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Bovine bile as a bio-surfactant pre-treatment option for anaerobic digestion of high-fat cattle slaughterhouse waste

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ABSTRACT

Bovine bile was assessed as a novel bio-surfactant pre-treatment to enhance anaerobic digestion of lipid-rich dissolved air flotation (DAF) sludge using biochemical methane potential (BMP) tests. Bile was dosed at arbitrary concentrations from 0.2–6 g/L. At 0.6 g bile/L, methane yield increased by 7.08%. Doses above 2 g bile/L produced negative impacts on SMP, kinetics and digestion profile. At 6 g/L bile produced a 6% decrease in specific methane production and up to 79% additional inhibitory duration, delayed time of peak methane production by up to 74%, and slowed total digestion time by up to 65%. Reaction kinetics declined linearly with respect to bile addition, reaching half the control value at 6 g/L bile concentration. Subsequent anaerobic toxicity assays between 1 and 6 g bile/L revealed that bile has an inhibitory effect under BMP testing at these higher doses. The economic viability of using bile as a bio-surfactant was assessed. In comparison to the current use of bile as a sale product to pharmaceutical companies, the addition of 0.2 g bile/L to existing slaughterhouse waste streams could increase the value of bile to 220% of its current sale value. The promising results of bile dosed at 0.6 g/L under BMP testing warrant further investigation into long-term impact of bile pre-treatments of high-fat slaughterhouse wastewater in semi-continuous digestion experiments.

1. Background & introduction

The high concentrations of fat, oil and grease (FOG) in red meat processing (RMP) water can be problematic in anaerobic digestion (AD) systems. Lipids affect digesters in many ways, including pipe blockages, crust formation and short-circuiting, sludge flotation and washout, and reversible inhibition of mass-transfer of nutrients induced by long-chain fatty acids (LCFA) [1]. This is particularly relevant when sludge is less active; situations where slaughterhouse waste is used in monodigestion or the AD technology does not incorporate temperature control and stirring. While FOG may be difficult to utilise as a substrate, altering the material with pre-treatment prior to entering an AD system may improve its bio-availability, and reduce either the frequency and or severity of complications [2].

Pre-treatment of a substrate involves the application of a treatment to the substrate prior to digestion in an attempt to improve substrate degradability [3]. The desired effect of this is to improve biogas yields, while improving or maintaining stable digester operation. While there have been many investigations into the pre-treatment of waste activated sludge, lipid pre-treatment has been a largely undeveloped field [4,5]. Pre-treatment options of particular interest include thermobaric, chemical, thermochemical, ultrasound, and biochemical methods. Of these, biochemical methods have been investigated the least, and literature regarding bio-surfactant pre-treatment methods is scarce [2].

Bio-surfactants are naturally-derived, typically non-toxic, and biodegradable surface active agents which improve the solubility of lipids into an aqueous solution, thereby increasing the interaction between microbial enzymes and lipids, and consequently enhancing hydrolysis, the rate-limiting step of anaerobic digestion [6–8]. However, this also increases the risk of foaming [9,10]. Saharan et al. [8] identified a number of potential bio-surfactants derived from microbiological and plant sources, although few have been investigated for application in anaerobic digestion. Some successful applications of bio-surfactants include use of 'BOD-balance' by Nakhla et al. [11], which is a combination of bio-surfactant and enzyme used by Damasceno et al. [12].

Investigation of BOD-balance by Nakhla et al. [11] as a pre-treatment to aid in the digestion of wastewater high in FOG yielded promising results. With a dose of 500 mg BOD-balance/L, the researchers measured no change in chemical oxygen demand (COD) solubilisation following pre-treatment, but did record a significant improvement in particulate COD (PCOD) soluble COD (SCOD) degradation. Bio-surfactant addition increased PCOD removal by 96%, and SCOD by 100%, while also increasing COD biodegradation rate coefficient of 164–238%. The authors note that the increase in PCOD removal is due

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to a reduction in surface tension induced by the bio-surfactant, which helps solubilize hydrophobic organics, including FOG and colloidals [11]. Unfortunately, there was little focus on methane production during the investigation by Nakhla et al. [11]. However, it was noted that bio-surfactant addition appeared to reduce methane yield.

Bile is a natural product which is formed in the liver and stored in the gall bladder. It is a by-product of meat processing, and while there are pre-existing markets in cosmetics, pharmaceuticals and biological media [13], bile may be of value in enhancing the anaerobic digestion of high-fat wastes and aid in the operation of on-site AD systems in red meat processing plants. *In vivo*, bile acts as a surfactant to reduce large fat globules into smaller globules and thereby increase the surface area to volume ratio, consequently increasing the surface area available for enzymatic degradation.

This article presents novel work conducted using bovine bile as a bio-surfactant pre-treatment of high-fat cattle slaughterhouse. The aim of the work was to assess the effectiveness of bile, a readily available by product of meat processing, in enhancing anaerobic digestion of abattoir wastewater using batch biochemical methane potential (BMP) tests.

