Ultrasonic pretreatment for anaerobic digestion of suspended and attached growth sludges

Peter Roebuck, Kevin Kennedy and Robert Delatolla

ABSTRACT

Anaerobic digestion (AD) is a proven technology for energy production from the stabilization and reduction of sewage waste. The AD and impact of ultrasonic pretreatment of four waste activated sludges (WASs) from conventional and three non-conventional municipal wastewater treatment plants were investigated. WAS from a conventional activated sludge (CAS) system, a rotating biological contactor (RBC), a lagoon, and a nitrifying moving-bed biofilm reactor (MBBR) were pretreated with ultrasonic energies of 800–6,550 kJ/kg total solids to illustrate the impact of sludge type and ultrasonic pretreatment on biogas production (BGP), solubilization, and digestion kinetics. The greatest increase in BGP over the control of pretreated sludge did not coincide consistently with greater sonication energy but occurred within a solubilization range of 2.9–7.4% degree of disintegration and are as follows: 5% ± 3 biogas increase for CAS, 12% ± 9 for lagoon, 15% ± 2 for nitrifying MBBR, and 20% ± 2 for RBC. The effect of sonication on digestion kinetics was inconclusive with the application of modified Gompertz, reaction curve, and first-order models to biogas production. These results illustrate the unique response of differing sludges to the same levels of sonication energies.

Key words | anaerobic digestion, degree of disintegration, municipal sewage sludge, pretreatment, sonication, ultrasound, waste activated sludge

Peter Roebuck
Kevin Kennedy
Robert Delatolla (corresponding author)
Department of Civil Engineering,
University of Ottawa,
161 Louis Pasteur, Ottawa, ON,
Canada
K1N 6N5
E-mail: robert.delatolla@uottawa.ca

INTRODUCTION

Biological treatment is the conventional means of treating municipal wastewaters. However, the conversion of soluble substrates into biomass results in a stream of waste activated sludge (WAS) that requires further management. Anaerobic digestion (AD) is an important sludge management tool that is used to degrade biomass, reduce waste volume, and stabilize the organic, putrescible content of the sludge (Appels *et al.* 2008a). As a by-product, AD produces a renewable, energy-rich biogas (BG) which can be used to reduce the energy requirements of the wastewater treatment processes of municipal plants (Shen *et al.* 2015).

AD is a multistage process involving hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Pavlostathis & Giraldo-Gomez 1991; Appels *et al.* 2008a). A cost intensive detraction to AD is its long hydraulic retention time (HRT) relative to aerobic digestion of sludge, resulting in larger footprints and higher capital costs. The rate limiting phase for the AD of sludge generated from municipal wastewater treatment is hydrolysis (Pavlostathis & Giraldo-Gomez 1991; Barber 2005). Digestion can be enhanced through the application of various pretreatment strategies designed to disintegrate the recalcitrant cell material of WAS resulting in a shorter hydrolysis phase and a smaller fraction of undigested material in the effluent (Bougrier *et al.* 2006; Carrère *et al.* 2010; Alqaralleh *et al.* 2015; Cano *et al.* 2015). This reduces the HRT requirements and increases biogas production (BGP).

Ultrasound is a promising pretreatment technology that has been demonstrated at full-scale to yield 3–10 kW of energy from increased methane production for every

kilowatt of applied ultrasonic power (Barber 2005; Xie et al. 2007; Cano et al. 2015). The hydromechanical sheer stresses induced by cavitation disrupt sludge flocs and rupture microbial cell walls, thus releasing intracellular material and soluble organic matter (Wang et al. 2005). Sonicated sludge can also indirectly improve AD by enhancing the buffering capacity of anaerobic phases sensitive to acid accumulation through increased system alkalinity and formation of bicarbonate. Methanogenic biomass can also be improved by 45–140% with sonication densities of 0.18–0.52 W/mL (Mao & Show 2007). Ultrasonic pretreatment has been shown to unfavourably reduce the dewaterability of pre-digested sludge, yet enhance the dewaterability after AD, thereby reducing the cost of residual solids treatment (Şahinkaya & Sevimli 2013).

The investigation of ultrasonic pretreatment on sludges collected from different municipal wastewater treatment plants (WWTPs) has resulted in a wide range of reported performance parameters such as optimal energies, solubilizations, and biogas increases (BGI) (Appels et al. 2008b; Cano et al. 2015). The current literature is lacking with respect to ultrasonic pretreatment research on sludges generated by lagoons and attached growth, biofilm technologies. The different structure of the biomaterial and, in particular, the extracellular polymeric substances (EPS) component of biofilm sludge, as compared to conventional suspended systems, may have an impact on the efficacy of sonication. For example, nutritional, environmental, and operational conditions have been shown to have an impact on the EPS matrix of a rotating biological contactor (RBC) and activated sludges (Martín-Cereceda et al. 2001).

In this work, we investigate the impact of sonication and AD on a conventional sludge and three alternative sludges that are not conventionally digested anaerobically. In particular, the impact of various energy levels of sonication on BGP and solubilization were quantified through soluble chemical oxygen demand (sCOD) analysis and bioassays. Linear and non-linear regression models based on cumulative BGP were applied to compare and interpret digestion results. These investigations provide necessary information for the application of ultrasonic pretreatment to alternative sludges to determine potential viability and BG response of the AD of varying sludges.

