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J. Rajesh Banu, Preethi, M. Gunasekaran, Vinod Kumar, Shashi Bhatia, Gopalakrishnan Kumar

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Enhanced biohydrogen generation through calcium peroxide engendered efficient ultrasonic disintegration of waste activated sludge in low temperature environment

Rajesh Banu J [a] , Preethi [b], Gunasekaran M [b], Vinod Kumar, Shashi Bhatia [d], Gopalakrishnan KUMAR [e*]

[a] *Department of Life Sciences, Central University of Tamil Nadu, Neelakudi, Thiruvarur, Tamil Nadu 610005, India*
[b] *Department of Physics, Anna University Regional Campus Tirunelveli, Tamilnadu 627007 India*
[c] *School of Water, Energy and Environment, Cranfield University, Cranfield MK43 0AL, United Kingdom*
[d] *Department of Biological Engineering. Konkuk University, South Korea*
[d] *School of Civil and Environmental Engineering, Yonsei University, Seoul 03722, Republic of Korea*

Corresponding Author:

Gopalakrishnan KUMAR, School of Civil and Environmental Engineering, Yonsei University, Seoul 03722, Republic of Korea.

Email: gopalakrishnanchml@gmail.com

Abstract

Waste activated sludge is a renewable source for biohydrogen production, whereas the presence of complex biopolymers limits the hydrolysis step during this process, and thus pretreatment is required to disintegrate the sludge biomass. In this study, the feasibility of utilizing waste activated sludge to produce biohydrogen by improving the solubilization by means of thermo CaO_2 engendered sonication disintegration (TCP-US) was studied. The optimized condition for extracellular polymeric substance (EPS) dissociation was obtained at the CaO_2 dosage of 0.05 g/g SS at 70 °C. The maximum disintegration after EPS removal was achieved at the sonic specific energy input of 1612.8 kJ/kg TS with the maximum solubilization and SS reduction of 23.7% and 18.14%, respectively, which was higher than the US alone pretreatment. Thus, this solubilization yields higher biohydrogen production of 114.3 mLH_2/gCOD in TCP-US sample.

Keywords: Solubilization; dissociation; disintegration; biohydrogen; hydrolysis; extracellular polymeric substance

1. Introduction

The increase in energy demand is foreseeable in the forthcoming decade owing to faster commercialization and industrialization. In the modern era, the rise in population and obligations leads to an increase in organic load in wastewater, which upon biological treatment, results in the generation of a tremendous amount of sludge (Liu et al., 2021). These contaminants posses higher risk to the environment if they are not appropriately handled (Liang et al., 2021). Moreover, the disposal of sludge consumes 60% of the total operational cost and becomes a global challenge (Liu et al., 2017; Wu et al., 2019). Contrastingly, the WAS consist of colloidal particles with an enormous amount of organics, and thus, abundant research has been made by utilizing WAS as an efficient substrate for energy generation (Luo et al., 2018; Yuan et al., 2019). The emergent crisis of energy throughout the world leads to the selection of this waste as substrate (Luo et al., 2019; Wei et al., 2017). Anaerobic degradation (AD) is an eco-friendly technology to utilize organic waste for transforming it into energy (Abdelsalam et al., 2017). This transformation occurs due to the synergic effect between microbes and organic matter (González-Arias et al., 2020; Rodríguez-Valderrama et al., 2020). The energy obtained through AD, has a calorific value between 22000-25000 kJ m^{-3} (Xiao et al., 2014). AD is a sequential complex biological process, and its efficiency is governed by hydrolysis (Rouches et al., 2016). In raw WAS, AD efficiency is insignificant, owing to the presence of sludge flocs (Janke et al., 2017; Yang et al., 2019;). Flocs are made of extracellular polymeric substances (EPS) that mask the microbes from harsh environments (Wang et al., 2019). The hydrolysis rate was enhanced by the proper selection of pretreatment methods, namely, physical, chemical, mechanical biological, combined, and two phase pretreatment, which disintegrate the WAS and increases successive energy production (Ariunbaatar et al., 2014; Kavitha et al., 2015a, Saranya, et al., 2015). While undergoing the pretreatment methods, the substrate is made accessible to microorganisms and hydrolytic enzymes by partially destroying the substrate structure.

Among the different chemical pretreatment methods, the advanced oxidation process (AOP) has gained wide attention since it serves efficiently in the reduction of sludge and organic matter recycling (Yang et al., 2019). Calcium peroxide is one such chemical which is used as an oxidant in different fields, such as environmental renovation and pharmaceutical (Zhang, et al., 2015). It is a flexible and eco-friendly form of inorganic peroxides with higher energy covalent bond of peroxides (Ma et al., 2007). While in contact with a hydrous medium, it forms $Ca(OH)_2$, O_2, and H_2O_2 (Thani et al., 2016). The H_2O_2 further generates oxidative radicals such as $^{\bullet}OH$, $HO_2^{\bullet}$ and $^{\bullet}O_2^-$ and the Ca^{2+} ions, which possess flocculating effects (Lu et al., 2017). The strong oxidant radicals cease the hydrophilic functional group and the calcium ions upset the binding affinity of the EPS matrix (Wu et al., 2018; Wu & Chai, 2016). Additionally, the presence of $Ca(OH)_2$ enhances the alkalinity and improves liquefaction (Li et al., 2015). The decrease in EPS content is related to the compact structure of flocs due to the compression of EPS by bridging Ca^{2+} ions. The heat treatment, along with CaO_2, can stimulate CaO_2 decomposition and helps in faster $^{\bullet}OH$ radical and H_2O_2 generation (Kaewdee et al., 2016). The combination of pretreatment method was found to lower operation conditions (Liu et al., 2019). Moreover, it provides the advantage of reducing the temperature, and creates a higher binding site for Ca^{2+} to sludge flocs, thereby enhancing exopolymer solubilization (Xiao et al., 2017). Wang and Li, (2016) used CaO_2 and microwave for the pretreatment of WAS and showed a positive synergic effect on solubilization with higher methane yield. The sludge degradation was enhanced by 25.8%, when the CaO_2 dosage enhanced to 0.02 g/gVS as in the study by Wang et al., (2022). Ultrasonication (US) is the mechanical disintegration method which was proven to be a more significant technology with an energy utilization of greater than 5000 kJ/kg TS. It acts by forming bubbles, due to cavitation and rise in temperature, thereby causing sludge disintegration (Cheng et al., 2018). Li et al., (2019) studied the synergic effect of CaO_2 and

US and found enhanced waste disintegration, thereby improving the degradable organic matter. Moreover, this combined action reduced the recalcitrant contaminant frequency. Likewise, another study by Han et al., (2021) reported that the solubilization is improved to 50.7% when the CaO_2 dosage is applied at 0.05 g/gVSS when combined with US.

In the present investigation, the thermal and CaO_2 effect is combined to explore the efficiency of EPS removal from WAS followed by the ultrasonication effect on sludge disintegration for enhanced biohydrogen production. The main objective of the study was: 1) to insight into sludge biomass EPS dissociation through thermal-CaO_2 without any cell cleavage, 2) assess the potential of disintegration by ultrasonication, 3) to evaluate the degradability and biohydrogen production of the pretreated sludge.

Materials and methods

2.1 Sludge sampling

The WAS was collected from a Pulp and paper mill industry at Tirunelveli, Tamilnadu, India. The samples were collected and stored at 4 °C to maintain the sludge concentration. Upon characterization, the values were determined as follows: Mixed liquor suspended solid (MLSS) = 7000± 100 mg/L, Total COD = 10000±200 mg/L and soluble COD = 100±50 mg/L, carbohydrate = 2±1, protein = 5±1

2.2 Floc disturbance by thermal-calcium peroxide

2.2.1 With CaO_2 alone at room temperature

The fragmentation of sludge flocs was done in a 500 mL sample in a conical flask volume of 1000 mL with the CaO_2 dosage ranging from 0.02-0.2 g/g SS. The conical flask was shaken at 120 rpm for a total period of 90 min. A total number of 18 samples were collected every 5 minutes and were centrifuged at 10000 rpm and the supernatant was subjected to analysis.

2.2.2 With CaO_2 at variable temperature

The EPS removal was enhanced by the effect of temperature varying 50-80 °C in 500 mL of sludge sample at the dosage ranging 0.01-0.1 g/g SS at 20 minutes for efficient disruption of EPS.

2.3 **Disintegration of sludge biomass by ultrasonication**

The EPS matrix dissociation by CaO_2 was followed by ultrasonic disintegration. US was carried out at 20 kHz and 200 W power. The process takes place in 500 mL of flocculated and deflocculated sludge in a beaker. 12 number of sample was collected at regular interval for a total period of 60 min and were centrifuged at 10000 rpm. The efficiency of the sludge disintegration was determined by soluble organic release, SS reduction, and biopolymer release in supernatant and was compared with the ultrasonication pretreatment alone at the same conditions. The sonic specific input energy for the US was arrived as described by Yukesh Kannah et al., (2019).

