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EFFECTS OF INOCULUM SOURCE ON THE BIOCHEMICAL METHANE POTENTIAL (BMP) OF THE ORGANIC FRACTION OF MUNICIPAL SOLID WASTE IN SOLID-PHASE BMP ASSAYS

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Abstract: The source and type of Inoculum plays an important role in BMP assays through adaptation to substrate and provision of the required microbial consortia required to effectively degrade landfilled municipal solid waste (LMSW). While biosolids from wastewater treatment plants have been the preferred choice for use in conventional BMP assays, studies have shown a potential for this source to be misrepresentative of the consortia of microbes required for the effective degradation of LMSW. Also, the conventional BMP assays often misrepresent typical landfill conditions in terms of moisture content and sample size. This study investigated the effects of inoculum source on methane generation of LMSW by comparing the performance of biosolids and a laboratory derived inoculum (LDI) using; a synthetic waste representing the organic fraction of municipal solid waste in solid-phase BMP conditions and cellulose in conventional slurry phase conditions respectively. Results showed a statistically significant superior performance of the LDI over biosolids in terms of methane generation potential, L_0 , and lag-phases for both slurry-based and solid-based moisture conditions. However, the difference in rate of methane production (R_m) between both inocula was found to be statically insignificant when degrading cellulose only. The highest coefficient of variation between duplicates was found to be 23% indicating good repeatability of methods used and validity of results obtained.

1 INTRODUCTION

Biodegradation of organic waste in landfills produces landfill gas consisting primarily of carbon dioxide (CO_2) and methane (CH_4). In Canada, landfills are estimated to release 27 million tonnes of CO_2eq which is about 25% of anthropogenic methane emissions (Climate Change 2012). Fugitive emission of CH_4 into the environment poses potential climate change impacts due its global warming potential being 34 times greater than that of CO_2 (IPCC 2013). However, to avoid such environmental impacts, CH_4 can be harnessed as a clean renewable source of energy if volumes are high enough, or fugitive emissions could be mitigated by oxidizing the CH_4 gas to CO_2 through bio-mitigation techniques such as landfill bio covers (Majdinasab and Yuan 2017). The need to accurately predict the amount of CH_4 generated from landfills cannot be over-emphasized. Be it for decisions on exploitation and profitability by landfill operators and investors, for regulatory compliance, or for carbon credit projects, the amount of methane available for recovery or emitted from landfills is key. Typically, models based on first-order kinetics that require input parameters such as the first-order rate coefficient (k ; year^{-1}) and methane generation potential (L_0 ; $\text{m}^3 \text{CH}_4/\text{tonne}$ of waste) are adopted for this purpose (Krause et al. 2016). These parameters are usually

obtained from published data (Eggleston et al. 2006), theoretical or experimental means, of which the slurry-phase biochemical methane potential (BMP) assay is widely used.

The biochemical methane potential (BMP) assay is a laboratory-based method, where a sample of waste is incubated under controlled conditions and the cumulative methane gas generation over a period is observed (Owen et al. 1979). Nevertheless, the BMP assay for landfilled municipal solid waste (LMSW) is still a challenge because of its heterogeneous nature. Key factors such as moisture content, sample size and type of inoculum that affect CH₄ gas production in these conventional assays have been reported to be potentially misrepresentative of natural biodegradation conditions and consortia of microbes found in a landfill (Pearse et al. 2018).

The source of inoculum used in BMP assays plays a vital role in overall BMP results (Elbeshbishy et al. 2012). Ideally, the inoculum used should contain representative bacteria specifically required to promote biodegradation of LMSW and production of maximum amount of CH₄ gas. Most conventional BMP assays use digested sewage sludge from anaerobic digesters treating municipal sewage sludge, however, previous studies have shown inoculum obtained from LMSW degradation to be more suitable and optimal than digested sewage sludge as inoculum for assays with LMSW as substrate (Wakadikar et al. 2013). In addition to using digested sewage sludge, these conventional BMP assays use a slurry-based approach which simulates an environment that is predominantly liquid versus a predominance of solids in a landfill. The serum bottles used, contain mostly a liquid content, therefore misrepresenting the higher solids content found in a landfill (Karanjekar 2013, Weaver 2013). Conventional assays also use sample sizes as little as 0.2g that could decrease replicability of results, lead to difficulties quantifying gas production and misrepresent landfill waste composition. To address these shortcomings of conventional BMP methods and improve on its representation of natural landfilled conditions, a high-solids modified assay termed the landfilled biochemical methane potential (LBMP) was proposed (Pearse et al. 2018).