MATERIALS AND METHODS

Sludge sources

Sludge was collected from four municipal WWTPs operating suspended and attached growth, biofilm biological treatment technologies. The inoculum and four waste sludges were characterized for total chemical oxygen demand (tCOD), sCOD, total solids (TS), and volatile solids (VS) (Table 1). The first source of sludge for this study was thickened WAS (TWAS) that was collected from the Robert O. Pickard Environmental Centre (ROPEC) conventional activated sludge (CAS) facility, located in Ottawa, Canada. The plant is designed to treat an average of 545,000 m^3/day with a solid retention time (SRT) of 5–7 days. ROPEC was not operated to achieve nitrification. The bacteriological seed for AD (inoculum) was the same for all biogas tests during the study. Inoculum was collected from the mesophilic anaerobic digestors of the ROPEC facility operating at a 48/52% mixture of primary sludge and TWAS with an SRT of 20 days. The second source of sludge in this study was harvested from the Water Pollution Control Plant in Wendover, Canada and was sludge

Table 1 | Characteristics of inoculum and harvested sludges

Source	tCOD (mg/L)	sCOD (mg/L)	TS (%)	VS (%)	VS/TS (%)
Inoculum	21,200 ± 400	11,300 ± 100	1.48 ± 0.01	0.88 ± 0.01	59.5 ± 0.7
CAS	73,200 ± 1,100	15,400 ± 300	6.67 ± 0.02	4.43 ± 0.03	66.4 ± 0.5
Lagoon	24,100 ± 1,200	1,200 ± 200	3.64 ± 0.05	1.51 ± 0.03	42.0 ± 1.0
RBC	25,200 ± 200	420 ± 10	3.02 ± 0.02	1.70 ± 0.01	56.3 ± 0.5
MBBR	20,800 ± 2,200	515 ± 3	2.18 ± 0.02	1.53 ± 0.01	70.2 ± 0.8

Values are sample mean ± standard deviation ($n \geq 3$).

produced by a biofilm treatment system. This facility conducts secondary, biological treatment through the use of three RBCs with a maximum capacity of 1,260 m^3/day of municipal sewage. As the system is an attached growth technology, the SRT values are unknown. The sludge of the RBC technology is produced through the erosion, abrasion, and potential sloughing off excess biofilm mass from the rotating contactors. The third source of sludge was collected from the municipal WWTP at Masson-Angers, Canada that consists of four aerated lagoons in series to treat a combined maximum annual volume of 230,000 m^3 without achieving nitrification. Settled waste sludge was harvested from the fourth lagoon in the multilagoon system (Delatolla & Babartusi 2005). Sludge removal records from the lagoons indicate that the age of the harvested sludge was 5 years. The fourth source of sludge was a temporary, post-carbon-removal, nitrifying moving-bed biofilm reactor (MBBR) pilot system that was fed the final effluent from the Masson-Angers lagoon (Young *et al.* 2016). As the MBBR pilot is an attached growth technology, the SRT of the system is unknown. Sludge of the nitrifying MBBR technology is produced through the erosion of biofilm carriers that are kept in constant motion in the MBBR basins (Karizmeh *et al.* 2014; Forrest *et al.* 2016).

Sonication

RBC, lagoon, and MBBR sludge samples were gravity settled, centrifuged, and diluted with supernatant to an initial concentration of 6.5% TS in order to replicate the solids concentration of the TWAS samples collected from the CAS facility. All samples, including the TWAS, were further diluted to 4.5% TS with a buffer/micronutrient medium (Table S1, available with the online version of this paper) to maintain pH and ensure anaerobic growth would not be limited by a lack of trace nutrients (Kennedy & Droste 1985). Sonication was performed with a 450 Branson Digital Sonifier (Emerson Industrial, Connecticut), with a probe diameter of 13 mm, operating at 20 kHz and peak capability of 400 W. Sonication power (E_S, in kJ/kg) was quantified with specific energy (Equation (1)):

$$E_S = \frac{Pt}{vTS_o} \tag{1}$$

where P is the power (J), t is the duration of sonication (sec), v is the sample volume (L), and TS_o is the initial total solids (g/L).

Samples were sonicated in 200 mL batches for 1, 2, 5, and 10 min which correspond to specific energies of 800 ± 40, $1,550 \pm 130$, $3,770 \pm 300$, and $6,550 \pm 530$ kJ/kg TS. These values bracket the range of sonication power as reported by Bougrier *et al.* (2005) which defines low power ($E_S < 1,000$ kJ/kg TS) as the level at which disintegration is limited to floc disruption and high power (1,000 kJ/kg TS $< E_S < 7,000$ kJ/kg TS) as the level at which cell lysis occurs. Beyond $E_S = 7,000$ kJ/kg TS, there is little reported benefit to increasing BG production with the rate of solubilisation being reported to decrease (Bougrier *et al.* 2005). pH stability due to sonication was verified for each sludge. Samples were sonicated without temperature control to mimic conditions of full-scale applications. Temperature increases were uniform for all sludge types resulting in an 8 °C increase for 1 min of sonication, 14 °C increase for 2 min, 32 °C increase for 5 min, and 50 °C increase for 10 min.

Solubilization was quantified through the degree of sludge disintegration (DD) calculated as the ratio of sCOD increase after sonication to the total possible sCOD increase (Equation (2)). Sonication did not affect tCOD, and thus tCOD is consistent for each sludge type after sonication.

$$DD = \frac{sCOD - sCOD_0}{tCOD - sCOD_0} \tag{2}$$

where sCOD is soluble COD, $sCOD_0$ is the initial soluble COD of untreated sludge, and tCOD is total COD.

Bioassays

The bioassay tests measured BGP according to the procedure by Owen *et al.* (1979) as single stage mesophilic (35 °C) assays on sludges of 4.5% TS to test the effect of sonication on BGP. As mentioned above, the samples were diluted to 4.5% TS with a buffer/micronutrient medium, to maintain pH and ensure anaerobic growth would not be limited by a lack of trace nutrients (Kennedy & Droste 1985). CAS samples were digested in 500 mL reactor vessels containing 300 mL substrate and 60 mL inoculum. Due to

sample volume limitations, all other assays were conducted at a 1:10 scale to the CAS assays. RBC, lagoon, and MBBR tests were conducted in replicates of 4 in 50 mL reaction vessels containing 30 mL substrate and 6 mL inoculum. Bottles were purged with N_2 gas for 2 min, closed with butyl rubber stoppers, and sealed with an aluminium crimp. Samples were incubated and shaken at 35 °C in a Psycrotherm controlled environment incubator shaker (New Brunswick Scientific Co. Inc., USA). BGP was sampled daily and measured by manometer.

CAS sample assays were conducted in triplicate at 35 °C utilizing the AMPTS II (Bioprocess Control, Sweden). Quantity of produced BG was automatically logged every hour by the system.

