Journal of Environmental Chemical Engineering 12 (2024) 113353

Contents lists available at ScienceDirect

Journal of Environmental Chemical Engineering

journal homepage: www.elsevier.com/locate/jece

Surfactant-assisted thermal hydrolysis of sewage sludge: Biomolecule extraction and its potential for protease production

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ARTICLE INFO

Keywords:
Sewage sludge
Thermal hydrolysis
Surfactants
Proteases
B. Licheniformis
Biodegradability

ABSTRACT

Efficient management of sewage sludge from wastewater treatment plants (WWTPs) are crucial to obtain industrial value-added products and reducing environmental impact. Thermal hydrolysis, coupled with surfactants to enhance solubilisation, offers a promising approach for sewage sludge treatment. This study assessed the impact of four surfactants: sodium dodecyl sulphate (SDS), sodium dodecylbenzenesulfonate (SDBS), cetyl trimethyl ammonium chloride (CTAC), and tetraethylammonium chloride (TAC) on thermal hydrolysis of sewage sludge. The addition of anionic surfactants (SDS and SDBS) significantly increased biomolecule production, with proteins, humic-like substances, carbohydrates, and DNA concentrations reaching 6018 ± 28 mg/L (306 mg/gVSS$_o$), 2496 ± 103 mg/L (127 mg/gVSS$_o$), 1822 ± 4 mg/L (93 mg/gVSS$_o$), and 389 ± 3 mg/L (20 mg/gVSS$_o$), respectively, after 155 min of thermal hydrolysis. Low levels of surfactants (10 and 50 mg/gVSS$_o$) also led to a substantial increase in the readily biodegradable fraction of hydrolysed sewage sludge, reaching up to 90 %. Additionally, surfactant-assisted hydrolysed sewage sludge proved to be a favourable substrate for protease production using *Bacillus licheniformis*. The optimal enzymatic activity of 678 ± 14 U/mL was achieved when CTAC was added at a concentration of 10 mg/gVSS$_o$. In summary, this study evaluated an innovative approach to enhance the solubilisation of biomolecules from sewage sludge using surfactant-assisted thermal hydrolysis. This methodology not only positively affected biodegradability but also proved to be suitable for potentially producing valuable industrial products like proteases through fermentation with *B. licheniformis*, thus offering a new strategy for the sustainable treatment of sewage sludge.

1. Introduction

The management of sewage sludge, a by-product of wastewater treatment plants (WWTP), poses significant challenges due to its costly disposal [1]. Current methods such as soil fertilisation, landfilling, and incineration [2] have limitations and do not fully exploit the potential for obtaining high-added value products from this resource. Hydrothermal processes have emerged as an alternative for sewage sludge treatment, offering advantages such as improved dewaterability, enhanced digestibility for methane production, and the release of valuable biopolymers [3–6]. However, these processes generally require high energy consumption, involving temperatures and pressures of up to 200 °C and 60 bar, necessitating the exploration of alternative approaches to reduce these parameters [7–9]. To address this challenge, various chemical and oxidising promoters have been applied in the thermal hydrolysis process, including persulfate reagents [10], hydrogen peroxide [9], alkaline treatments [11], and surfactants [12].

Surfactants have emerged as a promising solution for the solubilisation of sewage sludge by interacting with the solid-liquid interface, facilitating the release of biopolymers and thus improving other treatment methods [13,14]. Previous studies have assessed the potential of anionic surfactants, including sodium dodecyl sulphate (SDS) and sodium dodecylbenzene sulfonate (SDBS), and cationic surfactants like cetyl trimethyl ammonium bromide (CTAB) and dodecyl trimethyl ammonium bromide (DTAB), to enhance the management and treatment of sewage sludge. However, the use of cetyl trimethyl ammonium chloride (CTAC) and tetraethylammonium chloride (TAC) has not been evaluated. Additionally, these studies mainly focused on dewaterability, organic release in terms of chemical oxygen demand (COD) and ammonium ($NH4^+$-N), and its use as a substrate in anaerobic digestion [15–18]. Nevertheless, the impact of surfactants on the biodegradability of the hydrolysed sewage sludge and its potential toxicity was not assessed, nor was its direct suitability as a fermentation medium for producing high-value compounds [19,20]. In this regard, the

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https://doi.org/10.1016/j.jece.2024.113353
Received 18 March 2024; Received in revised form 12 June 2024; Accepted 15 June 2024
Available online 18 June 2024

bioproduction of proteases was analysed due to their widespread application in various industries such as food, pharmaceuticals, tanneries, textiles, and detergents since they are able to hydrolyse peptide bonds in proteins. Additionally, proteases constitute 60 % of the total enzyme market, with annual sales ranging from 1.42 to 1.8 billion euros, and the detergent industry stands as the largest consumer with sales reaching 0.95 billion euros [19,21]. *Bacillus licheniformis*, a Gram-positive bacterium, was selected as the model microorganism for obtaining proteases due to their technical and economic advantages, including its versatile enzymatic repertoire and its ability to efficiently break down various substrates, thriving under diverse nutrient conditions [22,23].

Thus, Moreno et al. [24] conducted a study on protease production using *B. licheniformis* with hydrothermally-treated sewage sludge as a substrate. Their focus was on evaluating fermentation inhibitors, initial pH under oxidising and inert atmospheres, and inoculum level on protease production. However, there is still a gap in understanding how surfactants contribute to the solubilisation of biopolymers during the thermal hydrolysis of sewage sludge, especially under moderate operating conditions, which are below the commonly reported ranges of 160–200 °C and 40–60 bar [4,6]. Furthermore, the impact of these conditions on biodegradability and their potential utility as a fermentation medium for protease production requires further exploration.

Therefore, this work aimed to evaluate the use of surfactant-assisted thermal hydrolysis at moderate conditions (125 °C and 20 bar) to solubilise biopolymers from sewage sludge, including proteins, humic-like substances, carbohydrates, and deoxyribonucleic acid (DNA). These conditions were selected to ensure the sterilisation of the treated sewage sludge (> 121 °C) [25] and to prevent the formation of Maillard compounds, which are known to negatively affect the biodegradability of sewage sludge at temperatures exceeding 140 °C [26].

Four surfactants were analysed, including two anionic: SDS and SDBS, and two cationic: CTAC and TAC due to their capacity to reduce surface tension, thereby interacting with extracellular polymeric substances (EPS) and cell walls within the sewage sludge [27–29]. Respirometry assays were conducted to evaluate the biodegradability and potential toxicity of surfactant-assisted hydrolysed sewage sludge. Additionally, the bioproduction of proteases from different surfactant-assisted hydrolysed sewage sludges using *B. licheniformis* was also examined.

2. Material and methods

2.1. Sewage sludge and surfactants

For this study, a thickened sewage sludge from a WWTP in Asturias (Spain) was employed, with the following characteristics (referred in mean values): total chemical oxygen demand (TCOD): 37169 ± 438 mg O_2/L, soluble chemical oxygen demand (SCOD): 490 ± 30 mg O_2/L, soluble proteins: 21 ± 4 mg/L, soluble humic-like substances: 69 ± 6 mg/L, soluble carbohydrates: 16 ± 2 mg/L, DNA: 7 ± 1 mg/L, total suspended solids (TSS): 27.2 ± 0.1 g/L, volatile suspended solids (VSS): 19.7 ± 0.1 g/L, colour number (CN): 0.013 ± 0.001, pH: 6.83 ± 0.01. Surfactants used to assist thermal hydrolysis are detailed in Table 1.

2.2. Surfactant-assisted thermal hydrolysis experimental setup

To carry out surfactant-assisted thermal hydrolysis experiments, a PARR 4520 series reactor with a propeller stirrer was employed. This reactor, with a capacity of 1 L, was filled to 70 % to ensure safety conditions. In order to maintain inert conditions, an N_2 atmosphere with a flow rate of 2000 mL/min was used, passing through a humidifier before being introduced into the reactor. A proportional–integral–derivative (PID) controller was used to regulate the temperature of the reactor and the humidifier, as well as the gas flow. Pressure was controlled by a backpressure reducer. To ensure a homogeneous concentration of the surfactant, 100 mL of sewage sludge was centrifuged. The surfactant was then added to the liquid phase (supernatant) and mixed until completely dissolved. Subsequently, this solution was carefully mixed with the solid phase and the remaining sewage sludge (600 mL). The sewage sludge mixture was then introduced into the reactor and heated to 125 °C at 20 bar. Once these conditions were reached, the sewage sludge was left in the reactor for 4 h. This temperature was chosen to strike a balance between achieving sludge sterilisation (typically at 121 °C [25,30]), preventing the formation of Maillard compounds that can hinder biodegradability [26], reduce energy consumption, and obtaining a sufficient amount of biopolymers for subsequent fermentation and proteolytic enzyme production. Samples were collected over time to monitor the evolution of biopolymers. After collection, the samples were centrifuged at 10000 × g for 20 min, and the liquid phase was separated for further analysis. Each surfactant was used at a concentration ranging from 10 to 150 mg/gVSS_o (where VSS_o represents the VSS content of the raw sludge, measured at 19.7 ± 0.1 g/L). Additionally, thermal hydrolysis in the absence of surfactants was performed as a control.

