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Biochemical methane potential and biodegradability of complex organic substrates

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ABSTRACT

The biomethane potential and biodegradability of an array of substrates with highly heterogeneous characteristics, including mono- and co-digestion samples with dairy manure, was determined using the biochemical methane potential (BMP) assay. In addition, the ability of two theoretical methods to estimate the biomethane potential of substrates and the influence of biodegradability was evaluated. The results of about 175 individual BMP assays indicate that substrates rich in lipids and easily-degradable carbohydrates yield the highest methane potential, while more recalcitrant substrates with a high lignocellulosic fraction have the lowest. Co-digestion of dairy manure with easily-degradable substrates increases the specific methane yields when compared to manure-only digestion. Additionally, biomethane potential of some co-digestion mixtures suggested synergistic activity. Evaluated theoretical methods consistently over-estimated experimentally-obtained methane yields when substrate biodegradability was not accounted. Upon correcting the results of theoretical methods with observed biodegradability data, an agreement greater than 90% was achieved.

1. Introduction

Today, a diverse range of organic substrates is subjected to the process of anaerobic digestion – waste stabilization is the primary objective, and livestock manure, a substrate which amounts to over a billion tons produced per year in the United States, is probably the main waste treated via this process in the world (Kellogg et al., 2000). Given that the primary sources of biomethane in livestock operations come from animal enteric fermentation and uncovered raw manure-stabilization lagoons (Amon et al., 2001), the benefits of anaerobic digestion of animal manure are evident. Manure-associated greenhouse gas (GHG) emissions comprise a significant contribution to total GHGs released by the US agricultural sector, with biomethane, from conventional livestock practices, estimated at 8% of the total anthropogenic biomethane emissions (USEPA, 2010). Along with mitigating biomethane gas emissions, anaerobic digestion of animal manure has the potential to reduce farm-generated odors, improve crop-based nutrient management, and produce local, renewable energy. Food residues and waste activated sludge (WAS) are additional examples of organic wastes stabilized through anaerobic digestion. In Germany there are over 500 anaerobic digestion facilities for the treatment of the organic fraction of municipal solid waste (Kübler et al., 2000) and over 4000 on-farm digesters. A growing number of on-farm digester operations throughout New York State are currently co-digesting livestock manure with a range of easily-degradable food residues, such as cheese whey and wastes from ice cream and onion operations (Gooch and Pronto, 2009). Waste activated sludge, generated by municipal wastewater treatment plants (WWTP) in amounts that reach over 10 million dry tons per year in the European Union (Appels et al., 2008), is usually digested on-site with concomitant production of electricity and heat. In Germany, the use of short rotation crops for bioenergy generation has been increasing, and in 2007 had an agricultural area of 500,000–550,000 ha dedicated exclusively to produce energy crops to sustain its 3750 biogas plants (Rosch et al., 2009).

To anticipate the overall impact and methane yields of such a diverse range of substrates on large-scale, continuous-flow anaerobic digesters, long- and short-term, laboratory-scale experimental methods have been developed. Long-term studies (i.e. 1–2 years) conducted in bench-scale, continuous-flow reactors, are designed to emulate the conditions of commercial-scale digesters and study their overall performance over time. Short-term (i.e. 1–2 months), batch-mode anaerobic digestion tests, such as the biochemical methane potential (BMP) assay, are primarily intended to determine methane yields and biodegradability of substrates.

In addition, a considerable number of theoretical approaches has also been developed. In the early stages of anaerobic digestion, stoichiometrical-based methods that predicted the major final products of fermentation were developed, (Symons and Buswell, 1933; McCarty, 1972). Most recent approaches are more complex models that simulate the biochemical and physicochemical reactions of anaerobic digestion to predict the major transient and final products of the fermentation process (Angelidaki et al., 1999;

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Nomenclature

BMP	Biochemical methane potential (equivalent to B_o), mL CH_4/g VS added
B_o	Observed SMY, mL CH_4/g VS added
B_u	Ultimate SMY, mL CH_4/g VS added
COD_D	Degradable chemical oxygen demand, mg/L
COD_T	Total chemical oxygen demand, mg/L
f_D	Substrate biodegradable fraction, decimal
n	Number of moles of gas
P	Absolute pressure of gas, kPa
R	Universal gas constant, 8.3145 L kPa/K mol
SMY	Specific methane yield, mL CH_4/g VS added
T	Temperature of gas, K
V	Volume of gas, m^3
WSMY	Weighted specific methane yield, mL CH_4/g VS added

Molecular formulae

Carbohydrates (as glucose) $C_6H_{12}O_6$
Lipids (as palmitic acid) $C_8H_{16}O$
Proteins (average protein molecular formula) $C_{16}H_{24}O_5N_4$
Volatile fatty acids (as acetic acid) CH_3COOH

Batstone et al., 2000). Regardless of the theoretical method used, its accuracy will largely depend on the knowledge of the substrate composition, and particularly, on its biodegradable fraction. Thus, the need for a simple, quick, and accurate method to estimate biomethane yields and biodegradability of organic substrates is apparent. In this study, the biomethane potential of more than 30 substrates, including mono- and co-digestion samples, was determined using the BMP assay. Based on the substrate characteristics and observed biomethane yields, their biodegradable fraction was determined. Similarly, the co-digestion of dairy manure with several organic substrates was evaluated for its potential to increase methane production over conventional manure-only digestion methods. In addition, the feasibility of using two common theoretical methods to estimate the biomethane yields of complex substrates was evaluated. Selection of substrates was based on their frequency of inclusion in anaerobic digesters in New York State and to cover a wide range of material biodegradabilities and chemical compositions.

2. Methods

2.1. Experimental methods

The anaerobic digestion of substrates was performed in batch mode using the biochemical methane potential (BMP) assay. The methane potential of substrates was evaluated based on their specific methane yield (SMY) – defined here as the total volume of methane produced during the digestion period per amount of substrate initially added (i.e. mL CH_4/g VS added).

2.1.1. Biochemical methane potential assay

The BMP protocol followed in this study was based on the principles described by Owen et al. (1979) and revised by others (Chynoweth et al., 1993; Hansen et al., 2004). Briefly, known amounts of substrate and an active anaerobic inoculum were added to 250-mL serum bottles. pH was measured, and bottles were gassed with N_2 and sealed immediately using rubber septa and aluminum crimp caps. Once sealed, the bottles were placed in an incubator and maintained at a constant mesophilic temperature (35 ± 1 °C). In each BMP trial, two additional bottles containing only inoculum were included to account for background (i.e. endogenous) methane production. Mixing was performed manually to each bottle every 2 days during the entire incubation period. The duration of the BMP assay was specifically determined for each substrate, and the test was ended when the cumulative biogas curve reached the plateau phase, usually after 30 days.

