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Combined ultrasonication and thermal pre-treatment of sewage sludge for increasing methane production

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Abstract

This paper focuses on the combination of ultrasonic and thermal treatment of sewage sludge (SS). The combination involved ultrasonicating a fraction of the sludge and thermal treatment at various temperatures and this resulted in solubilization of proteins and carbohydrates, and so contributing to increased COD solubilization. During the treatment, SCOD, soluble proteins and carbohydrates increased from 760 mg/L to 10,200 mg/L, 110 mg/L to 2,900 mg/L and 60 mg/L to 630 mg/L, respectively. It was found ultrasonication of only a fraction of the sludge (>20%) followed by thermal treatment led to significant improvement compared to thermal and ULS treatments applied on their own. At 65°C, the kinetic of solubilization was improved and the hyper-thermophilic treatment time could be reduced to a few hours when ultrasonication was used first. A linear correlation (R^2 = 95%) was found between the SCOD obtained after ultrasonication pre-treatment and anaerobic biodegradability. The combined treatment resulted in 20% increase in biogas production during the anaerobic digestion of the pre-treated sludge.

Keywords: Waste Activated Sludge (WAS); ultrasonication; thermal pre-treatment; anaerobic biodegradability.

Introduction

Large amount of surplus biological sludge is generated during activated sludge process. Cost for sludge treatment and disposal may take as high as 50% of total cost for a wastewater treatment plant. [1] Anaerobic digestion is commonly accepted as an ideal method to stabilize sludge for safe disposal and utilization. [2] It has the advantages of low biomass yield, high stabilization degree as well as production of methane gas. [3] It is known that the digestible organic fraction in Waste Activated Sludge (WAS) is only about 30-45% (w/w) of biomass in conventional anaerobic treatment, while the methane production can improve markedly by disintegrating the WAS cells to release the intracellular organics using chemical or mechanical disruption methods. [4]

WAS mainly consists of intact microorganisms and their secretions forming particles larger than 0.1 µm that cannot be directly assimilated by the microorganisms. Hydrolysis of the cells must first take place before the soluble materials released can be converted to methane gas in the anaerobic digester. Cell lysis of the microorganisms limits the rate of hydrolysis which further limits the rate of the whole anaerobic process. [5] Furthermore, during the activated sludge process, bacterial cells form flocs which structure is enhanced by extracellular polymeric substances (EPS). This complex structure protects microorganisms from being degraded and makes the cell lysis even harder.

Pre-treatment of WAS has been proven to disrupt sludge structures, causing solubilisation of organics and accelerate subsequent anaerobic digestion. [6-9] One of the pre-treatment method is an anaerobic or aerobic biological method that requires either thermophilic (around 55°C) or hyper-thermophilic (between 60 and 70°C) conditions (Table 1) which typically result in an increase in hydrolysis activity, increase of biodegradable COD and pathogen destruction. [4-10-11] The increase in hydrolytic activity was demonstrated by Hasegawa et al. [12] who reported 40% VSS

71 solubilization due to the pre-treatment. Production of biogas after anaerobic digestion of the
72 microaerobically-pretreated sludge was increased by 1.5X when compared with the sludge
73 without pre-treatment. Destruction of 75% organic solids from excess waste activated sludge was
74 obtained at full scale, by combining a conventional municipal activated sludge process with a
75 thermophilic aerobic sludge digester (65°C, HRT of 2.8 days).[13] However, depending on the type
76 of sludge (primary, secondary or mixture of both) the residence of this type of treatment is
77 generally 2 days or longer.
78 Another relatively new pre-treatment of WAS is ultrasonication. Huge hydro-mechanical shear
79 force generated by cavitation bubbles during ULS is believed to be the predominant effect for
80 sludge disintegration. [7-14] In contrast with thermal methods, ULS is, comparatively, a very rapid
81 method that causes solubilization of both extracellular and intracellular substances leading to an
82 increase in soluble microbial products. [15] Microorganisms in WAS degrade the organic matter by
83 producing hydrolytic enzymes that are released into the media. Therefore, a physical treatment
84 such as ULS should be useful to disrupt the flocs, release the enzymes and at the same time
85 improve the thermal pre-treatment, but information on combination of these two pre-treatment
86 methods is still scarce in the literature. Since ultrasonication is an energy-intensive process, its
87 major disadvantage is its high energy consumption. [16] Therefore, the ultrasonication of a fraction
88 of WAS would be an interesting option to study and the objective of this paper was to investigate
89 how combination of ULS and thermal pre-treatments can enhance methane production. The
90 specific objectives were to determine the optimum temperature and duration of thermal pre-
91 treatment of WAS, and to determine how ultrasonication could improve solubilization of COD,
92 proteins and carbohydrates when it is applied before thermal treatment. Another objective was to

investigate the solubilization of WAS when only a fraction of it was ultrasonicated and to determine the impact on solids destruction and biogas production.

[Table 1]

Materials and methods

Sludge samples

Mixture of primary sludge and thickened waste activated sludge (ratio around 1:1 based on dry solids) were collected from Ulu Pandan municipal wastewater reclamation plant (Singapore). Properties of the sludge used in this study are listed in Table 2.

[Table 2]

Analytical methods

The measurement of pH (Jenway) was accurate to within ±0.02 units. The Total Solids (TS), Volatile Solids [17], Total Suspended Solids (TSS), Volatile Suspended Solids (VSS), Soluble Chemical Oxygen Demand (SCOD) and Total Chemical Oxygen Demand (TCOD) were measured in triplicate as described in Standard Methods.[18] Their coefficient of variation [19] for ten identical samples was 2.7%, 3.8%, 2.8%, 4.8%, 1.9 and 1.6%, respectively.

Proteins concentration was determined in triplicate by Lowry's method [20] using bovine serum albumin (Sigma-Aldrich) as standard and a UV/VIS scanning spectrophotometer (Shimadzu, UV-1800) against the blank at a wavelength of 750 nm. The coefficient of variance was within 2.8% for ten identical samples. As the precise chemical formula of the proteins detected was not determined, the percentage of soluble COD represented by protein had to be estimated by assuming a stoichiometric conversion factor of 1.5 which is derived from the typical formula of proteins ($C_{16}H_{24}O_5N_4$) presented in Rittmann and McCarty.[21] Carbohydrates concentration was determined in triplicate by sulphuric-phenol method [22] using D-Glucose (Merck) as standard and the same UV spectrophotometer against the blank at a wavelength of 485 nm. To convert into COD, 1g carbohydrates assumed as $C_6H_{12}O_6$ is equivalent 1.07 g COD.[23] The coefficient of variance was within 6.8% for ten identical samples. Soluble fractions of above-mentioned parameters were obtained from by filtrating supernatant fraction of centrifuged sludge (10,000 rpm for 10 mins) through a 0.45 μm membrane filter. Ammonia-Nitrogen was measured in triplicate using Nessler method [18] by reading the absorbance at 425 nm. The COV was equal to 6.6% for ten identical samples. Soluble Phosphorus (as PO_4^{3-}) was analyzed using the vanadomolybdophosphoric acid colorimetric method described in Standard Methods.[18] The absorbance was read on the same spectrophotometer at 470 nm and the coefficient of variation for three replicates was 0.6%. Particle size distribution was measured with particle size analyzer (Shimadzu, model SALD-3101) according to laser diffraction. The median diameter was used to quantify the particle size distribution. By definition it is the particle size such that 50% of the particles are larger and 50% are smaller than that value.

Sludge disintegration degree (DD_{COD}) was used in this study to express the ratio of solubilized COD to the maximum possible COD solubilization and can be used to quantify the sensitivities of different sludge to ultrasound treatment: [24]

$$DD_{COD} = \frac{\mathrm{SCOD_T} - \mathrm{SCOD_O}}{\mathrm{SCOD_{NaOH}} - \mathrm{SCOD_O}} \quad (1)$$

Where $SCOD_T$ is the Soluble COD of treated sample, $SCOD_{NaOH}$ is the Soluble COD of sample immersed in 1M NaOH (ratio 1:1) at 90°C for 10 minutes and $SCOD_O$ is the soluble COD of the raw sample.

Combined ultrasonic and thermal treatments

Experiments were carried out to investigate the effect of ultrasonication and thermal treatments separately and in combination. The ultrasonication tests were carried out in batch mode using an ultrasonicator (Misonix, Q700) with a frequency of 20 kHz and a maximum power input of 700W. A solid tip probe (#4208) with a diameter of 19.1 mm and maximum amplitude of 60 μm was immersed 1-2 cm below the surface of the sludge. 80% of the maximum amplitude was used and the corresponding power input was around 140W. Ultrasonication energy input was quantified using both the ultrasonication time and the specific energy input calculated as follows: [16]

$$SEI = \frac{Total\ energy\ input\ (J)}{Total\ solids\ in\ sample\ (g)} = \frac{P*t}{V_{sludge}*TS} \quad (2)$$

Where P is Power input of ultrasonicator (W), t is the time of ultrasonication (s), V_{sludge} is the volume of treated sludge (L) and TS is the Total solids concentration of treated sludge (g/L).

During ultrasonication the temperature was monitored and refrigerated below 30°C with ice-water mixture if necessary.

For the combined treatment, ULS-treated sludge (at 5,000 kJ/kg TS unless stated otherwise) and non treated sludge were mixed at a specific ratio in plastic capped tube (total volume was 10 mL) and placed in a water bath (Haake DL30) without shaking for 24 hours to study the thermal treatment. The temperatures tested for the combined ULS-thermal treatment were 25°C (ambient), 35°C, 45°C, 55°C, 65°C, 75°C and 85°C.