The objective of this study was to determine the effects of inoculum source on cumulative methane generation potential L_0 of LMSW using the LBMP approach. To meet the study objectives, firstly, the performance of a laboratory derived inoculum was compared against digested sewage sludge from a wastewater treatment plant in slurry-based degradation conditions using a known substrate (microcrystalline cellulose) and a sample size larger than those commonly found in literature. Secondly, the performance of both inocula was compared in a solid-phase LBMP assay. For consistency between experiments and to determine replicability of the BMP methods, synthetic waste (of a known composition) simulating the organic fraction of municipal solid waste (OFMSW) composition was used.

2 MATERIALS AND METHODS

2.1 Materials and Characterisation Results

Two types of inocula were used in this study; inoculum in the form of digested sewage sludge obtained from the Bonnybrooks wastewater treatment plant in Calgary, which treats about 500,000 cubic meters of wastewater per day. The laboratory-derived inoculum (LDI) was obtained from a mixture of OFMSW inoculated with biosolids that had been allowed to degrade for over a period of 100 days in a previous BMP experiment.

Both inocula were first compared using microcrystalline cellulose (Thermo Fisher Scientific, CAAAA17730-36); a substrate with known biodegradability and then with synthetic waste representing the organic fraction (i.e., food, yard waste & paper) of residential MSW (OFMSW) in Calgary. Paper waste consisted of corrugated cardboard, used office paper, newsprint and magazines. Office paper and corrugated cardboard wastes were sourced from blue recycling bins on the University of Calgary (UofC) campus while newsprints and magazines were sourced from free publication stands in the UofC. To maintain consistency of samples, equal proportions of various cardboard (thickness of 0.3mm), magazines and newsprints available were collected. Yard waste consisted of tree branches, grass clippings and leaves. Clippings were obtained from mowing activities on the UofC campus, leaves and branches were sourced from a composting site on the UofC campus. Leaves and grass clippings samples were still green and obtained before the Fall season. Typical representative food waste from Canadian researchers (Ara et al. 2014, Shahriari et al. 2012) consisting of cooked white rice, cooked pasta, carrot, apple, banana peel, corned beef, dog food and

cabbage were used. All items were obtained fresh from a grocery store and prepared prior to the experiment. To minimize variations in samples, items were sourced from the same store and same produce supplier. Prior to use in the experiment, the waste was characterized to determine potential inhibition of methane production, elemental composition, and the inoculum was characterised to determine its quality and suitability for use in BMP experiments. All physical and chemical parameters were analysed using existing standard methods. Moisture content (MC) values of $46.27 \pm 0.04\%$, $79.73 \pm 0.03\%$ and $88.85 \pm 0.08\%$ were obtained for waste, biosolids and LDI respectively. The volatile solids (VS) content of the waste was $88.87 \pm 0.49\%$, while that of the biosolids and LDI were $66.87 \pm 0.05\%$ and $67.39 \pm 1.39\%$ respectively. The pH and C/N ratio for the waste was 6.82 ± 0.33 and 33 respectively, for the biosolids, the values were 7.54 ± 0.10 and 6 respectively while that of the LDI was 7.20 ± 0.40 and 12 respectively.

2.2 Experimental Design and Experimental Methods

Batch experiments were conducted in duplicates. A one-factor-at-a-time (OFAT) design was used due to its simplicity. Other factors considered in the experiment include; temperature (35°C), particle sizes of waste ($< 10\text{ mm}$), inoculum to substrate ratio (0.4 on VS basis) and initial pH of waste and inoculum maintained between 7.0 - 8.5 as shown in Table 1. The coefficient of variation (CV) was used to determine the variability between duplicate results. Controls were also run for each treatment with moisture and inoculum only, to determine background contribution of the inoculum to the overall CH_4 production.