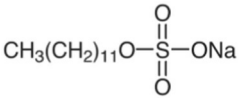
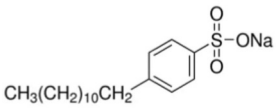
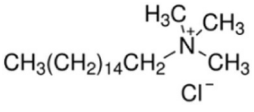
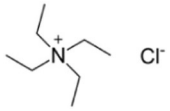
2.3. Sewage sludge analytical methods

For the measurement of soluble proteins and humic-like substances, a Lowry modified method was applied, using bovine serum albumin (BSA) and humic acid as standard compounds [31]. The phenol-sulfuric acid method was used to quantify soluble carbohydrates using D-glucose as the standard [32]. For DNA determination the diphenylamine method was employed, using DNA calf thymus as standard [33]. TCOD, SCOD, TSS, VSS and pH measurement was conducted following the Standard Methods [34]. To determine CN, spectral absorption coefficients (SAC) were measured at three different wavelengths: 436, 525, and 620 nm. Eq. 1 was then used to calculate the CN of the samples.

$$CN = \frac{SAC_{436}^2 + SAC_{525}^2 + SAC_{620}^2}{SAC_{436} + SAC_{525} + SAC_{620}} \quad (1)$$

For the determination of soluble proteins, humic-like substances, carbohydrates, DNA and CN, a Thermoscientific Genesys 150 UV–visible spectrophotometer (Thermo Fisher Scientific, USA) was used. TCOD and SCOD quantification were performed using a DR2500 spectrophotometer (Hach Company, USA). pH determination was conducted with a

Table 1
Properties of the selected surfactants to assist thermal hydrolysis.

Properties	SDS	SDBS	CTAC	TAC
Chemical structure				
CAS number	151–21–3	25155–30–0	112–02–7	56–34–8
Molecular weight (g/mol)	288.38	348.48	320.0	164.7
Type of surfactant	Anionic	Anionic	Cationic	Cationic

Basic pH meter, Sension+ PH3 (Hach Company, USA).

Biodegradability was determined using a BM-EVO analyser (SURCIS S.L., Spain), which measures the variation in readily biodegradable COD over time. Further details on the equipment setup can be found in Pola et al. [35], with the only modification being in the VSS concentration (3.5 g/L).

All measurements were performed in triplicate and the data are shown as mean values.

In order to examine if the addition of the surfactants can cause the precipitation of the biopolymers [15], a Fourier transform infrared spectroscopy (FTIR) was performed to the solid phase obtained after the surfactant-assisted thermal hydrolysis of the sewage sludge. A Varian 670-IR spectrometer (Agilent Technologies, USA) was used with an attenuated total reflectance accessory (ATR) was placed in the sample compartment. Data spectra were recorded between 4000 and 600 cm^{-1} in the mid-infrared region. For the collection of the signals a total of 32 scans were performed with a resolution of 4 cm^{-1}.

2.4. Production of proteases from surfactant-assisted hydrolysed sewage sludge

2.4.1. Microorganism

B. licheniformis (CECT20) was maintained at −20 °C (in glycerol at 40 % [v/v]). Subcultures of this strain were subsequently streaked onto nutrient broth (NB) agar plates containing 5 g/L beef extract, 10 g/L peptone, 5 g/L NaCl and agar (2 % w/v) and then preserved at 4 °C for 48 h.

2.4.2. Inoculum

The *B. licheniformis* was inoculated into a 500 mL Erlenmeyer flask containing 100 mL of NB medium (same components mentioned above, except agar) using a loopful from a fresh NB agar plate. During the incubation period, the flask was shaken at 150 rpm for 12 h at 37 °C in an orbital shaker. The growing cells were then used to inoculate shake flasks containing the surfactant-assisted hydrolysed sewage sludges for protease production.

2.4.3. Batch fermentation from surfactant-assisted hydrolysed sewage sludge

Batch fermentations were carried out in 250 mL Erlenmeyer flasks containing 50 mL of the surfactant-assisted hydrolysed sewage sludge inoculated with 10 % (v/v) biomass harvested from NB inoculum cultures by centrifugation at 12,500 rpm for 10 min. Incubation was performed at 37ºC with an orbital agitation speed of 150 rpm. Samples were performed periodically collected to determine bacterial growth and biomolecule concentration. Biomass was separated by centrifugation at 12,500 rpm for 10 min, and the cell-free supernatants were stored at −20 °C until further analysis. Duplicate fermentations were performed for these experiments.

2.4.4. Analytical methods to monitor fermentation

Bacterial growth was determined spectrophotometrically as optical density at 600 nm. For this, cultures were removed by centrifugation at 8000 rpm for min.

In order to measure the enzymatic activity of proteases, the azocasein method was employed [36]. One enzyme activity unit was defined as the amount required to increase absorbance by 0.1 at 420 nm within 1 h. Biopolymers were also measured to determinate their consumption during the fermentation process. Thus, soluble protein, humic-like substances and carbohydrates were measured according to the methodology described in Section 2.3.

2.5. Statistical analysis

Significant differences in solubilised biomolecules after thermal hydrolysis and surfactant-assisted thermal hydrolysis, as well as protease activity after batch fermentations were analysed using a one-way analysis of variance (ANOVA) at a significance level of 5 % in Excel software. If the ANOVA coefficient (F) value exceeded the critical F-value, indicating significant differences between groups ($p < 0.05$), a *post-hoc* test (Tukey's HSD) was applied to evaluate significant differences between group pairs.

3. Results and discussion

Preliminary assays were performed to evaluate the effect of the surfactants at a concentration of 100 mg/$gVSS_o$ on the solubilisation of biopolymers from sewage sludge in the absence of thermal hydrolysis (Figure S1 in the Supplementary Information). The mixtures of sewage sludge and surfactants were left to interact for 60 min. The results showed that the surfactants favoured the release of biopolymers, with the highest solubilisation of these achieved when SDS and SDBS were used, resulting in biopolymer concentrations of 4140 ± 188 mg/L (210 mg/$gVSS_o$) and 3980 ± 96 mg/L (202 mg/$gVSS_o$), respectively. Besides, these anionic surfactants were particularly effective in the solubilisation of proteins compared to humic-like substances, carbohydrates and DNA. Thus, the concentration of proteins obtained with SDS were around 3, 10.1 and 16.4 times higher than those obtained for humic-like substances, (925 ± 72 mg/L; 45 mg/$gVSS_o$), carbohydrates (272 ± 38 mg/L; 14 mg/$gVSS_o$) and DNA (169 ± 5 mg/L; 9 mg/$gVSS_o$). This can be explained considering the strong denaturing effect of SDS on proteins through a combination of hydrophobic and electrostatic interactions, thus enhancing their release compared to the other biopolymers [37,38].

Besides, the anionic surfactants were particularly effective in the solubilisation of proteins compared to the cationic ones. Thus, the concentration of proteins obtained with SDS and SDBS were around 2.7 and 3.1 times higher than those obtained when CTAC (979 ± 37 mg/L; 50 mg/$gVSS_o$) and TAC (870 ± 28 mg/L; 44 mg/$gVSS_o$) were used. This can be explained considering that the binding of SDS and SDBS with proteins were stronger compared to that of CTAC and TAC and the formation of micelle-like aggregates was not as clear as with anionic surfactants [39,40].

Regarding humic-like substances and carbohydrates, SDS and SDBS were also more effective than CTAC and TAC, but the differences were less marked. Thus, for humic-like substances, the concentrations attained with SDS and SDBS were approximately 1.2 times and 1.8 times higher than those obtained with CTAC (789 ± 42 mg/L; 40 mg/$gVSS_o$) and TAC (517 ± 38 mg/L; 26 mg/$gVSS_o$). In the case of carbohydrates, the concentrations obtained with SDS and SDBS were approximately 1.3 times higher than those obtained with CTAC (190 ± 20 mg/L; 10 mg/$gVSS_o$) and TAC (190 ± 6 mg/L; 10 mg/$gVSS_o$). These differences in the solubilisation of humic-like substances and carbohydrates may stem from structural differences within these biopolymers [15,41]. It was reported that CTAC neutralized charges on EPS, facilitating the release of enclosed biopolymers [42]. However, unbound micelles and monomers of CTAC interact with functional groups on humic-like substances and carbohydrates of opposite charge, potentially forming complexes and precipitating onto the solid phase of sewage sludge [15,43]. This phenomenon will be further discussed in subsequent sections focusing on surfactant-assisted thermal hydrolysis.

Additionally, a one-way ANOVA was conducted to assess whether there were statistically significant differences among the concentrations of solubilised biomolecules (dependent variable), with each of the surfactants used (4 levels) as a factor. Since the ANOVA indicated significant differences ($p < 0.05$), a Tukey's test was performed, obtaining statistically significant differences between all pairs of surfactants.

3.1. Surfactant-assisted thermal hydrolysis

3.1.1. Anionic surfactants (SDS and SDBS)

Figs. 1 and 2 illustrate the solubilisation of proteins (a), humic-like

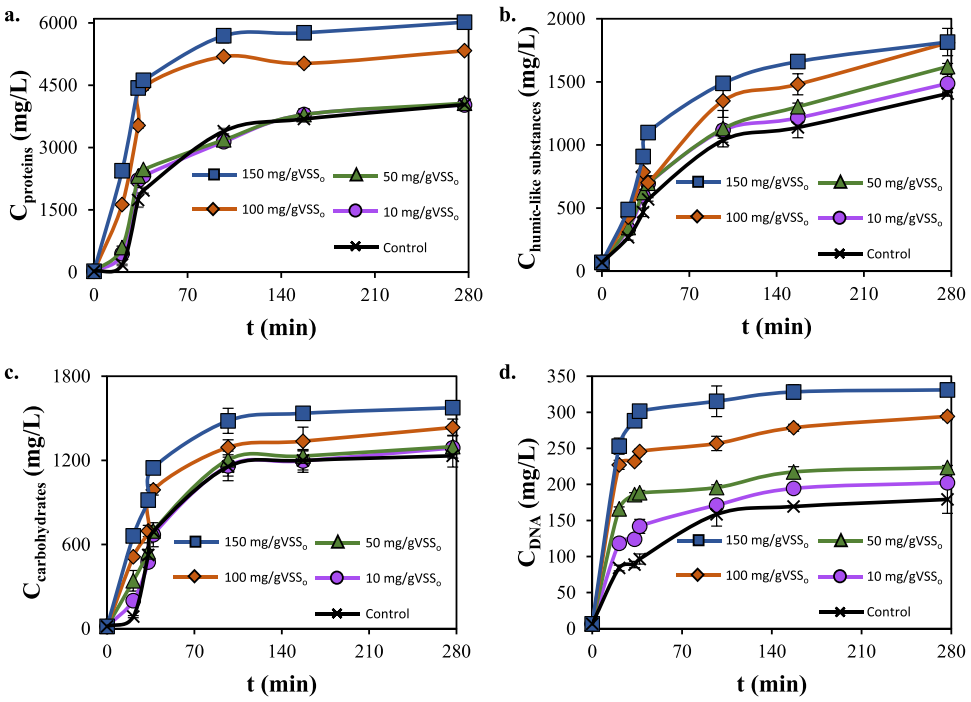


Fig. 1. Evolution of proteins (a), humic-like substances (b), carbohydrates (c) and DNA (d) using SDS-assisted thermal hydrolysis at different concentrations, and in non-assisted thermal hydrolysis (control).

