Bio-stimulation of anaerobic digestion by low intensity ultrasonication

Vinay Kumar Tyagi, Aron Loh Shu Tien, Banafsha Ahmed and Arti Gautam

ABSTRACT

Low intensity ultrasonication (US) was applied to stimulate the biological activities in anaerobic digestion (AD) process. The enhancement in methane production was used to investigate the sono-biostimulation effects on the process performance. The 32% higher CH_4 production was observed over control at best US intensity and irradiation time of 0.0028 W/mL and 120 s, respectively. The sono-biostimulation effects in terms of higher CH_4 generation over control lasted for 45 h. The increase in the concentration of NH_4^+-N and K^+ considered the indication of cell lysis under applied US conditions. At best US intensity and irradiation time, the NH_4^+-N and K^+ fraction in the medium remained similar as of control, which indicated that no cell lysis occurred. The preliminary findings of the study showed that low intensity US can be a promising solution to enhance the process efficiency in terms of higher methane production with minimal energy requirement.

Key words | anaerobic digestion, bio-stimulation, low intensity, methane, ultrasonication

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INTRODUCTION

Anaerobic digestion (AD) is considered to be the most attractive process for organic wastes stabilization across the world. AD, a mature technology, turns organic matter into two valuable products: (a) energy-rich biogas and (b) nutrient rich digestate, with or without minimum energy requirement (Tyagi *et al.* 2018). However, the major limitations of AD is slow and partial degradation of organic wastes (sludge, organic fraction of municipal solid waste, and lignocellulosic wastes), i.e. low degradation efficiency (20–50%) and longer retention time (20–50 days). These limitations can be overcome by pre-treatment of the organic substrates by means of thermal (conventional and microwave), chemical (acid, alkali, peroxidation, ozonation) or mechanical processes (high-pressure homogenizer, ultrasonication etc.). Pretreatment is an interesting option to achieve the high organic matter solubilization, to increase acidogenic and methanogenic biodegradability, and subsequent improve the methane (CH_4) production (Tyagi & Lo 2011; Liu *et al.* 2019; Wang *et al.* 2019). Among the pretreatment technologies studied, ultrasonication (US) has gained wide attention as an effective mechanical method for organic waste due to excellent performance, technical and operational stability, and environment friendly processing (Tyagi *et al.* 2014a). Ultrasonication has been studied widely to enhance the solubilization of organic feedstocks before anaerobic digestion (Pilli *et al.* 2011). However, earlier studies also reported that controlled US for a short period can enhance the metabolic activities of the biomass, which may help to improve the overall process efficiency (Schlafer *et al.* 2002; Kwiatkowska *et al.* 2011; Tyagi *et al.* 2013, 2014a). The enhancement of biological activities by the means of low intensity US has been reported due to several reasons: defensive response to the induced mechanical stress, increased permeability of cell wall and cell membrane, improved mixing conditions and liquid–solid interface, which therefore enhances the mass transfer rate (Chisti 2003).

Most of the research on ultrasonication has been focused mainly on the pre-hydrolysis of the substrate through solubilization of the organic matter. However, little attention has been paid to examine the beneficial effects of US on AD process performance. The objective of this work was to investigate the efficiency of US to enhance the process efficiency by stimulating the biological activities. The study was carried out in following steps: (1) to determine the best US intensity (W/mL) and exposure time (seconds, s) based on the amount and rate of methane generated; (2) to determine the occurrence of cell lysis, based on the ammonium, and potassium concentration in

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the medium, at variable US intensity and exposure time; and (3) to determine the time for which the effect of biostimulation lasts on the biomass after ultrasonication.