250 mL Wheaton borosilicate glass bottles were used as incubation, biogas and displaced water collection bottles. These bottles were capped with a Wheaton phenolic screw cap, open top, sealed with a butyl septum. The biogas collection bottles were initially filled with a saturated barrier solution (DI water containing 36.7% NaCl) to prevent the dissolution of CO_2 prior to gas volume measurements. Before placing bottles in the incubators, the headspace of the bottles was sparged and flushed with a mixture of 80% N_2 and 20% CO_2 gas until O_2 concentrations in the bottles were less than 1% and the headspace achieved anaerobic conditions.

The produced biogas was collected and measured using the principle of water displacement as shown in Figure 1. Headspace samples were obtained from reactors and analysed using an SRI low thermal conductivity detector (TCD) gas chromatograph equipped with two columns; molecular sieve (MoleSieve 13x) and a HayeSep-D. The column oven temperature was ramped from 50°C and 140°C and helium was used as the carrier gas. The gas chromatograph used a sample size of 5mL, and a peak simple software was used to analyse the gas samples after a run time of 12mins. Peer reviewed and widely applied BMP protocols by Holliger et al. (2016) and Angelidaki et al. (2009) were adopted with some modifications to inoculum source, sample size/reactors and moisture conditions to fit the purpose of this study. The cumulative methane production from the experiment was converted to STP dry conditions of 0°C and 100 kPa (accounting for water vapor pressure) and normalized per VS using Equation 1. Headspace volumes were not taken into consideration since the entire headspace of each reactor was replaced with flush gas.

$$[1] \quad B_T = \frac{\sum_{t=0}^{t=T} V_t G_t - \sum_{t=0}^{t=T} V_{Dt} G_{Dt}}{VS}$$

where B_T = BMP at time T; V_t = Volume of gas displaced at time t; G_t = Fraction of CH_4 recorded in reactor at time t; V_{Dt} = Volume of gas displaced at time t in control reactors; G_{Dt} = Fraction of CH_4 recorded in control reactors at time t; VS = Mass of volatile solids.

2.3 Data Analysis

The final BMP reading from the experiment was taken as the biochemical methane potential for the samples. The modified Gompertz equation (Equation 2), which is commonly used to model batch methanogenic data in anaerobic digestion studies (Hobbs et al. 2018, Moset et al. 2015), was used to fit the data using the numerical computing program, MATLAB R2018b (MathWorks Inc).

[2]

$$B_t = P \times \exp\left(\left(-\exp\frac{R_m \times e}{P} (\lambda - t) + 1\right)\right)$$

where B_t = cumulative methane production at time t (mL CH₄/gVS); P = Methane production potential (ml CH₄/gVS); R_m = specific rate constant (mL CH₄/gVS/d); λ = lag phase time (d); e = mathematical constant, 2.71828; t = time in days.

Table 1: Experimental design; variables and levels

Factor	Description	Value
Response/Dependent	Methane gas production	
Control/ Fixed	Temperature	35 °C (+/- 2 degrees)
	pH (initial)	7.0 - 8.5
	Particle size	< 10 mm
	Waste (g)	10
	Inoculum ISR	0.4
Manipulated/Independent (OFAT)	Treatment	Moisture condition
	Biosolids + Waste	Solid-phase
	LDI + Waste	Solid-phase
	Biosolids + Cellulose	Slurry-phase
	LDI + Cellulose	Slurry-phase