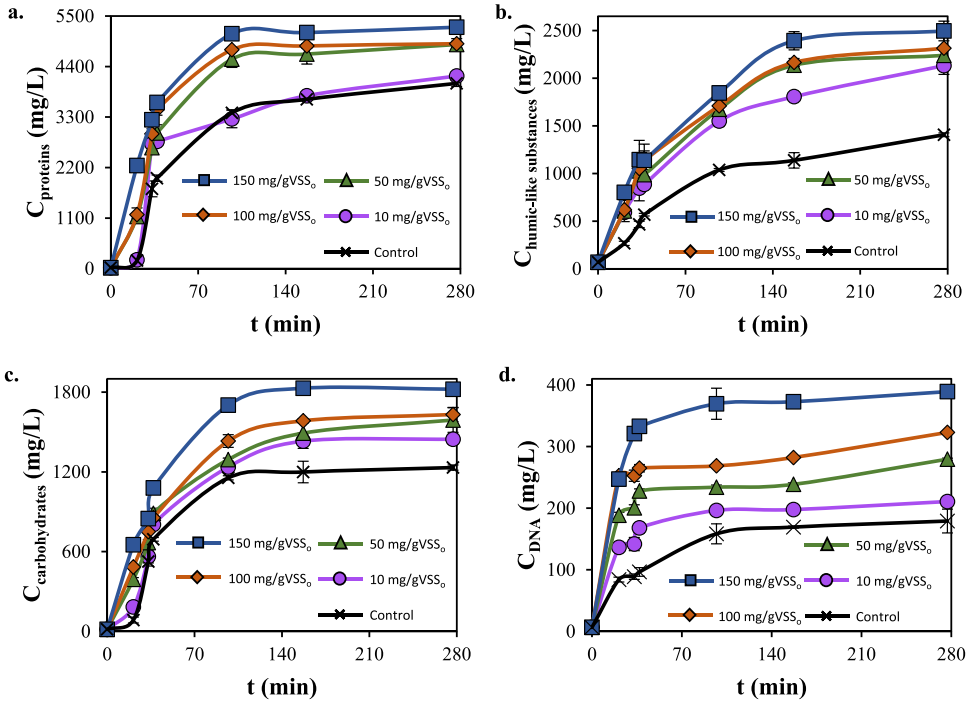


Fig. 2. Evolution of proteins (a), humic-like substances (b), carbohydrates (c) and DNA (d) using SDBS-assisted thermal hydrolysis at different concentrations, and in non-assisted thermal hydrolysis (control).

substances (b), carbohydrates (c), and DNA (d) using anionic surfactants (SDS and SDBS, respectively) as assisting agents in thermal hydrolysis of sewage sludge, at concentrations ranging from 10 to 150 mg/gVSS$_o$, together with the evolution of biopolymers in non-assisted thermal hydrolysis (control).

The solubilisation of proteins showed significantly higher levels compared to the control. SDS-assisted and SDBS-assisted thermal hydrolysis resulted in a substantial increase in protein concentration up to 95 min, with enhancements ranging from 40 % to 68 % (3293 ± 64 mg/L; 173 mg/gVSS$_o$) at SDS and SDBS concentrations of 100 and 150 mg/gVSS$_o$, respectively. This observation is consistent with the findings reported by Deo et al. [44], who suggested that SDS concentrations exceeding 4 mM (which corresponded to 59 mg/gVSS$_o$ in this study) created small hydrophobic microdomains within globular zein

proteins, thereby improving their solubility. This interaction involves the hydrophobic backbone of the proteins and the hydrophobic chain of SDS, resulting in the unfolding of insoluble proteins. Additionally, SDS disrupts hydrophobic interactions between amino acid residues in proteins, leading to denaturation and unfolding of the proteins into a linear, rod-like shape with a uniform negative charge [44,45].

However, SDS concentrations of 10 and 50 mg/gVSS$_o$ did not lead to an improvement in proteins solubilisation. In contrast, a SDBS concentration of 50 mg/gVSS$_o$ increased protein concentration by 17 %, but this was not effective at 10 mg/gVSS$_o$.

Humic-like substances exhibited consistent increases until 275 min into the experiment, with solubilisation enhancements of approximately 6 %, 15 %, 28 %, and 29 % when SDS was added at concentrations of 10, 50, 100, and 150 mg/gVSS$_o$, respectively, compared to the control (1407 ± 20 mg/L; 72 mg/gVSS$_o$). With SDBS assistance, humic-like substances reached their peak concentration (2496 ± 103 mg/L; 127 mg/gVSS$_o$) at 275 min with the addition of 150 mg/gVSS$_o$ of SDBS. This concentration was 75 % higher than the control and 37 % higher than that observed with SDS-assisted thermal hydrolysis at the same reaction time. This suggested that the presence of a phenyl ring linking the hydrocarbon chain and hydrophilic group in SDBS enhanced the hydrophobicity of the surfactant, effectively weakening the binding between EPS and cells compared to SDS. This led to improved solubilisation of humic-like substances, which were predominantly founded in EPS [18,46].

Carbohydrates exhibited a similar trend to soluble proteins, showing increases of up to 12 % and 28 % across SDS concentrations ranging from 10 to 150 mg/gVSS$_o$, compared to the control concentration which was 1155 ± 68 mg/L (59 mg/gVSS$_o$) at 95 min into the assay. For SDBS, the carbohydrates concentration reached a maximum value of 1829 ± 44 mg/L (93 mg/gVSS$_o$) with the addition of 150 mg/gVSS$_o$ of SDBS at 150 min. This value represented a 53 % increase over the control and was 19 % higher than that observed with SDS-assisted thermal hydrolysis at the same time.

For SDS-assisted and SDBS-assisted thermal hydrolysis, DNA concentration initially rose rapidly within the first 37 min of treatment and then remained stable. The concentrations obtained were from 1.7 to 3.5 times higher than that of the control (97 ± 7 mg/L; 5 mg/gVSS$_o$) for SDS and SDBS additions between 10 and 150 mg/gVSS$_o$, respectively.

On viewing the results, a synergistic effect between anionic surfactants and thermal hydrolysis in the solubilisation of sewage sludge biopolymers can be suggested. While SDS facilitated the solubilisation of all biomolecules, particularly proteins, SDBS demonstrated a more consistent enhancement for all biopolymers present in sewage sludge.

In this context, the solubilisation of sewage sludge can be divided into two steps: firstly, anionic surfactants weakened the binding of EPS to sewage sludge flocs, and subsequently, thermal hydrolysis promoted cell lysis, releasing intracellular materials such as proteins, carbohydrates, and primarily DNA [8].

This synergistic effect was further supported by the decrease in VSS and pH, and the increase in CN (Figures S2 to S4 in the Supplementary Information). Thus, the CN value was approximately 2.7 times higher than that of the control with a SDBS concentration of 150 mg/gVSS$_o$ at 95 min (Figure S3b in the Supplementary Information). This increase correlated with the higher concentration of humic-like substances observed, characterized by a dark brown colour due to the presence of moderate molecular weight carboxylic and phenol groups [46].

In terms of biopolymer production, anionic surfactant-assisted thermal hydrolysis of sewage sludge proved to be suitable when compared with other studies using hydrothermal techniques. Thus, the maximum values obtained in this study, corresponding to 6017 ± 28, 2495 ± 107, 1821 ± 4, and 389 ± 4 mg/L (306, 127, 93, and 20 mg/gVSS$_o$) for proteins, humic-like substances, carbohydrates, and DNA, respectively, were from 1.1 to 4 times higher than those obtained by Urrea et al. [8] when using wet oxidation at 190ºC and 65 bar. These values were similar to those obtained by García et al. [4], albeit under higher thermal hydrolysis conditions (160ºC and 40 bar). Therefore, the combination of moderate conditions (125°C and 20 bar) with surfactants promoted the disruption of EPS and cells, leading to the release of biopolymers and potentially resulting in energy savings.

Additionally, a one-way ANOVA was performed with anionic surfactants (SDS and SDBS) concentration considered as a factor (with 5 levels) and the solubilisation of biopolymers after 4 h of treatment as the dependent variable. The analysis revealed significant differences ($p < 0.05$). Subsequently, *post hoc* analysis using Tukey's test confirmed differences between pairs of concentrations groups across SDS (Table S1 in the Supplementary Information) and SDBS (Table S2 in the Supplementary Information) treatments. For proteins, significant differences ($p < 0.05$) were found in all SDS pair groups except for the pairs of 10–50 mg/gVSS$_o$, and the control. For humic-like substances and DNA, only the pairs of 100–150 mg/gVSS$_o$, and 10–50 mg/gVSS$_o$, and the control did not exhibit significant differences ($p < 0.05$) between them. Carbohydrates pairs showed significant differences, except for the pairs 100–150 mg/gVSS$_o$, and 10–50–100 mg/gVSS$_o$, and the control.