MATERIAL AND METHODS

Inoculum and substrate: The inoculum used as seed for the batch assays was collected from the anaerobic digester treating sewage sludge (primary and waste activated sludge mix) at a local wastewater treatment plant in Singapore. The sludge was stored at 4 °C before use. The physico-chemical characteristics of the seed sludge used were: pH 7.10 ± 0.2, total solids (TS) 14.50 ± 0.15 g/L, volatile solids (VS) 10.70 g/L ± 0.12 g/L, total suspended solids (TSS) 13.60 ± 0.20 g/L, volatile suspended solids (VSS) 10.10 ± 0.20 g/L, total chemical oxygen demand (tCOD) 15.41 ± 0.28 g/L, soluble COD (sCOD) 0.57 ± 0.13 g/L.

Starch was used as a substrate in this study. The starch solution of 4 g/L strength was prepared by mixing the starch powder in distilled water. The inoculum to substrate ratio was kept 1:1 (225 mL + 225 mL) in a 500 mL bottle. Once the substrate was prepared, it was purged with nitrogen gas to remove any oxygen present in the substrate.

Ultrasonication experiment: A laboratory scale ultrasonicator (QSonica Model Q700, 20 kHz, 700 W, USA) was used for the experiments. For each experiment, different volume of seed sludge (inoculum) was sonicated in order to achieve the desired US intensity (W/mL). The sludge was sonicated in a beaker, continuously stirred (200 revolutions per minute, rpm) using a magnetic stirrer. The ultrasound probe (13 mm diameter, 7 cm length) was placed at the middle of the sludge, which was immersed at a depth of 4 cm.

For the optimization of US intensity the anaerobic seed sludge was sonicated at five US intensities of 0.0007, 0.0015, 0.0021, 0.0028, and 0.0035 W/mL (power amplitude: 1%) for 30 s. The exposure period was optimized using the optimum US intensity of 0.0028 W/mL (power amplitude: 1%) at different US times of 15, 30, 45, 60, 120, 180, 240, and 300 s.

BMP assay: The biomethane potential (BMP) tests were carried out in an automatic methane potential test system (AMPTS) (Bioprocess Control AB, Lund, Sweden). The experiments were performed in triplicates at 37 °C. One set of blank (Inoculum only), to quantify the methane production from the inoculum only, and one set of control (non-sonicated inoculum + substrate), were included in each experiment. Methane production from blank was subtracted from methane production of the sonicated and control tests to eliminate background methane overestimation.

Figure 1 shows the experimental plan of the study.

Analytical methods: The CH_4 production (flow and volume), soluble COD (sCOD), NH_4^+-N and K^+ were