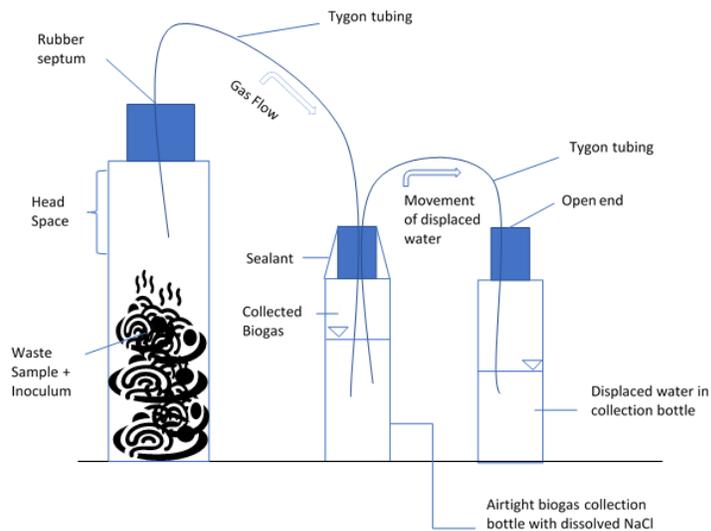


Figure 1: Schematic of experimental set up

Student t - tests were used to evaluate difference between means of two independent groups on the continuous dependent variable cumulative CH₄ production. Regression analysis was conducted for the fitted curves to validate the models. A high (close to 1) coefficient of determination, R² and adjusted R², indicated a strong model and better prediction of the response.

3 Results and Discussion

3.1 The Performance of LDI Over Biosolids Using Microcrystalline Cellulose

Figure 2 and Figure 3 show the methane gas concentration profiles and time dependent methane yield after 133 days of incubation. The initial aim was to run the experiments until gas production ceased or increased by 1% or less over 3 consecutive days. However, after 133 days of running, gas production still occurred, albeit at a very slow rate, and the experiment was stopped. A significant difference in CH₄ composition in biogas was observed between the biosolids and LDI inoculated samples. Initially, both treatments had a short lag phase but as incubation continued, the treatment with biosolids experienced some toxic effects depicted by the fall and sudden rise again in CH₄ concentration. From the characterisation results, it was observed that the biosolids inoculum had more nitrogen than the LDI, which may be a possible cause of the toxic effects experienced through ammonia inhibition of the treatment. When ammonia is produced in the system it has been shown to affect the activities of methanogenic bacteria and thus would lead to reduced amount of CH₄ in the system (Akindele and Sartaj 2018), such as, that shown in Figure 2.

An accumulation of a burnt yellow like substance on the valves and tubes of a biogas collection bottle possibly associated with the accumulation of ammonia in the headspace was observed. According to research done by (Akindele and Sartaj 2018), CH₄ producing microbes are able to acclimatize to ammonia concentrations and thus CH₄ concentration began to pick up again in the reactors using biosolids as the inoculum. At the end of 133 days, both reactors produced similar concentrations of CH₄ in the biogas (> 50%). In the cumulative CH₄ production graph in Figure 3, it can be seen that the toxic effects experienced in the treatments with biosolids had a ripple effect on cumulative CH₄ yield; a long lag phase depicted by the convex shape of the cumulative CH₄ curve. The treatments with LDI showed a shorter lag phase and steady rise in CH₄ production until reactors were stopped, revealing no signs of inhibition in the reactors. Both inocula continued to produce CH₄ gas with the treatment with biosolids reaching that of LDI.

The cumulative CH₄ yield curves were modelled using the modified Gompertz equation, and the obtained model parameters P, R_m and λ from the fitted curves are shown in Table 2. There was a significant difference (p-value < 0.05) between the ability of biosolids and LDI to degrade cellulose. The methane production potential (P) of LDI (309 mLCH₄/gVS cellulose) was higher than that of biosolids (254.2 mLCH₄/gVS cellulose). Both CH₄ potentials fell within the range of values (175 to 412 mLCH₄/gVS cellulose) found in literature but a widely accepted range for the degradation of cellulose has been found to be between 303 to 412 mLCH₄/gVS cellulose, i.e., P values greater than 70% of the theoretical CH₄ yield of cellulose (415 mLCH₄/gVS cellulose) (Grosser 2018, Raposo et al. 2011). Using this criterion, The P values from using biosolids fall short and can be said to be less adapted to cellulose and might require a longer incubation period. This result agrees with previous findings and reiterates the opinion that inoculum sourced from WWTPs might not be optimal for all types of substrates (Wakadikar et al. 2013, Moset et al. 2015). To further validate this possibility, Moset et al. (2015) through their study, observed that interaction effects exists between source of inoculum and substrate indicating that inoculum may behave differently from one substrate to the next and it is therefore necessary to find an optimal inoculum adapted to the substrate being tested.