2. Materials and methods

2.1. Inoculum and substrate

Three batches of inocula were used in this experiment. The inoculum for the first BMP test using bile at 1–6 g/L was collected from the sludge recirculation pump servicing an anaerobic digester at a red meat processor. Due to unforeseen operational issues at the initial site of inoculum collection, the quality of the inoculum decreased markedly, and in subsequent testing was no longer able to produce > 80% of theoretical methane potential within 10 days when digesting cellulose. Consequently, subsequent batches of inoculum were collected from a new source at a wastewater treatment plant, prior to sludge thickening. Two separate samples were collected to conduct the second BMP testing bile at 0.2–1 g/L, and an anaerobic toxicity assay (ATA). Sludge was immediately transported back to the lab and stored in an incubator at 37 °C.

The substrate was dissolved air flotation (DAF) sludge, a concentrated source of FOG residues produced by the DAF process that is representative of the fatty material entering the anaerobic digestion system of red meat facilities [14]. Substrate was collected from the outlet of a DAF unit, and refrigerated at 4 °C until use. Avicel microcrystalline cellulose powder was used as a control substrate to measure sludge activity.

Bile was collected fresh from the abattoir and refrigerated at 4 °C until use. The characteristics of the inocula, substrates and bile used in this investigation are presented in Table 1.

Table 1
Characteristics of inocula, DAF sludge, cellulose and bile used in digestions.

	pH	VS (% of TS)	COD (mg/L)	FOG (mg/L)
Low-dose BMP: 0.2–1.0 g bile/L				
Inoculum	7.48	63.01	ND	ND
DAF sludge	4.40	98.32	469,000	85,000
High-dose BMP: 1–6 g bile/L				
Inoculum	6.86	76.86	ND	ND
DAF sludge	4.28	95.82	469,800	10,500
Anaerobic Toxicity Assay				
Inoculum	7.48	76.41	ND	ND
Cellulose	ND	95.38	ND	ND
Bio-surfactant				
Bile	6.74	81.7	ND	ND

ND = not determined; BMP = Biochemical methane potential; ATA = Anaerobic toxicity assay.

2.2. Pre-treatment of DAF sludge

Bile was dosed to reactors immediately prior to beginning the BMP digestion. Concentrations for bile addition were determined arbitrarily due to the novelty of the pre-treatment. Consequently, bile was dosed 0.2, 0.4, 0.6, 0.8, 1, 2, 3, 4, 5 and 6 g/L of final liquid volume prior to commencing the BMP test. Bile characteristics are presented in Table 1.

2.3. Biochemical methane potential and anaerobic toxicity assay

BMP tests were conducted using the Automated Methane Potential Test System II (AMPTS II; Bioprocess Control, Lund Sweden). Final reactor volume was 400 mL, with an inoculum to substrate ratio of 3:1 based on volatile solids (VS) to avoid overloading. Reactor temperature was maintained at 37 ± 1.0 °C in a water bath. Biogas was scrubbed of carbon dioxide using 3 M sodium hydroxide, and resulting methane was measured by the AMPTS II gas measurement unit. Cellulose controls were used to confirm sludge activity of > 80% of its theoretical maximum [15], and a bile control was used to account for methane yield from bile VS. Digestions were considered complete on the day that daily methane production dropped below 1% of the total methane production (T_{FIN}) [15]. Results are reported as normal millilitres (mL_N), normalised to 0 °C and 1 atm and corrected for water vapour.

An anaerobic toxicity assay was performed to elucidate the non-specific, overall inhibitory effect of high-dose bile addition. The ATA was performed using the AMPTS II as above, with cellulose as a standard substrate and bile was dosed at 1, 2, 3, 4, 5 and 6 g/L of final reactor volume. Inoculum to substrate ratio was 3:1 to be consistent with the BMPs. Kinetic analysis was used to quantify the effect of bile addition on methane formation rate kinetics, and inhibition with respect to lag phase, delay in reaching peak methane production, and time required to complete digestion (Table 3).

2.4. Analytical methods

Parameters included: pH, VS and total solids (TS) using standard method 2540G [16]. COD was measured using Merck colorimetric test kits type 5000–90000 mg COD/L with a Spectroquant Pharo 100 spectrophotometer. FOG content was measured using a Wilks Infracal II, with sample workup similar to the user manual. Briefly, sample material was acidified using HCl to pH < 2, shaken, mixed 10:1 with hexane, and shaken again for 1 min. Emulsified hydrophobic component was extracted and centrifuged at 18000*g* for 5 min to break the emulsion. Hexane component was measured on the Wilks Infracal II O&G unit. Samples requiring dilution for COD (1 in 10, V/V) and FOG (1 in 100–1000, V/V) analysis were diluted with distilled water prior to application to the analytical method.

2.5. Kinetic analysis

Kinetic analysis was applied to the collected data to determine the rate constant (*k, U*) of linear gas production and better estimate the lag period (λ) for each treatment to assess the degree of inhibition due to bile pre-treatment. Two equations were fitted to the data to acquire values for rate constants and lag periods. Eq. (1) was a standard growth curve logistic function, while equation 2 was a modified Gompertz equation [17]. Equations were fitted using SciPy optimization curve-fit routine [18]. In order for the equations to be applied, data must fit a sigmoid shape. With exception to the cellulose and controls, sigmoid-shaped graphs were achieved by excluding data obtained from days 0–3, with day 4 considered to be day 0 for subsequent curve fitting. This offset was then added back to the equation outputs to obtain the true value for variables such as inhibitory period and time of maximum production.

