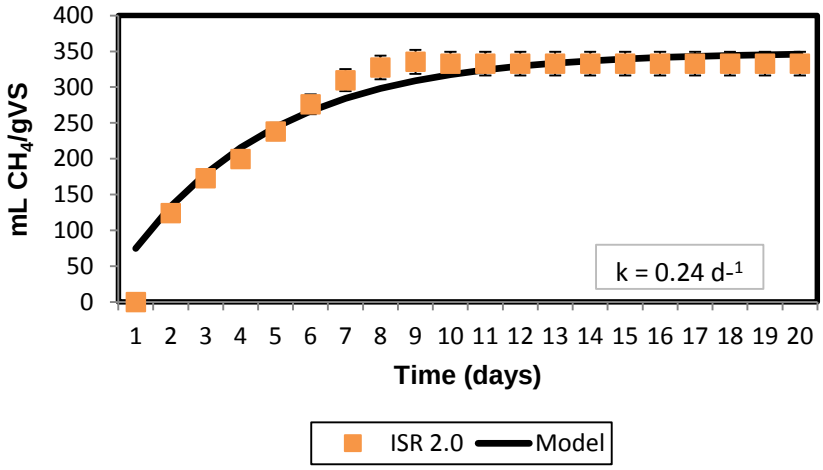

Fig. 3. Experimental data obtained from ISR 2.0 fit to the first-order kinetic model

Energy Estimation for the AaltoCell™ Process

As estimated by Vanhatalo *et al*. (2014), the MCC production process generates a total amount of 62,778 tons/year of wastewater, of which 3333 tons/year are dissolved sugars. Therefore, one can assume that there is a total weight of 3333 tonVS available for anaerobic digestion. The experimental BMP test results show that the ultimate methane potential using an ISR of 2.0 is 333 mL CH_4/gVS, which is equivalent to 333 m^3 CH_4/tonVS; therefore, an estimated amount of 1,109,889 m^3 of methane can be produced per year. Considering the higher heating value (HHV) of methane (55.5 MJ/kg) and its density at STP (0.716 kg/ m^3), the calculated amount of energy that can be generated in the MCC mill is 44,105 GJ/year.

The total amount of natural gas used per year in the MCC AaltoCell™ process is 5 GJ/BDt (Bone Dry tons); considering a mill capacity of 30,000 BDt of MCC per year (Vanhatalo *et al*. 2014), the annual natural gas demand amounts to 150,000 GJ/year. Therefore, the produced methane from the anaerobic digestion of the MCC wastewater can be used to replace 29.4% of the natural gas demand in a MCC mill.

A general theoretical calculation based on the sugar content of the wastewater can be estimated. Having a generated amount of 3,333,000 kg of sugars and knowing that 1 g of glucose is equivalent to 16,720 J, one can calculate a potential of 55,728 GJ/year. Another way of estimation is based on the theoretical yield of a molecule of glucose (Buswell equation), which renders a methane potential of 418 mL CH_4 per gram of glucose. This means 3333 tons of sugars produces 1,393,194 m^3, corresponding to 55,363 GJ/year.

A more specific calculation of the theoretical energy production is by application of the Buswell equation (Buswell and Mueller 1952) to the chemical formula of the wastewater determined by element analysis. As described in the "Anaerobic Degradability" section, the theoretical methane potential of the substrate wastewater was 364 mL of CH_4/gVS. This would therefore produce 1,213,212 m^3 of methane, which can potentially generate 48,211 GJ/year of energy.

The low deviations obtained between the theoretical and experimental energy yields (equal or lower to 21%) suggest that the proposed model in this study could effectively predict the energy production obtained in a reactor. These results could lead to a pilot scale operation that can be translated into industrial application and pave the way for a process change in wastewater treatment for pulp and paper mills. Benefiting from an

energy recovery of 29.4% of the total energy consumption of a MCC mill would mean economic benefits and lower carbon footprint. Moreover, the application of anaerobic digestion can reduce costs incurred in the treatment of the effluents, where there is less sludge produced, lower retention time, and overall less costs for waste management.

CONCLUSIONS

1. Results of this study demonstrated success of an effective batch mesophilic anaerobic digestion of wastewaters originating from the production of MCC at ISR 2.0, 1.0, and 0.8. Ultimate methane yields of ISR 2.0, 1.0, and 0.8 after 21 d of incubation showed slight variations with values of 332.9, 297.4, and 325 mL CH_4/gVS, respectively. However, methane production rate curves indicate that ISR 2.0 achieved its ultimate methane yield in 44% of the degradation time of ISR 1.0 and 0.8.
2. The ISR of 0.5 showed a process imbalance due to accumulation of acids originating from the higher concentration of dissolved sugars in the substrate and causing a complete cessation of methane production after day 5 and a final pH value of 5.6.
3. Both curves of methane potential and MPR as well as kinetic evaluations suggest that an ISR of 2.0 is the adequate to achieve an efficient maximum methane yield with a high degradability of 91.5%. The amount of inoculum is then sufficient to have the suitable methanogen that are able to rapidly transform the organic acids into methane and create an equilibrium in production and consumption of intermediate products.
4. The ISR plays a critical element in the BMP test, and working with high ISR is the way to obtain a reproducible kinetic constant.
5. Considering the volume and VS content of wastewater produced in a MCC mill using the AaltoCell™ process, by anaerobic digestion of their wastewater a total energy amount of 44,105 GJ/year can be produced, which can be used to replace 29.4% of the natural gas demand.

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