Observing the R_m and λ values of both treatments with biosolids and LDI, there was a statistically significant difference (p-value < 0.05) in λ values as treatments with LDI experienced little or no lag-phase compared to 84 days experienced in treatments with biosolids. Even with a significant numerical difference in R_m values, the difference was found not to be statistically significant (p-value > 0.05) indicating that even after the prolonged lag-phase, treatments with biosolids were able to pick up on CH₄ production. R_m and λ values were difficult to compare to literature values because these values would be affected by the ISR used in the experiments. The ISR value used in this study was set at 0.4 to minimize CH₄ contribution from inoculum which is lower than values used in literature.

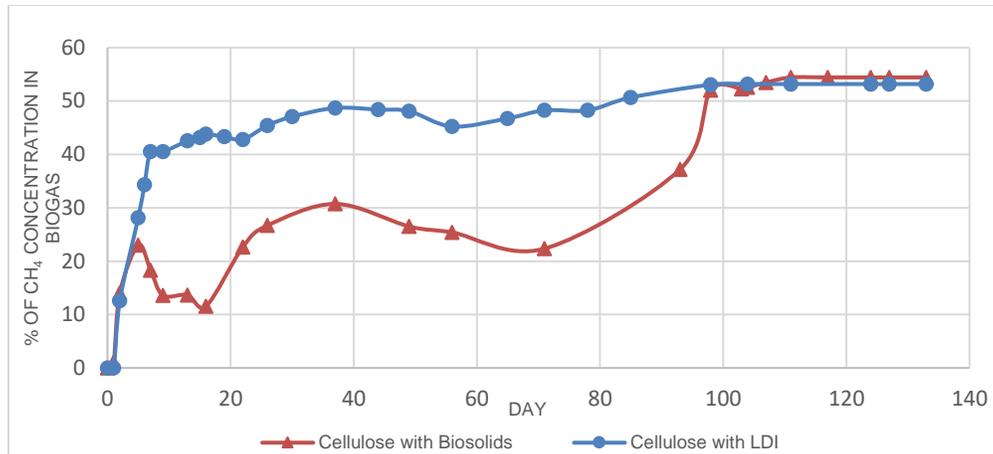


Figure 2: Methane gas composition in biogas over time for biosolids and LDI inocula using cellulose as substrate

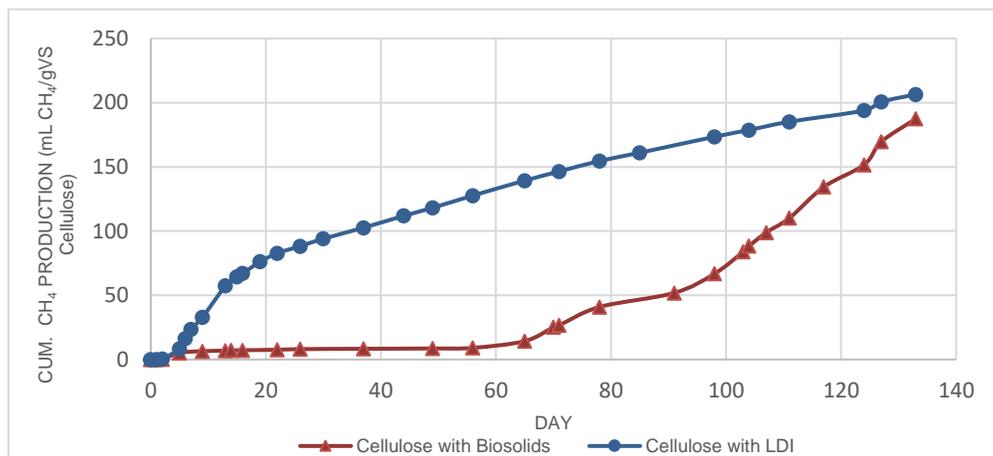


Figure 3: Cumulative methane production of biosolids and LDI inocula using cellulose as substrate