With SDBS-assisted treatment, proteins concentration levels were grouped differently, except for the pairs 50–100 mg/gVSS$_o$, as well as 10 mg/gVSS$_o$ and the control, that showed no significant differences between them. For humic-like substances statistically significant differences ($p < 0.05$) were observed between all pairs, except for 50–100 mg/gVSS$_o$ and 10–50 mg/gVSS$_o$. In the case of carbohydrates, only 50–100 mg/gVSS$_o$ pair did not show significant differences. For DNA, only the pair of 10 mg/gVSS$_o$ and the control did not show significant differences.

Thus, this analysis proved the effect of anionic surfactants on the solubilisation of biopolymers in the thermal hydrolysis treatment of sewage sludge.

3.1.2. *Cationic surfactants (CTAC and TAC)*

Figs. 3 and 4 show the evolution of biomolecules: proteins (a), humic-like substances (b), carbohydrates (c), and DNA (d) during cationic surfactant-assisted thermal hydrolysis using CTAC and TAC at concentrations ranging from 10 to 150 mg/gVSS$_o$ alongside the control experiment (without surfactant).

Proteins and DNA exhibited higher concentrations in the presence of CTAC compared to the control. Specifically, proteins and DNA solubilisation increased by 22 % and 19 %, respectively, with a CTAC concentration of 10 mg/gVSS$_o$ over 275 min. However, higher CTAC concentrations (100 and 150 mg/gVSS$_o$) had a detrimental effect on proteins solubilisation, decreasing by 7 % and 21 %, respectively, over the same period. Similarly, DNA solubilisation decreased by 27 % and 39 % at these higher concentrations.

The observed behaviour of proteins at low concentrations of CTAC suggested that the hydrophobic tail of this surfactant interacted with the hydrophobic side chains of the proteins, disrupting hydrogen-bond interactions and forming CTAC-protein micelles, which led to protein denaturation and subsequent solubilisation [15]. However, at higher concentrations of CTAC, these mixed micelles may adsorb or precipitate onto the surface of the surfactant-assisted hydrolysed sewage sludge, as further evidenced by the FTIR results (Section 3.2).

In contrast, carbohydrates showed greater levels of solubilisation compared to the control at lower CTAC concentrations, with a 44 % improvement with a CTAC addition of 10 mg/gVSS$_o$ for 275 min. However, as the CTAC concentration increased, the improvement in solubilisation gradually decreased, with only an 8 % improvement achieved at a CTAC concentration of 150 mg/gVSS$_o$. This decrease in carbohydrate solubilisation may result from the formation of potential complexes between these compounds and CTAC. This phenomenon is likely due to the combined effects of electrostatic and hydrophobic interactions, consistent with findings from other studies involving ionic surfactants. These studies indicated the ability of such surfactants to interact with polysaccharides of opposite charges [47].

Additionally, there were no significant variations in the

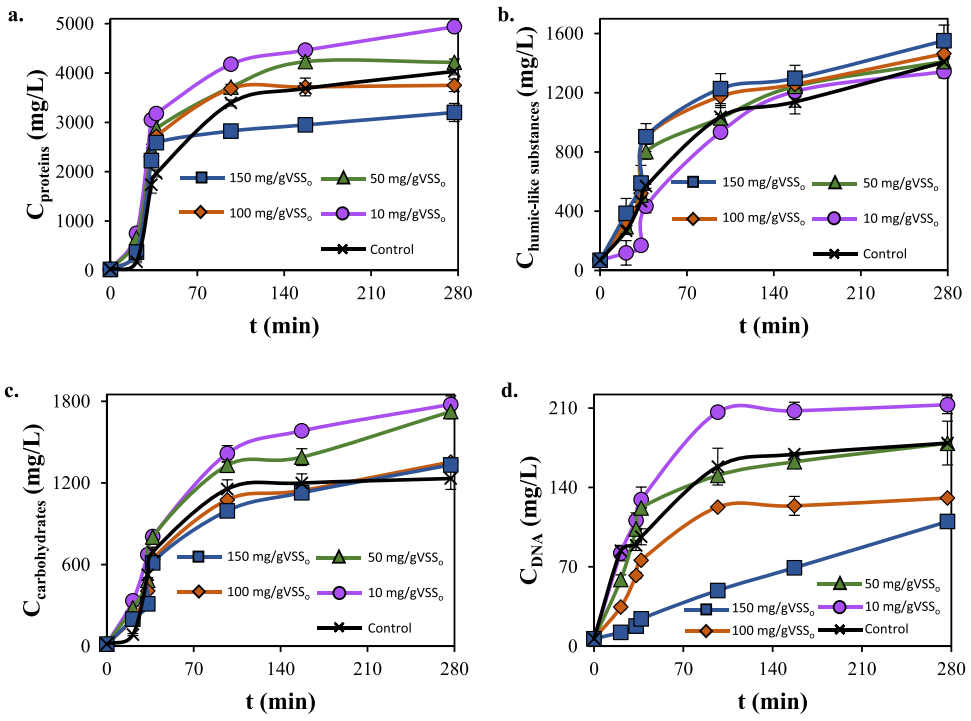


Fig. 3. Evolution of proteins (a), humic-like substances (b), carbohydrates (c) and DNA (d) using CTAC-assisted thermal hydrolysis at different concentrations, and in non-assisted thermal hydrolysis (control).

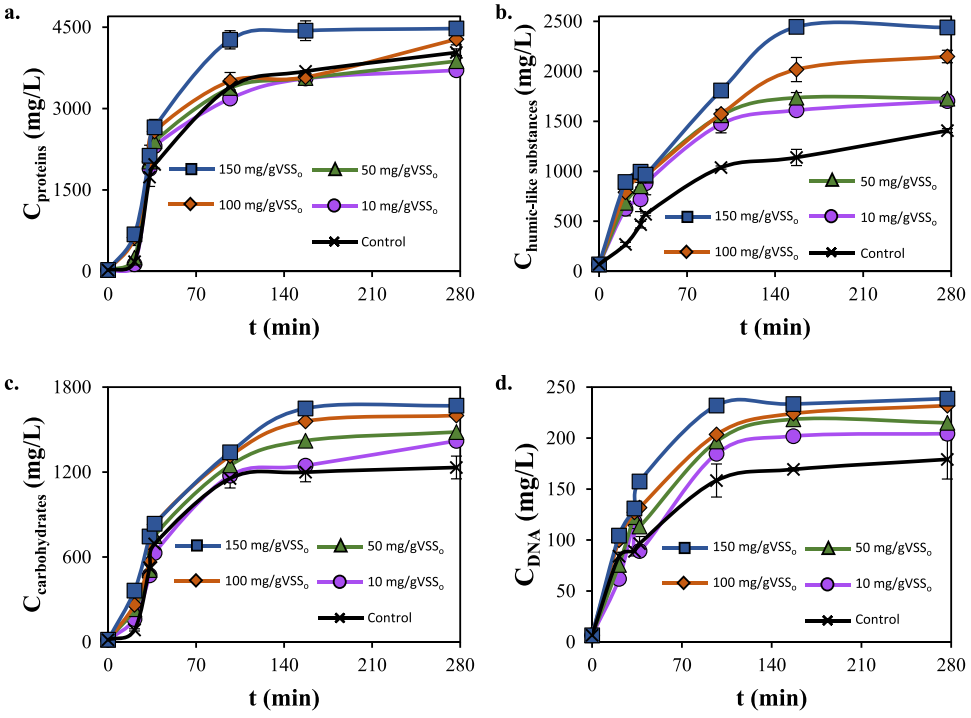


Fig. 4. Evolution of proteins (a), humic-like substances (b), carbohydrates (c) and DNA (d) using TAC-assisted thermal hydrolysis at different concentrations, and in non-assisted thermal hydrolysis (control).

solubilisation of humic-like substances compared to the control when CTAC was used as an assisting surfactant for thermal hydrolysis. This can be attributed to the complex nature of these compounds. Humic-like substances are amphiphilic, containing both hydrophilic (e.g., phenolic and carboxyl groups) and hydrophobic (e.g., carbonyl group) groups. When CTAC is added, the ionisation of the phenolic and carboxyl groups resulted in electronegative characteristics, allowing them to be adsorbed onto the electropositive CTAC micelles through electrostatic interactions. Furthermore, this dual nature may lead to the formation of aggregates between humic-like substances and CTAC micelles, consequently reducing their solubility [48]. These findings are consistent with the results obtained in the FTIR analysis.

For TAC-assisted thermal hydrolysis, higher concentrations enhanced the solubilisation of biopolymers, particularly humic-like substances and carbohydrates, which exhibited improvements of 71 % and 35 %, respectively, when using a dosage of 150 mg/VSS_o of TAC. The impact was less pronounced for proteins and DNA, with enhancements of approximately 11 % and 33 %, respectively. These results suggested that this surfactant facilitated the neutralisation of negative charges within the sewage sludge matrix, potentially leading to the liberation of EPS, primarily composed of humic-like substances and carbohydrates [8]. Therefore, increased addition of TAC may induce greater destabilisation of the bonds between EPS and cells, consequently resulting in the release of intracellular components under the influence of thermal energy during thermal hydrolysis.

The marked difference in the solubilisation of sewage sludge between TAC and CTAC can be attributed to the presence of the hydrocarbon chain in the latter [16].

The results of VSS and CN (Figure S2 and S3 in the Supplementary Information) indicated that low CTAC concentrations assisted in the solubilisation of sewage sludge by neutralising its negative surface charge. However, at higher concentrations of CTAC, a lesser reduction in VSS and even an increase compared to the control were observed. This suggested the complexation and precipitation of biomolecules [12,15,49].

In contrast, for TAC-assisted thermal hydrolysis, the decrease in VSS and the increase in CN with increasing surfactant concentration confirmed the charge neutralisation of EPS as discussed earlier.