2.1.2. Biological inoculum and nutrient requirements

The inoculum was obtained from a farm-based completely-mixed anaerobic digester operated at a 25-day hydraulic retention time (HRT), which co-digested dairy manure with an array of food residues (i.e. cheese whey, milk slop, and raw onions). The inoculum was harvested from the supernatant of the digester's effluent after 24 h of quiescent settling. No additional external nutrients/trace elements were added to the BMP bottles – it was assumed that basic nutrient requirements for anaerobic microorganisms were provided by the manure-based inoculum, as Gustafson (2000) found in significant amounts in dairy manure.

2.1.3. Biogas production measurement

Biogas production was determined indirectly, by measuring the cumulative pressure inside the bottles via pressure transducers. Pressure was continuously measured using a data acquisition (DAQ) system interfaced with a computer, and controlled via LabVIEW® (National Instruments Co., Austin, TX). In addition, a pressure-control bottle containing the equivalent volume of sample replaced by tap water was included to account for abiotic internal pressure variations due to temperature and atmospheric pressure changes. Similarly, temperature was monitored through thermocouples measuring gas-phase temperature changes in tap water-containing bottles. Finally, pressure data were converted to volume of biogas at standard temperature and pressure (STP), according to the ideal law of gases:

$$PV = nRT \tag{1}$$

where P, V, n, and T are respectively: absolute pressure (kPa), volume (m^3), moles, and temperature (K) of the gas; and R is the universal gas constant (8.3145 L kPa/K mol).

2.2. Analytical methods

All substrates were mixed and blended thoroughly to reduce particle size and create uniform and representative specimens. Total solids (TS), volatile solids (VS), and chemical oxygen demand (COD) (colorimetric dichromate closed reflux method) were determined according to Standard Methods (APHA, 1995). 10-day biochemical oxygen demand (BOD) tests were performed using a HACH BODTrak (HACH Co., Loveland, CO). Methane and carbon dioxide content in the biogas was determined with an SRI 8610C (SRI Instruments, Torrance, CA) gas chromatograph equipped with a thermal conductivity detector (TCD), using Helium as a carrier gas in a 0.3-m HaySep-D packed Teflon® column under isothermal conditions at 105 °C. Additional analyses to determine the precise chemical composition of dairy manure were conducted. Hemicellulose, cellulose, and lignin content were determined according to the neutral detergent fiber (NDF) and acid detergent fiber and lignin (ADF/ADL) analyses described by Mertens (2002) and Möller (2009), respectively. Total Kjeldahl nitrogen (TKN) concentration was determined according to the Standard Methods (APHA, 1995). Total ammonia-N (TAN) concentration was measured using an ion selective electrode (Thermo Fisher Scientific, Inc.). Total organic nitrogen was calculated by subtracting TAN from TKN. Total protein content was calculated based on the assumption that an average protein contains 16% organic N. Neutral lipids were determined according to method of Loehr and Rohlich (1962). Non-lignocellulosic carbohydrates (e.g. sugars, starch, pectin) were obtained by difference.

2.3. Methane yield estimation methods

The ability of theoretical methods to accurately estimate methane yields of complex substrates was evaluated by comparing the observed SMY (B_o) of selected substrates to the ultimate SMY (B_u). Only a brief description of the theoretical methods is presented here – the reader is referred to the original cited literature for further details.

Bioenergetics and Stoichiometry of Biological Reactions (McCarty, 1972) – the thermodynamic equilibrium of microbiologically-mediated reactions can be used to estimate cell yields and the overall stoichiometry associated with growth, and determine which fraction of a particular organic substrate (i.e. electron donor) will be used for energy and which fraction for synthesis of cellular material.

Buswell Formula (Symons and Buswell, 1933) – this equation simply represents a balanced redox reaction where the only products of anaerobic digestion are methane, carbon dioxide, and ammonia. In contrast to the method of McCarty, the Buswell Formula assumes that all the electrons donated are exclusively used for metabolic energy, i.e. cellular synthesis is neglected.

The two methods described above do not account for substrate biodegradability, or in other words, it is assumed that all the electrons from the donor are available for the electron acceptors. The ability of either method to accurately estimate biomethane yields primarily depends on two fundamental substrate characteristics, namely chemical composition and biodegradability. With the exception of the chemical composition of dairy manure, which was analytically determined in this study, and switchgrass, which was as reported by Lemus et al. (2002), the composition of all the substrates covered in this study was obtained from the Nutrient Data Laboratory (NDL) database (USDA, 2009). For both theoretical methods, calculations were based on the molecular formulae of the substrates' constituents, as stated previously. A more difficult parameter to estimate is substrate biodegradability. The rate at which substrates are degraded will be mainly determined by its physical and chemical properties as well as its susceptibility to produce inhibitory intermediate products throughout the bioconversion processes. Physicochemical characteristics, such as particle size, lignin content or degree of crystallinity of the lignocellulosic matrix, will mainly affect the kinetics of the hydrolysis step, while pH, un-ionized ammonia, or fatty acid (long- and short-chain) concentrations, could affect one or multiple step(s) of the anaerobic digestion process (i.e. hydrolysis, acidogenesis/β-oxidation, acetogenesis, and methanogenesis). Therefore, depending on the residence time, the rate of degradation of the substrate will determine its extent of biodegradability, and thereby its biomethane yield. Conventional methods described in the literature to estimate the biodegradability of organic substrates are experimental. In fact, the BMP assay is one of the most widely used analytical methods to determine the portion of substrate that can be biologically degraded under anaerobic conditions. This fraction can be estimated by the ratio between the degradable and total chemical oxygen demand:

$$f_D = \frac{\mathrm{COD}_D}{\mathrm{COD}_T} \tag{2}$$

where f_D is the substrate biodegradable fraction (decimal), COD_D is the degradable chemical oxygen demand (mg/L), and COD_T is the total chemical oxygen demand (mg/L). COD_D can be calculated from the observed specific methane yields (B_o) and the theoretical 350 mL of CH_4 (at STP) per g of COD stabilized (McCarty, 1964). Also, COD_T was determined analytically for each substrate (see Tables 1 and 2).