Table 2: Model parameters from curve fitting of final cumulative CH₄ yields of cellulose with biosolids and LDI

Treatment	Cellulose with Biosolids	Cellulose with LDI
P (mLCH ₄ /gVS cellulose)	254.2	309
L ₀ (mLCH ₄ /g cellulose)	123.49	150.11
R _m (mLCH ₄ /gVS cellulose/day)	3.171	8.227
λ (day)	84.16	4.5
R- Squared	0.9786	0.9663
Adj R- Squared	0.9766	0.9621

3.2 The Performance of LDI Over Digested Sewage Sludge Using OFMSW

Figure 4 and Figure 5 show the methane gas concentration profiles and time dependent methane yield after 30 days of incubation (day 37 to be precise). The initial aim was to run the experiments until gas production ceased or increased by 1% or less over 3 consecutive days or at least at the same length of time with the cellulose treatments. However, after 37 days of running, biogas production decreased significantly and CH₄

concentrations plummeted in the reactors with biosolids due to inhibition. It was also observed that the N₂ concentration in the biogas was high at 79%, leading to a conclusion of possible inhibition by ammonia. Even after 111 days of incubation, N₂ gas concentrations were still high in both duplicates with little or no CH₄ gas production. When the liquid extracts from the reactors were tested, it was found that the VFA levels were 11.7 ± 0.2 mg/L and pH value at 6.02 ± 0.2, which is lower than recommended for methanogenesis. Therefore, the performance of both inocula was only compared when all reactors were actively producing biogas and CH₄ concentrations were increasing while N₂ concentrations were decreasing.

A significant difference in CH₄ composition in biogas was again observed between the biosolids and LDI inoculated samples similar to the treatments with cellulose. However, in this case, the biosolids treatment had a pronounced lag phase right from the beginning of the incubation and hardly increased. At the end of 37 days, the CH₄ concentration in the biosolids treatments was at 10% while that in the LDI treatments had increased to about 50%. In the cumulative CH₄ production graphs in Figure 4, the toxic effects experienced in the treatments with biosolids occurred past day 37 of incubation until the reactors were stopped. The treatments with LDI showed a lag phase of approximately 2 days and CH₄ production continued to rise steadily till day 37. The CH₄ yield curves were not modeled since the results were only compared until day 37 of incubation.

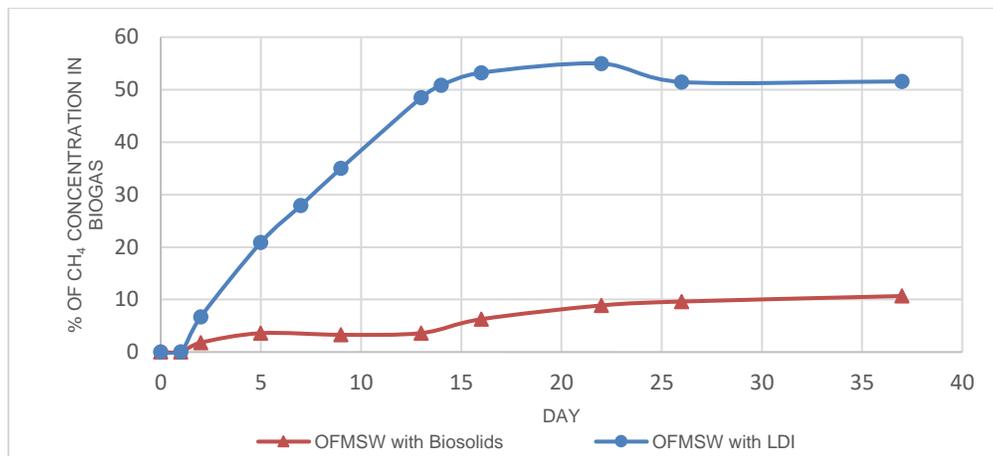


Figure 4: Methane gas composition in biogas over time for biosolids and LDI inocula using OFMSW as substrate