Furthermore, a one-way ANOVA was conducted with the concentrations of the cationic surfactants (CTAC and TAC) as a factor (each with 5 levels), and the concentrations of biopolymers after 4 h of treatment as dependent variable. This analysis revealed statistically significant differences ($p < 0.05$), except for DNA concentration in CTAC treatment, where no significant differences were observed. Subsequent Tukey's test confirmed these findings, with designated letters ($a > b > c > d$) indicating distinct pairs of concentration groups in the CTAC and TAC treatments.

The results indicated variability in protein concentration with CTAC treatment (Table S3 in the Supplementary Information). Most pairs showed significant differences, except for 50 mg/$gVSS_o$ and the control. For humic-like substances, the pairs 50–100–150 mg/$gVSS_o$, and the control were grouped together, indicating no significant differences among them. However, 10–50–100 mg/$gVSS_o$, and the control pairs formed a separate group. Similarly for carbohydrates, the pairs at 10–50 mg/$gVSS_o$ were in distinct groups, while those at 100–150 mg/$gVSS_o$, and the control were grouped together.

TAC (Table S4 in the Supplementary Information) treatment also revealed distinct protein concentration groups between the pairs: 10–150 mg/$gVSS_o$, 50–100 mg/$gVSS_o$, 50–150 mg/$gVSS_o$, control-50 mg/$gVSS_o$ and control-150 mg/$gVSS_o$. For humic-like substances, each concentration level showed significant differences except for the 10–50 mg/$gVSS_o$ pair. Carbohydrates and DNA showed significant differences between control-50 mg/$gVSS_o$, control-100 mg/$gVSS_o$ and control-150 mg/$gVSS_o$ pairs.

This analysis confirmed the influence of CTAC and TAC on biomolecule solubilisation of hydrolysed sewage sludge.

3.2. FTIR analysis

The FTIR spectra of each surfactant and the solid phases after thermal hydrolysis and surfactant-assisted thermal hydrolysis using a concentration of 150 mg/VSS_o for each surfactant are provided in Figure S5 of the Supplementary Information. Firstly, analysing the FTIR spectrum of the solid phase after thermal hydrolysis, distinct characteristic bands can be distinguished. The broad band observed within the range of 3000–3600 cm^{-1} corresponds to the stretching of O-H bonds of water molecules. Additionally, the band at 2922 cm^{-1} represents the asymmetric stretching of C-H bonds in methyl groups, while the band at 2852 cm^{-1} is likely attributed to the symmetric stretching of C-H bonds in methylene groups, commonly associated with lipids or fatty acids [50]. The presence of -C═O bonds in amide I is reflected by bands located around 1630 cm^{-1}, and bands around 1530 cm^{-1} are attributed to the C-N stretching in amide II, which is characteristic proteins [50]. The bands at around 1430 and 1230 cm^{-1} are assigned to the symmetric stretching of carboxylate groups of humic-like substances, and the C-O stretching in carboxylic acids, respectively. Additionally, the spectral peak observed in the wavenumber range of 1170–1000 cm^{-1} indicates the presence of C−C, C−O−C, and O−H bonds, typically associated with the presence of polysaccharides or carbohydrates [47,50–52].

The FTIR spectra of each surfactant (SDS, SDBS, CTAC, and TAC) can also be found in Figure S5 of the Supplementary Information. First, focusing on anionic surfactants, the FTIR spectrum of SDS exhibits distinct characteristic peak assignments. The peak at 3466 cm^{-1} corresponds to the stretching of H-OH bonds, while the peaks at 2916 cm^{-1} and 2849 cm^{-1}, correspond to the stretching of CH_2 groups. The peak at 1467 cm^{-1} is due to the bending of CH_2 groups, which are indicative of a polar environment [53,54]. Additionally, the peak at 1216 cm^{-1} corresponds to the skeletal vibration involving the S-O bond of the bridge, the peak at 1079 cm^{-1} corresponds to the stretching of C-C bonds, and the peak at 824 cm^{-1} corresponds to the asymmetric bending of CH_2 group and C-H bonds [53,54].

Regarding to the FTIR spectrum of SDBS, several characteristic bands are observable. The broad band spanning the range of 3000–3600 cm^{-1} corresponds to the stretching vibrations of O-H bonds involved in hydrogen bonding, indicative of the presence of hydrophilic groups in SDBS that contribute to its surfactant properties. The band at 2923 cm^{-1} is attributed to the asymmetric stretching of C-H bonds in the SDBS alkyl chain, specifically indicating the presence of the dodecyl group within the surfactant molecule [54]. Additionally, the band at 2853 cm^{-1} corresponds to the symmetric stretching of C-H bonds in the methylene groups of the alkyl chain [55]. The presence of the benzene ring in SDBS is evident in the bands ranging from 1600 to 1500 cm^{-1}, associated with the stretching vibrations of C═C bonds in the aromatic structure [56]. Furthermore, the band around 1041 cm^{-1} indicates the bending vibrations of C-H bonds in the aromatic ring [55]. Moving on to FTIR spectra of CTAC, a broad band within the spectral range of 3000–3600 cm^{-1}, can be observed, corresponding to the stretching vibrations of the O-H bonds engaged in hydrogen bonding [54]. This implies the presence of hydrophilic groups within CTAC, thereby contributing to its surfactant properties. Furthermore, pronounced absorptions at 2913 and 2847 cm^{-1} are attributable to the C−H stretching vibrations of the methyl and methylene groups of CTAC [57]. The bands located at 1631 and 1469 cm^{-1} represent the asymmetric and symmetric stretching vibrations of N^+-CH3, respectively [51,58]. Additionally, the band at 961 cm^{-1} corresponds to the out-of-plane -CH3 vibration [54,58]. Lastly, for TAC, the C-H and C-N vibrations were identified at 1392–1490 cm^{-1} and 1001 cm^{-1}, respectively. Additionally, the wide band observed around 3373 cm^{-1} can be attributed to the O-H stretching of water molecules, as TAC exhibits a highly hydrophilic and hygroscopic nature [59].

In the FTIR spectra of the solid phase after the SDS-assisted thermal hydrolysis, the peaks at 2922, 2852, and 1006 cm^{-1} showed a slight increase in intensity. A similar increase was also observed in the spectral region from 1216 to 824 cm^{-1} bands. However, no significant peak shifts or new peaks were identified. Therefore, the presence of SDS in the solid phase seems improbable. Likewise, in the FTIR spectrum of the solid phase after the SDBS-assisted thermal hydrolysis, only the peaks at 2922 and 2852 cm^{-1} exhibited slight change in intensity No significant peak shifts or new peaks were evident. Thus, the presence of SDBS in the solid phase also appears unlikely. Conversely, when CTAC was used as a surfactant, the peaks at 2922 and 2852 cm^{-1} shifted slightly to lower wavenumbers, and a significant increase in the intensity of these peaks was observed, indicating a higher abundance of hydrocarbon chains. This increase may be attributed to the presence of this surfactant in the

solid phase. The band at 1439 cm^{-1}, attributed to the symmetric stretching of carboxylate groups characteristic of humic-like substances, shifted to a higher wavenumber (1451 cm^{-1}). Furthermore, the peak at 1630 cm^{-1}, corresponding to -C═O bonds in amide I due to proteins, also slightly shifted to a lower wavenumber. Additionally, a notable increase in the intensity of the peaks at 1530 cm^{-1}, attributed to the C-N stretching in amide II of proteins, and at 1008 cm^{-1} assigned to the presence of C–C, C–O–C, and O–H bonds due to the presence of polysaccharides or carbohydrates, was observed [15,54,57,58]. This implies the complexation and precipitation of proteins, carbohydrates and humic-like substances. This behaviour aligns with the decrease observed in biomolecules in the liquid phase of the hydrolysed sewage sludge. In this context, Li et al. [15] conducted a study investigating how CTAB improved sludge dewaterability, demonstrating complexation between biomolecules and CTAB, a surfactant similar to CTAC. Finally, in the FTIR spectrum of the solid phase of the TAC-assisted thermal hydrolysis, no notable shifts or variations in the position and intensity of the peaks were observed, thus suggesting that TAC is unlikely to be present in the solid phase.

3.3. *Biodegradability of hydrolysed sewage sludge and surfactant-assisted hydrolysed sewage sludge*

A positive effect of the surfactants on the solubilisation of sewage sludge using thermal hydrolysis was observed. However, limited information is available on the impact of these surfactants in the biodegradability of the resulting hydrolysates. To gain insight into this impact, the composition of the soluble organic fraction of the hydrolysates was analysed at the end of treatment, as shown in Fig. 5a, c, e and g. This fraction, quantified as SCOD, can be subdivided into non-