Table 1
Physical and biochemical characteristics of the mono-digestion samples.

Mono-digestion samples	BOD (g/kg)	COD (g/kg)	TS (g/kg)	VS (g/kg)	BOD/COD	VS/TS	VS/COD
Raw manures							
Raw dairy manure	45.8	128.9	124.0	102.1	0.36	0.82	0.79
Manure separated liquid	33.2	71.0	57.5	40.5	0.47	0.71	0.57
Food residues							
Cheese whey	64.9	128.3	71.4	59.8	0.53	0.84	0.53
Plain pasta	188.7	934.3	422.6	407.7	0.20	0.97	0.44
Meat pasta	205.8	562.8	381.8	340.6	0.37	0.89	0.61
Used vegetable oil	ND	2880.0	991.0	988.8	ND	1.00	0.34
Ice cream	ND	266.8	113.8	109.1	ND	0.96	0.41
Fresh dog food	ND	530.4	132.2	125.6	ND	0.95	0.24
Cola beverage	ND	121.5	93.6	88.7	ND	0.95	0.73
Cabbage, raw	ND	90.9	78.6	72.0	ND	0.92	0.79
Potatoes, raw	53.5	261.8	177.4	163.5	0.20	0.92	0.63
Invasive aquatic plants							
Frogbit (Oneida lake)	32.9	49.5	51.8	38.7	0.67	0.75	0.78
Water Chestnut (Oneida river)	40.4	46.2	89.0	74.2	0.87	0.83	1.61
Eurasian milfoil (Oneida lake)	26.4	27.8	106.1	66.7	0.95	0.63	2.40
Water celery (Oneida lake)	27.9	33.6	92.9	47.0	0.83	0.51	1.40
Chara (Tully lake)	27.9	31.5	148.8	37.7	0.89	0.25	1.20
Others							
Switchgrass	88.6	706.7	930.1	904.9	0.13	0.97	0.68
Corn silage	ND	ND	217.3	200.7	ND	0.98	0.91
Corn leachate	50.7	122.3	49.2	35.4	0.42	0.72	0.29
Mouthwash	ND	160.5	130.2	118.4	ND	0.91	0.74
Suspended fat, oil and grease (FOG)	155.5	600.1	267.2	229.7	25.9	0.86	0.38
Settled fat, oil and grease (FOG)	97.0	290.0	128.4	112.6	33.4	0.88	0.39

ND: not determined.

Table 2
Physical and biochemical characteristics of the co-digestion samples.

Co-digestion samples (M = dairy manure)	Mix ratio (VS basis)	BOD (g/kg)	COD (g/kg)	TS (g/kg)	VS (g/kg)	BOD/COD	VS/TS	VS/COD
M:Cheese whey	90:10	45.5	103.2	83.2	68.4	0.44	0.82	0.66
M:Cheese whey	75:25	46.4	100.3	68.5	57.7	0.46	0.84	0.58
M:Plain pasta	90:10	91.8	158.5	132.0	116.7	0.58	0.88	0.75
M:Plain pasta	75:25	97.2	293.6	222.5	211.4	0.33	0.95	0.67
M:Meat pasta	90:10	70.4	151.2	101.9	89.8	0.47	0.88	0.59
M:Meat pasta	75:25	98.8	233.0	148.5	136.9	0.42	0.92	0.59
M:Used vegetable oil	75:25	ND	922.0	263.6	235.4	ND	0.89	0.26
M:Dog food:ice cream	50:25:25	ND	317.0	106.9	96.9	ND	0.91	0.31
M:Cola beverage	75:25	38.6	122.4	102.7	83.8	0.32	0.82	0.68
M:Potatoes	75:25	58.0	122.0	134.4	114.3	0.48	0.85	0.94
M:Switchgrass	75:25	17.1	413.6	308.0	284.4	0.04	0.92	0.69
M:Mouthwash	75:25	51.1	168.6	110.5	86.0	0.30	0.78	0.51
M:Cola:mouthwash	75:12.5:12:5	53.5	140.7	108.8	86.8	0.38	0.80	0.62

ND: not determined.

3. Results and discussion

3.1. Characterization of substrates

The substrates investigated in this study cover a wide range of material biodegradabilities and chemical compositions, and include mono-digestion (digestion of a single substrate) and co-digestion (digestion of more than one substrate) samples. All co-digestion samples consisted of substrates co-digested with dairy manure. Main physical and chemical characteristics of the mono- and co-digestion samples are presented in Tables 1 and 2, respectively. Additionally, the chemical composition of selected substrates as obtained from the NDL database (Section 2.3) is shown in Table 3. As described in Section 2.2, the chemical composition of dairy manure was determined analytically in this study.

3.2. Experimental parameters

Rate (and extent) of methane production are maximized when the right pool of enzymes and microorganisms for degrading a particular substrate are present in the medium in sufficient concentrations. The inoculum used in this study was obtained from a well-established on-farm anaerobic digester acclimated to degrade lignocellulosic materials as well as easily-degradable carbohydrates, and a fraction of proteins and lipids. Therefore, it was assumed to be microbiologically adequate for degrading the diverse range of substrates proposed for the BMP assays. Similarly, the amount of inoculum used in the test bottles was determined on the basis of the amount of organic substrate available for degradation, i.e. an inoculum-to-substrate (I/S) ratio (VS basis), which is equivalent to the inverse value of the food-to-microorganism (F/M) ratio. In this study, preliminary trials (data not shown) were conducted with dairy manure to determine appropriate substrate concentrations and I/S ratios for the assay. It was concluded that for manure concentrations $\geqslant 3$ g VS/L, a minimum I/S ratio of 0.5 was required to ensure process start-up during the first 3 days of the assay. These results are supported by Hashimoto (1989), who also found a minimum ratio of 0.5 when digesting wheat straw at concentrations of 10–40 g VS/L. Furthermore, Hashimoto showed that maximum methane production rates were achieved at I/S ratios $\geqslant 2$. Similarly, studies conducted by Owen et al. (1979) and Chynoweth et al. (1993) suggested I/S ratios of 1 and 2, respectively. In contrast, Fernández et al. (2001) concluded that I/S ratios as low as 0.03 were sufficient to achieve maximum degradation of brewery spent grains at a concentration of 70 g/L (56 g VS/L). In this study, an I/S = 1 was used to maximize degradation rates and ensure that the methane potential was achieved.

Table 3
Chemical composition of selected substrates (% VS basis).