Eq. (1): Growth curve logistic equation

$$B = \frac{B_0}{1 + e^{-k(t-t_0)}} \quad (1)$$

From Eq. (1): B is the cumulative specific methane potential (SMP; mL CH_4/g VS) at time t (days); B_0 is the maximum SMP achieved by end of digestion; k is the rate constant; t_0 is the time at which maximum production rate occurs. The function is weighted using standard deviation to achieve a better fit.

Eq. (2): Modified Gompertz equation [17].

$$B = B_0 e^{-e^{\left(\frac{U_e}{B_0}(\lambda - t)+1\right)}} \quad (2)$$

From equation (2): B is the cumulative SMP at time t; B_0 is the maximum SMP achieved by end of digestion; U is the kinetic constant of methane production rate; λ is the duration of lag phase in days, used here to represent inhibition. Equation is unweighted.

2.6. Statistical analyses

One factor analysis of variance (ANOVA) was used to detect a difference between groups in BMP tests. Due to small sample sizes of $n = 3$, the non-parametric equivalent, the Kruskal-Wallis test was employed in an attempt to improve the resolution of the statistical investigation. In the event that both the ANOVA and Kruskal-Wallis tests were significant with $P < .05$. T-tests were used to further investigate between groups, with the non-parametric Mann-Whitney test used to help account for low sample sizes. Where P values are given, all four of these tests have returned a significant result, and the T-test result has been reported.

3. Results and discussion

3.1. Biochemical methane potential of bile-treated DAF sludge

3.1.1. BMP of DAF sludge treated with bile at 0.2–1 g/L dosage

Addition of bile at 0.2–1 g/L improved biogas production from the outset of digestion (Fig. 1). With respect to the final methane yield attained by the control, bile treatments achieved equivalent methane yield 4 ± 0.71 days earlier and yields corresponded to the theoretical maximum yield from fat of 1014 mL/g VS. Impact to rate kinetics were negligible (Table 2).

Addition of bile at dosage of 0.2–1 g/L produced a significant increase in SMP in the range of 5.71%–7.08% with $P < .05$ in all cases. Although bile addition increased SMP at these doses, a dose-response relationship was not demonstrated. The increase in biogas production from the bile control was negligible, and coupled with the lack of dose-response relationship, this indicated the increase in SMP achieved by the digesters was not related to the additional VS or COD in the form of bile. It may be possible that 0.2 g bile/L was sufficient to saturate the fatty material, aid solubility of fats, and subsequently improve digestion such that further increases in bile dose would produce minimal improvement.

3.1.2. BMP of DAF sludge treated with bile at 1–6 g/L dosage

Bile addition at dosage of 1–6 g/L had negligible influence on biogas production. However, the impact on lag phase and T_{FIN} was significant and was prolonged with increasing doses of bile (Fig. 2; Table 3). An inhibitory duration of 7.1 ± 0.2 days was determined for the controls using the Gompertz equation. Addition of bile further increased the inhibitory duration by 10% ± 3% (3 g bile/L), 14% ± 3% (4 g bile/L), 37% ± 4% (5 g bile/L), and 79% ± 6% (6 g bile/L) (Fig. 2; Table 3). Similar outcomes were recorded by Feitkenhauer and Meyer [19] in which alcohol sulfate, an anionic surfactant was added to batch digestions. Although the concentration of surfactant used by Feitkenhauer and Meyer [19] was much lower at 50–500 mg/L, the researchers observed significantly prolonged inhibition. Lag phase was doubled in the lowest dose, and time required to finish digestion was also extended. The curves displayed in Fig. 2 are the average of 3 replicates, with error bars removed to improve clarity. Standard deviations ranged from 3 to 11 mL_N CH_4/g VS with an average of 8 mL_N CH_4/g VS.

Peak methane production rate was achieved by 10.1 ± 0.1 days in the controls, with similar results in the 1 and 2 g bile/L groups. Increased dosage produced statistically significant delays at 13 ± 2% (3 g/L), 19 ± 2% (4 g/L), 39 ± 2% (5 g/L) and 74 ± 3% (6 g/L) respectively (Table 3). Completion of digestion followed a similar trend. At doses of 0 and 1 g bile/L trials were complete after 17 ± 0 days, with a negligible increase at 2 g/L to 17.7 ± 0.6 days. At doses of 3–6 g bile/L, T_{FIN} was significantly delayed by 16 ± 7% (3 g/L), 22 ± 7% (4 g/L), 37 ± 7% (5 g/L), and 65 ± 6% (6 g/L) (Table 3). Reaction kinetics declined linearly with respect to bile addition ($R^2 = 0.9634$). From the logistic equation, a rate constant of $k = 0.73 \pm 0.01$ was determined for the controls. Addition of bile impacted rate constants by −3 ± 3% (1 g bile/L), −7 ± 1% (2 g bile/L), −22 ± 3% (3 g bile/