Analytical methods

TS and VS were measured as per standard method 2540 (APHA 2012). Samples for tCOD analysis were homogenized and measured using HACH method 10212. To mitigate the potential interference of filamentous bacteria causing bias during filtration, sCOD was separated by centrifuging sludge samples at 8,000 g for 20 min and measured using HACH method 8000.

Data analysis

Three non-linear models for the estimation of performance parameters were compared to empirical data for BGP (Table 2). The following models were shown by Donoso-Bravo *et al.* (2010) as being effective at modelling production

**Table 2** | Models for the determination of BGP parameters

Model	Equation		Reference
GM	$B = B_o \cdot \exp\left(-\exp\left(\dfrac{R_m e}{B_o}(\lambda - t) + 1\right)\right)$	(3)	Lay *et al.* (1996)
RC	$B = B_o\left(1 - \exp\left(-\dfrac{R_m(\lambda - t)}{B_o}\right)\right)$	(4)	Redzwan & Banks (2004)
FO	$B = B_o(1 - e^{-k_H t})$	(5)	Pavlostathis & Giraldo-Gomez (1991)

parameters in batch systems. Experimental ultimate biogas production (B_o), maximum production rate (R_m), and lag time (λ) parameters were determined as described by Lay *et al.* (1996). Cumulative biogas yield, B (mL/g VS) and time of digestion t (h) are independent variables. The modified Gompertz (GM) Equation (3) has been used successfully to model biogases in multiple AD systems. The transference function or Reaction Curve (RC) model (Equation (4)) is based on control principles by considering the process as a system receiving inputs and generating outputs to predict maximum gas production. A first-order (FO) kinetic model (Equation (5)) based on substrate degradation is used to find the coefficient of the limiting rate (k_H), which, for AD, is assumed to be hydrolysis. Non-linear optimization and statistical analysis were performed. Comparisons of data were conducted through *t*-tests, one-way analysis of variance (ANOVA), and Tukey's honest significant difference (HSD) range distribution, with *p*-value less than 0.05 indicating significance.

RESULTS AND DISCUSSION

Sludge particulate solubilization

Sonication pretreatment solubilizes particulate and cellular material to shorten the limiting hydrolysis phase of AD, thus potentially increasing the overall rate of treatment and potentially the extent of stabilization. The degree of sludge disintegration can be used to determine the extent of solubilization caused by pretreatment. The present study demonstrates that solubilisation increased with sonication time for all sludge samples analysed in this study (Figure 1). A one-way ANOVA comparing the differing sludge types and DD was conducted for 1 and 10 min levels of sonication. The differences in sludge type had a significant effect on mean DD at 1 min [$F_{(3, 50)} = 14.05$, $p < 0.0001$] and at 10 min [$F_{(3, 50)} = 171.9$, $p < 0.0001$]. Post hoc comparison using the Tukey's HSD test indicates that there is only a significant difference between 1 min RBC sludge ($DD_m = 1.36\%$, SD = 0.32) and the three other samples of 1 min MBBR ($DD_m = 3.76\%$, SD = 0.97), 1 min lagoon ($DD_m = 3.77\%$, SD = 0.27), and 1 min CAS ($DD_m = 4.60\%$, SD = 1.70). There is no significant difference in 1 min DD
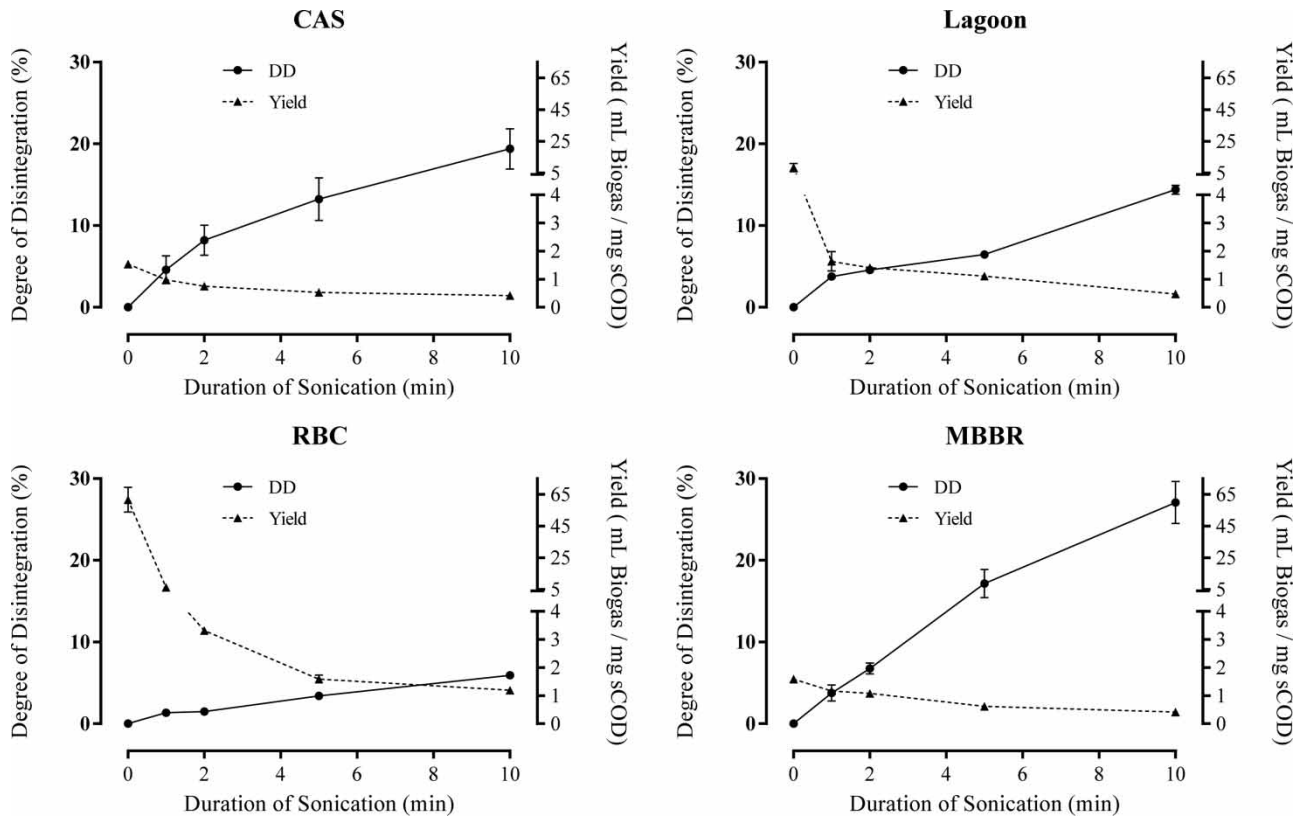
Figure 1 | Solubilization and yield (Y_{sCOD}) vs. sonication times for all sludges.