Anaerobic biodegradability

Prior to anaerobic biodegradability tests the sludge was pre-treated with ULS at 5,000 kJ/kg TS followed by thermal treatment at 65°C. Biochemical methane potential (BMP) assay were conducted according to Owens et al. [25] in 120 mL serum bottles to quantify the anaerobic degradability of sludge samples. For the BMP assays 10 mL of substrate (raw or treated sludge), 20 mL of degassed acclimatized inoculum and 30 mL medium were added to serum bottles. Mixture of 20% CO_2 and 80% N_2 were used to purge each bottle for three minutes to create absolute anaerobic environment. Two blanks containing the inoculum and the medium were run in parallel, and the methane produced was subtracted from the methane produced in the bottles containing the samples. All bottles were incubated in an orbital shaker at 35°C. The biogas volumes were regularly measured using a wetted glass syringe and reported at atmospheric pressure and a temperature of 35°C.[26] The composition of biogas was analyzed with gas chromatography (Agilent Technologies 7890A GC system) with two thermal conductivity detectors (TCD) and an Agilent HayeSep C 3.0 m X 1/8" X 2.0 mm packed column. The flow

rate was controlled at 45 mL/min and the temperatures of injector, oven and detector were 120°C, 115°C and 150°C, respectively.

Results and discussion

Preliminary tests on the effects of ultrasonication alone

In this section the effects of ultrasonication alone were investigated. Various parameters such as soluble carbohydrates, proteins, SCOD, phosphorus and ammonia are shown in Figure 1.

[Figure 1]

Figure 1A shows that ULS has a significant impact on soluble biopolymers with an increase in SCOD, proteins and carbohydrates concentrations to 5.5 g/L, 1.6 g/L and 500 mg/L, respectively. The increase in soluble carbohydrates was, relatively, less obvious because the sludge contained thickened waste activated sludge which is rich in proteins from bacterial cells and EPS. Figure 1B shows that ultrasonication had a significant effect on the soluble phosphorus concentration, which means that ULS was able to break open the cells and release phospholipids from cell membrane and phosphorus from the DNA into the bulk liquid. The concentration of ammonium in the supernatant was also analyzed, and it was found that it slightly increased from 120 to 170 mg/L during the first 5 minutes of treatment, but afterwards it remained constant. It is possible that

some proteins in the sludge were broken down or that ammonium from the cytoplasm was released in the supernatant due to the action of ULS.

Figure 1D shows the evolution of various groups of particles based on their size. Cavitation bubbles caused by ultrasound are known to disrupt floc structures and reduce floc size. Particles larger than 100 µm or cells flocs and aggregates are readily disrupted by ULS within the first minutes with the number of large flocs reduced from 26% to 12% and then below 5% as the SEI reached 10,000 kJ/kg TS. At the same time, the number of colloidal particles or small flocs (13-100 µm) also dropped significantly due to physical disruption, while the amount of single cells, small colonies and possibly cell debris (2-13 µm) started to increase markedly from 10% to 50%. This was consistent with Lehne et al. [27] who found that an obvious floc size reduction took place below a SEI of 3,000kJ/kg TS. Interestingly, beyond 10,000 kJ/kg TS higher SEIs became inefficient. The slow and steady increase of intra-cellular materials and EPS (<2 µm) showed that ULS could indeed disrupt cells walls and solubilise EPS even at low SEIs which was consistent with the evolution of soluble biopolymers, ammonia and phosphorus. This contradicts Lehne et al. [27] who suggested that cell lysis did not take place until a SEI of 3,000 kJ/kg TS was applied. Overall, it can be concluded that ULS was more efficient towards large flocs.

Preliminary tests on the combination of ULS and thermal treatments

In our preliminary tests it was observed that the temperature could increase up to 70°C during ULS if a small volume of sample (<50 mL) was used and if the sample was not cooled down. Using a pulse mode could reduce the heat generated, but was not efficient to solubilise more COD

(data not shown). It was then decided to investigate the effect of ULS and heat separately and by combining ULS and thermal treatment in sequence. The sequence of thermal-ULS was tested and it was found the thermal energy could lyze cells which then released soluble materials such as colloids and proteins leading to an increase in SCOD concentration (data not shown). However, during the subsequent ultrasonication the SCOD, soluble proteins and carbohydrates concentration increased only by 700 mg/L, 60 mg/L and 145 mg/L, respectively, which was deemed insignificant. It was postulated that the propagation of ultrasound waves was hindered and could not reach intact cells. Therefore, we focused on the ULS-thermal sequence in this study.

First, we ultrasonicated a specific percentage of the sludge (0-25-50-75-100%) and measured the SCOD concentration obtained after mixing with the non-treated fraction. The sludge was then incubated at a specific temperature without mixing and at neutral pH, and it can be seen in Figure 2 (top) that after 24 hours incubation at 30°C the SCOD increased to ~3 g/L for the 25% ULS-treated sludge and decreased for the higher ratios presumably because SCOD was consumed by mesophilic microorganisms at 30°C, and converted to CO_2.

Interestingly, the situation was very different at 55°C as shown in Figure 2 (bottom). When the sludge was not ultrasonicated and placed at 55°C (100% raw) the SCOD increased to 5.35 g/L, whereas with 25% ULS-treated sludge the SCOD increased to 7.1 g/L. The improvement of solubilization of the sludge due to the combination of thermal and ultrasonication is in line with previous studies, [28] but to our knowledge this is the first study where only a fraction of the sludge is ultrasonicated during the combined ULS thermophilic pre-treatment.

Moreover, the use of ultrasonication on 25% of the sludge improved even further the performance of thermal treatment. This was due to the disruption of flocs and the breakdown of cells

247 containing intra-cellular hydrolytic enzymes. This is in line with researchers who showed that
248 enzymes could be extracted from WAS using ULS.[29] However, it was demonstrated that the free
249 enzymatic activity present in the liquid phase was almost negligible, being immobilised on flocs
250 (connected to the polymeric extracellular substances) or attached to the cellular walls by ionic and
251 hydrophobic interactions.[30-31] Therefore, a physical treatment is useful to disrupt the flocs and
252 release the enzymes. Yu et al. [32] showed that ultrasonic pre-treatment enhanced enzymatic
253 activities and promoted the shifts of extracellular proteins, polysaccharides and enzymes from
254 inner layers of sludge flocs (i.e., pellet and tightly bound EPS) to outer layers (i.e., slime) and this
255 increased the contact and interaction among extracellular proteins, polysaccharides and enzymes
256 that were originally embedded in the sludge flocs, resulting in improved efficiency in the
257 subsequent aerobic degradation.
258 Heat and ultrasonication can also be used to rupture cells and release the intra-cellular proteases
259 which can hydrolyze proteins in the sludge. Nabarlatz et al. [29] also found that the activity of
260 extracellular protease in activated sludge tank was much lower than that of intracellular protease,
261 therefore, it is sensible to use ULS prior to thermal treatment. At 100% ULS-treated sludge the
262 final SCOD concentration reached almost 8 g/L. However, at higher ratios of ULS-treated sludge
263 the improvement of ultrasonication became marginal.
264
265 [Figure 2]
266

267 ***Effect of temperature during the ULS-thermal tre-treatment of sludge***

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269

Based on the previous experiment a 25% ratio was used to determine the optimum temperature for the enzymatic treatment. Figure 3 shows that the higher the temperature, the more COD was solubilized (up to ~11 g/L). Higher SCOD (up to 14 g/L SCOD) could be obtained depending on the initial solid concentration of the sludge (data not shown). Temperatures greater than 65°C resulted in a marginal increase. It was also found that mixing during the thermophilic treatment resulted in a 20% increase in SCOD concentration (data not shown).

To investigate further the effect of heat, a sample was autoclaved (121°C for 20 min) and the final SCOD was only 6,700 mg/L. As the temperature rises slowly in an autoclave, the enzymes were still active but could have been deactivated at high temperatures (>85°C) which limited the extent of solubilization compared to a milder thermal process. This showed further that heat was not the only phenomenon taking place. The solubilization of WAS by heat-treatment can be induced by sludge lysis and further cryptic growth (lysis-cryptic growth).[33] In the lysis-cryptic growth, sludge reduction is achieved because some portions of lysates are consumed for the catabolism and finally emitted as CO_2. This was confirmed using our sludge as a CO_2 production of 4.4 mL and 6 mL was recorded after 1 hour incubation at 55°C and 65°C, respectively. After 24 hours, the cumulative CO_2 production reached 9.9 mL and 10.2 mL, respectively, indicating the consumption of SCOD for the growth of both thermophilic and hyper-thermophilic bacteria.

[Figure 3]

Yan et al. [34] used a simple heat-treatment process (700 ml was incubated at 60 °C, 120 rpm for 24 h in a 1 l Erlenmeyer flask) and also showed that there was rapid increase in population of thermophilic bacteria at the early stage of heat-treatment and the emergence of protease-secreting

bacteria. Hasegawa et al. [12] showed that the hyper-thermophilic aerobic microbes were identified as belonging to *Bacillus*.

Therefore, the potential for increased performance is inherent in the sludge itself,[35] and although heat treatment is beneficial for solubilization, long thermal treatment are not interesting from a process point of view but also because some of the lyzate is consumed by thermophilic bacteria and lost as CO_2 and cannot be used to produce methane.

Combination of different ratios of sludge treated by ULS prior to thermal pre-treatment at 55°C

In this experiment a specific percentage of sludge (0, 5, 10, 20, 50 and 100%) was ultrasonicated, then mixed it with the remaining non-ultrasonicated fraction and incubated in a water bath to study the kinetics of solubilization at 55°C and 65°C. As 75°C and 85°C were shown to result in a marginal SCOD increase in the previous section, these temperatures were not tested further in details in this study. Carbohydrates and proteins are two predominant biopolymers in EPS structure which also contributes a great part of COD to sludge.[32] Therefore, the solubilization of carbohydrates and proteins provide essential information about disintegration of sludge structure. The soluble COD, proteins and carbohydrates concentrations obtained at 55°C are shown in Figure 4.