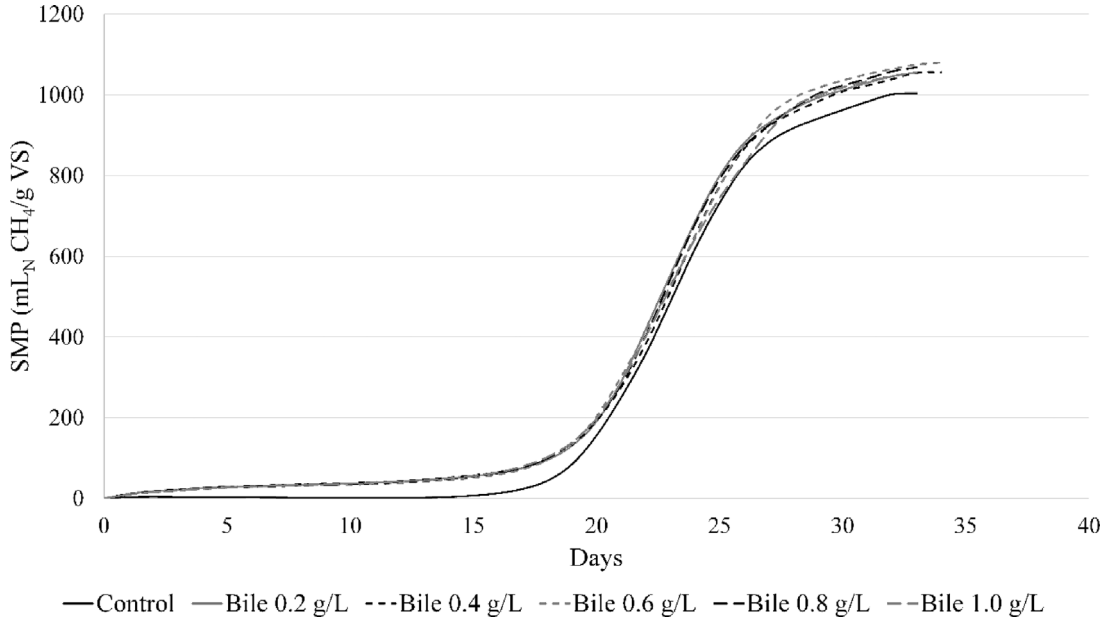

Fig. 1. Effect of low-dose bile on SMP and anaerobic digestion profile of DAF sludge.

Table 2
Kinetics modelling of SMP curves from low-dose bile BMP using a standard growth curve logistic equation and a modified Gompertz equation.

Units	Inhibition (λ) Days	T_0 Days	Finish day Days	B_0 NmL CH_4/g VS	*k*	U NmL CH_4/g VS/day	R^2 Logistic	R^2 Gompertz
Cellulose[a]	3.2 ± 0.1	5.8 ± 0.1	14 ± 1.2	348 ± 2	0.81 ± 0.04	71 ± 2	0.997	0.998
Control	19.2 ± 0.1	23.2 ± 0.0	33 ± 0.6	999 ± 7	0.53 ± 0.01	136 ± 2	1.000	0.999
Bile 0.2 g/L	19.1 ± 0.1	23.1 ± 0.1	33 ± 0.6	1056 ± 22	0.51 ± 0.01	139 ± 3	0.999	0.998
Bile 0.4 g/L	19.2 ± 0.1	23.4 ± 0.1	34 ± 0.0	1056 ± 3	0.48 ± 0.01	135 ± 9	0.999	0.997
Bile 0.6 g/L	19.1 ± 0.1	23.3 ± 0.2	33 ± 0.6	1080 ± 12	0.50 ± 0.01	135 ± 4	1.000	0.997
Bile 0.8 g/L	19.1 ± 0.1	23.3 ± 0.1	33 ± 0.6	1068 ± 4	0.50 ± 0.01	136 ± 4	0.999	0.998
Bile 0.1 g/L	19.0 ± 0.1	23.2 ± 0.3	33 ± 1.7	1056 ± 12	0.50 ± 0.03	129 ± 4	1.000	0.997

[a] Cellulose is provided as a reference for comparison, logistic equation was unweighted to achieve curve fit.

L), −27 ± 4% (4 g bile/L), −40 ± 2% (5 g bile/L), and −52 ± 5% (6 g bile/L) (Table 3).

Bile dosed at 1 and 2 g/L produced negligible impact on inhibitory duration. Given the minimum inhibitory concentrations of oleic acid (C18:1) from the literature of 0.443 mM [20], to 2.4 mM [21], and the composition of beef tallow of 37–47% C18:1 [22,23], the FOG load of 1245 mg/L equates to 1.73–2.15 mM C18:1. This is well within the reported range of inhibitory concentrations, and given sufficient solubilisation, LCFA inhibition should result.

While doses of 1 and 2 g bile/L appeared to have negligible impact on inhibitory duration, doses between 3 and 6 g bile/L induced significant inhibition. At doses of 3, 4, 5 and 6 g/L, inhibition increased by 9.8%, 14.1%, 36.6% and 78.87% respectively. The inhibition observed was consistent with descriptions of inhibition by LCFA adsorption, as the inhibition was reversible and overcome to produce roughly equivalent methane yields in all doses with exception to 6 g/L [24]. At 6 g bile/L, methane yield was reduced by an average of 6% ($P < .05$, $n = 3$).

The methane yields from the BMP trialling doses of 0.2–1 g bile/L were much greater than those obtained from the BMP trialling doses of 1–6 g bile/L. Yields in the low-dose BMP were very close to the theoretical maximum yield from fat of 1014 mL/g VS, and was likely due to the much greater FOG content of the DAF sludge used. Conversely, the yields from the high-dose BMP were much lower, which was consistent with a much lower FOG content.