between MBBR, lagoon, and CAS at this minimum level of sonication. At the highest applied level of sonication (10 min), results indicate that DD of 10 min CAS ($DD_m = 19.39\%$, SD = 2.47), 10 min lagoon ($DD_m = 14.39\%$, SD = 0.54), 10 min RBC ($DD_m = 5.92\%$, SD = 0.34), and 10 min MBBR ($DD_m = 27.07\%$, SD = 2.57) are all significantly different from each other. This suggests that sludge source can be a cause of significant variation in disintegration; the effect becoming more pronounced at higher levels of sonication. This is contrary to previous research that simply modeled DD linearly by the single variable of sonication time alone (Zhang *et al.* 2007).

In this study, BG yield was measured as BG produced per mass of sCOD consumed during digestion. As DD increased and more sCOD was available for digestion, the yield decreased (Figure 1). A comparison of yield (Y_{sCOD}) at 0 and 1 min sonication for each sludge type was conducted by the Student's *t*-test to determine the impact of low-powered sonication. There was a significant Y_{sCOD} decrease ($p < 0.0001$) between 0 and 1 min across

all sludge types: CAS (0 min $Y_{sCOD} = 1.54$, SD = 0.11; 1 min $Y_{sCOD} = 0.98$, SD = 0.09), lagoon (0 min $Y_{sCOD} = 8.36$, SD = 2.56; 1 min $Y_{sCOD} = 1.64$, SD = 0.34), RBC (0 min $Y_{sCOD} = 61.64$, SD = 7.81; 1 min $Y_{sCOD} = 6.18$, SD = 0.60), and MBBR (0 min $Y_{sCOD} = 1.59$, SD = 0.04; 1 min $Y_{sCOD} = 1.17$, SD = 0.05). The decrease in biogas yield continued as the degree of sonication was increased to 10 min. In particular, the RBC sludge showed the greatest effect as biogas yield decreased 10- and 60-fold from 62 to 6.2 and from 62 to 1.2 for 1 and 10 min sonication times.

The statistically significant difference in Y_{sCOD} at various sonication intensities may be caused by either the release of recalcitrant COD or by the inhibition to BGP. Similar effects have been noted in high temperature thermalizations greater than 170 °C (Carrère *et al.* 2010; Kim & Lee 2012). The cause in thermal pretreatments is thought to be the caramelization or burning of substrates and the conversion of carbohydrates and amino acids through Maillard reactions to melanoidins that are toxic or impossible to

degrade (Şahinkaya & Sevimli 2013). Although the bulk temperature of 1 min sonicated samples increased by only 8 °C, sonication can still cause high temperature effects in the sludge through the extreme local conditions of cavitation where the bubbles can have temperatures up to 5000 K (Flint & Suslick 1991; Tiehm *et al.* 2001). It was previously reported that similar inhibition to BGP was caused by high power sonication thought to arise from melanoidin formation or release of inhibitory long chain fatty acids from bacterial cell membranes (Appels *et al.* 2008b; Kim & Lee 2012). However, all four sludges used in this study show a significant decrease in Y_{sCOD} when pretreated with the lowest limit of ultrasonic power (1 min, $E_S = 800$ kJ/kg TS), which is lower than the power threshold of 1,000 kJ/kg TS required for cell lysis (Bougrier *et al.* 2005). Inhibition due to sonication is therefore more likely to be caused by melanoidin formation from high temperature cavitation rather than the release of inhibitory compounds from cell membranes.

BGP and effect of sonication

Four waste sludges from various sources and of differing natures were digested under similar conditions with similar initial solids concentrations. The effect of sonication on cumulative BGP normalized per mass of sludge (mL BG/g sludge), digested for 0, 1, 2, 5, and 10 min of sonication, is illustrated in Figure 2. Cumulative BGP normalized per mass of VS is also illustrated in Figure 2 to compare to conventional normalization methods. This study investigates the BG potential of various sludge types, of which differing VS fractions are inherent characteristics. Hence, the following discussion focuses on the BG produced normalized by sludge mass. It should be noted, however, that the BGP presented normalized per mass of sludge and normalized per mass of VS demonstrate similar responses to sonication. By mass, the ultimate BGP was statistically greater than the control for all sonicated samples. In the subsequent section, modelling is conducted with BGP data normalized by mass VS.

For BG produced from CAS sludge, there was not a significant effect of sonication on BGP with a 95% confidence ($p < 0.05$) for the five conditions [$F_{(4, 10)} = 2.623$, $p = 0.0985$]; however, a t-test performed on 0 min and 1 min BGP at a lower confidence of 90% determined there is a significant difference [$t_{(3)} = 2.967$, $p = 0.0592$] of ultimate BGP between the control, 0 min ($BGP_m = 7.6$, $SD = 0.2$), and 1 min ($BGP_m = 8.00$, $SD = 0.09$) sonication levels representing a 5% ± 3 increase in BGP over the control. While the DD increased with increased sonication for CAS (Figure 1), BGP from 1 min sonicated samples did not differ significantly ($p > 0.1$) from 2, 5, and 10 min sonicated samples and concomitantly the biogas yield (Y_{sCOD}) based on soluble COD decreased.