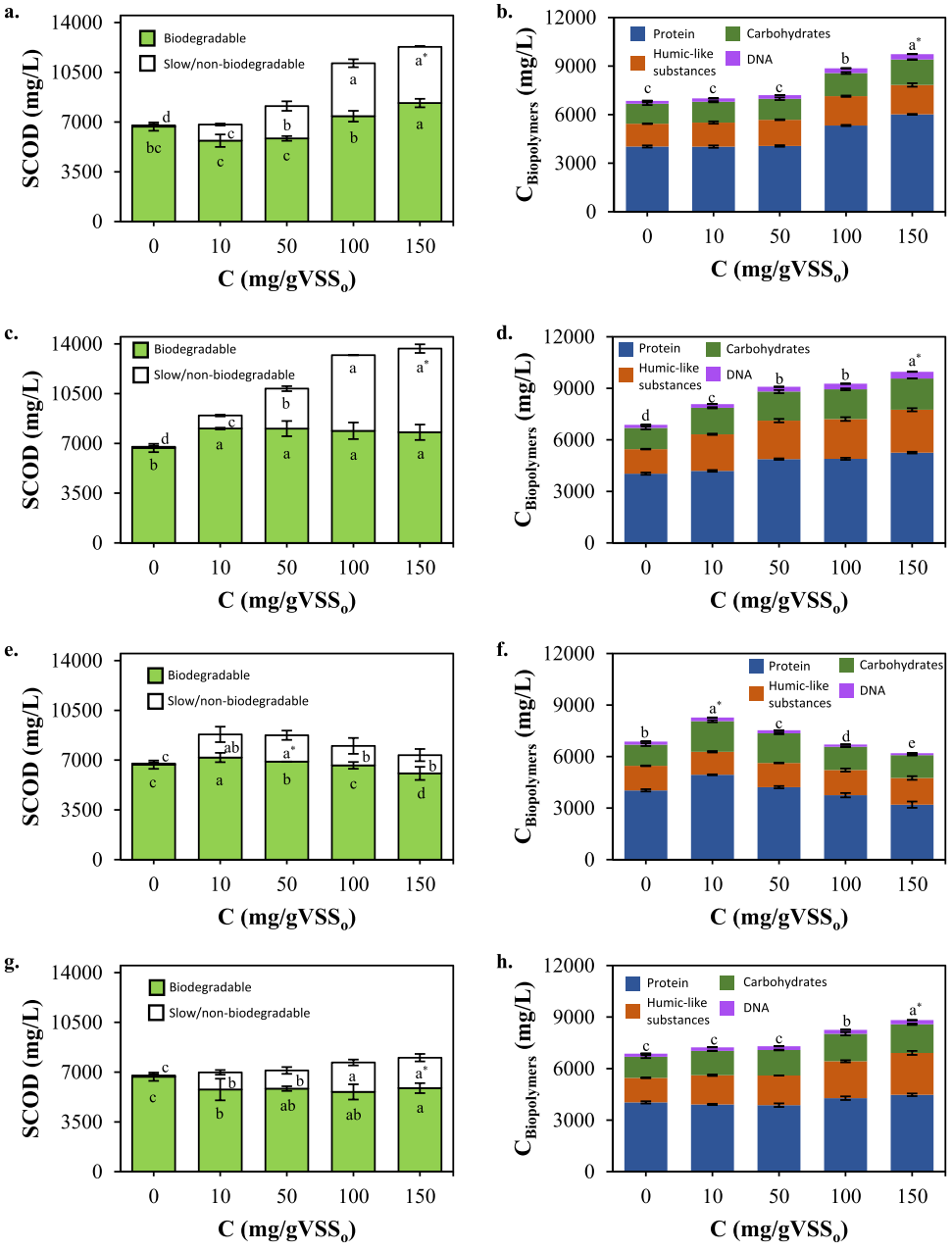


Fig. 5. Biodegradable and slow/non-biodegradable fractions after non-surfactant assisted thermal hydrolysis and thermal hydrolysis assisted by SDS (a), SDBS (c), CTAC(e) and TAC (g). Total concentration of solubilised biopolymers: proteins, humic-like substances, carbohydrates and DNA after non-surfactant assisted thermal hydrolysis and thermal hydrolysis assisted by SDS (b), SDBS (d), CTAC (f) and TAC (h). * Distinct letters indicate significant differences ($p < 0.05$).

biodegradable, slowly biodegradable, and readily biodegradable COD. The non-biodegradable fraction represents the portion of the soluble organic fraction that cannot be degraded by heterotrophic microorganisms. The slowly biodegradable fraction encompasses larger molecules such as proteins or complex carbohydrates that are released by raw sludge flocs and require hydrolysis before they can be biodegraded by the biomass. On the other hand, the readily biodegradable fraction consists of compounds that are directly available for bioassimilation by microorganisms, including volatile fatty acids, simple sugars, amino acids, and smaller proteins and carbohydrates, all resulting from the hydrolysis of larger biomolecules [35,60–62]. For comparison purposes, the concentration of SCOD and the total concentration of the biopolymers after thermal hydrolysis treatment were also depicted in Fig. 5b, d, f and h.

The results indicated that the non-surfactant assisted hydrolysed sewage sludge contained a significantly high proportion of readily biodegradable fraction, constituting approximately 99 % of the SCOD, while the slowly/non-biodegradable fraction was only around 1 %. This suggested that the longer reaction times (275 min) facilitated the hydrolysis and breakdown of larger molecules into smaller sizes suitable for bioassimilation by microorganisms [4]. In this sense, Romero et al. [30] observed that thermal hydrolysis of sewage sludge at 120 °C led to a 44 % reduction in molecules with a molecular size ranging from 15 to 150 kDa when the reaction time increased from 1 h to 4 h. Conversely, molecules <15 kDa increased by 41 % under the same temperature and reaction time due to the breakdown of larger molecules.

The use of lower temperatures and pressures (125ºC and 20 bar) resulted in a slightly higher value of readily biodegradable COD than that obtained by Pola et al. [35] when hydrothermal treatment of sewage sludge was conducted at 180 °C and 80 bar, yielding approximately 92 %.

In terms of the impact of surfactants on biodegradability, the addition of anionic surfactants, such as SDS and SDBS, led to a noticeable increase in the slowly/non-biodegradable fractions with increasing surfactant concentration. Notably, at a concentration of 150 mg/gVSS$_o$, the slow/non-biodegradable fraction reached 32 % and 43 % of the SCOD for SDS and SDBS, respectively. These results indicated that the introduction of anionic surfactants had a detrimental effect on the biodegradability of hydrolysed sewage sludge, which was particularly pronounced in the case of SDBS due to the presence of both the sulphonate group and benzene ring. It has been reported that the fatty alcohol sulphates (AS), including SDS, can be readily degradable under aerobic conditions when *Pseudomonas* and *Bacillus* are the dominant types of bacteria in a consortium [63]. The biodegradation of AS involves the enzymatic cleavage of the sulphate ester bonds to produce inorganic sulphate and a fatty alcohol [64]. Nevertheless, in the case of linear alkyl benzene sulphonates (LAS), such as SDBS, their removal in WWTPs is incomplete and they persist under anaerobic conditions [64]. The pathway of breakdown of LAS implies the degradation of the linear alkyl chain first, followed by the sulfonate group, and ultimately the benzene ring, requiring molecular oxygen, thus explaining why it is unlikely to be degraded anaerobically [65–67]. Consequently, the presence of the aromatic ring resulted in a slower degradation rate and a higher proportion of the slowly/non-biodegradable fraction in the SDBS-assisted hydrolysed sewage sludge. Additionally, the increase in the concentration of humic-like substances could also contribute to the increase in the slow/non-biodegradable fraction due to their low biodegradability [46].

The addition of cationic surfactants had a lesser effect on the biodegradability of the hydrolysed sewage sludge compared to the anionic ones. Specifically, in the case of CTAC surfactant, an increase in its concentration did not lead to a higher proportion of the slowly/non-biodegradable fraction, which consistently accounted for approximately 19 % of the SCOD regardless of the concentration used (Fig. 5e). This can be explained by considering the complexation and precipitation of biomolecules caused by CTAC, as indicated in section 3.3.1, resulting in the presence of the surfactant in the solid phase of the hydrolysed sewage sludge. This phenomenon also contributed to a decrease in SCOD and the total concentration of biomolecules (Fig. 5f).

Considering TAC (Fig. 5g), when 10 mg/gVSS$_o$ was used, 83 % of the SCOD corresponded to the readily biodegradable fraction, and only a 10 % reduction was observed when 150 mg/gVSS$_o$ was used. This slight impact can be attributed to the partially biodegradable nature of TAC, which was reported to have a biodegradation rate of 75 % [68]. Additionally, the slowly/non-biodegradable fraction may also be influenced by the rise in complex compounds, particularly humic-like substances, as commented earlier, which exhibit low biodegradability. In the case of quaternary ammonium salts, such as CTAC and TAC, the pathway for the biodegradation implies the cleavage of the C_{alkyl}-N bonds (N-dealkylation and N-demethylation) [64,69].

When comparing the influence of CTAC and TAC to SDS and SBD on the biodegradability at low concentrations (10 mg/gVSS$_o$) no markedly changes were found in all cases, with values around 15 % for the slow/non-biodegradable fraction of the SCOD.

Additionally a one-way ANOVA was conducted with the surfactant concentrations: 0 (as the control), 10, 50, 100, and 150 mg/gVSS$_o$ as the factor (5 levels), with biodegradable and slow/non-biodegradable fractions as dependent variables. Thus, significant differences ($p < 0.05$) were observed in both fractions. Subsequently, Tukey's HSD *post hoc* test was performed to evaluate differences between pair of surfactant concentrations (Fig. 5a, c, e, and g).

Regarding SDS, significant differences were observed in the biodegradable fraction, except for specific pairs (0–10, 0–50, 0–100, and 10–50 mg/gVSS$_o$). For the slow/non-biodegradable fraction, all group pairs showed significant differences except for 100–150 mg/gVSS$_o$. In the case of SDBS, a significant difference was determined in the biodegradable fraction for the 0 mg/gVSS$_o$ pair compared to other samples. For the slow/non-biodegradable fraction, significant differences were present in all group pairs except for 100–150 mg/gVSS$_o$. Considering CTAC, significant differences were observed in the biodegradable fraction among all pairs, except for 0–100 mg/gVSS$_o$. For the slow/non-biodegradable fraction, certain pairs: 10–50, 10–100 and 100–150 mg/gVSS$_o$ did not show significant differences between them. For TAC, in the biodegradable fraction, the pairs 0 and 10, 50, 100 and 150 mg/gVSS$_o$ and 10–150 mg/gVSS$_o$ showed significant differences. For the slow/non-biodegradable fraction, only the pairs 10–50 and 100–150 mg/gVSS$_o$ did not showed significant differences.

The same analysis was conducted to assess the impact of surfactant concentrations (factor with 5 levels) on the total biomolecule concentration (dependent variable), revealing significant differences ($p < 0.05$). Besides, Tukey's HSD *post hoc* test demonstrated distinct groups denoted by different letters ($a > b > c > d > e$) in Fig. 5b, d, f, and h.