2.4 **Bioacidogenesis**

Bioacidogenesis is used to evaluate the effect of pretreatment on hydrolysis and acidification. The fermentation was carried out in a 250 mL reactor with US alone and TCP-US sample as substrate and anaerobically digested sludge as inoculum. The ratio of substrate to inoculum was maintained at 9:1, and 50 mM of 2- bromoethane sulfonic acid (BESA) was added to the samples and heated to 102 °C to control the action of methanogens. The headspace was purged with nitrogen and tightly sealed with stopper to eradicate oxygen and kept in a shaker at 120 rpm for 72 h.

2.5 Biohydrogen generation

Biohydrogen potential assay is used to assess the efficiency of recovered biohydrogen by utilizing pretreated sludge as substrate. The biohydrogen production efficiency of US alone, and TCP-US pretreated samples were analyzed in two reactors at 35 °C. In the reactor, 70% of the pretreated sample, 25% of inoculum, and about 5% of nutrient solution was added and

preheated to 100 °C as described by Yukesh Kannah et al., (2019). After even mixing, the headspace was purged with nitrogen and septum was used to seal the mouth of the reactor. It is then kept in an orbital shaker at 120 pm and 35 °C temperature. The displacement of the syringe measured the produced biohydrogen. It occurs due to the pressure produced inside the reactor, which pushes the syringe upward, and the displaced syringe volume was noted. A gas chromatograph with a thermal conductivity detector measured the hydrogen content in produced gas.

2.6 Analytical methods

Mixed liquor Suspended solids (MLSS), TCOD, and SCOD were measured by standard methods. Biopolymers concentration and DNA release in the sludge samples was measured by the procedure described by Merrylin et al., (2014).

3. Results and discussion

3.1 EPS dissociation by the action of CaO_2 at normal temperature

EPS represents the chief fraction of organics used to determine the floc structure and strength. The sludge biodegradation is augmented by the EPS dissociation in the sludge matrix. Moreover, the EPS removal is strongly influenced by the pretreatment method. The major components of EPS are protein, DNA, and carbohydrate, which are adsorbed on the surface of the sludge. The biopolymer concentration varies based on the environmental conditions, and EPS serves as a protective layer surrounding the cell membrane of the microbes (He et al., 2015; Zhang et al., 2016). CaO_2 is an efficient oxidant that deliberately degraded to H_2O_2, O_2, and $Ca(OH)_2$ in the aqueous phase, and thus it attains more attention in case of sludge hydrolysis. The EPS are hydrated and negatively charged layer; thus, the oxidation by CaO_2 leads to the release of sludge EPS to the soluble phase by releasing the water entrapped into the sludge flocs. Moreover, the addition of CaO_2 improves the action of

hydrolytic and acid producing enzymes and improves the hydrolysis and acidification of sludge.

Fig 1 a shows the optimization of CaO_2 dosage for exopolymer destruction. From the figure, it is evident that the protein and carbohydrate release increases from 36.29 and 24.5 mg/L to 109.07 and 76.2 mg/L respectively between the dosage ranging 0.02-0.08 g/g SS. This may be due to the disruption of EPS matrix and the release of protein and carbohydrate present in exopolymer matrix. The presence of radicals start up the oxidation process, and the Ca^{2+} ions bridge between the EPS layer and hydrolyse the biomolecules by acting on peptide bonds and carbohydrate to generate free monomers in soluble phase. Beyond the dosage of 0.08 g/g SS till 0.2 g/g SS, the biopolymer release decreases from 109.07-27.3 mg/L (protein) and 76.2 - 11.34 mg/L (carbohydrate) respectively. This decrease at higher dosage is due to the soluble organic degradation by the redundant generation of $^{\bullet}OH$ radicals, and due to the presence of Ca^{2+} ions, leading to the reflocculation of sludge flocs.

In order to assess the deflocculation of WAS, it is mandatory to assess the soluble EPS release, which is used as one of the markers to assess the floc disruption. Again, from the figure, it is seen that the soluble EPS also follows the similar trend as that of biopolymer release. At the initiation of the experiment without any addition of deflocculant, the soluble EPS was 13.55 mg/L. From the figure, it is evident that the increase in CaO_2 dosage of 0.02-0.08 g/g SS, increases the soluble EPS release from 60.79-185.29 mg/L. It happens due to the production of strong oxidants that oxidize the hydrophilic functional group in EPS and the presence of Ca^{2+} cations, bridges the negative charge in the EPS layer and compress to release the compounds to the soluble phase. Beyond this dosage, the increase in dosage concentration leads to the higher generation of oxidizing radicals and further, the reflocculation of sludge occurs due to the presence of more and more Ca^{2+} cations and thus decreases the soluble EPS to 38.64 mg/L at the CaO_2 dosage of 0.2 g/g SS .

The harsh condition in deflocculation persuades the cell destruction and release the intracellular DNA and thus it is also used as an efficient marker in order to assess the EPS extraction prior to the cell lysis. The DNA release was enhanced from 0.879- 44.25 mg/L when the dosage increased from 0-0.08 g/g SS. The step by step increment of DNA shows that the deflocculation is efficient till this dosage. The sharp increment were seen at the dosage between 0.08 – 0.1 g/g SS, which shows the initiation of cell lysis. Further increment in CaO_2 dosage increases the DNA concentration due to the presence of phosphate prolific backbone, which resist the effect of oxidative radical attack and the degradation of DNA is limited. In contrast, the biopolymers were easily hydrolysed when the CaO_2 concentration enhances (Niu et al., 2016). It is concluded from the above facts that the optimum CaO_2 concentration for deflocculation is considered to be 0.08 g/g SS.

3.2 Variation of CaO_2 concentration at varying temperature on sludge EPS matrix disruption

The degree of hydrolysis enhances with the increase in CaO_2 concentration and treatment time. Usually, the temperature increase improves the digestibility of the substrate and hydrolysis rate, thus enhancing energy production. The increase in temperature enhances the CaO_2 decomposition and thereby increases the generation of reactive $^{\bullet}OH$ radicals from H_2O_2. During the thermal treatment, the generation of refractory contaminants is removed by CaO_2 (Kaewdee et al., 2016). While combining the thermal and oxidant pretreatment, the treatment efficiency is higher even at the lower addition of oxidants, and at lesser operating condition. While considering temperature, the lower temperature is much favourable since the higher temperature needs enormous energy as well as it produces larger inhibitory substances (Farno et al., 2015; Zhen et al., 2017). The generation of Ca^{2+} ions at varying temperature causes biopolymer solubilization by forming a bridge between the EPS layers (Xiao et al., 2017). The strong action of oxidative radicals and cations on sludge flocs causes stress, thereby releasing exopolymer substances.

The effect of temperature on soluble EPS, soluble COD and DNA is depicted in Fig 1b. In raw sludge, the DNA, soluble EPS, and soluble COD concentration were observed to be 0.879 mg/L, 13.55 mg/L, and 100 mg/L, respectively. While treating the WAS with varying concentrations of CaO_2 at different temperature, the soluble EPS, soluble COD and DNA increases. As shown in figure 1b, the soluble EPS, soluble COD, and DNA increases to 196.2 mg/L, 429.03 mg/L and 41.42 mg/L at 50 °C with the CaO_2 dosage of 0.07 g/g SS. At 60 °C, a higher release of 58.5 mg/L of DNA and soluble COD, soluble EPS release of 484.25 mg/L and 241.91 mg/L, respectively, were achieved at CaO_2 dosage of 0.06 g/g SS. As the temperature increases, the shift in dosage were seen. The increase in temperature, increases the activity of oxidative radicals and thus lower dosage of CaO_2 is enough for efficient disruption of EPS matrix. The maximum release of 69.05 mg/L, 673.44 mg/L, and 321.18 mg/L, respectively was observed at 70 °C at 0.05 g/g SS of CaO_2 dosage. The combined effect of thermal and CaO_2 dosage leads to higher decomposition, and the production of $^{\bullet}OH$ radicals, H_2O_2, and alkali enhance the disruption of EPS and facilitates the release of organics into the soluble phase (Zhen et al., 2017). Likewise, in a study by He et al., (2021), the combination of heat and CaO_2 at 60 °C and 12.5 mg/g dry solids substantially increases the dewatering capacity of sludge due to the decomposition of tightly bound EPS. While increasing the temperature to 80 °C, the decrement in the concentration of CaO_2 dosage does not cause any significant difference in soluble EPS, soluble COD, and DNA concentration. Beyond optimum dosage, the reduction in EPS concentration and soluble COD shows the mineralization of released components. Based on the above discussion, the CaO_2 dosage of 0.05 g/g SS at 70 °C was optimum for the sludge floc matrix disruption.

3.3 Low thermal CaO_2 induced ultrasonic biomass disintegration.

The probable effect of sonication on biomass disintegration after thermal CaO_2 dissociation of EPS in sludge biomass was evaluated. The three aspects were explored in order to assess

the efficiency TCP-US and US alone at varying specific energy input, are (i) solubilization, (ii) solid change, and (iii) intracellular polymer release.