Samples	Mix ratio (VS basis)	VFA	Protein	Lipids	Hemicelluloses	Cellulose	Lignin	Sugars, starch, pectin
Raw dairy manure	–	3.50	5.7	16.1	9.6	32.6	13.8	16.5
Cheese whey	–	0	13.4	5.7	0	0	0	80.9
Plain pasta	–	0	16.5	3.4	0	0	0	80.1
Meat pasta	–	0	19.3	14.0	0	0	0	66.7
Used vegetable oil	–	0	0	100	0	0	0	0
Ice cream	–	0	8.3	38.6	0	0	0	53.1
Fresh dog food	–	0	0.0	0.0	0	0	0	0
Cola beverage	–	0	0.7	0.2	0	0	0	99.1
Cabbage, raw	–	0	17.8	1.4	0	36.2	0	44.6
Potatoes, raw	–	0	10.5	0.8	0	9.5	0	79.2
Switchgrass	–	0	0.7	0	42.2	48.8	8.3	0
Corn silage	–	0	13.8	5.0	0	11.5	0	69.7
Suspended FOG	–	0	0	100	0	0	0	0
Settled FOG	–	0	0	100	0	0	0	0
M:Cheese whey	90:10	3.2	6.5	15.1	10.5	29.4	12.5	23.0
M:Cheese whey	75:25	2.6	7.6	13.5	8.8	24.5	10.4	32.6
M:Plain pasta	90:10	3.2	6.8	14.9	10.5	29.4	12.5	22.9
M:Plain pasta	75:25	2.6	8.4	13.0	8.8	24.5	10.4	32.4
M:Meat pasta	90:10	3.2	7.0	15.9	10.5	29.4	12.5	21.5
M:Meat pasta	75:25	2.6	9.1	15.6	8.8	24.5	10.4	29.1
M:Used vegetable oil	75:25	2.6	4.3	37.1	8.8	24.5	10.4	12.4
M:Cola beverage	75:25	2.6	4.4	12.2	8.8	24.5	10.4	37.2
M:Potatoes	75:25	2.6	6.9	12.3	8.8	26.8	10.4	32.2
M:Switchgrass	75:25	2.6	4.4	12.1	19.3	36.7	12.4	12.4

3.3. Experimental methane yields

A summary of the average specific methane yields (SMY) of all substrates analyzed in this study is presented in Fig. 1. Despite biodegradability limitations discussed in the next section, it is apparent that substrates high in lipids and easily-degradable carbohydrates (e.g. used oil, ice cream) have the highest SMY. On the other hand, lignocellulosic substrates, such as switchgrass and most of the substrates co-digested with manure, show the lowest SMY. Noticeably, most invasive freshwater aquatic plants exhibit a high SMY. From the co-digestion samples, the mixture of manure with fresh dog food and ice cream waste presented the highest methane yield, which is expected due to the high methane yield found in both mono-digestion experiments with these two substrates.

3.3.1. Previous studies

Dairy manure is probably one of the most thoroughly and frequently studied substrates in anaerobic digestion. As such, it constitutes an ideal substrate for biomethane potential comparisons. However, dairy manure is highly variable in nature, as it originates from a wide range of dairy operations, involving different animal breeds, ages, diets, as well as management practices. Interestingly, there seem to be a considerable good agreement among the SMYs (B_o) reported in the literature and those presented in this study, as we discussed below.

The B_o of manure found in this study was based on a total of 47 individual BMP assays performed on manure samples collected from six different dairy farms at various times of the year. The average and range of distribution of B_o was respectively, 243 ± 60 and 127–329 mL of CH_4 per g VS added. The average B_o found in this study compares well to the overall average value reported by the IPCC (1997) of 240 mL CH_4/g VS added. Also, it is within the range of distribution reported by Vedrenne et al. (2008) of 204–296 mL CH_4/g VS added, and compares quite well with the value reported by El-Mashad and Zhang (2010) of 241 mL CH_4/g VS added. Particularly remarkable is the fact that the average B_o obtained in this study also compares well with the B_o of 241 mL CH_4/g VS added, determined by Hoffmann et al. (2008) from a study with four CSTRs and throughout three different HRTs. A rather lower B_o was reported by Moller et al. (2004) in BMP studies, i.e. 148 ± 41 mL CH_4/g VS added; however, it is still within the