Regarding SDS, significant differences were observed across most pairs, except for 0–10, 0–50 and 10–50 mg/gVSS$_o$. For SDBS, only the pair 50–100 mg/gVSS$_o$ did not show significant differences. Considering CTAC, significant differences were found for all pairs.

In the case of TAC, the pairs of 0–10, 0–50 and 10–50 mg/gVSS$_o$ showed similarities but were different from other pairs.

This analysis highlighted how surfactant concentrations influenced the biodegradable and slow/non-biodegradable fractions, as well as the total biomolecule concentrations, illustrating distinct patterns across different surfactant types and concentrations.

3.4. Production of proteases byB. licheniformis from hydrolysed sewage sludge

The potential application of hydrolysed sewage sludge as substrate for the production of proteolytic enzymes using *B. licheniformis* was evaluated. In the case of surfactant-assisted hydrolysed sewage sludge, concentrations of 50 mg/gVSS$_o$ for SDS and SDBS, and 10 mg/gVSS$_o$ for CTAC and TAC were selected in order to obtain a balance between the quantity of biopolymers and the readily biodegradable fraction since high values of slowly/non-biodegradable fractions can potentially

impede microbial growth [70,71].

Fig. 6 illustrates the production of proteases by *B. licheniformis* and biomolecule consumption during the fermentation process using hydrolysed sewage sludge and surfactant-assisted hydrolysed sewage sludge as substrates. Additionally, microbial growth during this process can be found in Figure S6 of the Supplementary Information.

As previously indicated (Section 3.1), the hydrolysed sewage sludge contained significant quantities of dissolved biopolymers, specifically proteins (carbon and nitrogen source) and carbohydrates (carbon source). These biopolymers can be assimilated by *B. licheniformis* to produce extracellular proteases [72]. In all cases, maximum growth occurred at 24 h of fermentation with the highest value observed for the SDBS-assisted hydrolysed sewage sludge. Additionally, the highest consumption of biomolecules at 24 h (61 ± 4 %) was achieved with this substrate, compared to values of 46 ± 3 %, 50 ± 3 %, 42 ± 2 %, and 23 ± 4 %, for SDS-assisted, CTAC-assisted, TAC-assisted hydrolysed sewage sludge and in absence of surfactant, respectively. Thus, this 61 % decrease was associated with an assimilation of 674 ± 45 mg/L of carbohydrates, 3537 ± 70 mg/L of proteins and 566 ± 36 mg/L g of humic-like substances, corresponding to 54.7 %, 68.2 % and 40.2 % of their initial values, respectively. It was found that, in general, *B. licheniformis* first assimilated carbohydrates and proteins for both microbial growth and protease production, and the consumption of humic-like substances began between 12 h and 24 h. This suggested an easier metabolism of carbohydrates and proteins compared to humic-like substances, which aligned with what other authors have reported regarding the suitable use of *B. licheniformis* in the biodegradation of waste proteins and wastewaters of food industry [73].

The production of enzymes increased significantly up to 24 h, with a more gradual increase observed from that point until 72 h. The addition of surfactants, particularly SDBS and CTAC, led to a notable increase in enzymatic activity with values around 1.5 and 1.6 times higher compared to the non-surfactant assisted hydrolysed sewage sludge (450 ± 7 U/mL) at 72 h. It should be noted that the content of biomolecules was higher in SDBS (50 mg/gVSS$_o$) and CTAC (10 mg/gVSS$_o$) with total concentrations of 9089 ± 235 mg/L and of 8280 ± 141 mg/L, compared to SDS (50 mg/gVSS$_o$), TAC (10 mg/gVSS$_o$), and in the absence of surfactant, where the values were 7256 ± 157 mg/L, 7249 ± 90 mg/L, and 6982 ± 85 mg/L, respectively. Specifically, the concentrations of compounds more easily assimilated: carbohydrates and proteins in SDBS-assisted and CTAC-assisted hydrolysed sewage sludge was 28 % and 23 % higher, respectively, than those obtained with SDS and TAC.

The comparison of enzymatic activity between different works is complicated due to variations in fermentation conditions, including temperature, pH, inoculum, culture media and microorganisms, among others. However, it is noteworthy that higher values were obtained with the SDBS-assisted (700 ± 7 U/mL) and CTAC-assisted (678 ± 14 U/mL) hydrolysed sewage sludge, either exceeding or closely similar to the

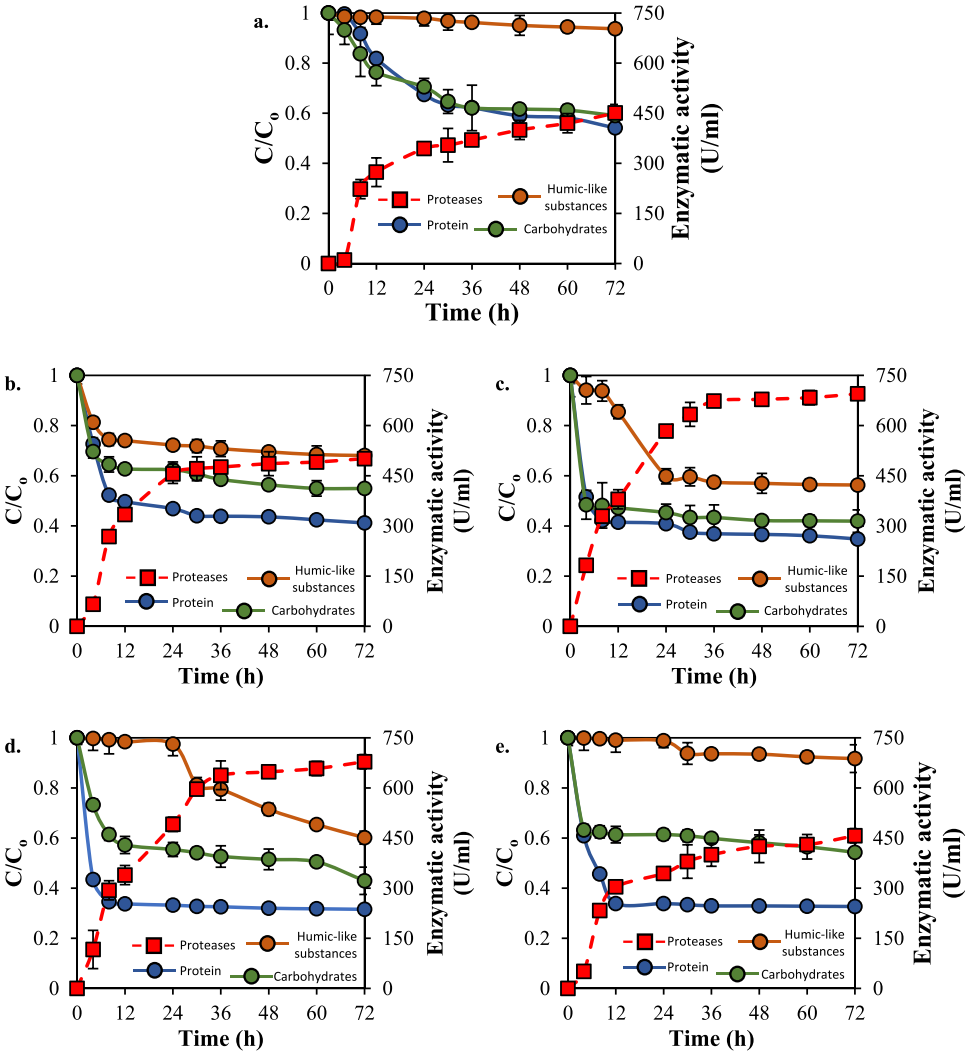


Fig. 6. Production of proteases by *B. licheniformis* and consumption of biomolecules: proteins, humic-like substances, and carbohydrates in non-surfactant assisted hydrolysed sewage sludge (a) and hydrolysed sewage sludge assisted by SDS (b), SDBS (c), CTAC (d), and TAC (e).

values reported in other studies using various substrates. For instance, N. Reddy et al. [19] achieved protease enzyme activity of approximately 11 U/mL by employing de-oiled neem seed cake as the substrate and *B. licheniformis* as the selected microorganism. Additionally, N. Annamalai et al. [74] obtained an enzymatic activity of approximately 473.4 U/mg by utilising marine debris as the substrate and *Bacillus firmus* CAS 7 as the bacterial strain for protease production. Furthermore, Moreno et al. [24] achieved a protease enzymatic activity of 903 U/mL, when sewage sludge treated in an oxidising atmosphere (wet oxidation) at 140°C was fermented with 30 % (v/v) inoculum of *B. licheniformis*.

Therefore, the use of SDBS and CTAC as surfactants has shown positive results in enhancing the solubilisation of sewage sludge and facilitating protease production. Particularly, CTAC stands out due to its lower concentration requirement, which could potentially lead to resource savings.

A one-way ANOVA indicated significant differences between groups ($p < 0.05$), with different substrates used as a factor (5 levels) and protease activity as the dependent variable. Subsequent Tukey's HSD *post hoc* test (Table S5 in the Supplementary Information) revealed that the pairs SDBS (50 mg/L) - CTAC (10 mg/L) did not exhibit significant differences but were significantly different from the other groups. Similarly, the pairs SDS (50 mg/L) - TAC (10 mg/L) and hydrolysed sewage sludge did not differ significantly from each other but showed significant differences compared to the previous group: SDBS (50 mg/L) - CTAC (10 mg/L).