3.3.1 Soluble organic release and solubilization

The soluble organic release is an index used to assess the efficiency of the disintegration process. The eminent soluble organic release entails the higher degree of disintegration of organics (Kavitha *et al.*, 2015b). The intracellular components are released to the soluble phase by the action of sonication, where the collision of cavitation bubbles causes the continuous breakage of cell walls. Fig 2a illustrates the soluble organic release in flocculated (US alone) and deflocculated (TCP-US) sludge samples concerning the sonic specific energy at varying power levels. In sonic disintegration, the input power is the major factor that initiates the specific energy of the medium. Specific energy stages a chief function in maintaining the economic process of sonic pretreatment since the decrease in specific energy saves the operating cost for the pretreatment of sludge biomass (Pham *et al.*, 2009). As observed in the figure, it follows two different phases: phase I is rapid increment, and phase II is steady increment. In Phase I, the soluble organic release increased from 100-1030 mg/L and 100-2370 mg/L, respectively at the specific energy input of 0-1881.6 kJ/kg TS in US alone and 0-1612.8 kJ/kg TS in TCP-US samples. The increment in TCP-US sludge could be explicated by the availability of enough surface area for US treatment due to the EPS removal and the intracellular component discharge to the aqueous phase by the action of sonication. The sludge biomass cells get cleaved due to the hydromechanical shear force generated by the cavitation bubble collision during ultrasonic disintegration. Likewise, the sCOD solubilization of 1-9.6% and 1-22.2%, respectively, were observed at the specific energy input of 0-1612.8 kJ/kg TS in US alone sample and 0-1382.4 kJ/kg TS in TCP-US sample. At optimum specific energy of 1612.8 kJ/kg TS and 1881.6 kJ/kg TS, the solubilization was found to be 23.7% and 10.3 % for TCP-US and US alone samples respectively, as observed

in Fig 2b. In phase II, the soluble organic release of 1050-1140 mg/L and 2410-2550 mg/L, respectively, for US and TCP-US sludge samples were achieved at the specific energy input ranging 2016-8064 kJ/kg TS and 1728-6912 kJ/kg TS. It shows the stable release after the optimal specific energy input, which indicates that most of the organics were released already and reached the saturated state. Likewise, the solubilization increases from 10.5-11.4% and 24.1-25.5%, respectively, for the specific energy input of 2016-8064 kJ/kg TS and 1728-6912 kJ/kg TS for US alone sample and TCP-US sludge samples. In a study by Salsabil *et al.* (2009), the higher specific energy input of 108,000 kJ/kg TS, shows up with only a solubilization of 10%. Likewise, in another study by Zhao *et al.*, (2019), the specific energy input of 66873.2 kJ/kg TS yields the maximum solubilization of 950 mg/L in waste activated sludge. They observed that the sCOD release is higher in the combination of lower power density with higher sonication time. Whereas in this study, 1612.8 kJ/kg TS of ultrasonic specific energy achieved the maximum solubilization of 23.7%, which demonstrates the efficiency of TCP-US pretreatment.

3.3.2 Solid changes and biomolecule release

Solid change is considered a major factor assessing the suspended solid reduction potential of sludge throughout the biomass disintegration. The change in suspended solids efficiency impacts the solubilization of sludge and energy depletion during sonication (Pilli et al., 2016). Thus, it is essential to assess the optimum point of SS reduction. Fig 3a depicts the SS reduction of flocculated (US alone) and deflocculated (TCP-US) sludge samples. From the figure, it was noted that the SS reduction trend also demonstrates two distinct phases as that of soluble organic release. In TCP-US sludge samples, the phase 1 extend from 0-1612.8 kJ/kg TS and phase II extend from 1728- 6912 kJ/kg TS respectively whereas in US alone sample the phase I and phase II extends from 0-1881.6 kJ/kg TS and 2016-8064 kJ/kg TS respectively. In phase I, while increasing the specific energy from 230.4-1152 kJ/kg TS, the

enhancement in SS reduction from 6730-5870 mg/L with the SS reduction percentage of 3.85-16.14 % was observed in TCP-US sample, and the SS reduction from 6880-6480 mg/L with the reduction percentage of 1.71-7.42 % was observed in US alone sample at the specific energy input between 268.8-1344 kJ/kg TS. The maximum reduction of 6440 mg/L and 5730 mg/L with reduction efficiency of 8 % and 18.14% were achieved in US alone and TCP-US samples at 1881.6 and 1612.8 kJ/kg TS. The rapid increment till the maximum reduction is attributed to the rapid soluble organic release of easily hydrolyzable matter persuaded by the cavitation caused by the sonication. Specifically, the increase in SS reduction was observed in TCP-US as compared to US alone at lesser specific energy input. This shows that the EPS dissociation eases the sludge disintegration potential by sonication and thus reduces the solids present in the sludge. Phase II shows the steady state of SS reduction since most of the solids were disintegrated in phase I itself, and very little amount of solids remained for disintegration in phase II. In phase II, the SS reduction percentage ranges between 8.28-9.14 % in US alone at the specific energy input between 2016-8064 kJ/kg TS and 18.57-19.71 % in TCP-US at the specific energy input between 1728-6912 kJ/kg TS. Based on this outcome, it is clear that the maximum specific energy of 1612.8 kJ/kg TS is optimum for SS reduction.

The major biopolymer present in the sludge is protein and carbohydrate. Biopolymer release for US and TCP-US samples are shown in Fig 3b. The release of protein and carbohydrate in TCP-US is higher with 1185, and 711 mg/L at the specific energy input of 1612.8 kJ/kg TS whereas the US alone sample shows the release of 575 and 345 mg/L at the specific energy input of 1881.6 kJ/kg TS. The flocculated sample release was lower even at the higher specific energy input since the EPS layer restricts the solubilization. The higher release in TCP-US is due to the effect of thermal CaO_2, which enhances the surface area for the effect

of sonication, where the generation of cavitation cause cleavage of the sludge cell biomass and thus release intracellular components to the soluble phase.

3.4 Impact of sludge pretreatment on anaerobic fermentation

The hydrolysis and the acidogenic VFA production phase of US alone and TCP-US pretreatment of WAS during anaerobic fermentation are illustrated in Fig 4. Complex polymers like protein and carbohydrates are present in a relatively more enormous amount in WAS. In hydrolysis phase, these protein and carbohydrate polymers are disintegrated into smaller monomers, such as amino acids and sugars, by the action of a hydrolytic enzyme (Shanthi et al., 2018). In acidogenic fermentation phase, the produced compounds were converted to VFA by the action of acetogenic fermentative bacteria. The higher production of VFA in pretreated substrate disintegrates the organic matter more rapidly and enhances hydrogen production than unpretreated samples (Kavitha et al., 2016). The figure shows that the protein, carbohydrate, and VFA at 0^{th} hour in US alone sample were 575, 345, and 115 mg/L, respectively. At 72^{nd} hour, the protein and carbohydrate were reduced to 88, 66 and 881 mg/L, respectively and it leads to the enhancement in VFA production by 881 mg/L. Likewise, in TCP-US sample, the protein, and carbohydrate decreased from 1185-119.2 mg/L and 711-89.4 mg/L, respectively, at 0-72 h. The decrease in biopolymer increases the VFA production from 237-1924.4 mg/L at 0-72 h. This indicates fermentative bacteria's efficient intake of soluble components (Kavitha et al., 2016). Biohydrogen generation is highly associated with the production of VFA during the acidogenic phase (Xia et al., 2015). Initially, during the experiment, pH was maintained at 7, and at the end of the experiment, pH shifted towards 5 due to the VFA production. The higher generation of VFA in TCP-US is due to the phase-separated pretreatment by removing EPS followed by a disintegration process, which makes soluble organic components available and is utilized efficiently by

fermentative microbes. Based on the results, it can be concluded that the TCP-US pretreated samples enhance the fermentation more efficiently than US alone pretreated samples.

3.5 Biohydrogenesis

The influence of US alone and TCP-US pretreated samples on hydrogen production were assessed by biohydrogen potential assay and illustrated in Fig 5. The hydrogen production consists of three phases: Lag phase, exponential phase, and steady phase. In both the samples, the lag phase occurs till 0.2 days, the phase where the adaptation of microbes to the newer environment occurs, and thus the biohydrogen release is negligible. As time increases, the exponential phase starts, and the increase in hydrogen production was observed in all the samples till 5 days. From the figure, it was observed that the maximum hydrogen production of 49.2 and 114.3 mL H_2/gCOD, respectively, were achieved in US alone and TCP-US samples on 5th day. This occurs due to the action of pretreatment, which enhances the nutrient supply to the hydrogen producers. Additionally, the inoculum is rich in cellulolytic bacteria, which helps in the enhancement of biohydrogen. The methanogenic action were restricted by preheating the samples at 100 °C since they cannot handle higher temperatures, whereas the hydrogen producers are spore producing and can withstand this temperatures. Thus, hydrogen is produced in an effective manner. The higher hydrogen generation in TCP-US samples is due to the hydrolysis of organics and efficient utilization of these organics by acetogenic bacteria due to phase separated pretreatment. The produced hydrogen is higher than the value obtained in the study by Preethi et al., (2021), where the sodium persulfate mediated bacterial pretreatment was adopted for treating WAS. Beyond 5th day, the steady phase commences, where the hydrogen production becomes almost stable due to the decline in released organics and was found to be 50.8-54.4 and 117-126.1 mL H_2/gCOD, respectively, in US alone and TCP-US samples from 5.5th day to 10th day. The kinetic parameters were derived using the Gompertz model for US alone, and TCP-US pretreated samples and showed that the

correlation coefficient R^2 was in 0.998-0.999 range and thus suggest it to be a good fit. Thus by analyzing these results, it is confirmed that biohydrogen production is much higher in TCP-US samples.