While it is standard for BMP experiments to be conducted at an I:S ratio of 2:1, Li et al. [25] identified that the optimum I:S ratio for FOG digestion lies between 4:1 and 1.33:1. For this work, an I:S ratio of 3:1 was used with the intent to limit overloading and subsequent inhibition typically associated with FOG digestion. As 4:1 was the highest ratio investigated by Li et al. [31], I:S ratios greater than 4:1 may further optimise FOG digestion, increasing SMP and/or reducing inhibition.

3.2. Evaluation of bile inhibition using anaerobic toxicity assay

The ATA produced small changes in digestion profile (Fig. 3). As bile dose increased from 0 to 6 g/L, inhibition increased by up to 16.67% with a good linear correlation to dose ($R^2 = 0.94$). The time required to reach T_0 was delayed by up to 10%, and also correlated well with bile dosage ($R^2 = 0.88$). Bile dosage showed no effect on time required to complete the digestion. Similarly, the rate constant, *k*, showed little change between the control and 4000 mg/L dose, but began to reduce with doses of 5000 and 6000 mg/L by 3% and 5% respectively (Table 4). The curves displayed in Fig. 3 are the average of 3 replicates, with error bars removed to improve clarity. Standard deviations ranged from 1 to 17 mL_N CH_4/g VS with an average of 5 mL_N CH_4/g VS.

In comparison to the results produced by the ATA, where sludge response at doses of 0–6 g bile/L was linear, the response observed in the BMP is much more typical of a logarithmic growth curve, in which 6 g/L is beginning to severely delay the process (Fig. 4). This variation could be a result of the much greater sludge quality in the ATA.

The anaerobic toxicity tests demonstrate that bile had an inhibitory effect during BMP testing at doses of 3–6 g bile/L. As evidenced by Girault et al. [26] and Martin-Gonzalez et al. [27], once an anaerobic consortium had overcome initial LCFA inhibition, the rate of biogas production increases to a similar rate as the controls. While the ATA was digesting cellulose, the recovery of the rate kinetics reproduced in the ATA indicated that bile produced reversible inhibition, while a dose of 6 g bile/L induced the first signs of decline in reaction kinetics. However, in the BMP, the rate kinetic began to decline significantly from addition of 3 g/L. This inhibition could be caused by susceptibility to free fatty acids [28], or bile [29], but possibly due to the compounding effect of both. Bile is known to be toxic to various bacteria, in particular, gram-positive bacteria [29]. While population composition varies, gram-positive can account for a considerable fraction of active anaerobic biomass [30]. It is therefore possible that bile toxicity could have played a major role in reducing the rate of biogas production.

3.3. Comparison of bile pre-treatment at low (0.2–1 g/L) and high (1–6 g/L) doses

The high-dose trial utilised a FOG-acclimatised inoculum, with a substrate relatively low in FOG content. The resulting impact of bile addition was found to be largely negligible or negative depending on the dose. In comparison, the low-dose trial used an inoculum that was unaccustomed to high-fat substrates, and was combined with a substrate much higher in FOG content, yet produced an increase in SMP.

Table 3
Kinetics modelling of SMP curves from high-dose bile BMP using a standard growth curve logistic equation and a modified Gompertz equation.

Units	Inhibition (λ) Days	T_0 Days	Finish day Days	B_0 NmL CH_4/g VS	*k*	U NmL CH_4/g VS/day	R^2 Logistic	R^2 Gompertz
Cellulose[a]	1.3 ± 0.1	2.9 ± 0.1	10.0 ± 0	312 ± 6	1.30 ± 0.13	102 ± 4	0.991	0.998
Control	7.1 ± 0.2	10.1 ± 0.1	17.0 ± 0	765 ± 11	0.73 ± 0.01	121 ± 6	0.999	0.999
Bile 1 g/L	7.3 ± 0.1	10.3 ± 0.1	17.0 ± 1	764 ± 12	0.71 ± 0.02	121 ± 6	0.999	0.995
Bile 2 g/L	7.0 ± 0.2	10.1 ± 0.1	17.7 ± 0.6	761 ± 3	0.68 ± 0.01	109 ± 5	0.993	0.994
Bile 3 g/L	7.8 ± 0.2	11.4 ± 0.2*	19.7 ± 1.2*	756 ± 7	0.57 ± 0.02*	96 ± 4*	0.997	0.994
Bile 4 g/L	8.1 ± 0.2	12.0 ± 0.2*	20.7 ± 1.2*	755 ± 8	0.53 ± 0.02*	88 ± 4*	0.994	0.989
Bile 5 g/L	9.7 ± 0.3*	14.0 ± 0.2*	23.3 ± 1.2*	741 ± 3	0.44 ± 0.01*	75 ± 4*	0.989	0.987
Bile 6 g/L	12.7 ± 0.4*	17.6 ± 0.3*	28.0 ± 1*	720 ± 11*	0.35 ± 0.02*	65 ± 3*	0.986	0.986

* Statistically significant at $P < .05$, $n = 3$.
[a] Cellulose is provided as a reference for comparison, logistic equation was unweighted to achieve curve fit.