It can be seen that the thermophilic treatment alone resulted in a final SCOD of 7.8 g/L, whereas a significant increase to 8 g/L, 8.7 and 9.3 g/L was observed when 20%, 50% and 100% of sludge was ultrasonicated prior to the thermal treatment, respectively. Below 20% of ULS-treated sludge there was a small effect as indicated by close SCOD values. The results indicated that as the

316 percentage of ULS increases, more cells are broken down and more intracellular materials are
317 released into the bulk as shown by higher SCOD concentrations. However, the effect of ULS was
318 not linear, meaning that 100% ULS treated did not result in twice the solubilization of 50% ULS-
319 treated sludge. This shows that treating 100% of sludge by ULS is not an interesting option,
320 however, 20% and above had a positive impact on the subsequent thermal treatment.
321 It was also found that ultrasonication increased the COD solubilization rate of the overall pre-
322 treatment. For instance, the thermophilic treatment took 24 hours to reach 7.8 g/L SCOD, whereas
323 only 3 hours thermal treatment was required when 100% sludge was ultrasonicated. This
324 demonstrated that the thermal treatment time can be significantly reduced by combining ULS.
325
326 **[Figure 4]**
327
328 It can be seen from Figure 4B and 4C ULS improved the rate of proteins and carbohydrates
329 solubilization compared to the thermophilic treatment alone. The concentration increased during
330 the first six hours of thermophilic treatment and decreased afterwards due to the consumption of
331 nitrogen and carbohydrates by thermophilic bacteria. Proteins and carbohydrates solubilization
332 might have continued after 6 hours, but it could not compensate for the uptake by opportunistic
333 thermophilic microorganisms, resulting in a net decrease after 6 hours of treatment. This net
334 decrease was, however, not observed in SCOD concentration (Fig. 4A) as COD analysis
335 encompassed various biopolymers including proteins and carbohydrates, and also lipids,
336 phosphates, ammonia, humic and fulvic acids that were solubilized.
337

Combination of different ratios of sludge treated by ULS prior to thermal pre-treatment at 65°C

The soluble COD, proteins and carbohydrates concentrations obtained at 65°C are shown in Figure 5. As expected, the extent and rates of COD, proteins and carbohydrates solubilization was enhanced at 65°C compared to 55°C. This is due to improved cell lysis and possibly higher enzyme activity. Yu et al. [32] had indeed also showed that enzymatic activities (proteases, α-amylase, α-glucosidase, alkaline-phosphatase and acid-phosphatase) were markedly increased after ultrasonication. In terms of final SCOD concentration, 100%ULS was equivalent to 1 hour hyper-thermophilic treatment. Both conditions resulted in ~5 g SCOD/L. When 100% of sludge was ultrasonicated, less than 1 hr of hyper-thermophilic condition was required to reach 8 g SCOD/L. However, 24 hrs were required to reach that level in individual hyper-thermophilic pre-treatment. Therefore, ULS shortened significantly the hyper-thermophilic treatment. Sahinkaya and Sevimli [28] reported that the SCOD increased from 55 to 3,500 mg/L after 10 minutes ultrasonication (1.5 W/mL) at 80°C for 1 hour which was found to be the optimum temperature. Ultrasonication alone resulted in a concentration of 2,250 mg/L. This confirmed the better results using a combination of ultrasound and thermal treatments. However, these concentrations were much lower than in this study due to lower TS level (4 g/L) in the raw sludge.

[Figure 5]

It can be seen that the extent of protein solubilization increased as the percentage of ULS-treated sludge increased. This is in line with the previous observations at 55°C. However, at 65°C, the effect of ULS was more dominant as shown by a significantly higher solubilization rate at

361 percentages as low as 10%. Interestingly, there was no net decrease in soluble proteins
362 concentrations at 65°C in contrast to what was observed at 55°C. This indicates that the rate of
363 proteins solubilization was higher than the rate of proteins degradation and consumption by
364 hyper-thermophilic bacteria. Soluble carbohydrates, however, were consumed by hyper-
365 thermophilic bacteria as indicated by a net decrease in concentration after 6 hours. The existence
366 of such hyper-thermophilic bacteria was previously documented and was found to belong to
367 *Bacillus*. [12]The net decrease was insignificant for the 100% ULS-treated sample showing that
368 ULS could also inhibit to some extent the growth of the hyper-thermophilic bacteria and avoid the
369 consumption of soluble carbohydrates.
370

371 ***TSS and VSS removals during ULS and thermal pre-treatment***

372

373 [Table shows the TSS and VSS removal during the individual and combined pre-treatments. It
374 can be seen that ULS alone resulted in TSS removals lower than 10%, while the thermal treatment
375 resulted in TSS removals in the range 20-23%. When 50% of the sludge was ultrasonicated and
376 treated at 65°C, then a maximum of 27% TSS and VSS removal was obtained. Treating 100% of
377 the sludge by ultrasonication did not increase this removal, confirming that ultrasonication of a
378 high proportion is not required.
379 Yu et al. [32] obtained 11.8% TSS reduction after an ultrasonication treatment (10 min, 3 kW/L) of
380 WAS and attributed this to the release of soluble organic carbon sources and extracellular
381 enzymes, and the enhanced contact between them. The sludge reduction for TSS was 30.9% after
382 aerobic degradation (compared with 20.9% in the control) showing that the ultrasonic pre-

383 treatment could significantly enhance aerobic digestion efficiency and the extent of sludge
384 biodegradability.
385
386
387
388
389 **[Table 3]**

390 ***Effect of the combined pre-treatment on anaerobic biodegradability***

391
392 The effect of ULS alone on the anaerobic biodegradability was tested first. As shown in Figure 6
393 (a), the biodegradability of all the ultrasonicated sludge was higher than the control. The ultimate
394 biodegradability increased with increasing specific energy input. However, ultrasonication at high
395 specific energy input may not be economical. It was found that 9 kJ/g TS ultrasonication
396 improved the sludge biodegradability by 14.8%, whereas at a SEI 7 times higher (i.e. 63 kJ/g TS),
397 the biodegradability increased by 31.8% which was only 2.2 times higher compared to the
398 improvement induced by 9 kJ/g TS ultrasonication.
399 In order to evaluate the possibility to use SCOD data to predict the biodegradability, the sludge
400 ultimate biodegradability and SCOD concentrations were plotted in Figure 6 (b). Linear
401 regression was found to be the most suitable model to describe the relationship. The coefficient of
402 determination (R^2) was 94.83%, indicating a strong correlation which is in line with Bougrier et
403 al. [36] who observed that biogas increase in ultrasonicated sludge originated mainly from the
404 soluble fraction.
405

406

407 Since it was found that higher SCOD were obtained by combining ULS and thermal treatment (up
408 to 14 g/L), a higher methane production was expected to be found using the combined pre-
409 treatment. Several combinations of pre-treatments were tested and the BMP results are shown in
410 Figure 7. A small percentage (5%,~5,000 kJ/kg TS) of ULS-treated WAS was combined with the
411 thermal treatment at 65°C for 24 hours and it was found that the methane production increased by
412 20%. This was higher than previous studies [28] where 13.6% increase in methane was obtained
413 after 1 minute ultrasonication (1 W/mL) and 1 hour thermal treatment at 80°C. It was also found
414 that methane production was greater with the combination compared to ultrasonication of 5% or
415 even 100% alone. The methane percentage in the biogas was up to 6% higher indicating a higher
416 calorific value due to the combined pre-treatment. However, a lag-phase of 8-12 days was
417 observed following the combined treatment which may be the result of a higher SCOD and its
418 components which the anaerobic inoculum was not acclimated to. Gavala et al. [37] found that
419 there are indigenous microorganisms in primary sludge capable of methane production and
420 incubation at 70°C for 1 day or more as a pre-treatment resulted in their inactivation.
421 Furthermore, they found that the thermal pre-treatment of both primary and secondary sludges led
422 to increased hydrogen levels that can inhibit methane production. [26] Our anaerobic inoculum
423 may have not contained enough hydrogenotrophic species which led to some inhibition and the
424 observed lag-phase.
425 Moreover, this combination (+20%) was more efficient that ULS alone at high SEI (100%, 9,000
426 kJ/kg TS) as shown in Figure 6(a) (+14.8%). This is due to the COD solubilization obtained after
427 the pre-treatment. After 100% ULS typical SCOD concentrations are in the range 4-5 g/L (Fig.
428 1A), whereas the combination of ULS and thermal treatment resulted in 10-11 g/L SCOD.

ULS is a fast method, but relatively inefficient to solubilise COD and it is expensive to treat 100% of WAS. Thermal treatment is efficient to solubilise COD, but is a slow process. Thermal treatment could be a viable option to consider if waste heat is available on site. It was found that these disadvantages can be alleviated when both methods are combined together, while methane production is improved. Further work is needed to find an optimum combination.

[Figure 7]

Conclusion

In this paper we investigated the pre-treatment of sewage sludge using ultrasonic and thermal treatments. Ultrasonication had a marked effect on particles with size greater than 100 microns (flocs) and in the range 13-100 microns (cells, colonies or colloids) at specific energy input lower than 10,000 kJ/kg TS. The optimum temperature during the thermal treatment was found to be 65°C. It was found the combination of ULS (30 sec., 5,000 kJ/kg TS) and thermal treatments resulted in greater solubilization of COD (760 to 10,200 mg/L), proteins (115 to 2,900 mg/L) and carbohydrates (60 to 660 mg/L) than individual treatments. During ultrasonication treatment alone (30 sec., 5,000 kJ/kg TS), SCOD, soluble proteins and carbohydrates concentrations increased to 4,700 mg/L, 1,000 mg/L and 500 mg/L, respectively. The ultrasonication of 50% of the sludge followed by the incubation at 65°C could increase the SCOD from 760 mg/L to 9,300 mg/L. It was also found that ultrasonication increased the COD solubilization rate of the subsequent thermal treatment at 65°C and treatment time could then be reduced to a few hours (1-6 hours) instead of 24 hours or several days. The SCOD obtained after ultrasonication pre-

452 treatment and its anaerobic biodegradability was found to be linearly correlated (R^2 = 95%). The
453 combined treatment resulted in 20 % increase in biogas production.
454

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456

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459

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461

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FIGURE CAPTIONS

Figure 1. Effect of ultrasonication: Specific Energy Input on (A) soluble proteins, carbohydrates and COD, (B) soluble phosphorus concentration, (C) soluble ammonia concentration, (D) evolution of various groups of particles based on the size. The ammonia and phosphorus concentration remained constant beyond 6,000 kJ/kg TS.