4. Conclusion

The dissociation of EPS prior to the sonic disintegration enhances the biomass lysis and hydrogen production remarkably due to the EPS removal at 0.05 g/g SS CaO_2 and 70 °C. Moreover, the biomass disintegration was augmented at the sonic specific energy of 1612.8 kJ/kg TS. The heat and the $^{\bullet}OH$ radicals produced by CaO_2 destruct the EPS layer. Further, the sonication process disintegrates the cell and releases intracellular components. Thus, TCP-US pretreated sample shows improved solubilization of 23.7%, which aids in the enhancement in hydrogen production to 114.3 mL H_2/gCOD on 5^{th} day during the exponential phase as compared to the US alone sample.

References

1. Abdelsalam, E., Samer, M., Attia, Y. A., Abdel-Hadi, M. A., Hassan, H. E., & Badr, Y. (2017). Effects of Co and Ni nanoparticles on biogas and methane production from anaerobic digestion of slurry. *Energy Conversion and Management*, *141*, 108–119. https://doi.org/https://doi.org/10.1016/j.enconman.2016.05.051
2. Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., & Lens, P. N. L. (2014). Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Applied Energy*, *123*, 143–156. https://doi.org/https://doi.org/10.1016/j.apenergy.2014.02.035
3. Cheng, C., Zhou, Z., Qiu, Z., Yang, J., Wu, W., & Pang, H. (2018). Enhancement of sludge reduction by ultrasonic pretreatment and packing carriers in the anaerobic side-stream reactor: Performance, sludge characteristics and microbial community structure. *Bioresource Technology*, *249*, 298–306.

https://doi.org/https://doi.org/10.1016/j.biortech.2017.10.043

4. Farno, E., Baudez, J. C., Parthasarathy, R., & Eshtiaghi, N. (2015). Impact of temperature and duration of thermal treatment on different concentrations of anaerobic digested sludge: Kinetic similarity of organic matter solubilisation and sludge rheology. *Chemical Engineering Journal*, *273*, 534–542. https://doi.org/https://doi.org/10.1016/j.cej.2015.03.097
5. González-Arias, J., Gil, M. V., Fernández, R. Á., Martínez, E. J., Fernández, C., Papaharalabos, G., & Gómez, X. (2020). Integrating anaerobic digestion and pyrolysis for treating digestates derived from sewage sludge and fat wastes. *Environmental Science and Pollution Research*, *27*(26), 32603–32614. https://doi.org/10.1007/s11356-020-09461-1
6. Han, J.H. and Nam, S.Y., 2021. Sewage sludge solubilization using ultrasonic combined with calcium peroxide. *Journal of the Korea Organic Resources Recycling Association*, *29*(4), pp.41-46. https://doi.org/10.17137/korrae.2021.29.4.41
7. He, D.-Q., Wang, L.-F., Jiang, H., & Yu, H.-Q. (2015). A Fenton-like process for the enhanced activated sludge dewatering. *Chemical Engineering Journal*, *272*, 128–134. https://doi.org/https://doi.org/10.1016/j.cej.2015.03.034
8. He, D., Bao, B., Sun, M., Chen, J., Luo, H. and Li, J., 2021. Enhanced dewatering of activated sludge by acid assisted Heat–CaO_2 treatment: Simultaneously removing heavy metals and mitigating antibiotic resistance genes. *Journal of Hazardous Materials*, *418*, p.126248. https://doi.org/10.1016/j.jhazmat.2021.126248
9. Janke, L., Weinrich, S., Leite, A. F., Terzariol, F. K., Nikolausz, M., Nelles, M., & Stinner, W. (2017). Improving anaerobic digestion of sugarcane straw for methane production: Combined benefits of mechanical and sodium hydroxide pretreatment for process designing. *Energy Conversion and Management*, *141*, 378–389. https://doi.org/https://doi.org/10.1016/j.enconman.2016.09.083

10. Kaewdee, P., Chandet, N., Rujijanagul, G., & Randorn, C. (2016). Multicatalytic properties of nanoparticle CaO_2 synthesized by a novel, simple and economical method for wastewater treatment. *Catalysis Communications*, *84*, 151–154. https://doi.org/https://doi.org/10.1016/j.catcom.2016.06.031

11. Kavitha, S., Adish Kumar, S., Kaliappan, S., Yeom, I. T., & Banu, J. R. (2015). Achieving profitable biological sludge disintegration through phase separation and predicting its anaerobic biodegradability by non linear regression model. *Chemical Engineering Journal*, *279*, 478–487. https://doi.org/https://doi.org/10.1016/j.cej.2015.05.051

12. Kavitha, S., Jayashree, C., Adish Kumar, S., Kaliappan, S., & Rajesh Banu, J. (2014). Enhancing the functional and economical efficiency of a novel combined thermochemical disperser disintegration of waste activated sludge for biogas production. *Bioresource Technology*, *173*, 32–41. https://doi.org/https://doi.org/10.1016/j.biortech.2014.09.078

13. Kavitha, S., Rajesh Banu, J., Vinoth Kumar, J., & Rajkumar, M. (2016). Improving the biogas production performance of municipal waste activated sludge via disperser induced microwave disintegration. *Bioresource Technology*, *217*, 21–27. https://doi.org/https://doi.org/10.1016/j.biortech.2016.02.034

14. Kavitha, S., Saranya, T., Kaliappan, S., Adish Kumar, S., Yeom, I. T., & Rajesh Banu, J. (2015). Accelerating the sludge disintegration potential of a novel bacterial strain Planococcus jake 01 by $CaCl_2$ induced deflocculation. *Bioresource Technology*, *175*, 396–405. https://doi.org/https://doi.org/10.1016/j.biortech.2014.10.122

15. Li, X., Liu, Y., Xu, Q., Liu, X., Huang, X., Yang, J., Wang, D., Wang, Q., Liu, Y., & Yang, Q. (2019). Enhanced methane production from waste activated sludge by combining calcium peroxide with ultrasonic: Performance, mechanism, and implication. *Bioresource Technology*, *279*, 108–116.

https://doi.org/https://doi.org/10.1016/j.biortech.2019.01.115

16. Liang, T., Elmaadawy, K., Liu, B., Hu, J., Hou, H. and Yang, J., 2021. Anaerobic fermentation of waste activated sludge for volatile fatty acid production: recent updates of pretreatment methods and the potential effect of humic and nutrients substances. *Process Safety and Environmental Protection*, *145*, pp.321-339. https://doi.org/10.1016/j.psep.2020.08.010

17. Liu, H., Xiao, H., Fu, B., & Liu, H. (2017). Feasibility of sludge deep-dewatering with sawdust conditioning for incineration disposal without energy input. *Chemical Engineering Journal*, *313*, 655–662. https://doi.org/https://doi.org/10.1016/j.cej.2016.09.107

18. Liu, X., Wu, Y., Xu, Q., Du, M., Wang, D., Yang, Q., Yang, G., Chen, H., Zeng, T., Liu, Y., Wang, Q., & Ni, B.-J. (2021). Mechanistic insights into the effect of poly ferric sulfate on anaerobic digestion of waste activated sludge. *Water Research*, *189*, 116645. https://doi.org/https://doi.org/10.1016/j.watres.2020.116645

19. Liu, X., Xu, Q., Wang, D., Yang, Q., Wu, Y., Yang, J., Liu, Y., Wang, Q., Ni, B.-J., Li, X., Li, H., & Yang, G. (2019). Enhanced Short-Chain Fatty Acids from Waste Activated Sludge by Heat–CaO2 Advanced Thermal Hydrolysis Pretreatment: Parameter Optimization, Mechanisms, and Implications. *ACS Sustainable Chemistry & Engineering*, *7*(3), 3544–3555. https://doi.org/10.1021/acssuschemeng.8b05799

20. Lu, S., Zhang, X., & Xue, Y. (2017). Application of calcium peroxide in water and soil treatment: A review. *Journal of Hazardous Materials*, *337*, 163–177. https://doi.org/https://doi.org/10.1016/j.jhazmat.2017.04.064