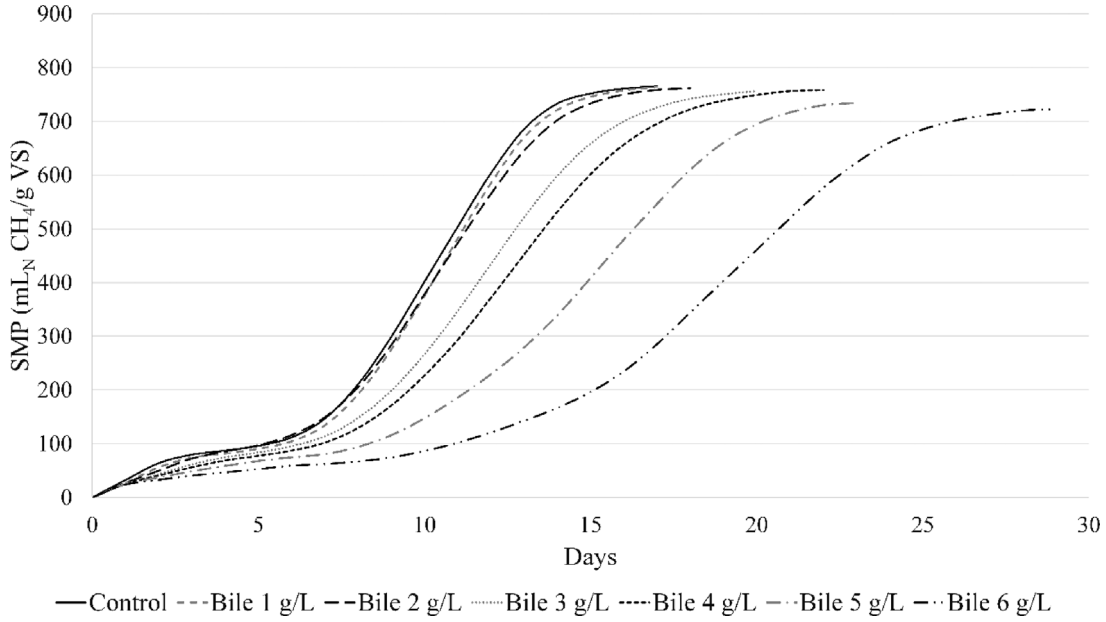

Fig. 2. Effect of high-dose bile on SMP and anaerobic digestion profile of DAF sludge.

The data indicate that there is potential for beneficial outcomes from low-dose bile addition. However, the data also support that the influence of bile on the digestion process at these lower doses is also reliant on other factors, indicated by the varying result of the 1 g/L trial, which overlaps both BMP investigations. It is likely that variation in inoculum or substrate is responsible for this inconsistency. Subsequently, the cellulose controls for each BMP and the ATA were compared (Fig. 5). The sludge used for the low-dose investigation, while slower to complete digestion, produced 9% and 12% more biogas than the inoculum used for the ATA and high-dose BMP respectively. It is likely that the superior sludge quality used in the low-dose BMP was responsible for the positive response to bile addition observed in the low-dose BMP.

Bile is a complex mixture of components, with a range of critical micellar concentrations (CMC). Surfactant compounds within bile, sodium deoxycholate, sodium chenodeoxycholate and sodium cholate, have CMCs of 5.3, 7.0 and 18.4 mM respectively [31]. Of the bile doses trialled, 5 g/L and 6 g/L have potential bile salt concentrations above 5.3 mM, with 5.6 and 6.7 mM respectively. Measured differences in inhibition, SMP, finish time and reaction kinetics, as shown in Figs. 2 and 4, and Tables 3 and 4 indicate that degradative effects are appearing as early as 3 g/L, with a potential bile salt concentration of 3.6 mM. While these effects don't appear to correlate with CMC, these degradative effects do appear to become more severe at concentrations above the CMC of sodium deoxycholate, and micelle formation may induce more significant inhibitory effects.

3.4. Economic considerations of bile pre-treatment of wastewater at meat processing facilities

Bile is a by-product of red meat processing and was used in this study to assess the relative merit as onsite treatment of processing wastewater to improve anaerobic digestion. The current use of bile at

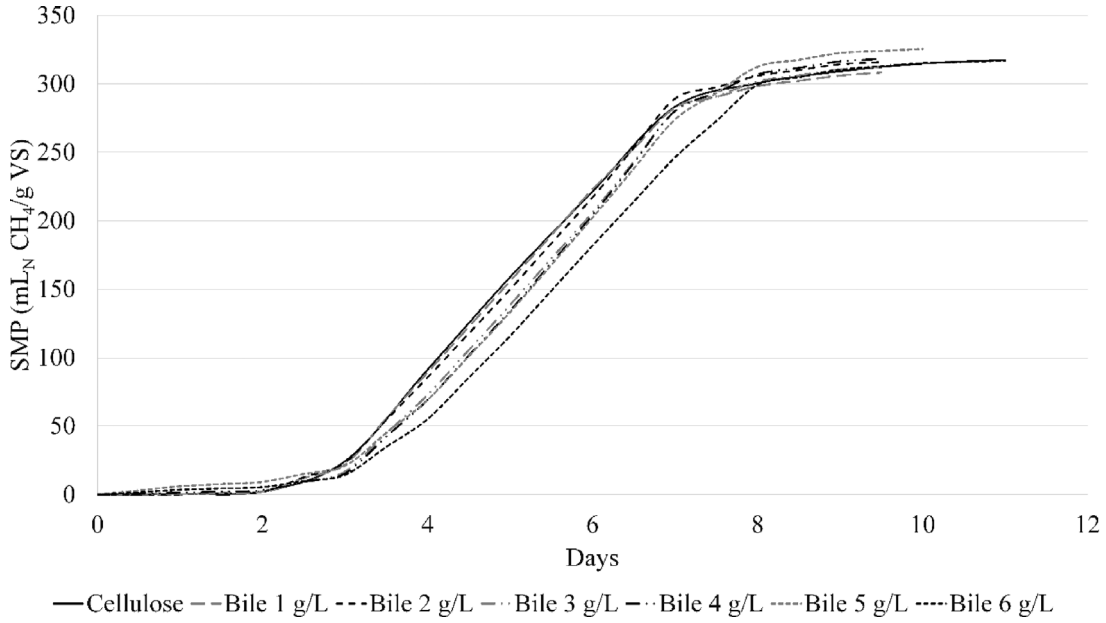

Fig. 3. Anaerobic toxicity assay with cellulose as a standard substrate, and bile as the substance in question.