Figure 2. Effect of ultrasonication of 0, 25, 50, 75 and 100% of sludge (30 sec, ~5000 kJ/kg TS) followed by thermal treatment at 30°C (top) and 55°C (bottom).

Figure 3. Effect of the incubation temperature on the SCOD during the thermal treatment following ultrasonication of 25% of sludge (~ 5,000 kJ/kg TS).

Figure 4. Evolution with time of the SCOD (A), soluble proteins (B) and carbohydrates (C) during the thermal treatment at 55°C following the ultrasonication of 0, 5, 10, 20, 50 and 100% of sludge (~ 5,000 kJ/kg TS).

Figure 5. Evolution with time of the SCOD (A), soluble proteins (B) and carbohydrates (C) during the thermal treatment at 65°C following the ultrasonication of 0, 5, 10, 20, 50 and 100% of sludge (~ 5,000 kJ/kg TS).

Figure 6. (a) Anaerobic digestion tests of control and ultrasonicated sludge (b) Linear fitting of sludge biodegradability and SCOD concentration after ULS pre-treatment.

Figure 7. Cumulative methane production after several combinations of ULS and thermal pre-treatments.

TABLE CAPTIONS

Table 1. thermal pre-treatment methods

Table 2. Properties of the sewage sludge used in this study. NM= not measured.

Table 3. TSS and VSS removal during the combined ULS/thermal pre-treatment.

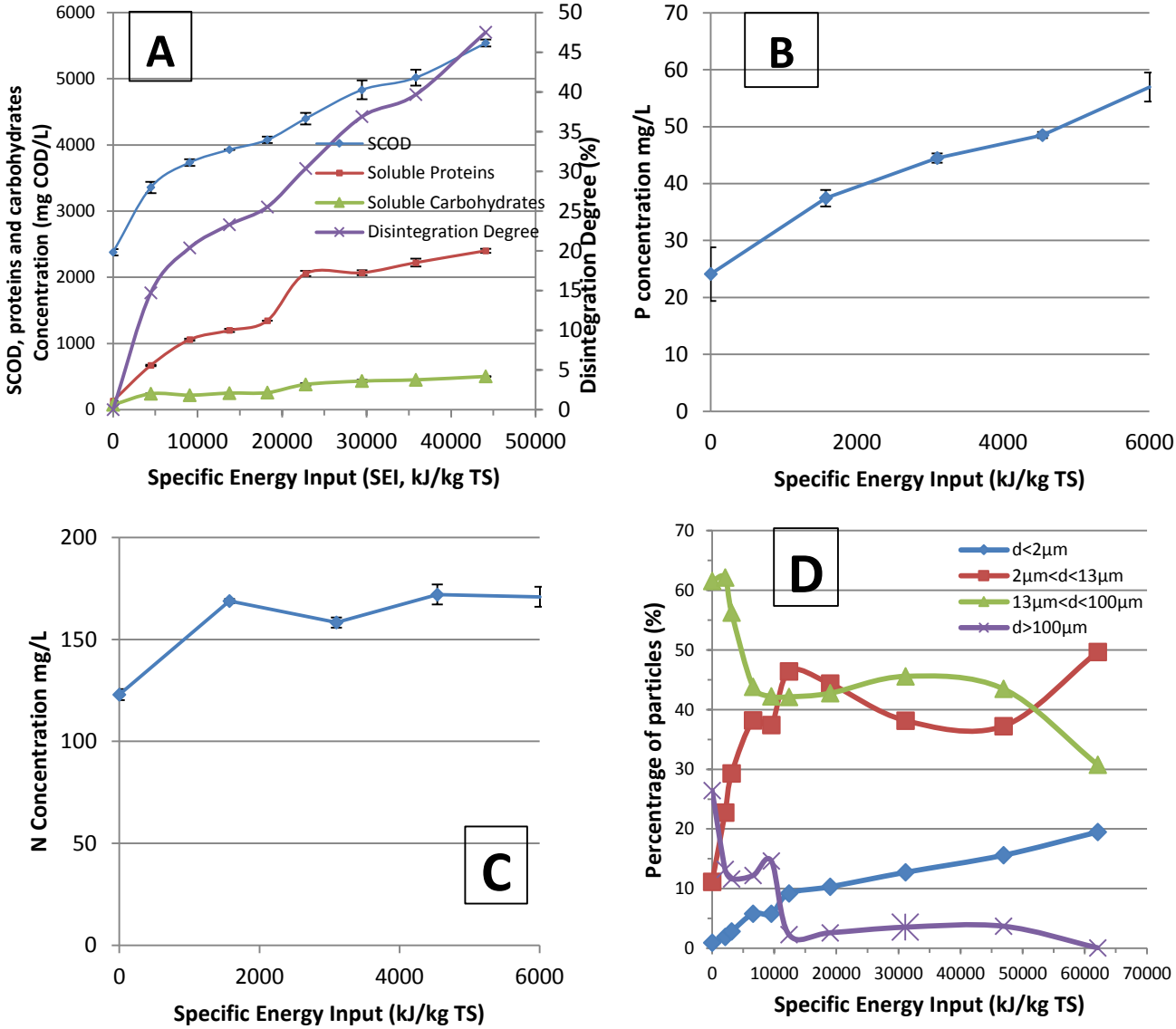
A
SCOD, proteins and carbohydrates Concentration (mg COD/L)
Disintegration Degree (%)
SCOD
Soluble Proteins
Soluble Carbohydrates
Disintegration Degree
Specific Energy Input (SEI, kJ/kg TS)
B
P concentration mg/L
Specific Energy Input (kJ/kg TS)
C
N Concentration mg/L
Specific Energy Input (kJ/kg TS)
D
Percentrage of particles (%)
d<2µm
2µm<d<13µm
13µm<d<100µm
d>100µm
Specific Energy Input (kJ/kg TS)

Fig. 1

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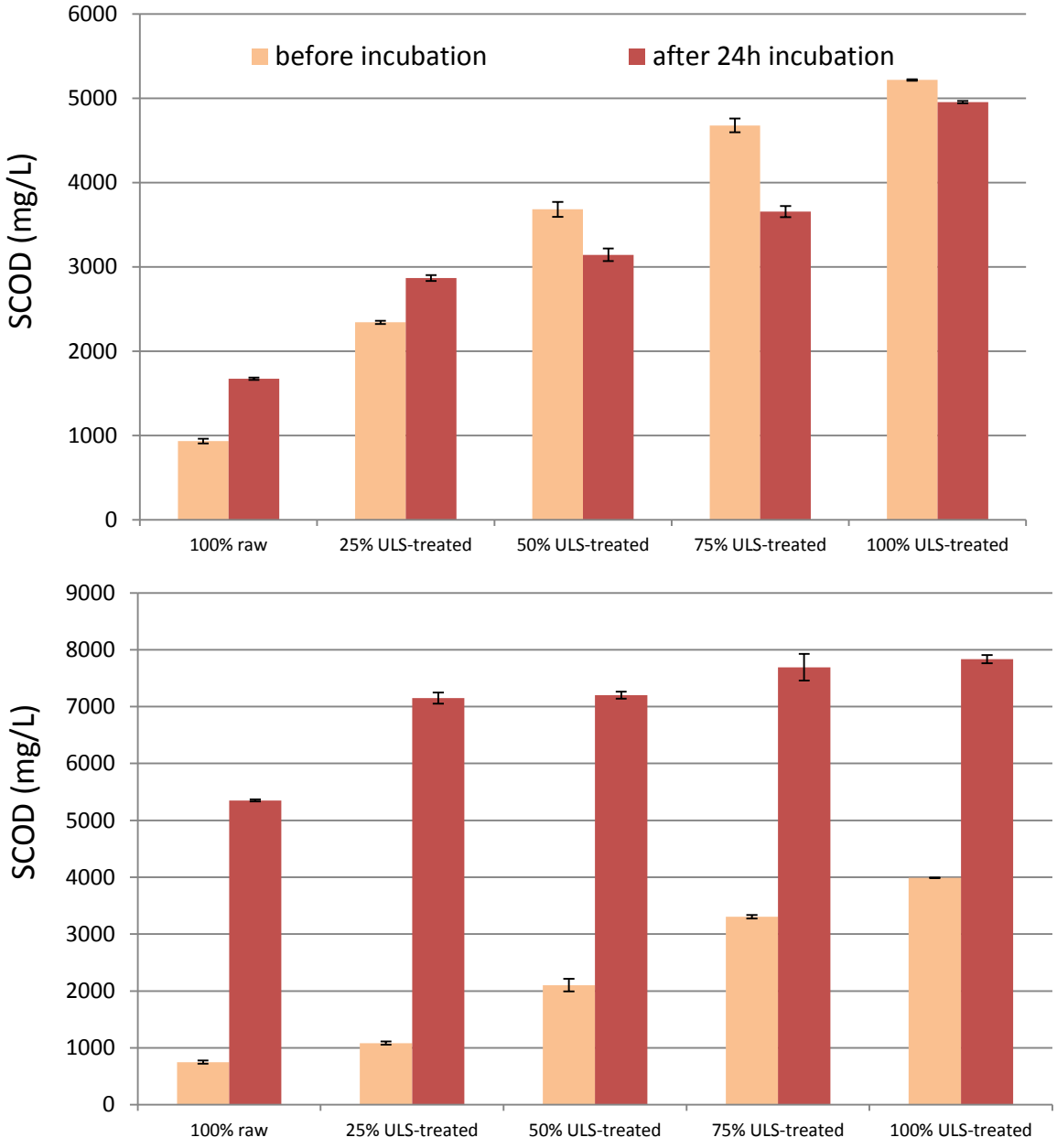