21. Luo, J., Wu, L., Zhang, Q., Fang, F., Feng, Q., Xue, Z., Cao, M., Peng, Z., Li, C., & Cao, J. (2019). How Do Biocides That Occur in Waste Activated Sludge Affect the Resource Recovery for Short-Chain Fatty Acids Production. *ACS Sustainable Chemistry &*

Engineering, *7*(1), 1648–1657. https://doi.org/10.1021/acssuschemeng.8b05420

22. Luo, J., Zhang, Q., Wu, L., Feng, Q., Fang, F., Xue, Z., Li, C., & Cao, J. (2018). Improving anaerobic fermentation of waste activated sludge using iron activated persulfate treatment. *Bioresource Technology*, *268*, 68–76. https://doi.org/https://doi.org/10.1016/j.biortech.2018.06.080

23. Ma, Y., Zhang, B.-T., Zhao, L., Guo, G., & Lin, J.-M. (2007). Study on the generation mechanism of reactive oxygen species on calcium peroxide by chemiluminescence and UV-visible spectra. *Luminescence*, *22*(6), 575–580. https://doi.org/https://doi.org/10.1002/bio.1003

24. Merrylin, J., Kaliappan, S., Kumar, S. A., Yeom, I.-T., & Banu, J. R. (2014). Enhancing aerobic digestion potential of municipal waste-activated sludge through removal of extracellular polymeric substance. *Environmental Science and Pollution Research*, *21*(2), 1112–1123. https://doi.org/10.1007/s11356-013-1976-3

25. Niu, T., Zhou, Z., Ren, W., Jiang, L.-M., Li, B., Wei, H., Li, J., & Wang, L. (2016). Effects of potassium peroxymonosulfate on disintegration of waste sludge and properties of extracellular polymeric substances. *International Biodeterioration & Biodegradation*, *106*, 170–177. https://doi.org/https://doi.org/10.1016/j.ibiod.2015.10.021

26. Pham, T. T. H., Brar, S. K., Tyagi, R. D., & Surampalli, R. Y. (2009). Ultrasonication of wastewater sludge—Consequences on biodegradability and flowability. *Journal of Hazardous Materials*, *163*(2), 891–898. https://doi.org/https://doi.org/10.1016/j.jhazmat.2008.07.091

27. Pilli, S., Yan, S., Tyagi, R. D., & Surampalli, R. Y. (2016). Anaerobic digestion of ultrasonicated sludge at different solids concentrations - Computation of mass-energy balance and greenhouse gas emissions. *Journal of Environmental Management*, *166*, 374–386. https://doi.org/https://doi.org/10.1016/j.jenvman.2015.10.041

28. Preethi., Banu, J.R., Sharmila, V.G., Kavitha, S., Varjani, S., Kumar, G. and Gunasekaran, M., 2021. Alkali activated persulfate mediated extracellular organic release on enzyme secreting bacterial pretreatment for efficient hydrogen production. *Bioresource Technology*, *341*, p.125810. https://doi.org/10.1016/j.biortech.2021.125810

29. Rodríguez-Valderrama, S., Escamilla-Alvarado, C., Rivas-García, P., Magnin, J.-P., Alcalá-Rodríguez, M., & García-Reyes, R. B. (2020). Biorefinery concept comprising acid hydrolysis, dark fermentation, and anaerobic digestion for co-processing of fruit and vegetable wastes and corn stover. *Environmental Science and Pollution Research*, *27*(23), 28585–28596. https://doi.org/10.1007/s11356-020-08580-z

30. Rouches, E., Herpoël-Gimbert, I., Steyer, J. P., & Carrere, H. (2016). Improvement of anaerobic degradation by white-rot fungi pretreatment of lignocellulosic biomass: A review. *Renewable and Sustainable Energy Reviews*, *59*, 179–198. https://doi.org/https://doi.org/10.1016/j.rser.2015.12.317

31. Salsabil, M. R., Prorot, A., Casellas, M., & Dagot, C. (2009). Pretreatment of activated sludge: Effect of sonication on aerobic and anaerobic digestibility. *Chemical Engineering Journal*, *148*(2), 327–335. https://doi.org/https://doi.org/10.1016/j.cej.2008.09.003

32. Shanthi, M., Rajesh Banu, J., & Sivashanmugam, P. (2018). Effect of surfactant assisted sonic pretreatment on liquefaction of fruits and vegetable residue: Characterization, acidogenesis, biomethane yield and energy ratio. *Bioresource Technology*, *264*, 35–41. https://doi.org/https://doi.org/10.1016/j.biortech.2018.05.054

33. Thani, Q. A., Schaffer, B., Liu, G., Vargas, A. I., & Crane, J. H. (2016). Chemical oxygen fertilization reduces stress and increases the recovery and survival of flooded papaya (Carica papaya L.) plants. *Scientia Horticulturae*, *202*, 173–183. https://doi.org/https://doi.org/10.1016/j.scienta.2016.03.004

34. Wang, C., Wei, W., Dai, X. and Ni, B.J., 2022. Calcium peroxide significantly enhances

volatile solids destruction in aerobic sludge digestion through improving sludge biodegradability. *Bioresource Technology*, *346*, p.126655. https://doi.org/10.1016/j.biortech.2021.126655

35. Wang, D., Huang, Y., Xu, Q., Liu, X., Yang, Q., & Li, X. (2019). Free ammonia aids ultrasound pretreatment to enhance short-chain fatty acids production from waste activated sludge. *Bioresource Technology*, *275*, 163–171. https://doi.org/https://doi.org/10.1016/j.biortech.2018.12.055
36. Wang, J., & Li, Y. (2016). Synergistic pretreatment of waste activated sludge using CaO_2 in combination with microwave irradiation to enhance methane production during anaerobic digestion. *Applied Energy*, *183*, 1123–1132. https://doi.org/https://doi.org/10.1016/j.apenergy.2016.09.042
37. Wei, W., Zhou, X., Wang, D., Sun, J., & Wang, Q. (2017). Free ammonia pretreatment of secondary sludge significantly increases anaerobic methane production. *Water Research*, *118*, 12–19. https://doi.org/https://doi.org/10.1016/j.watres.2017.04.015\
38. Wu, B., & Chai, X. (2016). Novel insights into enhanced dewatering of waste activated sludge based on the durable and efficacious radical generating. *Journal of the Air & Waste Management Association*, *66*(11), 1151–1163. https://doi.org/10.1080/10962247.2016.1189858
39. Wu, B., Su, L., Dai, X., & Chai, X. (2018). Development of sludge-derived mesoporous material with loaded nano CaO2 and doped Fe for re-utilization of dewatered waste-activated sludge as dewatering aids. *Chemical Engineering Journal*, *335*, 161–168. https://doi.org/https://doi.org/10.1016/j.cej.2017.10.015
40. Wu, Y., Wang, D., Liu, X., Xu, Q., Chen, Y., Yang, Q., Li, H., & Ni, B. (2019). Effect of poly aluminum chloride on dark fermentative hydrogen accumulation from waste activated sludge. *Water Research*, *153*, 217–228.

https://doi.org/https://doi.org/10.1016/j.watres.2019.01.016

41. Xia, A., Cheng, J., Ding, L., Lin, R., Song, W., Su, H., Zhou, J., & Cen, K. (2015). Substrate consumption and hydrogen production via co-fermentation of monomers derived from carbohydrates and proteins in biomass wastes. *Applied Energy*, *139*, 9–16. https://doi.org/https://doi.org/10.1016/j.apenergy.2014.11.016

42. Xiao, K., Seow, W. Y., Chen, Y., Lu, D., Jiang, X., & Zhou, Y. (2017). Effects of thermal-Fe (II) activated oxone treatment on sludge dewaterability. *Chemical Engineering Journal*, *322*, 463–471. https://doi.org/https://doi.org/10.1016/j.cej.2017.04.055

43. Xiao, Y., Yuan, H., Pang, Y., Chen, S., Zhu, B., Zou, D., Ma, J., Yu, L., & Li, X. (2014). CO_2 Removal from Biogas by Water Washing System. *Chinese Journal of Chemical Engineering*, *22*(8), 950–953. https://doi.org/https://doi.org/10.1016/j.cjche.2014.06.001

44. Yang, J., Liu, X., Wang, D., Xu, Q., Yang, Q., Zeng, G., Li, X., Liu, Y., Gong, J., Ye, J., & Li, H. (2019). Mechanisms of peroxymonosulfate pretreatment enhancing production of short-chain fatty acids from waste activated sludge. *Water Research*, *148*, 239–249. https://doi.org/https://doi.org/10.1016/j.watres.2018.10.060

45. Yuan, Y., Hu, X., Chen, H., Zhou, Y., Zhou, Y., & Wang, D. (2019). Advances in enhanced volatile fatty acid production from anaerobic fermentation of waste activated sludge. *Science of The Total Environment*, *694*, 133741. https://doi.org/https://doi.org/10.1016/j.scitotenv.2019.133741