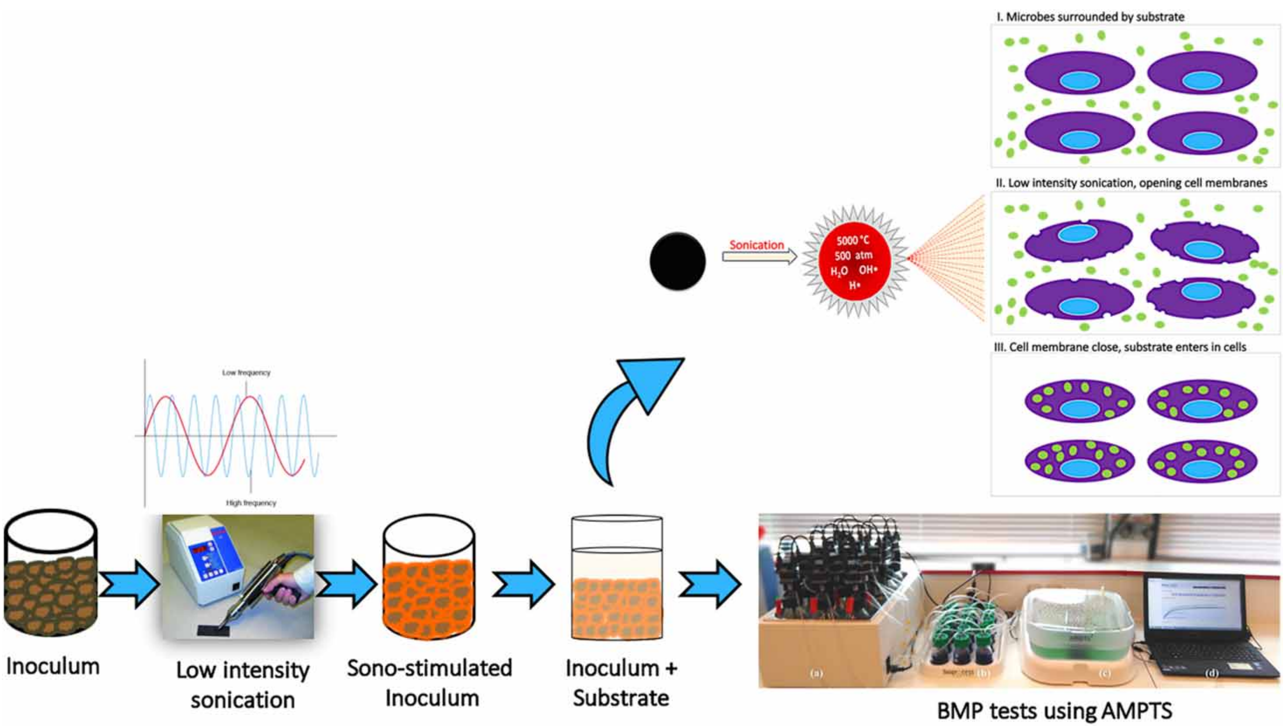

Figure 1 | Experimental plan with sono-biostimulation mechanism (Chisti 2003; Achkar *et al.* 2016).

analyzed to observed the effects of US. Sludge solubilization is characterised by measuring the increase in soluble COD (sCOD), NH_4^+-N and K^+. All the physico-chemical parameters were analyzed as per *Standard Methods* (APHA 2005). The biogas composition was measured in a gas chromatograph (Shimadzu GC-2014) with a packed column (Carbosieve SII- SUPELCO) and thermal conductivity detector (TCD) (Tyagi *et al.* 2014b).

RESULTS AND DISCUSSION

Best ultrasonication intensity

The findings in terms of percentage improvement (hourly) in CH_4 generation with respect to control show that CH_4 generation reached up to 60% higher than the control at US intensity of 0.0028 W/mL (Specific energy: 8.3 kJ/kg TS) and 30 s US time (Figure 2(a)). For the same US intensity and time, the overall CH_4 produced in five days digestion period was 20% higher than the control. The highest CH_4 yield of 113 mL $CH_4/gCOD_{added}$ was observed at US intensity of 0.0028 W/mL and time of 30 s, which was 32% higher than the control (86 mL $CH_4/gCOD_{added}$). Figure 2(b) shows that the enhanced bio-stimulation effect lasted for 28 hours (h). During this period, the CH_4 generation was 50% higher (0.0028 W/mL, 30 s) than the control. Stable cavitation occurred at low US intensity ($\leq$0.1 W/mL at 25 kHz), which instigated the enhancement of mass transfer and fluid mixing, and favorably affects the metabolic activities of microbiome (Kwiatkowska *et al.* 2011). However, high US intensity may lead to instantaneous cavitation, which could destroy the cell structure and subsequently inhibit biological activities. Based on these observations, 0.0028 W/mL US intensity was considered as the best intensity for all further experiments.