Sludge samples from the CAS system yielded lower increases in BGP with sonication in comparison to the BGP increases of the alternative sludges studied here. It should be noted that the CAS plant operated with a low SRT and as such the sludge is likely to contain less cell debris and less recalcitrant material than CAS systems operating at longer SRTs. The small differences in BGI from sonication suggest that the pretreatment was less effective on a more readily digestible CAS sludge or energy levels applied in this study were less than the threshold required to exceed floc disintegration and enter a cell lysis phase. This implies that the reaction of CAS sludge to sonication is unique with respect to the reactions of the alternative sludges to the same sonication energy levels.

An ANOVA conducted on the ultimate BGP of lagoon sludges indicated that sonication had a significant effect for all five applied sonication energy levels [$F_{(4, 15)} = 5.994$, $p = 0.0044$]. 1 min of sonication produced the greatest final quantity of BG ($BGP_m = 1.326$, $SD = 0.003$) for all lagoon sludges, increasing BGP over the control by 12% ± 9. A subsequent post hoc comparison using Tukey's HSD test found BGP from 1 min sonicated sludge was significantly greater ($p < 0.05$) than the control, 0 min ($BGP_m = 1.2$, $SD = 0.1$), and 10 min ($BGP_m = 1.19$, $SD = 0.02$) ultimate BGP, yet not significantly different ($p > 0.05$) than 2 min ($BGP_m = 1.28$, $SD = 0.04$) and 5 min ($BGP_m = 1.29$, $SD = 0.03$). The overall low ultimate BGP of 1.19–1.32 mL/g sludge suggests that a majority of the biodegradable organics were digested over the 5 years the sludge has accumulated at the benthic zone of the lagoon. As such, it is likely that the tCOD was refractory and not susceptible to sonication pretreatment.

An ANOVA conducted on ultimate BGP of RBC sludges indicated that sonication had a significant effect on ultimate BGP for this sludge type [$F_{(4, 15)} = 49.75$, $p < 0.0001$]. In
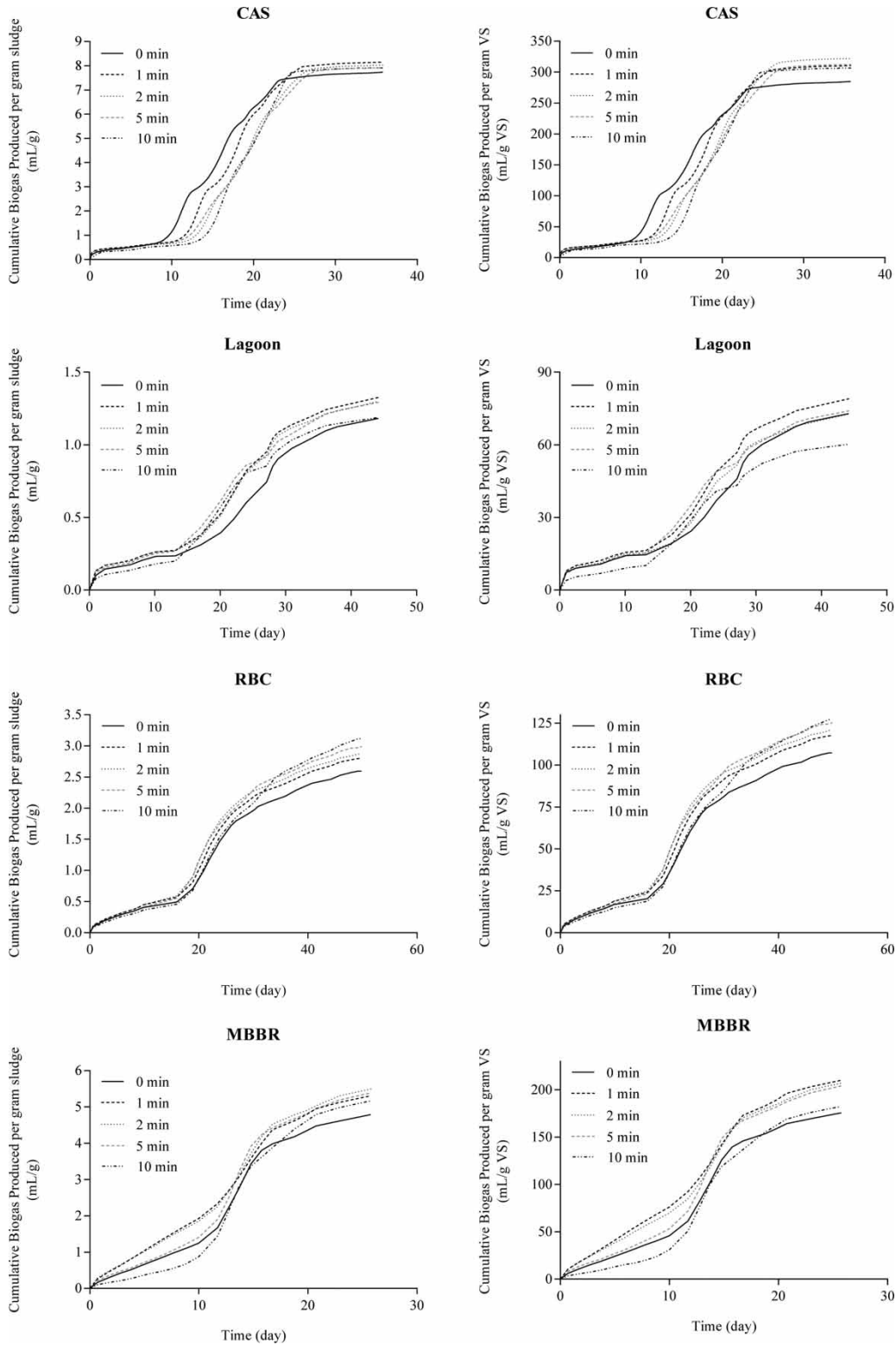
Figure 2 | Cumulative BGP for all sludge types and sonication times normalized by mass of sludge and by mass of VS.

this case, 10 min of sonication produced the greatest final volume of BG ($BGP_m = 3.12$, SD = 0.01) for RBC sludges, increasing BGP over the control by 20% ± 2. A post hoc comparison found BGP from 10 min sonicated sludge significantly greater ($p < 0.05$) than 0 min ($BGP_m = 2.59$, SD = 0.04), 1 min ($BGP_m = 2.80$, SD = 0.06), 2 min ($BGP_m = 2.87$, SD = 0.04), and 5 min ($BGP_m = 2.98$, SD = 0.09). This is the only case within the four sludge types tested where increased sonication energy correlated directly with increased BGP. Sonicating RBC sludge for 10 min exhibited the greatest increase in biogas, 20% ± 2 compared to the other sludges (Figure 3). The fact that biofilm sludge has a high component of extracellular polysaccharide material the sonication pretreatment may have a very positive impact on solubilizing the EPS and making it more readily available for digestion.