46. Yukesh Kannah, R., Kavitha, S., Sivashanmugham, P., Kumar, G., Nguyen, D. D., Chang, S. W., & Rajesh Banu, J. (2019). Biohydrogen production from rice straw: Effect of combinative pretreatment, modelling assessment and energy balance consideration. *International Journal of Hydrogen Energy*, *44*(4), 2203–2215. https://doi.org/https://doi.org/10.1016/j.ijhydene.2018.07.201

47. Zhang, A., Wang, J., & Li, Y. (2015). Performance of calcium peroxide for removal of

endocrine-disrupting compounds in waste activated sludge and promotion of sludge solubilization. *Water Research*, *71*, 125–139. https://doi.org/https://doi.org/10.1016/j.watres.2015.01.005

48. Zhang, J., Zhang, J., Tian, Y., Li, N., Kong, L., Sun, L., Yu, M., & Zuo, W. (2016). Changes of physicochemical properties of sewage sludge during ozonation treatment: Correlation to sludge dewaterability. *Chemical Engineering Journal*, *301*, 238–248. https://doi.org/https://doi.org/10.1016/j.cej.2016.04.151

49. Zhao, Y.-H., Zhang, B., Tao, J., Li, Q., & Lv, B. (2019). Optimization of Energy Consumption of the Ultrasonic Pretreatment on Sludge Disintegration. *IOP Conference Series: Materials Science and Engineering*, *592*(1), 12198. https://doi.org/10.1088/1757-899x/592/1/012198

50. Zhen, G., Lu, X., Kato, H., Zhao, Y., & Li uYou, Y. (2017). Overview of pretreatment strategies for enhancing sewage sludge disintegration and subsequent anaerobic digestion: Current advances, full-scale application and future perspectives. *Renewable and Sustainable Energy Reviews*, *69*, 559–577. https://doi.org/10.1016/j.rser.2016.11.187

Figures

Fig 1a Optimization of CaO_2 dosage for EPS destruction a) biopolymer (protein and carbohydrate) b) DNA and c) Soluble COD

Fig 1b Effect of temperature on soluble EPS, Soluble COD and DNA at varying dosage of CaO_2

Fig 2 Effect of a) flocculated sample (ultrasonication alone) and, deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment) on soluble organic release at varying sonic specific energy and (b) sCOD solubilization at varying sonic specific energy

Fig 3 Effect of varying sonic specific energy on a) flocculated sample (ultrasonication alone) and deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment) on SS reduction and b) biopolymer release at optimum specific energy in flocculated sample (ultrasonication alone) and deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment)

Fig 4 Effect of flocculated sample (ultrasonication alone) and deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment) on VFA release during anaerobic fermentation

Fig 5 Hydrogen production in flocculated sample (ultrasonication alone) and deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment)

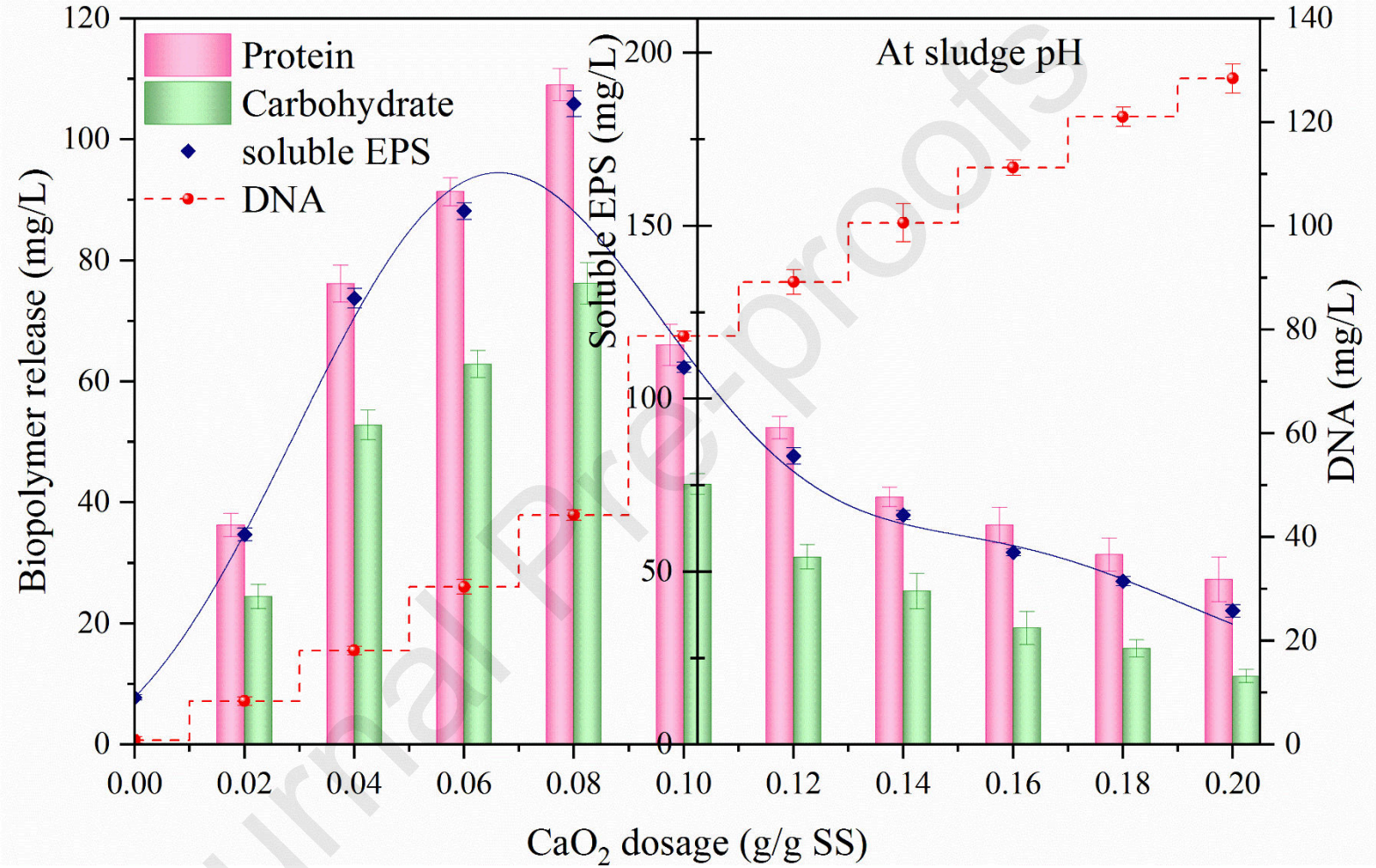


Fig 1a Optimization of CaO_2 dosage for EPS destruction a) biopolymer (protein and carbohydrate) b) DNA and c) Soluble EPS