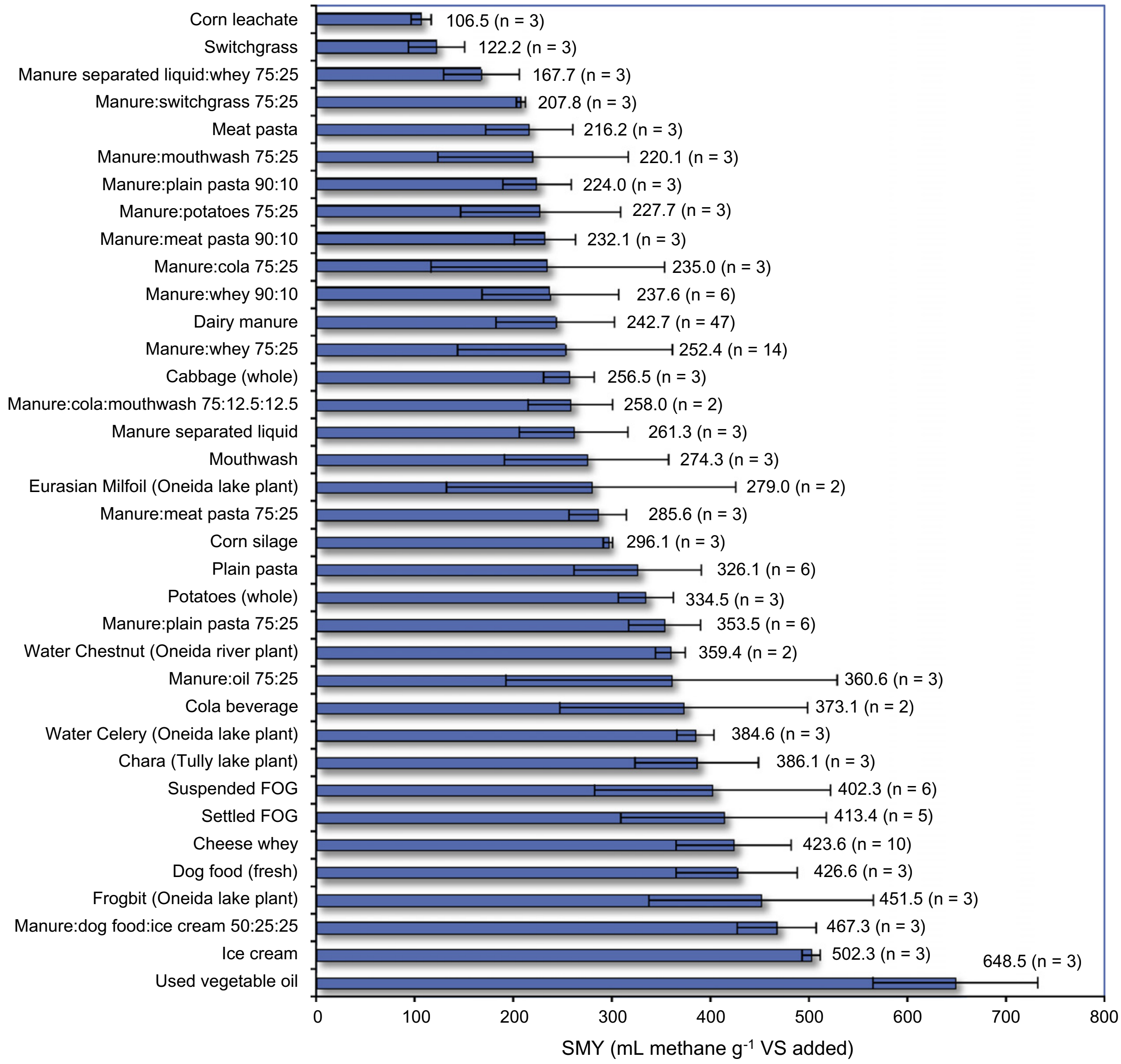

Fig. 1. Summary of the observed specific methane yields (B_o) at STP, as obtained from the BMP assay of some 30 mono- and co-digested substrates. The value outside the bars is the average B_o with the sample number in parenthesis. Error bars represent the standard deviation of B_o for each substrate.

range of distribution of the observed SMYs found in this study. All preceding B_o values were obtained at mesophilic conditions (~35 °C). At thermophilic conditions (55 °C), this study's average B_o also agrees quite well with the values reported in the literature for both batch and continuous studies. Nielsen et al. (2004), for example, reported a B_o of 227 mL CH_4/g VS added in batch operation, and 236–241 mL CH_4/g VS added in CSTRs operated at 15-day HRT. Likewise, Mladenovska et al. (2006) reported 233 and 238 mL/g VS added at thermophilic conditions in batch and CSTRs, respectively.

In spite of the intrinsic variability of dairy manure among the studies discussed above, their average biomethane potential are comparable, and this study constitutes no exception. Particularly interesting, is the fact that the aforementioned studies have been conducted under a wide range of experimental conditions, from batch to continuous mode, and from mesophilic to thermophilic range temperatures. The latter is especially important if BMP results are to be used for estimating approximate biomethane potential and biodegradability of specific substrates in large-scale, continuous-flow anaerobic digesters (see caveats discussed in Section 3.3.3).

3.3.2. BMP production curves

During a BMP assay, biogas production curves can follow a diverse array of patterns. These patterns are not by any measure trivial, but have meaningful implications. Biodegradability characteristics of substrates and production of inhibitory intermediate products will mainly control the kinetics of the different steps of anaerobic digestion and define the shape of the biogas production curve. This, can aid to identify important characteristics of substrates and anticipate digestion issues. Fig. 2 depicts four distinctive biogas production patterns from four different substrates during the course of a 40-day BMP assay. Fig. 2A depicts the cumulative biogas production of dairy manure, a slowly-degradable substrate due to its composition, which consists of approximately 60% lignocellulose (Table 3). It is apparent that the biogas production rate approaches zero near 25 days sludge residence time, which would indicate its biochemical biogas potential. Conversely, Fig. 2B shows the steep biogas production pattern of cheese whey – a substrate mostly composed of easily-degradable sugars (Table 3), which appears to achieve its maximum biogas potential in less than 15 days. Fig. 2C shows the BMP curve of used vegetable oil where biogas production appears to be highly inhibited during the first 12 days of digestion. Lipid-rich substrates are easily degradable (mostly short-chain fatty acids), but are prone to produce biochemical inhibition due to long-chain fatty acid (LCFA) accumulation coming from the hydrolysis of neutral lipids. Therefore, the limiting factor of biodegradability in this case is mainly attributed to LCFA accumulation and inhibition, rather than substrate recalcitrance as in the case of dairy manure, where the lignocellulosic matrix is primarily responsible for its low biodegradability. Fig. 2D illustrates corn silage, which as discussed next, it is one of the most biodegradable substrates, but which paradoxically exhibits a rather slow degradability rate, maybe due to the acidic characteristics of this substrate and insufficient buffering capacity.

3.3.3. Scopes and limitations of the BMP assay

Interpretation of the BMP assay results is of paramount importance. A valid concern exists regarding the suitability of using the results of the BMP assay, a laboratory-scale, batch test, to predict