An ANOVA conducted on ultimate BGP of MBBR sludges indicated that sonication again had a significant effect for all levels of sonication [$F(4, 10) = 19.33$, $p = 0.0001$]. The greatest increase of BGP production over the control, 15% ± 2, occurred for the 2 min sonicated sludge ($BGP_m = 5.49$, SD = 0.2), which was significantly greater ($p < 0.05$) than 0 min ($BGP_m = 4.79$, SD = 0.06) and 10 min ($BGP_m = 5.17$, SD = 0.07), yet not significantly different ($p > 0.05$) from 1 min ($BGP_m = 5.30$, SD = 0.04) and 5 min ($BGP_m = 5.4$, SD = 0.2) BGP. This similarity in the response of the MBBR sludge to the RBC sludge indicates that sonication may have a greater effect on sludge generated by biofilm technologies.
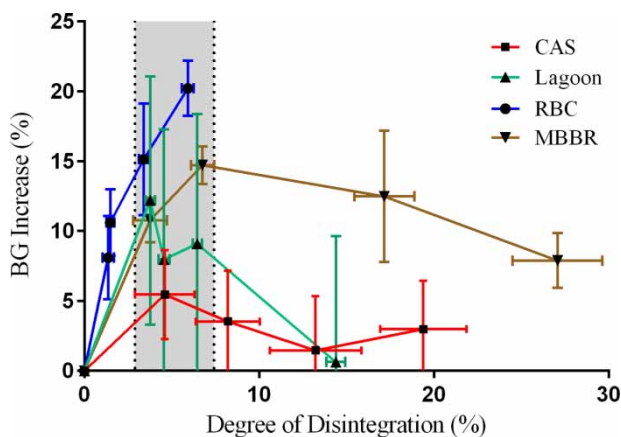
Figure 3 │ Biogas increase vs. solubilization as the degree of disintegration for all sludge types. Shaded area represents the region of maximum biogas increases for four sludge types tested.

The effects of sonication on solubilization of sludge and increase of BGP appear to vary according to the sludge source tested. Bougrier *et al.* (2005) reported the effective sonication energy ranges of floc disruption and cell lysis based on municipal secondary WAS using high-load aeration floatation-thickening to 1.85% TS. Other researchers using different secondary sludge sources found similar phases of floc disruption and cell lysis, yet at differing energy levels (Zhang *et al.* 2008). The present study shows that sludges react uniquely to ultrasound based on their source and treatment technology. This is likely due to the varying composition of the sludge, with the results demonstrating that biofilm sludge and hence high EPS content sludge are more responsive to ultrasound treatment; as demonstrated by the DD and BGP of the RBC and MBBR sludge. The ultrasound treatment in this study appears to effectively disintegrate and disrupt the EPS and embedded cells in biofilm sludge, thus increasing readily available substrate for digestion and ultimately BGP. The level of solubilisation due to sonication and peak BGP vary according to sludge type. However, peak BGP of all sludge types occurred when the DD was within a small range of 2.9–7.4% (Figure 3). The sonication energy level that achieved the peak BGP was unique for each different sludge type. Each sludge had a unique value of sonication energy for the point of diminishing returns where DD may increase, but BGP is inhibited. CAS sludge yielded lower increases in BGP after sonication in comparison to the BGP increases of the alternative sludges studied here. The small differences in increased BGP after sonication for CAS sludge suggest that the energy levels used were less than the threshold required to exceed floc disintegration and enter a cell lysis phase. This would make the reaction of CAS sludge to sonication unique with respect to the reactions of the alternative sludges to the same sonication energy levels. The alternative sludges also reacted uniquely to sonication in terms of BGP increases. These results could explain the large range of literature values for the increased BGP from sonication pretreatment of 6.3–53% (Table 3). Hence, variation in reported results of AD of sonicated WAS may be due to variations in treatment style, initial sCOD, sludge age, and influent concentrations that are unique to each source sludge studied (Wang *et al.* 2005). This could explain the wide variation in BGP increases.