Table 4
Anaerobic toxicity assay with cellulose and bile.

Units	Inhibition (λ) Days	T_0 Days	Finish day Days	SMP mL_N CH_4/g VS/day	*k*	U mL_N CH_4/g VS/day	R^2 Logistic	R^2 Gompertz
Cellulose	3.0 ± 0.1	5.1 ± 0.1	10 ± 0	318 ± 2	1.03 ± 0.05	83 ± 3	0.998	0.998
Bile 1 g/L	3.0 ± 0.1	5.0 ± 0.0	10 ± 0	309 ± 2	1.07 ± 0.03	84 ± 3	0.998	0.997
Bile 2 gL	3.1 ± 0.1	5.1 ± 0.0	10 ± 0	318 ± 9	1.05 ± 0.03	84 ± 3	0.998	0.995
Bile 3 g/L	3.3 ± 0.1	5.3 ± 0.0	10 ± 0	313 ± 2	1.07 ± 0.03	85 ± 3	0.998	0.995
Bile 4 g/L	3.4 ± 0.1	5.4 ± 0.0	10 ± 0	322 ± 1	1.06 ± 0.03	86 ± 3	0.998	0.995
Bile 5 g/L	3.5 ± 0.1	5.5 ± 0.0	10 ± 0	318 ± 1	1.04 ± 0.03	83 ± 3	0.998	0.995
Bile 6 g/L	3.5 ± 0.1	5.7 ± 0.0	10 ± 0	318 ± 1	1.00 ± 0.03	80 ± 3	0.998	0.993

Australian RMP facilities is as a sale product to pharmaceutical industries. The effort of collecting and preparing bile for this purpose is considerable. Although collection by specialised equipment can yield approximately 96% of bile, simple slashing of the gall bladder and draining bile by workers can result in loss of up to 50% is common [32]. Collected bile must then be heated to concentrate the bile to 75% solids. This carries the benefits of preserving the product and avoiding the cost of preservatives, while eliminating large quantities of water to reduce shipping costs [32]. At a throughput of 500 head of cattle per day for a medium sized Australian RMP facility, with a bile volume of 0.4 L and a solids content of 10%, this equates to 20 kg of solids available per day [32]. At a value of 25 AUD/kg (Dennis King, pers.comm. 1/11/2017) [37], bile is worth 500 AUD/day or 125,000 AUD/year (250 days) to the processor. Assuming 50% loss of bile, this value is reduced to 250 AUD/day or 62500 AUD/year. This return is so low that many processors consider the return on invested effort and energy does not warrant collection (Dennis King, pers.comm. 1/11/2017).

By comparison, dosage to the waste stream does not require concentration. At a throughput of 500 cattle, 200 L of bile is recoverable. While the maximum methane increase measured in this investigation was 7.08%, 0.6 g bile/L, the corresponding quantity would require 600 L of bile to treat 1 ML of wastewater. Alternatively, a dose of 0.2 g bile/L would require 200 L of bile, and is possible for an increase in methane yield of 5.71%, assuming 100% bile recovery.

In comparison with the financial implications considered in Harris *et al.* [14], an SMP of 759 m^3 CH_4/kg VS was measured for a DAF sludge containing 14.21% VS, presumed to be primarily FOG. For a 143 m^3 load of waste, the equivalent FOG load was calculated to be around 20.3 m^3, or, with a density of approximately 0.7861 g/mL, a rough mass of 15.96 tons of FOG solids. The treatment of this volume of wastewater with bile would require 28.6 L of bile. This mass, with an SMP of 759 m^3/kg VS would produce 12105119 m^3 CH_4. An additional 5.71% equates to 691202 m^3 CH_4. With an energy content of 35.75 MJ/m^3, this volume contains 19334 MJ. At 3.6 kWh/MJ, and an electrical conversion efficiency of 40% for a combined heat and power plant, this would result in approximately 2148 kWh of usable electricity. At a rate of 0.15 AUD/kWh, this would be worth 322 AUD. If used to offset natural gas, at a rate of 8.15 AUD/GJ, this would be worth 158 AUD. With respect to the volume of bile generated per day, around 200 L, the treatment of 1 ML of such waste would bring the value of bile up to around 1102 AUD/day based on these values. In comparison with the collection and preparation of bile for sale to the pharmaceutical industry, bile for wastewater treatment is dosed directly to the waste stream with no other treatment, reducing the effort and energy investment. Conclusion

This work identified that bile dosed at 0.6 g/L produced a 7.08% increase in methane yield. Higher doses of bile ranging between 3 and 6 g/L resulted in reduced methane yield, increased inhibition by up to 79%, and reduced reaction kinetics by 52%. The economic viability of using bile as a bio-surfactant was assessed. In comparison to the current use of bile as a sale product to pharmaceutical companies, the addition of 0.2 g bile/L to existing slaughterhouse waste streams could increase the value of bile, through biogas production, to 220% of its current sale value. The quality of inoculum and substrates were important factors when assessing the effect of bile as a pre-treatment option.