The effect of US intensity and exposure time on cell lysis was also determined. The increase in the concentration of ammonium and potassium were the indications of occurrence of cell lysis at the applied US intensity and exposure time. No sign of increase in ammonium and potassium was observed except a slight increase in sCOD (10–15%) from US intensity of 0.0021 W/mL onwards (Figure 2(c)). As sludge was exposed to the US, extracellular polymeric substances (EPS) were released due to the breakup of sludge flocs, which contributed to increase in soluble organic fraction into the liquid phase to some extent, which also depends on the US intensity and exposure time applied. Upon exposure to an adequately high US intensity, cell membranes were ruptured, and thus released intracellular material into the medium (Foladori *et al.* 2007). The concentration of nitrogenous compounds also increased: through the release of intracellular ammonia (Carrere *et al.* 2010), the solubilization of organic nitrogen (Bougrier *et al.* 2005) and ammonia formation from hydrolysis of proteins (Van de Moortel *et al.* 2017). Studies related to increase in potassium indicates that when the cells lysis takes place under high mechanical stress, the intracellular potassium released into the bulk liquid. Potassium efflux has been used as an indicator of cytoplasmic secretion under cell disintegration conditions (Wimmer & Love 2004; Liao *et al.* 2005).

Best ultrasonication time

The best US intensity of 0.0028 W/mL was applied at different irradiation times of 15, 30, 45, 60, 120, 180, 240 and 300 s. The enhancement in CH_4 generation compared to control revealed that the enhanced bio-stimulation effect lasted up to maximum 73 h (30 s exposure time) (Figure 3(a)). The CH_4 generation of sonicated reactors during higher bio-activity hours was compared with the CH_4 generation in control reactor for respective hours. The highest CH_4 generation of 61% was observed in the reactor sonicated for 120 s, wherein sono-biostimulation effect lasted for 45 h. For the same irradiation time, the overall CH_4 produced in 3.5 days digestion period was 32% higher (highest among the assays run) than the control. The highest CH_4 yield of 153 mL $CH_4/gCOD_{added}$ was observed at US intensity of 0.0028 W/mL and irradiation time of 120 s (specific energy: 24.3 kJ/kg TS), which was 42% higher than the control (108 mL $CH_4/gCOD_{added}$). In another experiment, the US exposure time above 120 s, i.e. 180, 240, and 300 s were tested. A cumulative reduction in CH_4 generation was observed for 180 s (22%), 240 s (54%) and 300 s (41%) in comparison with 120 s assay (data not presented), which may be due to detrimental effects of ultrasonication on anaerobic microbiome.

In order to realize the microbial inhibition, the effect of US intensity and exposure time on cell lysis were determined. No sign of increase in ammonium and potassium were observed up to 120 s, however, above 120 s an apparent increase in ammonium (2–5%) and potassium (16–38%) was observed (Figure 3(b)). Similarly, soluble COD was increased by 12–15% above 120 s US exposure period. This could be the result of excessive cavitation due to prolonged exposure, which may lead to cell wall destruction and subsequently decreased CH_4 generation.

Thus, an irradiation time of 120 s was observed to be the best in this study. This implies that the anaerobic activity in