Table 3 | Comparison of reported results for AD of sonicated sludge types

Sludge type	Ultrasonic conditions			AD conditions			Findings	Source
	Frequency (kHz)	Energy level	Duration	Scale	Temperature	Residence time (days)	Biogas (BGI) and/or methane (MI) increase	
Industrial WAS	20	0.33 W/mL	20 min	Batch	Mesophilic	40	104% MI	Chu *et al.* (2002)
						6	290% MI	
Municipal WAS	41	–	150 min	Semi-continuous	Mesophilic	8	41.6% BGI	Tiehm *et al.* (2001)
	20	–	30 min	Batch	Mesophilic	33	23% BGI	Onyeche *et al.* (2002)
	27	200–300 W/L	2.5–30 min	Batch	Mesophilic	19	10–20% BGI	Grönroos *et al.* (2005)
	20	7,000 kJ/kg TS		Batch	Mesophilic	16	40% BGI	Bougrier *et al.* (2005)
	31	10 W/cm²	90 sec	Pilot	Mesophilic	8	16% BGI	Nickel & Neis (2007)
	20	1 W/mL	1 min	Batch	Mesophilic	–	5.6% MI 6.3% BGI	Şahinkaya & Sevimli (2013)
	20	800 kJ/kg TS		Batch	Mesophilic	30	5% BGI	Current study
TWAS	20	0.52 W/mL	1 min	Batch	Mesophilic	16	53% BGI	Mao & Show (2007)
	25	1,020 W/L	–	Batch	–	8	40% BGI	Appels *et al.* (2008b)
1/3 Primary 2/3 WAS	20	13.7 W/cm²	1.5 sec	Full	–	30	45% BGI	Xie *et al.* (2007)
53% Primary 47% WAS	31	–	96 sec	Batch	Mesophilic	28	30% BGI	Tiehm *et al.* (1997)
75% Primary 25% WAS	20	11,000 kJ/kg TS		Semi-continuous	Mesophilic	20	31% BGI	Benabdallah El-Hadj *et al.* (2007)
					Thermophilic	15	16% BGI	
Not specified	–	–	–	14 Full	–	12–69	20–50% BGI	Barber (2005)
	20	10.8 kW/kg TS		Pilot	–	20	42% BGI	Pérez-Elvira *et al.* (2009)
Lagoon	20	800 kJ/kg TS		Batch	Mesophilic	30	12% BGI	Current study
RBC	20	6,550 kJ/kg TS		Batch	Mesophilic	30	20% BGI	
MBBR	20	1,550 kJ/kg TS		Batch	Mesophilic	30	15% BGI	

Modelling of BGP results

Three kinetic models previously used for methane production to describe the AD process and critical digestion performance parameters were tested against BGP normalized by mass of VS (Figure 2) (Donoso-Bravo *et al.* 2010). Overall, the models showed strong correlation ($r^2 = 0.920$–0.999)

with the data and are deemed useful for the accurate determination and comparison of design parameters. As an example, Figure 4 illustrates the results of the non-linear regression using the three models for 1 min sonication times. The complete results can be found in Table S2 (available with the online version of this paper). Non-linear regression results for 0, 2, 5, and 10 min sonications can be
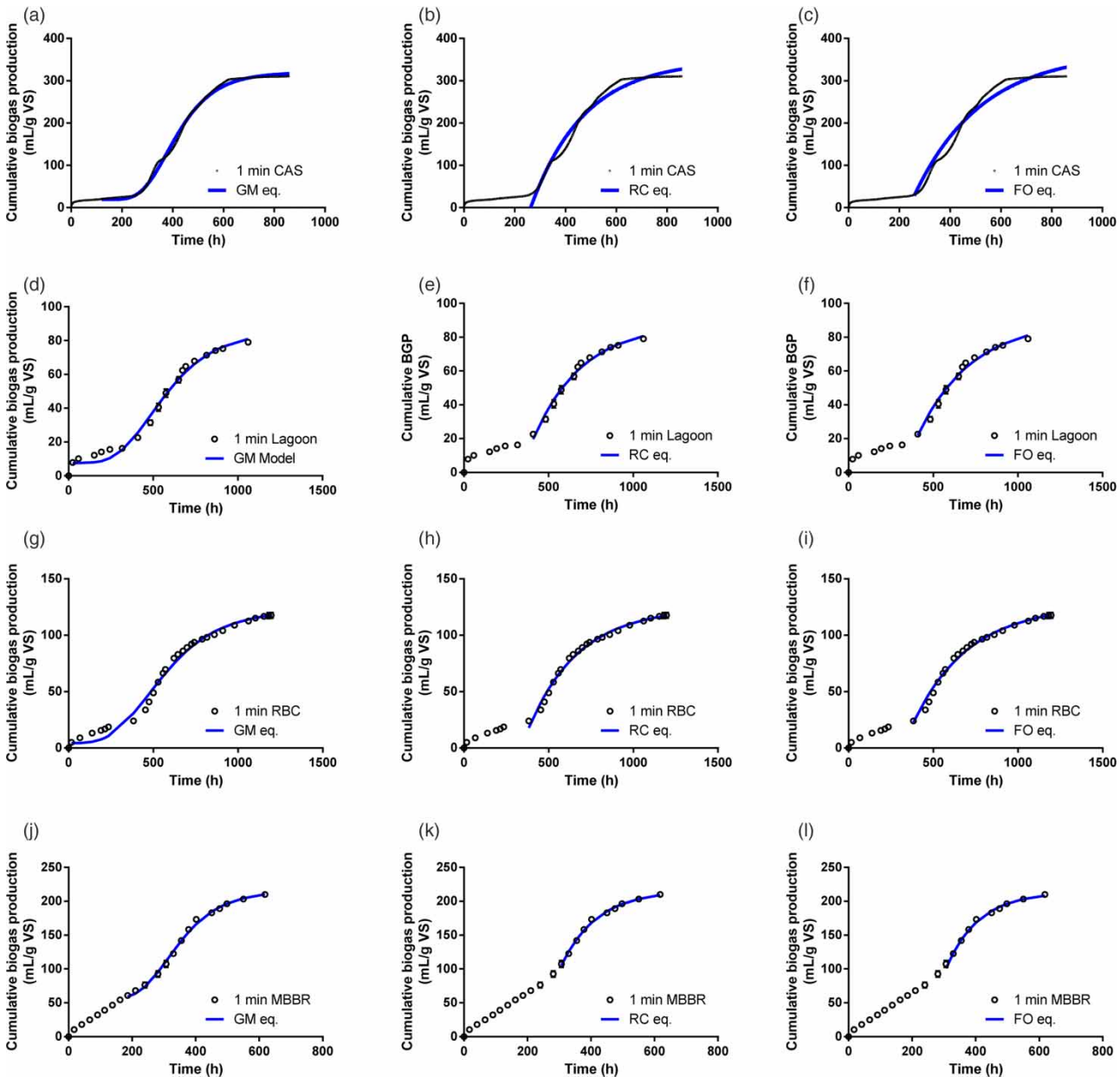
Figure 4 │ GM, RC, and FO models fit to mean cumulative BGP for 1 min of sonication for CAS (a)–(c), lagoon (d)–(f), RBC (g)–(i), and MBBR (j)–(l). Standard deviation of BGP is not included for CAS.

found in Figures S1–S4 (available online). The GM model had overall stronger fit ($r^2 \geq 0.961$) and could accommodate a larger data set that had significant lag times better when compared to RC (r^2: 0.920–0.995) and FO (r^2: 0.917–0.992), but tended to underestimate the maximum rate of BGP (slope) for the curve as noted by Donoso-Bravo *et al.* (2010).