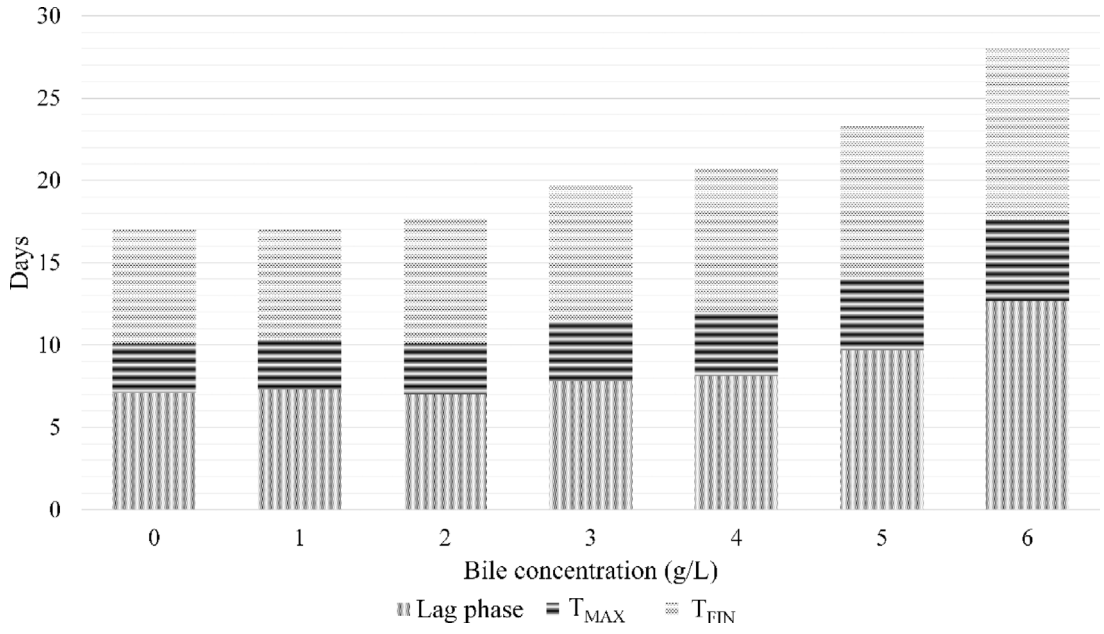

Fig. 4. Inhibition period with respect to bile addition in BMP testing. T_{MAX} represents the time between recovering from lag phase inhibition and achieving maximum methane production rate. T_{FIN} represents the time between achieving T_{MAX} and completing digestion with methane production < 1% of total yield.

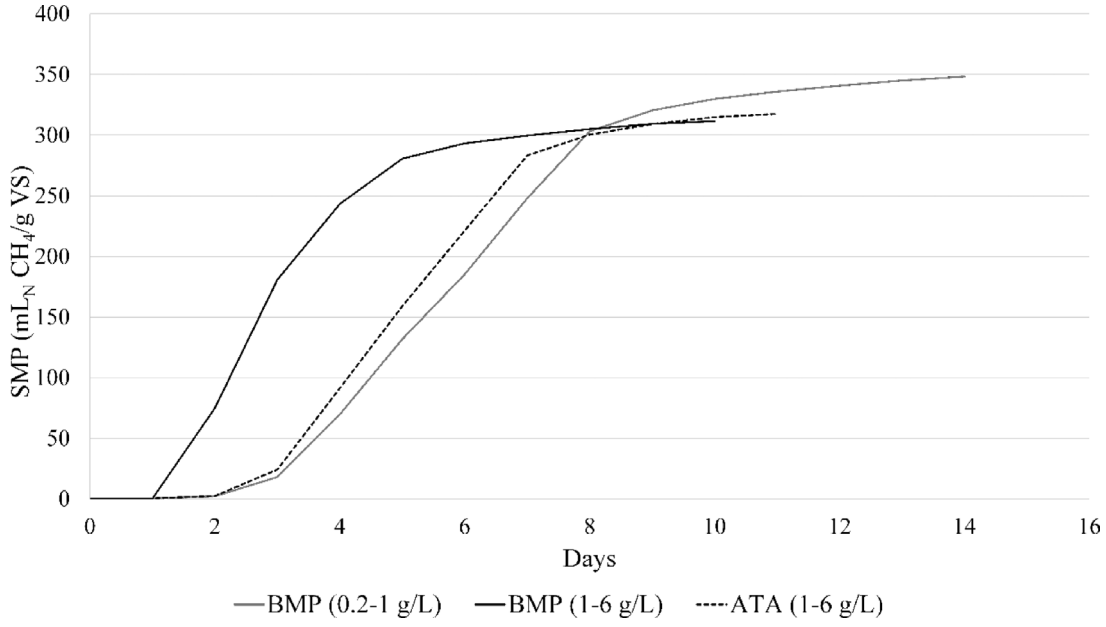

Fig. 5. Comparison of cellulose controls from BMP and ATA investigations.

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