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

Peter W. Harris*, Thomas Schmidt, Bernadette K. McCabe

National Centre for Engineering in Agriculture, University of Southern Queensland, Toowoomba, QLD, Australia

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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

* Corresponding author.
E-mail addresses: Peter.Harris@usq.edu.au (P.W. Harris), Thomas.Schmidt@usq.edu.au (T. Schmidt), Bernadette.McCabe@usq.edu.au (B.K. McCabe).

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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