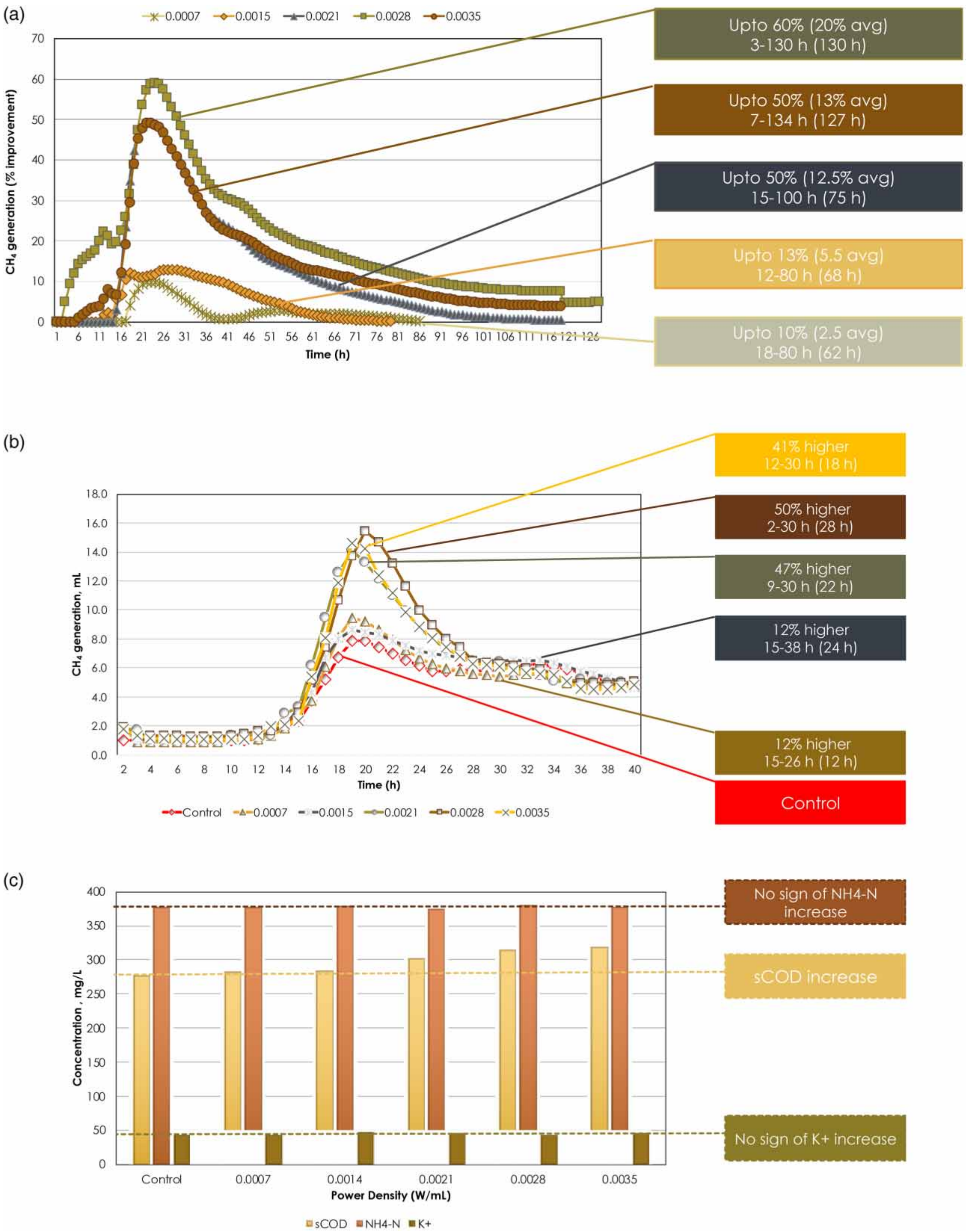

Figure 2 | Optimization of US intensity: (a) % improvement, (b) CH_4 generation, (c) biomass disintegration.

terms of enhanced CH_4 generation was maximum at an irradiation time of 120 s.

Low intensity sono-biostimulation in anaerobic digesters and reactors may offer a feasible option for the treatment of complex organic substrates like industrial and municipal sludges, organic fraction of municipal solid waste and ligno-cellulose based agricultural wastes treatment, where energy requirement is low in comparison with energy intensive pretreatment methods. Nevertheless, the quantification of benefits and a profound understanding of the mechanism