A one-way ANOVA was conducted on each model parameter to compare the effects of sonication on B_o, R_m, Lag, and k_H for 0, 1, 2, 5, and 10 min of sonication pretreatment. All 32 tests showed a significant effect of sonication on the individual design parameters at the $p < 0.05$ level. There is no case in which the control sludge without sonication pretreatment has the greatest modeled maximum (R_m) or overall (k_H) kinetic rate (Table S2). There is no case in which the highest BGP occurs at the same sonication level as the highest maximum (R_m) or overall (k_H) rate.

Ultimate BGP has already been discussed in the previous section. Since there are two functions that can model the R_m parameter, a comparison of the standard deviation of residuals ($S_{y.x}$) for GM and RC models was conducted over the same, truncated data set as used by the RC model to determine which model deviated the least from experimental values and could thus more accurately portray R_m. For CAS sludge, the more accurate parameter is derived from the GM model while RBC, Lagoon, and MBBR R_m are better predicted by the RC model. A comparison of R_m and k_H modelled parameters with DD were found to be significantly different ($p < 0.05$) based on separate ANOVA tests; yet, there is no clear effect of DD on R_m or k_H, which is concurrent with Donoso-Bravo *et al.*'s (2010) findings. It is possible that the increased initial sCOD generated from sonication pretreatment has no effect on the maximum rate of digestion or the overall apparent hydrolysis coefficient while still significantly being able to impact the ultimate BG yield.

During lag time before active digestion, the inoculum's hydrolytic microbes require time to adjust enzyme production to accommodate the available substrates. Since inoculum was not acclimatized to the substrates before digestion, a significant lag phase was expected, but is usually neglected in kinetic analysis due to highly variable and uncertain lag phase length in batch experiments (Lay *et al.* 1996). Experimental lag was determined by extrapolating a line from the point of R_m

to the axis with slope of R_m (Lay *et al.* 1996). However, CAS data show apparent diauxic characteristics where multi-phasic BGP is evident, separated by multiple, short duration plateaus in the profile (Figure 2 – CAS), making the aforementioned method unreliable and inconsistent since the maximum BGP rate could occur in different phases resulting in a non-sensical value for lag. In this case, a better separation between lag and growth phases was determined by the time when BGP first exceeded 2 mL/day. The BGP varies in the lag phase yet, when it reaches 2 mL/day, it does not decrease again. Using this estimation for the time when lag phase ends matches with the graphical data (Figure 2) and will be used to delineate between lag phase and the start of the active digestion phase, which ends when BGP again decreases below 2 mL/day.

The hourly resolution of CAS BGP data illuminated multiple production phases separated by mini-plateaus representing diauxic performance (Figure 2 – CAS). The CAS control sludge without pretreatment has four active phases of BGP separated by short lag times in 345 ± 2 h of active digestion and an FO modelled hydrolysis coefficient of $3.90 \cdot 10^{-3}$ h^{-1}. The 1, 2, and 5 min sonicated sludge digestions also have four phases of BGP. Their apparent hydrolysis coefficient did not differ significantly with that of the control. However, the 10 min sonicated sludge has three phases of BGP. When compared to the control, it results in a significantly shorter [$t(4) = 37.74$, $p < 0.0001$] active digestion time of 272 ± 2 h and a faster hydrolysis coefficient of $4.60 \cdot 10^{-3}$ h^{-1}. This indicates that sonication pretreatment is effective at homogenizing the most recalcitrant substrates that compose the fourth BGP peak into ones that can be digested with other more preferable, more easily digestible substrates. This effectively shortens active digestion time through the removal of an additional lag phase.

Overall, excepting 1, 2, and 10 min sonication pretreatment of MBBR sludge, sonication increases the maximum rate of digestion over non-sonicated sludge in the other 13 cases, but not in a clearly definable pattern. Ideal sonication energy may be unique for each sludge type and dependent on system requirements. Designing a system for both the greatest increase in kinetics and greatest BGP may not be possible.

CONCLUSIONS

In this work, sonication pretreatment was proved to significantly increase tCOD biogas yield in mesophilic batch assays of the four sludge types tested. It had a greater impact on increasing BGP from biofilm type sludge (15–20% for MBBR and RBC) as compared to conventional, suspended growth technologies (5–12% for CAS and Lagoon). The different sludge types tested responded uniquely to the same levels of sonication energies. An optimal specific energy for the greatest production of BG was not found that coincided for all sludges. Instead, optimal specific energy was unique for each sludge, but the peak BGP for all sludges occurred within a small solubilization range of 2.9–7.4% DD. Sonication pretreatment exhibited significant BGP inhibition relative to sCOD, even at the lowest applied energy levels of 800 kJ/kg TS. In most cases, there was no significant difference ($p < 0.05$) in increased BGP between low (1 min, 800 kJ/kg TS) and high (10 min, 6,550 kJ/kg TS) energy levels. The use of automated BG logging revealed diauxic growth patterns in the BGP of CAS WAS. The duration of the active phase of BGP decreased significantly in the AD of sludge sonicated for 10 min (6,550 kJ/kg TS) where the number of active phases of BGP was reduced from 4 to 3.

Three models were used to fit experimental data and determine ultimate BGP, maximum rate of digestion, lag time, and rate of hydrolysis coefficient. GM, RC, and FO models show strong correlations with sonicated waste sludge BGP. The GM model was useful for fitting experimental data with significant lag time. R_m and k_H parameters were successfully determined with RC and FO models when data sets were truncated to remove lag time. Sonication had no clear effect on R_m or k_H, which were shown to be poor indicators of the effect of sonication pretreatment on digestion kinetics. Overall, ultrasound impacted BGP, disintegration and digestion rates in ways that could not be easily correlated with the basic characterizations and models employed in this study.

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