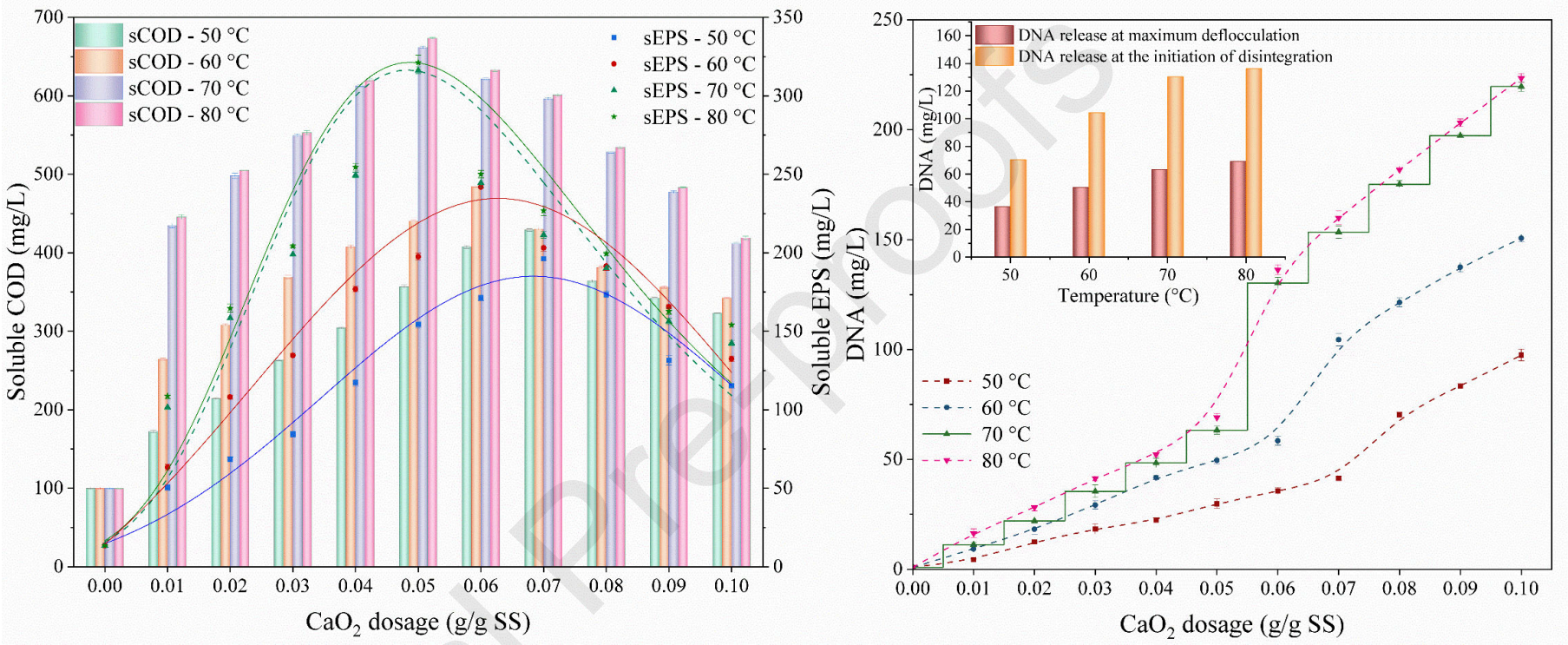
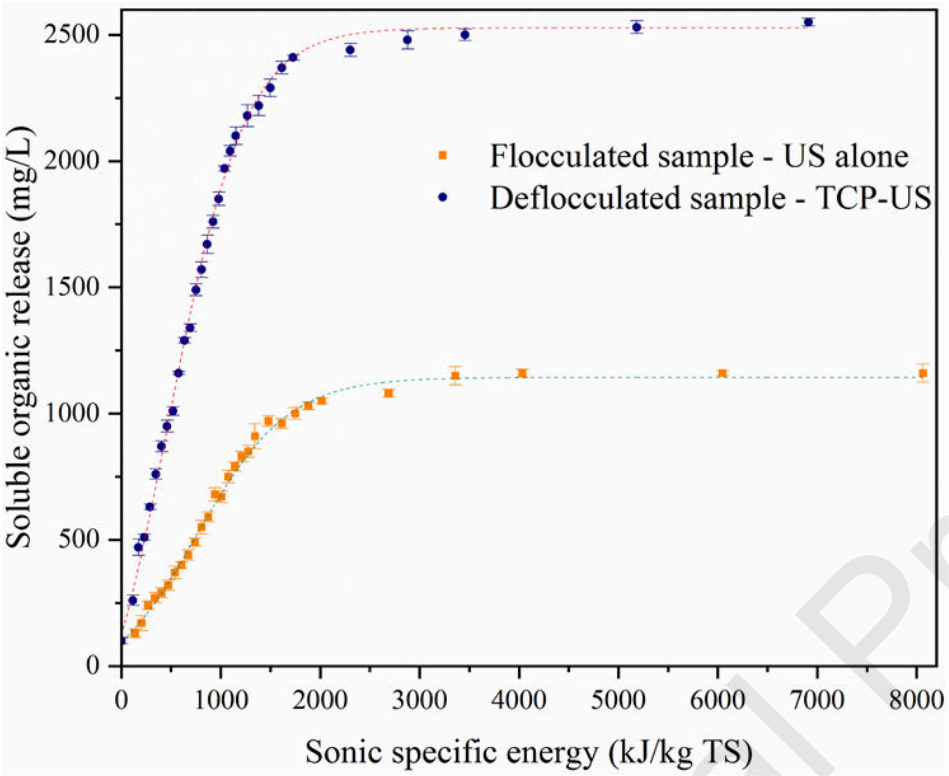
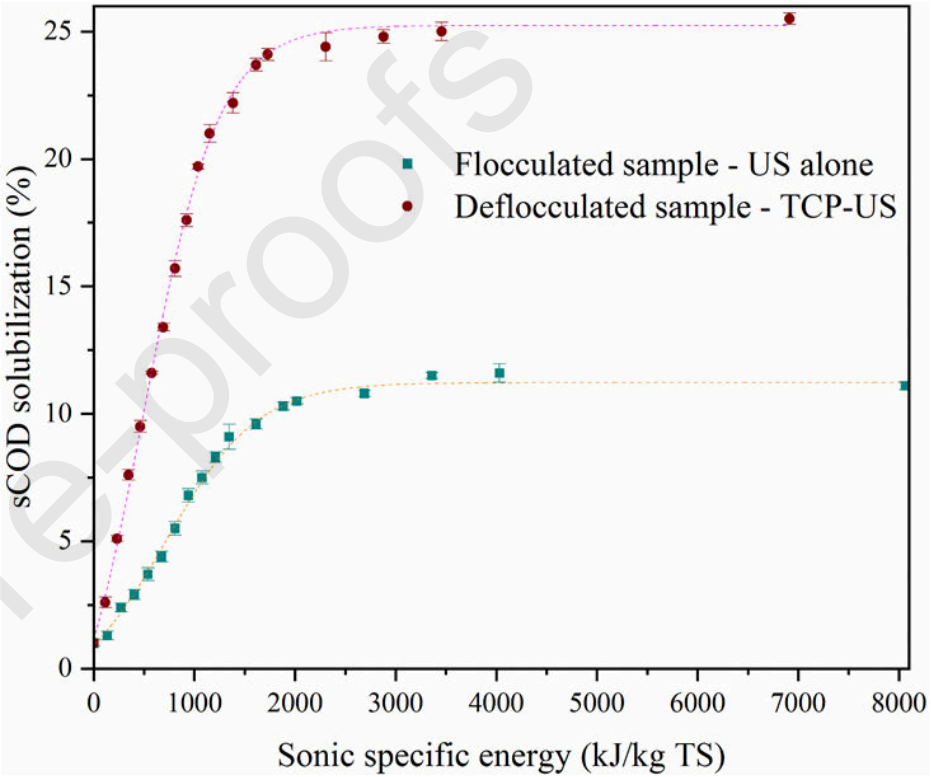


Fig 1b Effect of temperature on soluble EPS, Soluble COD and DNA at varying dosage of CaO_2



[a]



[b]

Fig 2 Effect of a) flocculated sample (ultrasonication alone) and, deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment) on soluble organic release at varying specific energy and (b) sCOD solubilization at varying sonic specific energy

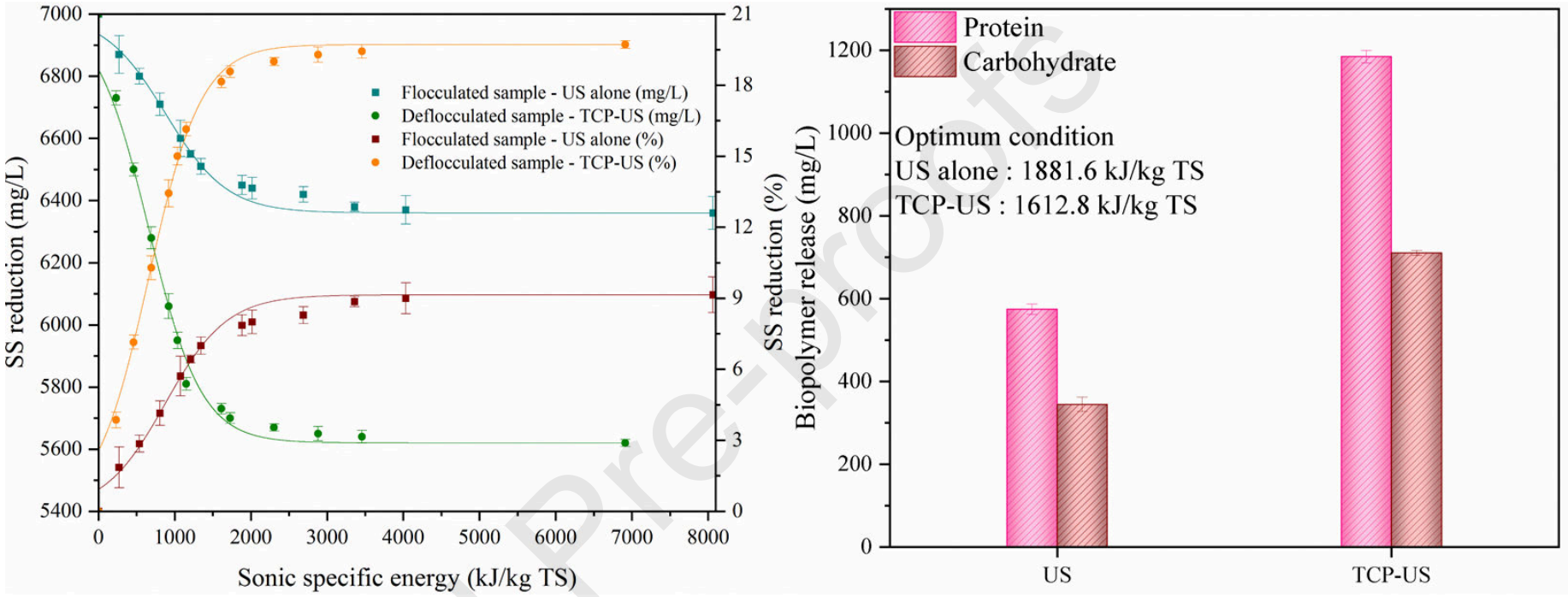


Fig 3 Effect of varying sonic specific energy on a) flocculated sample (ultrasonication alone) and deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment) on SS reduction and b) biopolymer release at optimum specific energy in flocculated sample (ultrasonication alone) and deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment)