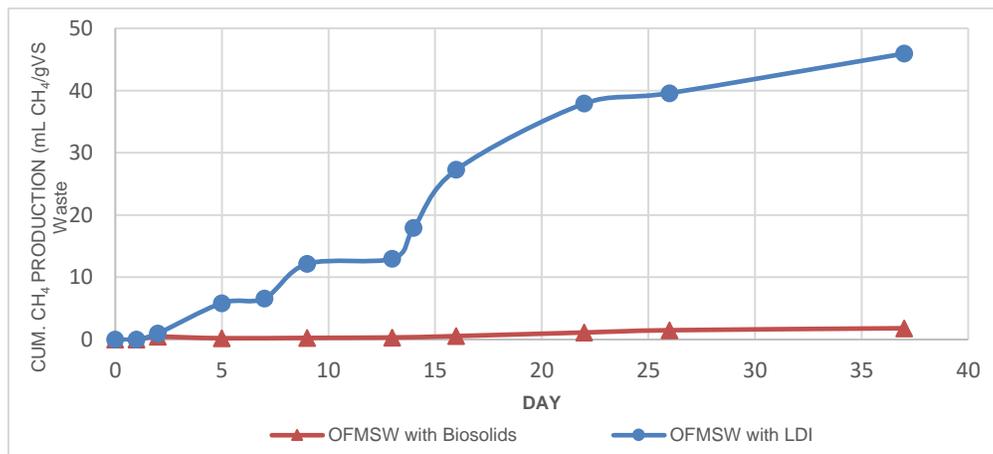


Figure 5: Cumulative methane production of biosolids and LDI inocula using OFMSW as substrate

Both results from the cellulose and OFMSW treatments agree with findings of Moset et al. (2015) and Wakadikar et al. (2013) and reiterates that inoculum sourced from WWTPs might not be optimal for all types of substrate, especially LMSW. This also indicates that inoculum sourced from a digester treating the same type of substrate to be incubated might be more superior to any other type of inoculum. However, this differs from the findings of (Bao 2011), where inoculum in the form of a laboratory-derived culture obtained by enrichment from a hand squeezed extract of decomposing residential waste was found to be less superior to biosolids. A study by Burrell et al. (2004); Staley et al. (2012), revealed that microbial communities in decomposed solids and leachate phases in LMSW are different, suggesting that bacteria groups in LMSW such as methanogens, acidogens and cellulolytic bacteria populate solids or leachate preferentially, and a combination of both solid particulates and planktonic phases must be considered for microbial diversity. For this reason, the hand squeezed extract from Bao (2011)'s study could have been lacking the necessary microbial population to effectively degrade LMSW, hence the disparity with this study. The highest coefficient of variation between duplicates was found to be 23% indicating good repeatability of methods used and validity of results obtained.

4 Conclusion

Overall, the LDI performed better than biosolids in terms of cumulative CH₄ production, CH₄ production potential and lag phases in both slurry-based and solid-based conditions. The difference in R_m values for both inocula were statistically insignificant, indicating that even after long inhibition periods, the biosolids inoculum was able to overcome toxic effects and produce CH₄ at a fast-enough rate. This was however only when cellulose was used as a substrate due to its simple and robust structure. In this study, even after an incubation period of 133 days, the CH₄ yield curves had still not plateaued for both sources of inocula, indicating that longer incubation times were required compared to those found in literature (usually between 30 to 60 days). A possible reason for this could be the chosen ISR of 0.4 used in this study, as higher ISRs would lead to an increased rate of CH₄ production and faster degradation of the substrate, however, there is a possibility of underestimating or overestimating CH₄ potential with higher ISRs (Moset et al. 2015).

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