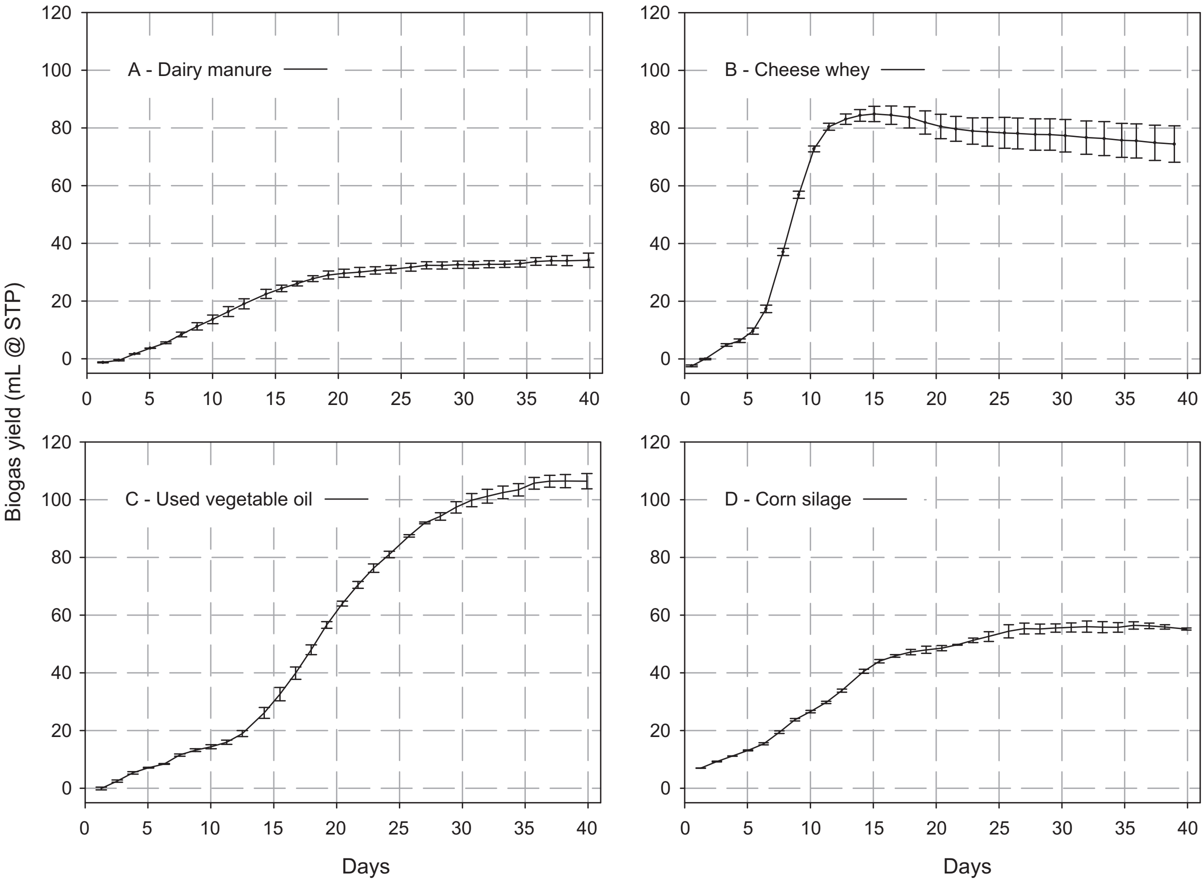

Fig. 2. BMP assay curves for a 40-day run showing four distinctive biogas production patterns (mL @ STP) from four different substrates; A: dairy manure, B: cheese whey, C: used vegetable oil, D: corn silage; error bars represent the standard deviation for the replicates.

the performance of continuous-flow, commercial-size anaerobic digesters. In addition to the physical differences in the fluid- and thermo-dynamic characteristics given by the reactor's scale and geometry, batch reactors and continuous-flow digesters essentially differ in their mode of operation. The way the reactor is fed has a fundamental impact on the thermodynamic equilibrium of the anaerobic process – and thus, on the food-web interactions. Semi- and continuous-flow digesters are characterized by dynamic changes due to periodic substrate feeding and product removal – thus, unless the digester undergoes shock loads or sudden environmental changes, process unbalance (and product accumulation) rarely occurs under steady-state conditions. In contrast, in a batch reactor, unless removed via biologically-mediated processes, substrates, microorganisms, enzymes, intermediate products, and (sometimes) final products are accumulated within the system. When the concentration of an intermediate product (particularly, volatile fatty acids and hydrogen) reaches the homeostatic threshold of a certain organism, or group of organisms, the thermodynamic balance is altered, and one or several metabolic reactions may be inhibited, causing no further product accumulation and delay of substrate degradation. In most cases, product inhibition is reversible, and as soon as thermodynamic conditions become favorable, reactions resume.

The BMP assay is designed to provide ideal anaerobic conditions and prevent any form of biochemical inhibition. To ensure this, three important conditions should be met throughout the BMP assay: (1) appropriate microbial community, enzyme pool, and nutrients are present; (2) environmental conditions are optimal; and (3) substrate and intermediate product concentrations are well below inhibitory/toxic levels. Nevertheless, product inhibition is difficult to prevent, and indeed occurs in some BMP assays. Fortunately, product inhibition primarily affects reaction kinetics, and thus, provided that adequate digestion time is allowed, stabilization of the substrate's biodegradable fraction and maximum methane yields should be achieved. However, a more difficult problem to foresee, which directly affects the biomethane potential, is trace element deficiency. This can occur during long-term anaerobic digestion of certain substrates lacking an essential element, such as cobalt in thin stillage (Agler et al., 2008). Accordingly, the BMP assay may not be a suitable test to predict biomethane yields, stabilization performance, or possible process failure due to shock loads and product inhibition, over long-term semi- and continuous-flow anaerobic digestion operations. BMP results should be limited to a relative interpretation of the substrate's methane potential, and not for an absolute estimation of daily biomethane yields or the overall performance and stability of large-scale digesters. The BMP assay is best suited when used to elucidate what types of substrates, from an array of potential substrates, have the highest biomethane potential. In addition, the assay can be used to estimate the potential ideal ratios between co-sub-