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644 Fig. 2

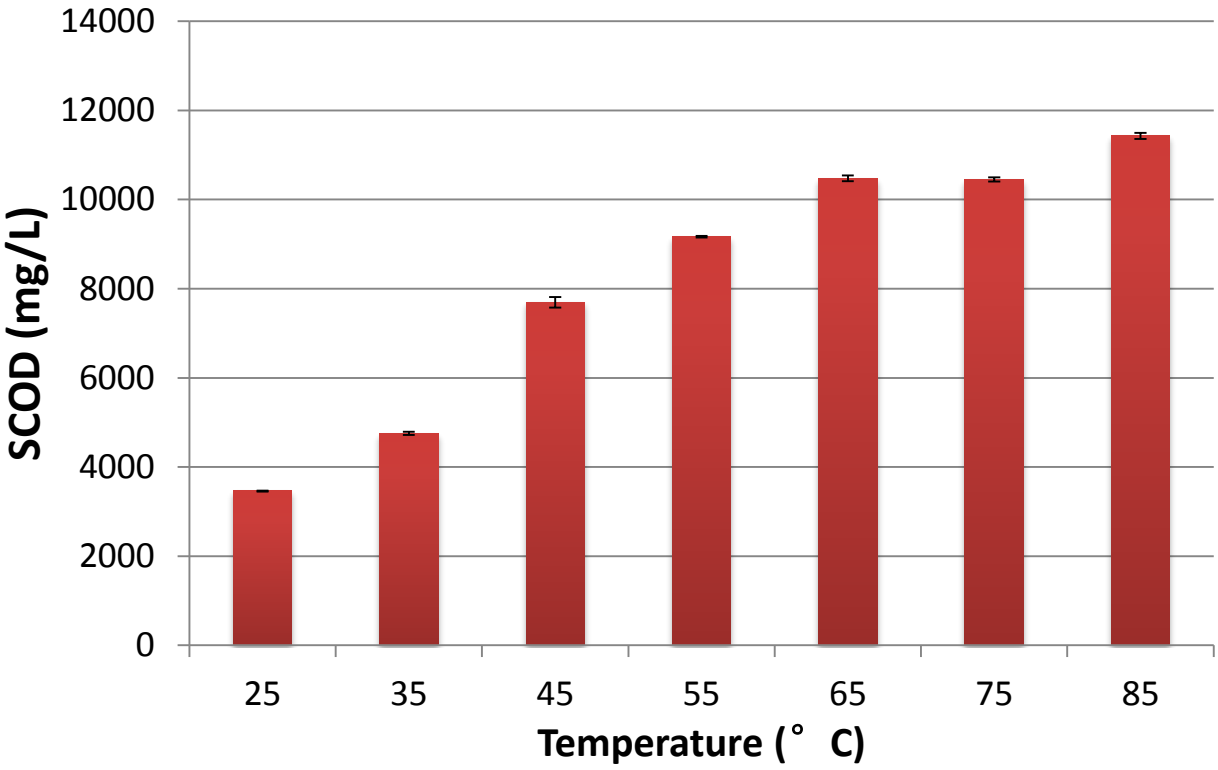
14000
12000
10000
8000
6000
4000
2000
0
SCOD (mg/L)
25
35
45
55
65
75
85
Temperature (° C)

Fig. 3

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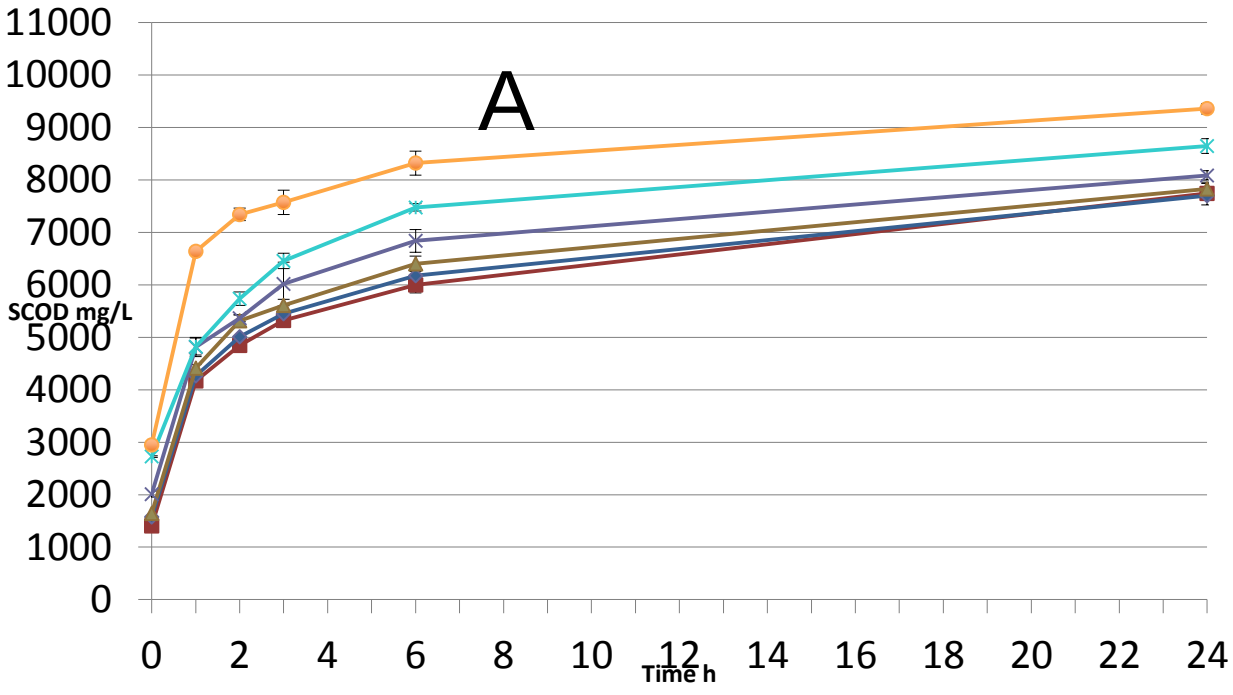

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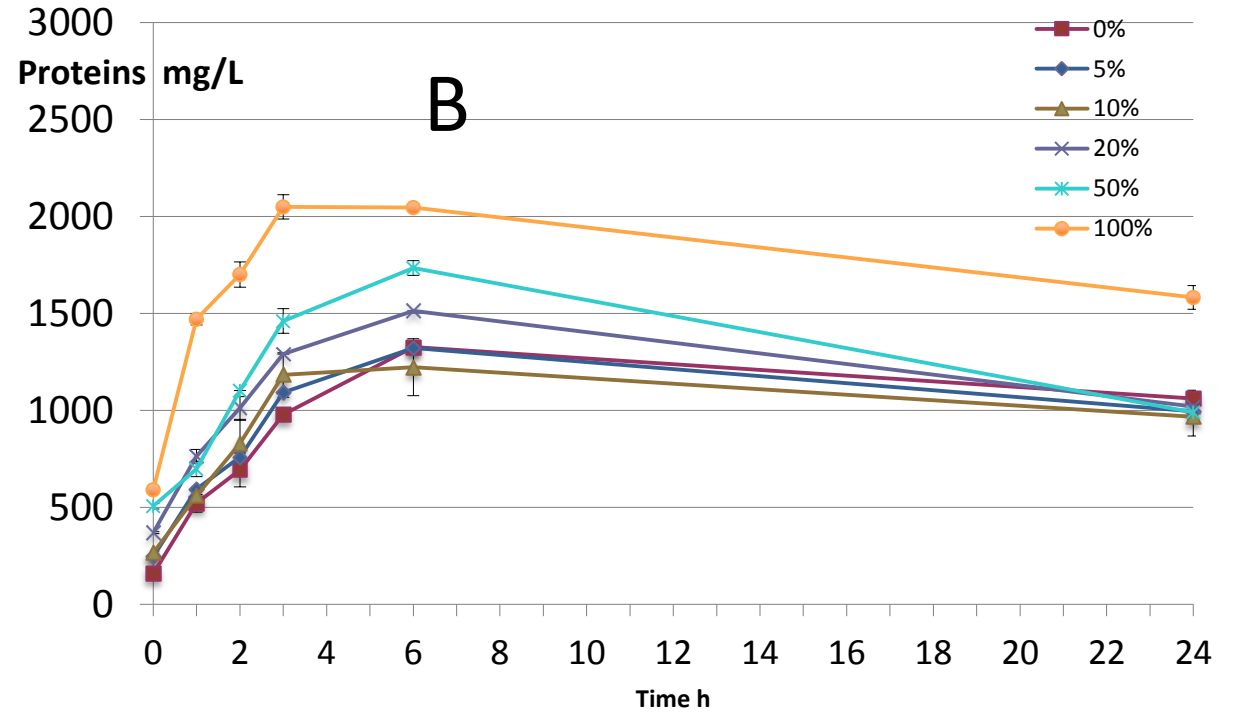