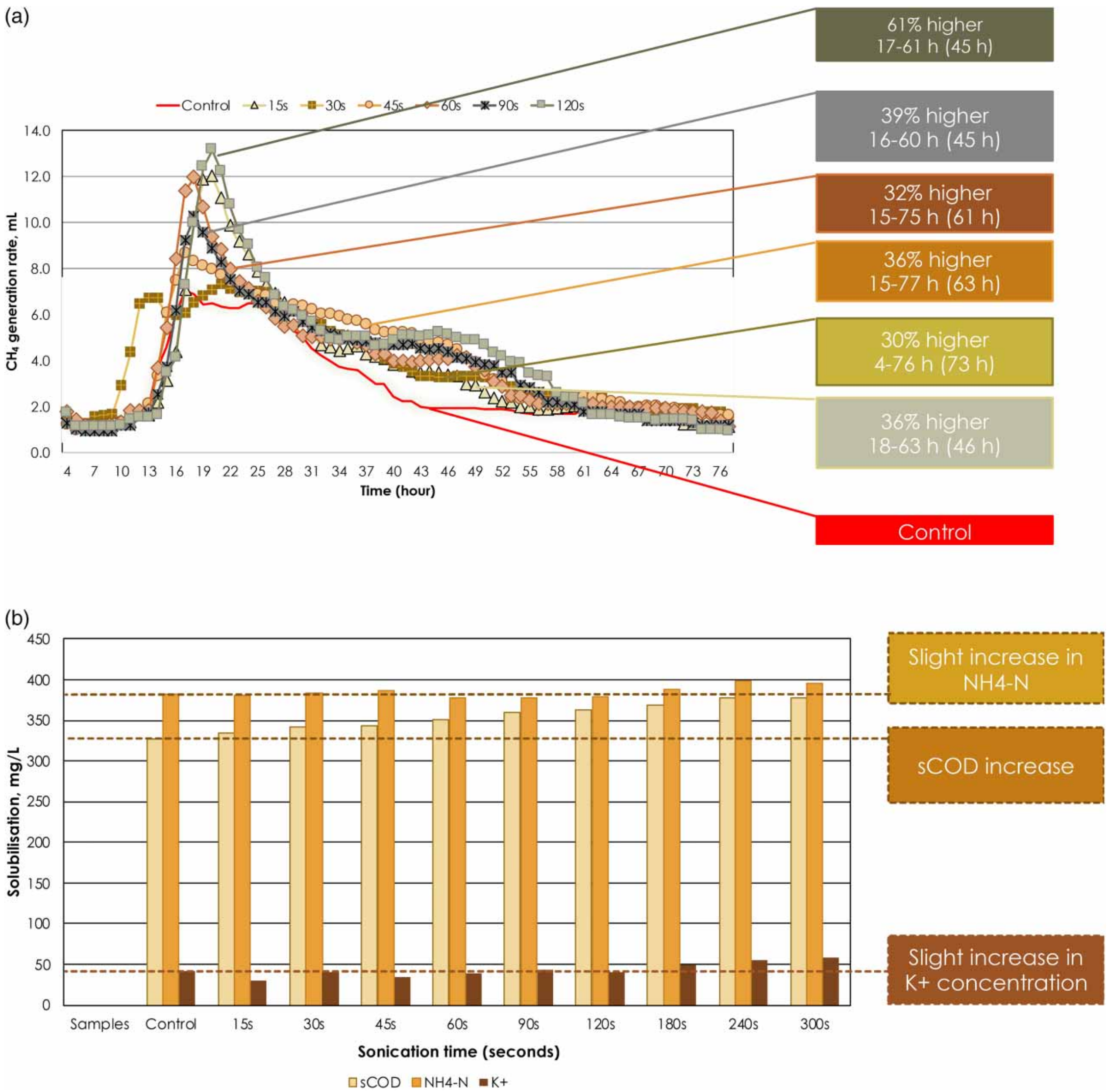

Figure 3 | Optimization of US time: (a) CH_4 generation rate, (b) effect on biomass disintegration.

of biological stimulation can be derived from further investigations.

CONCLUSIONS

The effects of low-intensity ultrasonication on the methanogenesis were investigated. The preliminary findings of the study showed that low intensity US can be a promising solution to enhance the process efficiency in terms of higher methane production with minimal energy requirement. Applied ultrasonication treatment was suitable to improve the process performance in terms of higher CH_4 generation over the control reactor. The best treatment condition was 0.0028 W/mL for 120 s. The sono-biostimulated effects were observed to last for 45 h after ultrasonication. No signs of increase in ammonium and potassium were observed, which revealed no cell lysis occurred under the best treatment conditions.

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