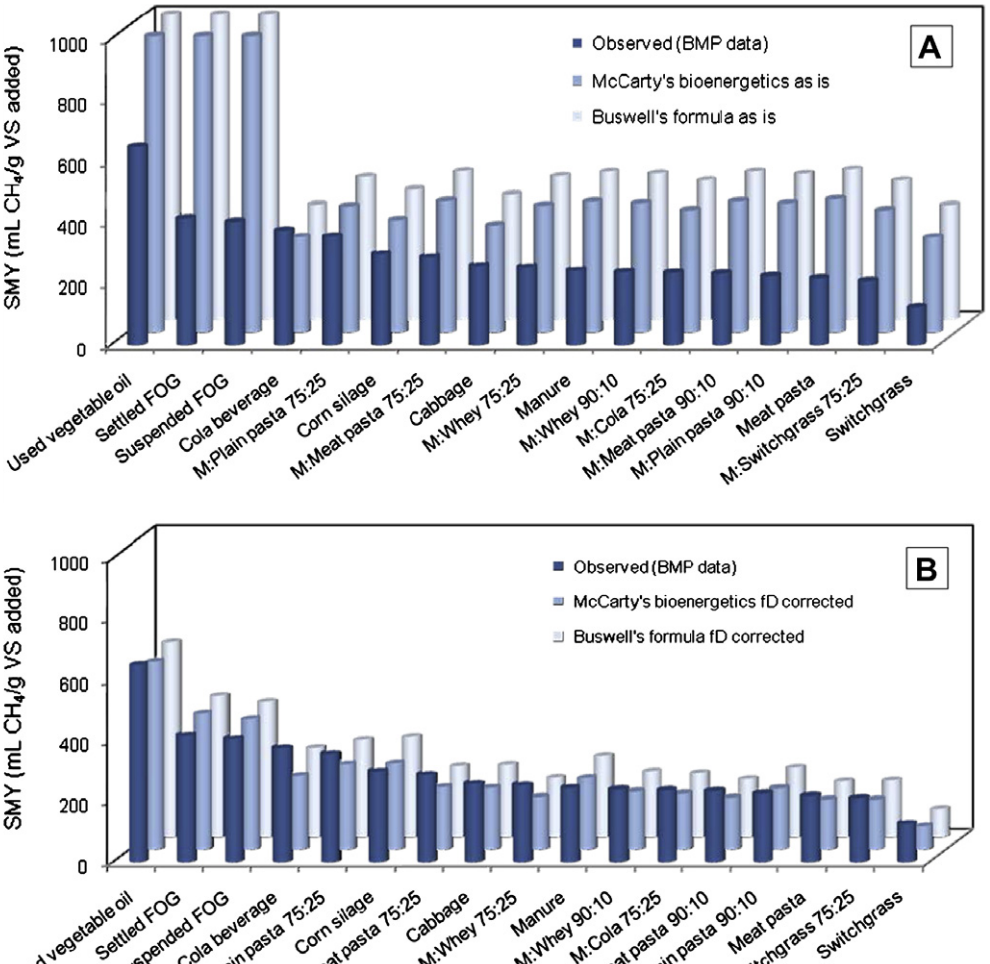

Fig. 3. Observed and estimated methane yields of 17 selected substrates using McCarty's bioenergetics and Buswell's formula; A: theoretical estimations as is, i.e. not accounting for substrate biodegradability, B: theoretical estimations corrected using the substrate biodegradable fraction (f_D) reported in Table 4.

strates when co-digestion is intended. Lastly, BMP assay results can be used to determine the extent of anaerobic biodegradability of substrates, and thus, relative residence times required for complete digestion.

3.4. Theoretical methane yields

A comparison between observed (B_o) and estimated (B_u) specific methane yields (SMYs) for 17 selected substrates is depicted in Fig. 3A. Estimated SMYs using McCarty's method were in average 11.6% lower than the values obtained using the Buswell Formula, and therefore, closer to the observed SMYs. This is expected, since McCarty's method accounts for the fraction of electron donor which is lost in cell protoplasm synthesis, while the Buswell Formula does not. In fact, Symons and Buswell (1933) reported that during the digestion of pure carbohydrates, an average of 12% of the total carbon fed was lost in the cell protoplasm which was not accounted for by their formula. Regardless of the method used, however, estimated methane yields are consistently higher than the observed ones. A potential source of difference may come from the use of theoretical substrate compositions, rather than actual, experimentally-determined substrate constituent's concentrations. However, data suggest that the largest contribution of difference is due to the fact that the two methods do not account for substrate biodegradability. The biodegradable fraction (f_D) was calculated using Eq. (2) and the substrates characteristics reported in Tables 1 and 2. The results are presented in Table 4. With the exception of the co-digestion of manure and plain pasta, data suggest that most degradable substrates are sugar- and starch-rich carbohydrates. The high biodegradability for co-digestion of manure and plain pasta could be due to experimental error, or more likely, due to a synergistic mixture as discussed in the next section. Furthermore, it is noticeable that the lower the substrate biodegradability is, the poorer the estimation is, i.e. recalcitrant lignocellulosic substrates and oil-rich substrates where product inhibition is likely to occur.

Table 4
The anaerobic biodegradability fraction (f_D) of selected substrates sorted by decreasing biodegradability. Data calculated using Eq. (2) and the experimental parameters presented in Tables 1 and 2.

Samples	Mix ratio (VS basis)	B_o (mL CH_4/g VS added @ STP)	COD_{CH4} (g COD/g VS added)	f_D (g COD/g COD)
Cola beverage	–	373.1	1.066	0.78
Corn silage	–	296.1	0.846	0.77
M:Plain pasta	75:25	353.5	1.010	0.68
Used vegetable oil	–	648.5	1.853	0.64
Cheese whey	–	423.6	1.210	0.64
M:Potatoes	75:25	227.7	0.651	0.61
Potatoes	–	334.5	0.956	0.60
Ice cream	–	502.3	1.435	0.59
Cabbage	–	256.5	0.733	0.58
Manure	–	242.7	0.693	0.55
M:Meat pasta	75:25	285.6	0.816	0.48
M:Plain pasta	90:10	224.0	0.640	0.48
Settled FOG	–	413.4	1.181	0.46
M:Cola	75:25	235.0	0.671	0.46
M:Whey	90:10	237.6	0.679	0.45
Suspended FOG	–	402.3	1.149	0.44
Plain pasta	–	326.1	0.932	0.41
M:Whey	75:25	252.4	0.721	0.41
M:Switchgrass	75:25	207.8	0.594	0.41
M:Meat pasta	90:10	232.1	0.663	0.39
Meat pasta	–	216.2	0.618	0.37
Fresh dog food	–	426.6	1.219	0.29
M:Oil	75:25	360.6	1.030	0.26
Switchgrass	–	122.2	0.349	0.24
Corn leachate	–	106.5	0.304	0.09

The importance of using substrate biodegradability information to estimate biomethane yields is demonstrated in Fig. 3B, which corrects the theoretical calculations depicted in Fig. 3A using the substrate biodegradability fractions reported in Table 4. In comparison, Fig. 3B exhibits a considerable better agreement between the theoretical and observed data for both theoretical methods. As shown in Fig. 4, after biodegradability data are included in the calculations both methods exhibit an agreement higher than 90% with the observed data, as determined by their coefficient of determination, i.e. $R^2 = 0.91$ and $R^2 = 0.93$ for McCarty and Buswell methods, respectively. The difference that prevails can be attributed to variation of the observed SMYs, as suggested by the standard deviation (SD) of B_o (Fig. 1), which in most cases is greater than 10%. It is also apparent that of both methods, the Buswell Formula produces results closer to observed biomethane values after correcting for substrate biodegradability – this is explained by the fact that McCarty's method accounts for cell synthesis, which is already factored in the observed biodegradability fraction. This leads to the conclusion that when biodegradability data is available, the Buswell Formula is the method of choice for estimating methane yields.