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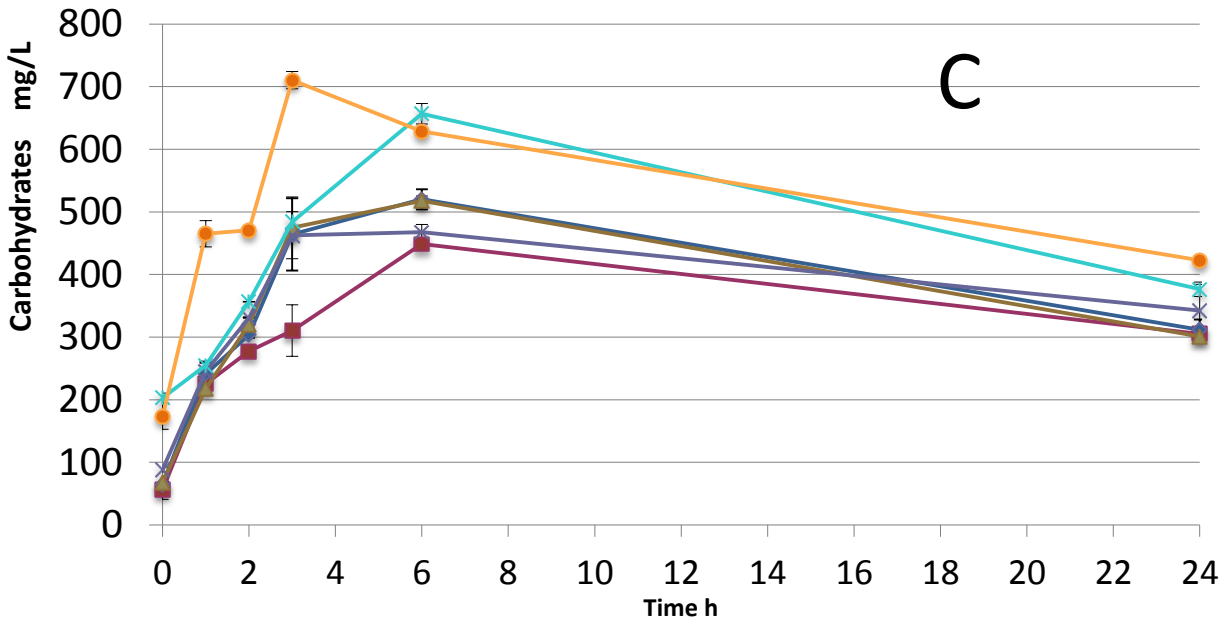
C
Carbohydrates mg/L
800
700
600
500
400
300
200
100
0
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2
4
6
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10
12
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16
18
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22
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Time h

Fig. 4

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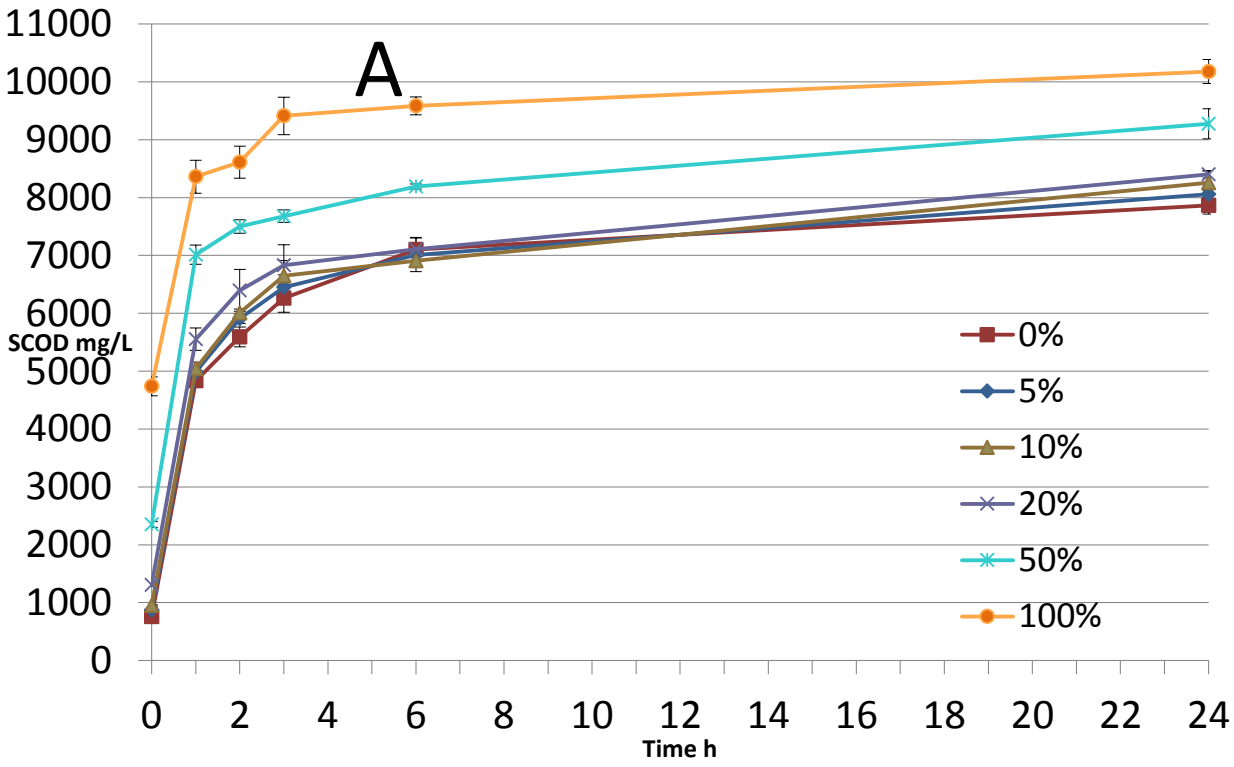

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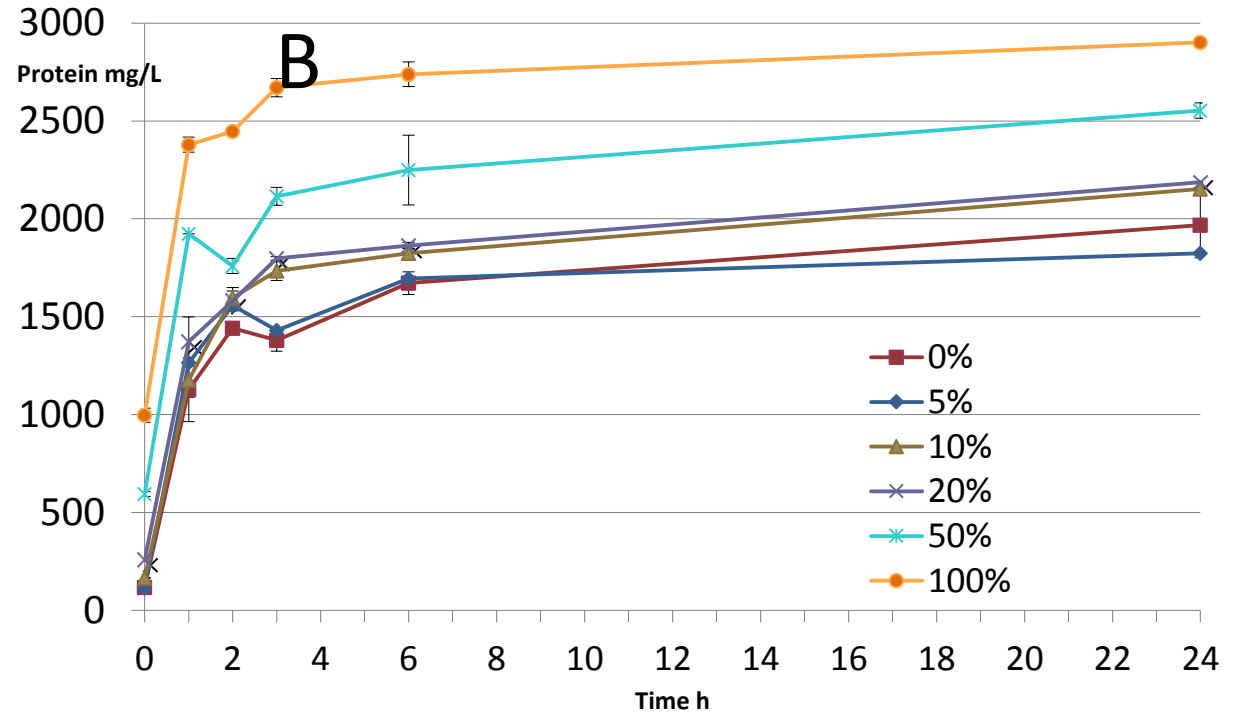