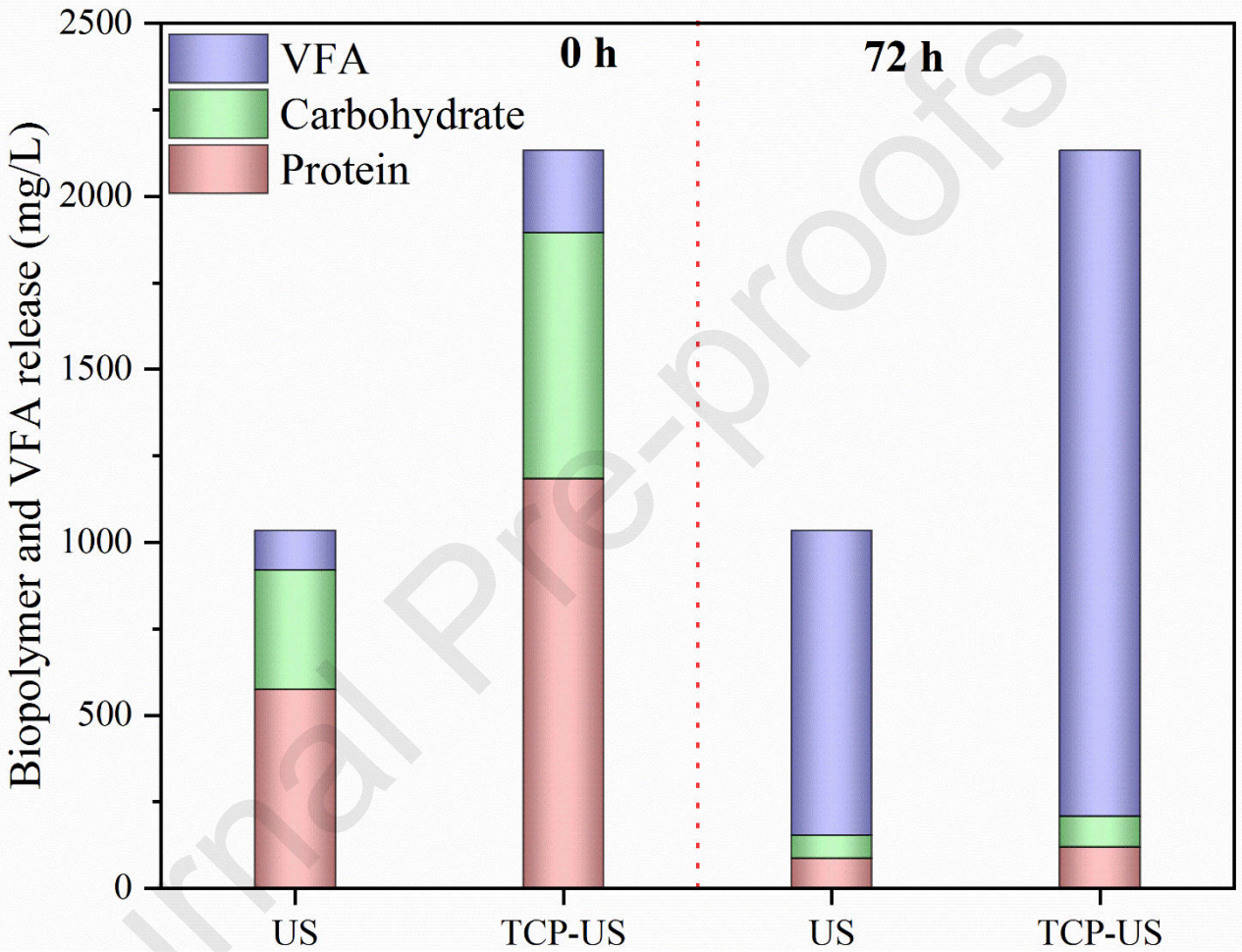


Fig 4 Effect of flocculated sample (ultrasonication alone) and deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment) on VFA release during anaerobic fermentation

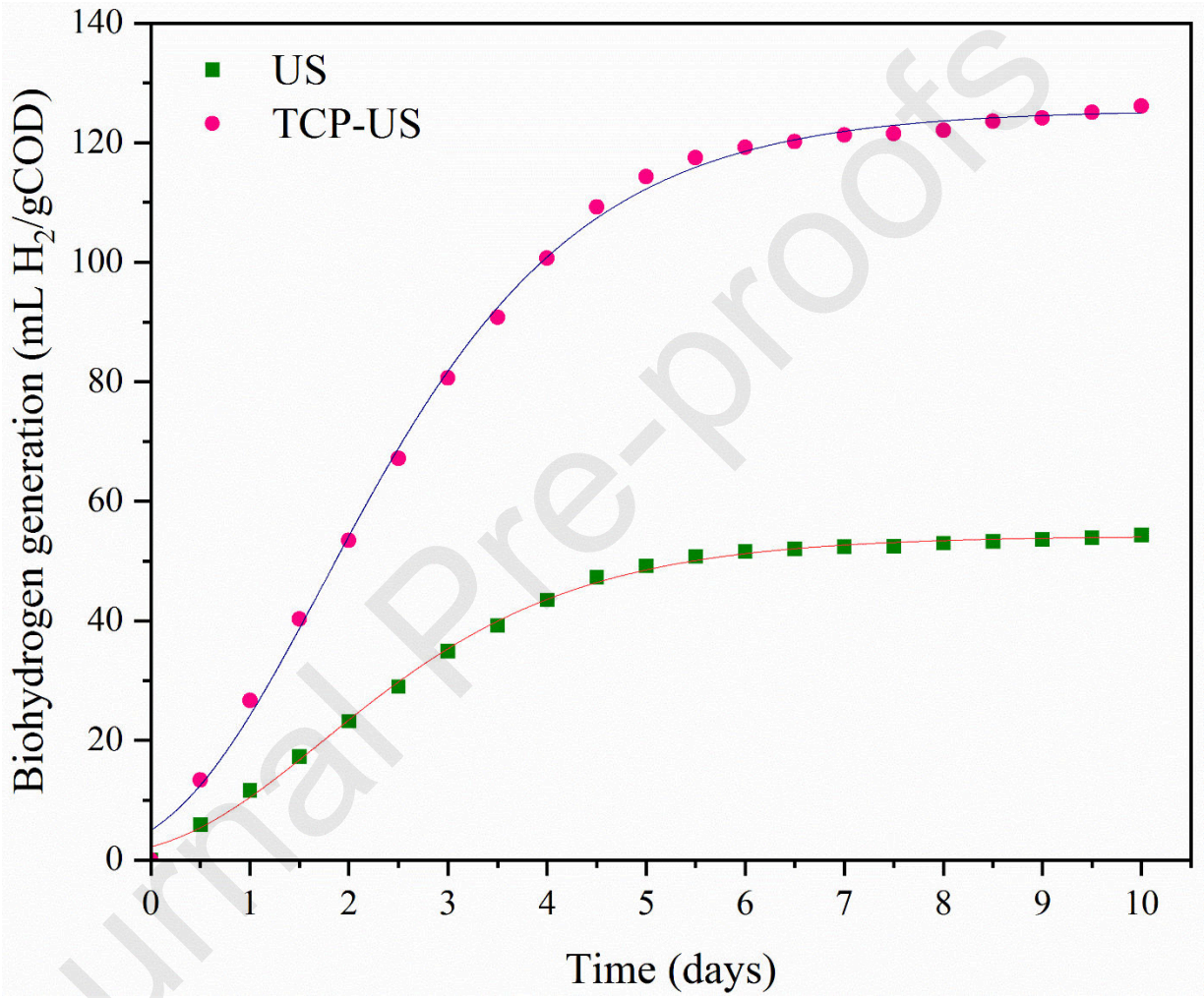


Fig 5 Hydrogen production in flocculated sample (ultrasonication alone) and deflocculated samples (low thermal CaO_2 – ultrasonication pretreatment)

CRediT authorship contribution statement

Rajesh Banu J: Conceptualization, Writing – original draft & editing. Gunasekaran M: Conceptualization, Writing – original draft, review & editing. Preethi: Conceptualization, Writing – original draft, review & editing. Shashi Bhatia: Writing – review & editing. Vinod Kumar: Writing – review & editing. Gopalakrishnan Kumar: Writing – review & editing, Funding acquisition, Project administration & Supervision.

Highlights:

- Waste activated sludge as an efficient substrate for biohydrogen production.
- The enzymatic hydrolysis is boosted by thermo CaO_2 provoked sonication pretreatment.
- Sonic specific energy of 1612.8 kJ/kg TS is efficient for biomass disintegration.
- Peak bioH_2 production of 114.3 mLH_2/gCOD was achieved via TCP-US pretreatment.