In summary, it is apparent that the use of stoichiometric methods together with biodegradability information is able to produce reasonable estimations of specific methane yields. The use of empirical methods, employing single and multiple regression models to estimate SMYs, has also been described (Gunaseelan, 2007). However, the use of purely empirical (as opposed to descriptive)

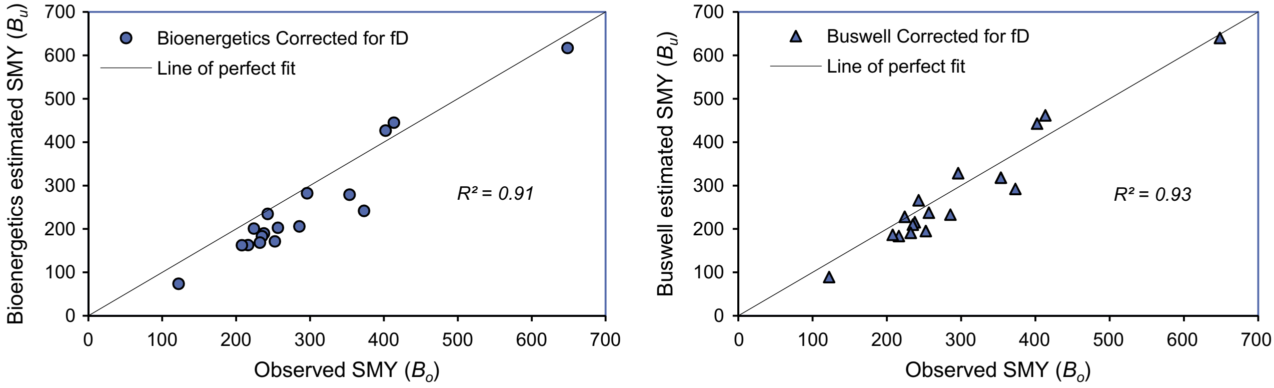

Fig. 4. Observed vs. estimated specific methane yields using McCarty's bioenergetics (left) and Buswell's Formula (right) after correcting for substrate biodegradability.

methods to estimate anaerobic digestion products will most likely compound the effects of substrate chemical composition, biodegradability and bioenergetics; disregarding the stoichiometry therein.

3.5. Effects of co-digestion on methane yields: synergistic substrate mixtures?

Co-digestion of certain substrates can produce synergistic or antagonistic effects. The synergistic effect of co-digesting swine manure with oil mill waste was reported by Angelidaki and Ahring (1997). Synergism would be seen as an additional methane yield for co-digestion samples over the weighted average of the individual substrates' SMY, namely the weighted specific methane yield (WSMY). Similarly, evidence of antagonism would be translated into a lower methane yield in the co-digestion samples as compared to the WSMY. Synergistic effects may arise from the contribution of additional alkalinity, trace elements, nutrients, enzymes, or any other amendment which a substrate by itself may lack, and could result in an increase in substrate biodegradability, and therefore, biomethane potential. Antagonistic effects can come from several factors, such as pH inhibition, ammonia toxicity, high volatile acid concentration, among others. Table 5 summarizes this analysis for co-digestion mixtures of dairy manure with food residue substrates, depicting the differences between the methane yields from co-digestion samples and the WSMYs calculated from mono-digestion methane yields. For example, the WSMY of manure co-digested with cheese whey is 288 mL/g VS; however, the observed SMY of this co-digestion sample was 252 mL CH_4/g VS. Since the negative differential in methane yield is within its SD (109 mL/g VS), it is not clear if this difference is indeed the result of an antagonistic effect (Table 5). The similarity of the B_o and WSMY values for the co-digestion of manure with switchgrass and its SD suggest that the co-digestion of these two substrates does not produce either synergistic or antagonistic effects. Furthermore, it is evident that a mixture of lignocellulosic substrates will not produce high methane yields unless some kind of pretreatment is applied. Data suggest, however, that the co-digestion of manure with both plain pasta and meat pasta is synergistic, since methane yields are 30% higher than the digestion of manure and pasta separately. Similarly, the positive differential suggests that the co-digestion of manure with oil is synergistic; however data in this case are not conclusive, since this differential is within the SD of B_o.

Table 5
Observed SMYs (B_o) from the co–digestion samples as compared to weighted SMYs (WSMY), calculated as the sum of the individual contributions of the mono-digestion samples.

Substrate	B_o (mL/g VS @ STP)	SD (mL/g VS @ STP)	WSMY (mL/g VS @ STP)	Differential (B_o – WSMY)
Manure	242.7	60.2	–	–
Co-substrates				
25% Cola	235.0	118.5	275.3	−40.3
25% Potatoes	227.7	81.1	265.7	−38.0
25% Cheese whey	252.4	109.0	287.9	−35.5
25% Mouthwash	220.1	96.5	250.6	−30.5
10% Plain pasta	224.0	34.7	251.0	−27.0
10% Cheese whey	237.6	69.3	260.8	−23.2
10% Meat pasta	232.1	31.0	240.0	−7.9
12.5% Cola and 12.5% mouthwash	258.0	42.8	262.9	−4.9
25% Switchgrass	207.8	4.5	212.6	−4.8
25% Used oil	360.6	168.1	344.1	16.4
25% Meat pasta	285.6	29.0	236.1	49.5
25% Plain pasta	353.5	36.6	263.6	89.9
25% Dog food and 25% ice cream	467.3	39.9	353.6	113.7

Due to the variability of the data it is not possible to draw definitive conclusions on the synergism and absence of antagonism observed in our co-digestion trials. Further co-digestion studies with dairy manure, a lignocellulosic substrate, and a co-substrate with a well-balanced composition of proteins, lipids, and easily-degradable carbohydrates should be conducted to elucidate which components within the co-digestion mixture show further degradation as compared to their mono-digestion condition.

4. Conclusions

Substrates highly rich in lipids and easily-degradable carbohydrates exhibited higher methane potential – more recalcitrant, lignocellulosic-materials presented lower methane yields. Experimental biomethane yields were consistently over-estimated by the theoretical methods evaluated; by including the experimentally-obtained biodegradability fraction in the calculations, an agreement of over 90% was achieved. Co-digestion of dairy manure with easily-degradable substrates increases the biomethane yields when compared to manure-only digestion; Synergistic biomethane yields were observed in a number of substrates co-digested with dairy manure; however, further testing is necessary to validate this conclusion.

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