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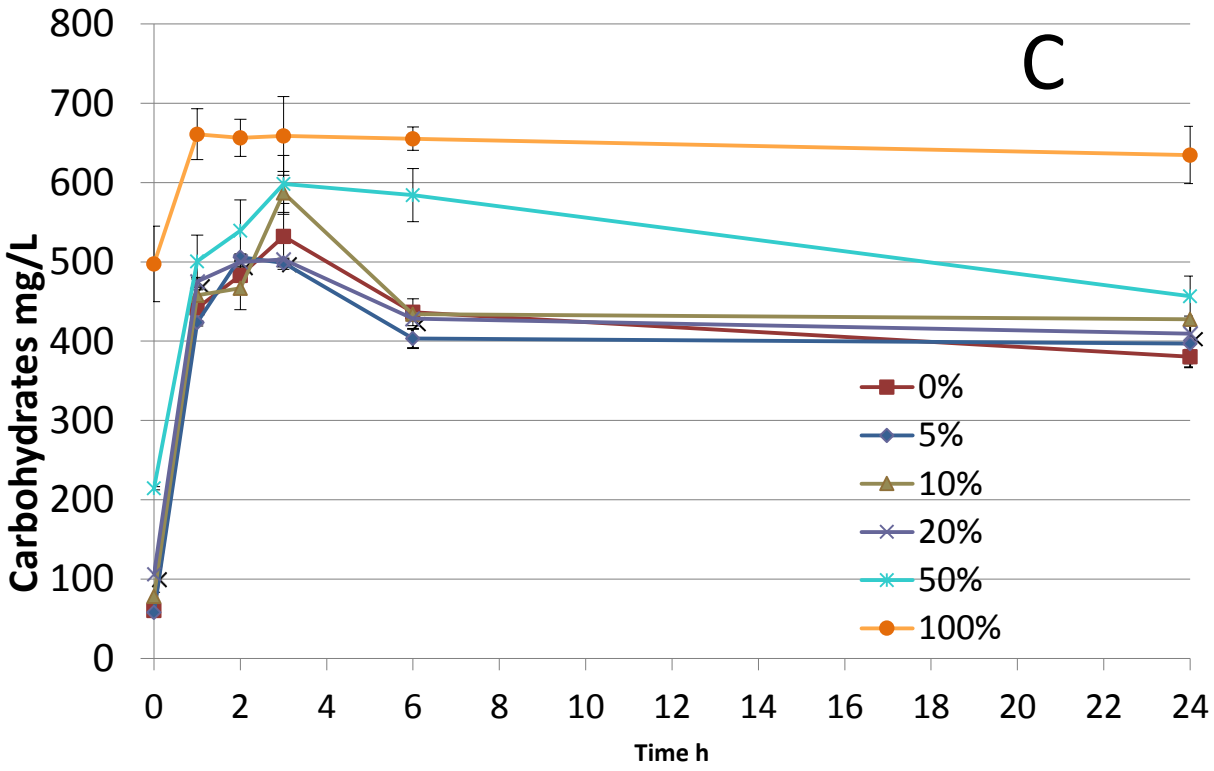

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690 Fig. 5

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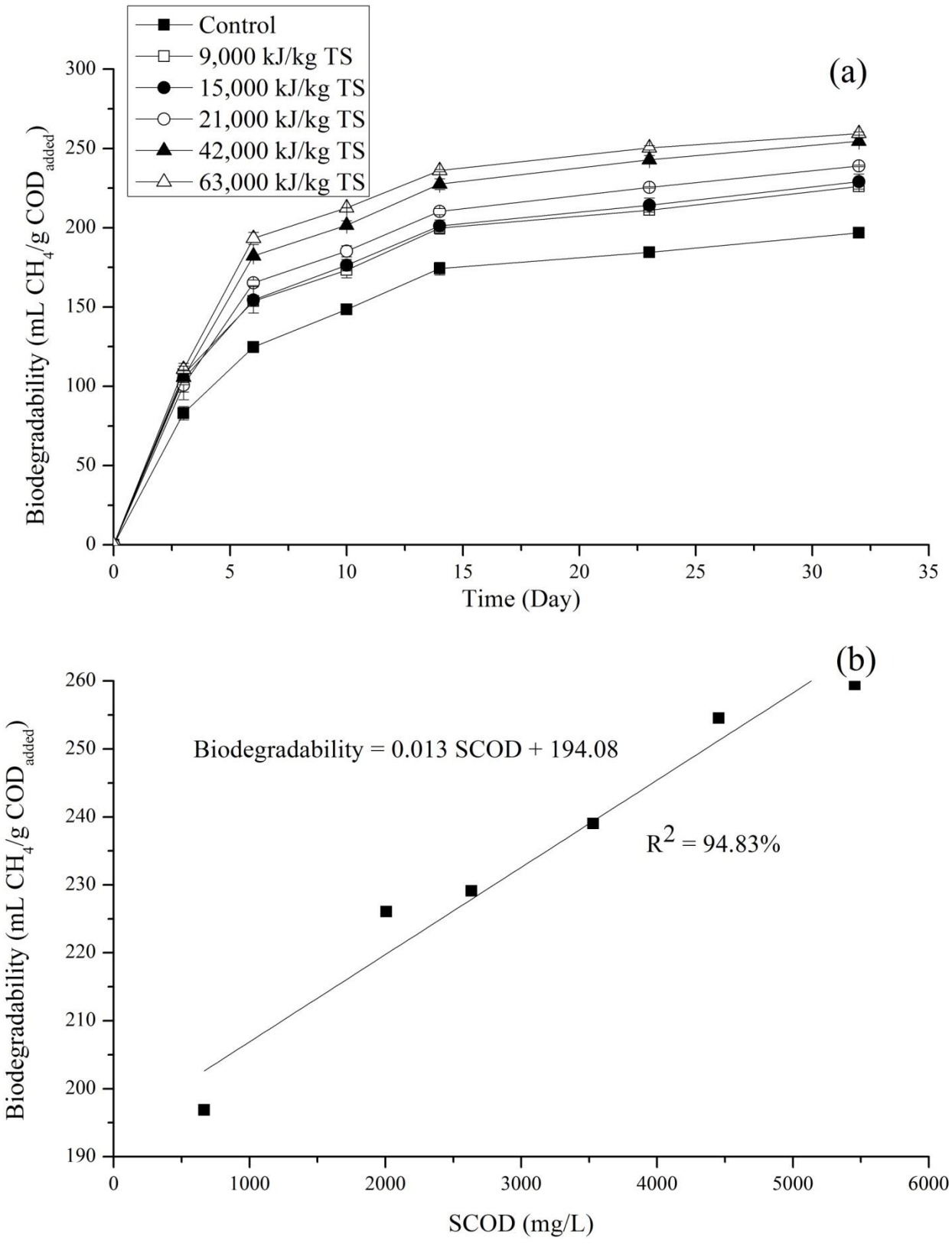

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706 Fig. 6

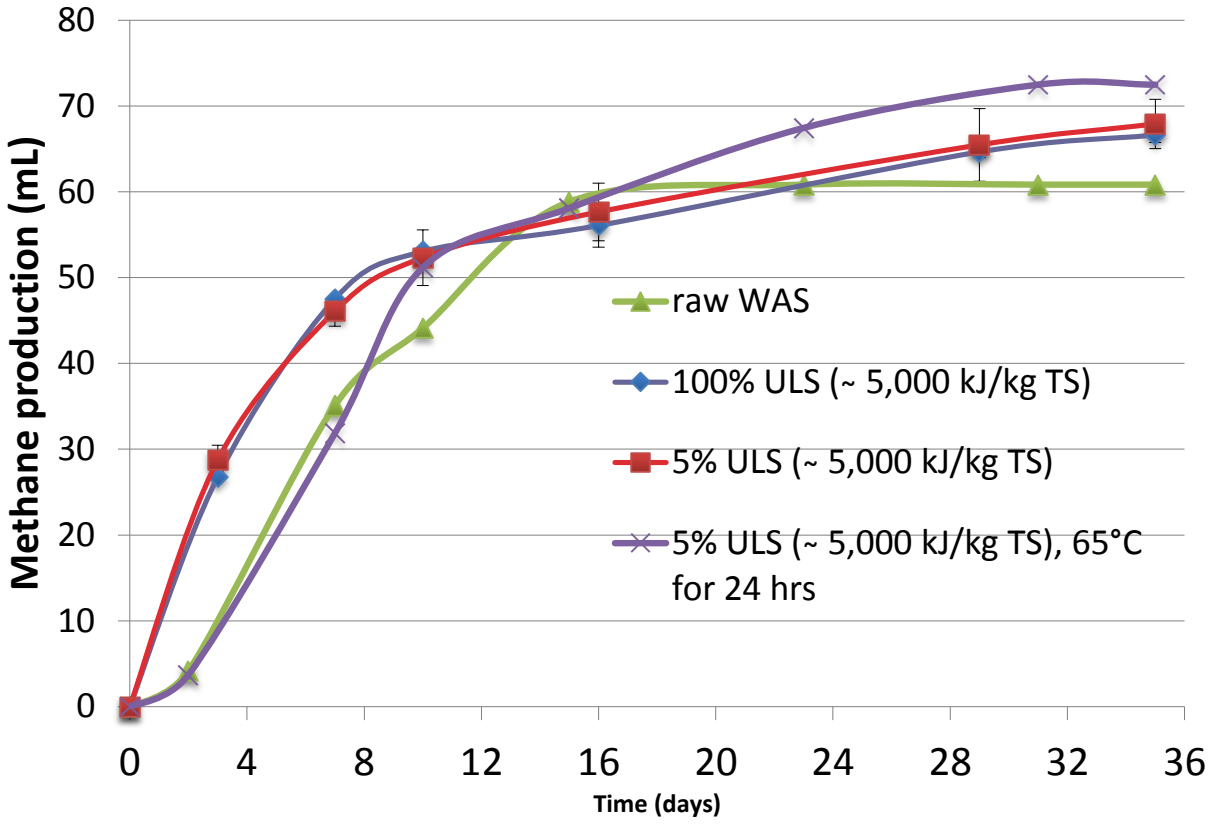
Methane production (mL)
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
28
32
36
Time (days)
raw WAS
100% ULS (~ 5,000 kJ/kg TS)
5% ULS (~ 5,000 kJ/kg TS)
5% ULS (~ 5,000 kJ/kg TS), 65°C for 24 hrs

Fig. 7

722 **Table 1**. thermal pre-treatment methods

Treatment conditions	Anaerobic digestion Conditions	Results	Reference
Microaerobic, 60–70°C, 1 day	Batch, 10 days 37°C	Increase of biogas production from 200 to 300 $mL.g^{-1}$ VS_{fed} (+50%)	[37]
Microaerobic 65°C, 1 day	CSTR, HRT: 21 and 42 days 35°C	Increase of COD removal (+30%) No methane production increase	[38]
70°C 7 days	Batch 37°C	Increase of CH_4 production from 8.30 to 10.45 $mmol.g^{-1}$ VS_{fed} (+26%)	[37]
70°C 7 days	Batch 55°C	CH_4 production of 10.9 $mmol.g^{-1}$ VS_{fed} (no influence)	[37]
70°C 4 days	Batch 37°C	Increase of CH_4 production from 21.2 to 24.7mmol g^{-1} VS_{fed} (+16%)	[37]
70°C 7 days	Batch 55°C	Increase of CH_4 production from 13.7 to 25.5 $mmol.g^{-1}$ VS_{fed} (+86%)	[37]
70°C 2 days	CSTR, HRT: 13 days (15 days without pretreatment) 55°C	Increase of CH_4 production from 40 to 55 $mL.L^{-1}$ d^{-1} (+28%)	[39]
70°C 9, 24, 48 h	CSTR, HRT: 10 days 55°C	Increase of CH_4 production from 0.15 to 0.18 $mL.g^{-1}$ VS_{fed} (+20%) Increase of energy production (+60–100%)	[40-41]
70°C 2 days	CSTR, HRT: 13 days (15 days without pretreatment) 55°C	Increase of CH_4 production from 13.6 to 20.1 $mmol.g^{-1}$ VS_{fed} (+48%)	[17]
50–65°C 2 days	CSTR HRT: 13–14 days 35°C	Increase of CH_4 production (+25%) compared to 35°C pretreatment	[42]

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732 **Table 2**. Properties of the sewage sludge used in this study.

Parameters (acronym, unit)	WAS	Anaerobic Inoculum
pH	5.9-6	7.3
Soluble Chemical Oxygen Demand (SCOD, mg/L)	670 - 1440	454 ± 8
Total Chemical Oxygen Demand (TCOD, g/L)	18 - 25	13.75 ± 0.53
Total Solids (TS, g/L)	13.6 -17.2	9.5 ± 0.3
Volatile Solids (VS, g/L)	10.7 - 13.4	7.1 ± 0.3
Total Suspended Solids (TSS, g/L)	12.4 - 15.9	9.3 ± 0.2
Volatile Suspended Solids (VSS, g/L)	10.3 - 13.0	7 ± 0.3
Ammonia (mg N/L)	122.97 ± 2.72	NM
Phosphate (mg PO_4^{3-}/L)	24.11 ± 4.71	NM

733 NM= not measured.
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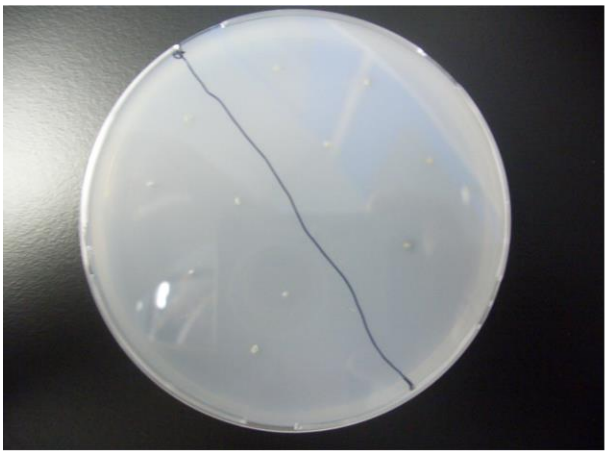
Table 3. TSS and VSS removal during the combined ULS/thermal pre-treatment.

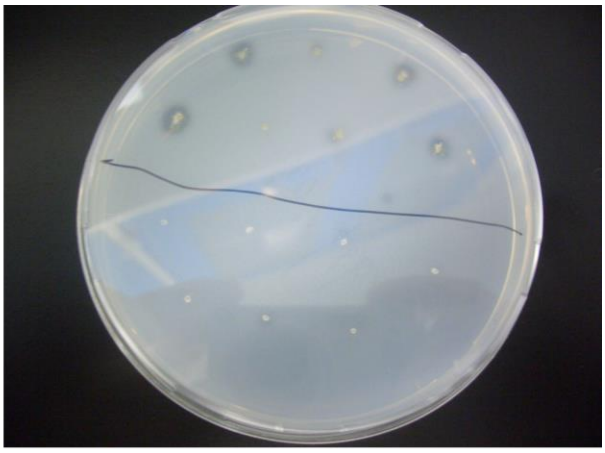
	TSS removal %	VSS removal %
Raw	0	0
ULS 20% (5,000 kJ/kg TS)	4.6	2.36
ULS 50% (5,000 kJ/kg TS)	7.28	5.91
ULS 100% (5,000 kJ/kg TS)	8.62	6.86
raw+55°C for 24hrs	20.5	19.15
raw+65°C for 24hrs	22.22	22.93
ULS 20% + 55°C for 24hrs	21.65	23.4
ULS 50% + 55°C for 24hrs	21.46	20.57
ULS 100% + 55°C for 24hrs	22.8	22.46
ULS 20% + 65°C for 24hrs	23.75	23.4
ULS 50% + 65°C for 24hrs	27.2	26.95
ULS 100% + 65°C for 24hrs	24.33	24.35

SUPPORTING INFORMATION

Figure 1S. (A) Samples (1 µL) taken after 6 hours of hyper-thermophilic pre-treatment at 65°C with and without ultrasonication and pipetted into wells on Petri dishes placed at 55°C. The Petri dishes contain several replicated wells. (B) Samples taken after 6 hours of enzymatic pre-treatment at 65°C with and without ultrasonication and pipetted into wells on Petri dishes placed at 37°C. (C) Samples taken after 24 hours of hyper-thermophilic enzymatic pre-treatment at 65°C with and without ultrasonication and pipetted into wells on Petri dishes placed at 37°C.



A WAS: 0 % ULS, 6 h at 65°C
Petri dish: 24 hrs at 55°C



WAS: 100 % ULS, 6 h at 65°C
Petri dish: 24 hrs at 55°C

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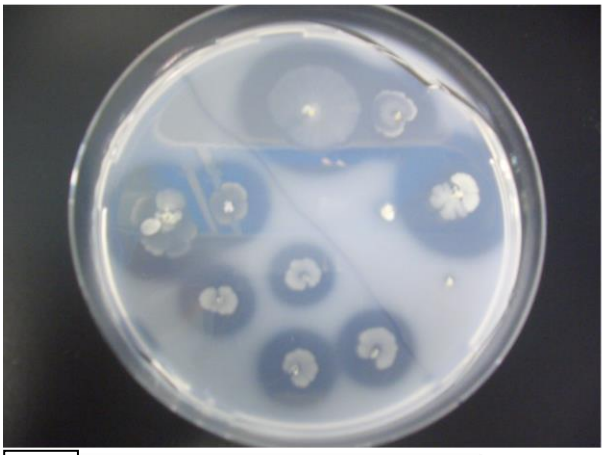
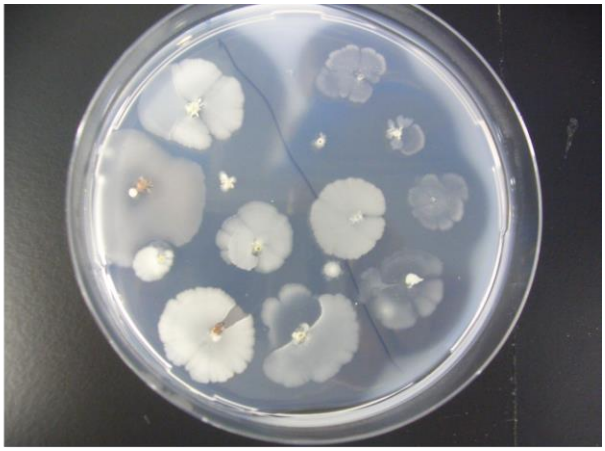


B WAS: 0 % ULS, 6 h at 65°C
Petri dish: 96 hrs at 37°C



WAS: 100 % ULS, 6 h at 65°C
Petri dish: 96 hrs at 37°C

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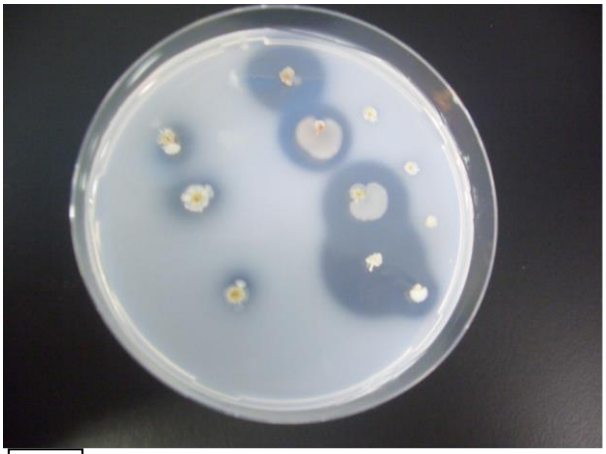
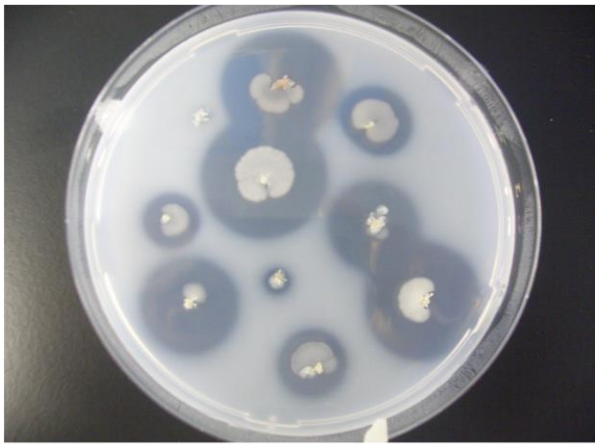


C WAS: 0 % ULS, 24 h at 65°C
Petri dish: 96 hrs at 37°C



WAS: 100 % ULS, 24 h at 65°C
Petri dish: 96 hrs at 37°C

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