4. Conclusions

This study demonstrated the beneficial effect of surfactants on thermal hydrolysis of sewage sludge. Thus, the addition of SDS, SDBS, and TAC at concentrations of 150 mg/gVSS$_o$ resulted in a significant improvement in biomolecule production, with increases of 44 %, 45 %, and 31 %, respectively, compared to thermal hydrolysis without surfactants at 275 min. In the case of CTAC, a 20 % increase in biomolecule production was observed at a CTAC concentration of 10 mg/gVSS$_o$, since higher concentrations led to a decrease in biomolecule yield due to their complexation and precipitation. However, the addition of SDS and SDBS at higher concentrations caused a notable increase in the slow/non-biodegradable fraction, with the maximum reaching 43 % when 150 mg/gVSS$_o$ of SDBS was used. Finally, SDBS-assisted and CTAC-assisted hydrolysed sewage sludges were found to be a suitable substrate for protease production using *B. licheniformis*. Thus, an enzymatic activity of around 700 U/mL was obtained after 72 h of fermentation, when CTAC and SDBS were added at concentrations of 10 mg/gVSS$_o$ and 50 mg/gVSS$_o$, respectively.

This research introduces an innovative approach for sewage sludge solubilisation through surfactant-assisted thermal hydrolysis, highlighting its potential for generating valuable industrial products such as proteases via fermentation with *B. licheniformis*.

CRediT authorship contribution statement

Paula Oulego: Writing – review & editing, Validation, Supervision, Resources, Methodology, Conceptualization. **Juan Fernando Moreno:** Visualization, Investigation, Data curation. **Luis Romero:** Writing – original draft, Visualization, Investigation, Data curation, Conceptualization. **Mario Diaz:** Supervision, Project administration, Funding acquisition. **Sergio Collado:** Validation, Resources, Methodology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

This work was co-financed by Spanish MCIU (Projects MCIU-19-RTI2018–094218-B-I00 and MCIU-22-PID2021–125942OB-I00) and Foundation for the Promotion of Applied Scientific Research and Technology in Asturias (Project SV-PA-21-AYUD/2021/51041). Luis Romero acknowledges an FPI grant from Spanish MICINN (PRE2019–091054). Juan F. Moreno also acknowledges the financial support from the Spanish Ministry of Universities through the FPU predoctoral aid programme (FPU20/06328).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jece.2024.113353.

References

[1] F. Battista, G. Strazzera, F. Valentino, M. Gottardo, M. Villano, M. Matos, F. Silva, MariaA. M. Reis, J. Mata-Alvarez, S. Astals, J. Dosta, R.J. Jones, J. Massanet-Nicolau, A. Guwy, P. Pavan, D. Bolzonella, M. Majone, New insights in food waste, sewage sludge and green waste anaerobic fermentation for short-chain volatile fatty acids production: a review, J. Environ. Chem. Eng. 10 (2022) 108319, https://doi.org/10.1016/j.jece.2022.108319.

[2] M. Bernardi, D. Cretenot, S. Deleris, C. Descorme, J. Chauzy, M. Besson, Performances of soluble metallic salts in the catalytic wet air oxidation of sewage sludge, Catal. Today 157 (2010) 420–424, https://doi.org/10.1016/j.cattod.2010.01.030.

[3] J.L. Urrea, S. Collado, P. Oulego, M. Díaz, Wet oxidation of the structural sludge fractions, J. Clean. Prod. 168 (2017) 1163–1170, https://doi.org/10.1016/j.jclepro.2017.09.033.

[4] M. García, J.L. Urrea, S. Collado, P. Oulego, M. Díaz, Protein recovery from solubilized sludge by hydrothermal treatments, Waste Manag. 67 (2017) 278–287, https://doi.org/10.1016/j.wasman.2017.05.051.

[5] L. Niero, F. Morgan-Sagastume, A. Lagerkvist, Accelerating acidogenic fermentation of sewage sludge with ash addition, J. Environ. Chem. Eng. 9 (2021) 106564, https://doi.org/10.1016/j.jece.2021.106564.

[6] O. Suárez-Iglesias, J.L. Urrea, P. Oulego, S. Collado, M. Díaz, Valuable compounds from sewage sludge by thermal hydrolysis and wet oxidation. A review, Sci. Total Environ. 584–585 (2017) 921–934, https://doi.org/10.1016/j.scitotenv.2017.01.140.

[7] Y. Khan, G.K. Anderson, D.J. Elliott, Wet oxidation of activated sludge, Water Res. 33 (1999) 1681–1687, https://doi.org/10.1016/S0043-1354(98)00387-X.

[8] J.L. Urrea, S. Collado, P. Oulego, M. Díaz, Formation and degradation of soluble biopolymers during wet oxidation of sludge, ACS Sustain Chem. Eng. 5 (2017) 3011–3018, https://doi.org/10.1021/acssuschemeng.6b02664.

[9] L. Romero, P. Oulego, S. Collado, M. Díaz, Advanced thermal hydrolysis for biopolymer production from waste activated sludge: kinetics and fingerprints, J. Environ. Manag. 342 (2023) 118243, https://doi.org/10.1016/j.jenvman.2023.118243.

[10] K.M. Lee, M.S. Kim, C. Lee, Oxidative treatment of waste activated sludge by different activated persulfate systems for enhancing sludge dewaterability, Sustain Environ. Res. 26 (2016) 177–183, https://doi.org/10.1016/j.serj.2015.10.005.

[11] Y. Xiang, Y. Xiang, L. Wang, Kinetics of activated sludge protein extraction by thermal alkaline treatment, J. Environ. Chem. Eng. 5 (2017) 5352–5357, https://doi.org/10.1016/j.jece.2017.09.062.

[12] R. Guan, X. Yuan, Z. Wu, H. Wang, L. Jiang, Y. Li, G. Zeng, Functionality of surfactants in waste-activated sludge treatment: a review, Sci. Total Environ. 609 (2017) 1433–1442, https://doi.org/10.1016/j.scitotenv.2017.07.189.

[13] H. Yuan, N. Zhu, F. Song, Dewaterability characteristics of sludge conditioned with surfactants pretreatment by electrolysis, Bioresour. Technol. 102 (2011) 2308–2315, https://doi.org/10.1016/j.biortech.2010.10.065.

[14] H. Wang, Q. Zhang, Z. Lei, H. Li, Y. Wu, X. Han, Y. Wang, Metagenomic analysis reveals the effect of benzalammonium bromide on methane production during sludge anaerobic digestion, J. Environ. Chem. Eng. 12 (2024) 112314, https://doi.org/10.1016/j.jece.2024.112314.

[15] Z. Li, Y. Li, D. Wang, L. Yuan, X. Liu, C. Pan, X. Zhang, Insights into cetyl trimethyl ammonium bromide improving dewaterability of anaerobically fermented sludge, Chem. Eng. J. 435 (2022) 134968, https://doi.org/10.1016/j.cej.2022.134968.

[16] L.F. Wang, D.Q. He, Z.H. Tong, W.W. Li, H.Q. Yu, Characterization of dewatering process of activated sludge assisted by cationic surfactants, Biochem Eng. J. 91 (2014) 174–178, https://doi.org/10.1016/j.bej.2014.08.008.

[17] X. Liu, J. Wang, E. Liu, T. Yang, R. Li, Y. Sun, Municipal sludge dewatering properties and heavy metal distribution: Effects of surfactant and hydrothermal

treatment, Sci. Total Environ. 710 (2020) 136346, https://doi.org/10.1016/j.scitotenv.2019.136346.
[18] H. Wang, S.C. Yang, W. Cai, W. Liu, A. Wang, Enhanced organic matter and nutrient release from waste activated sludge using ultrasound and surfactant synergetic pre-treatment, Bioresour. Technol. Rep. 6 (2019) 32–38, https://doi.org/10.1016/j.biteb.2019.01.017.
[19] N. Reddy, V. Deekonda, S. Seshagiri, R. Reddy, A.K. Gangula, Production, characterization and applications of proteases produced by Bacillus licheniformis, Acinetobacter pittii and Aspergillus niger using neem seed oil cake as the substrate, Ind. Crop Prod. 187 (2022) 115403, https://doi.org/10.1016/j.indcrop.2022.115403.
[20] I. Talhi, L. Dehimat, A. Jaouani, R. Cherfia, M. Berkani, F. Almomani, Y. Vasseghian, N.K. Chaouche, Optimization of thermostable proteases production under agro-wastes solid-state fermentation by a new thermophilic Mycothermus thermophilus isolated from a hydrothermal spring Hammam Debagh, Algeria, Chemosphere 286 (2022) 131479, https://doi.org/10.1016/j.chemosphere.2021.131479.
[21] I.A. Shaikh, B. Turakani, J. Malpani, S.V. Goudar, M.H. Mahnashi, R. Hamed Al-Serwi, M.M. Ghoneim, M. El-Sherbiny, B. Abdulaziz Mannasaheb, F. Alsaikhan, V. Sindagimath, A.A. Khan, U.M. Muddapur, S. Azzouz, T. Mohammed, S. M. Shakeel Iqubal, Extracellular protease production, optimization, and partial purification from bacillus nakamurai PL4 and its applications, J. King Saud. Univ. Sci. 35 (2023) 102429, https://doi.org/10.1016/j.jksus.2022.102429.
[22] J.G. dos Santos Aguilar, H.H. Sato, Microbial proteases: production and application in obtaining protein hydrolysates, Food Res. Int. 103 (2018) 253–262, https://doi.org/10.1016/j.foodres.2017.10.044.
[23] J. Parrado, B. Rodriguez-Morgado, M. Tejada, T. Hernandez, C. Garcia, Proteomic analysis of enzyme production by Bacillus licheniformis using different feather wastes as the sole fermentation media, Enzym. Micro Technol. 57 (2014) 1–7, https://doi.org/10.1016/j.enzmictec.2014.01.001.
[24] J.F. Moreno, P. Oulego, S. Collado, M. Díaz, Valorisation of waste activated sludge for protease production by Bacillus licheniformis, J. Clean. Prod. 445 (2024) 141282, https://doi.org/10.1016/j.jclepro.2024.141282.
[25] L. Guo, X.M. Li, X. Bo, Q. Yang, G.M. Zeng, D. xiang Liao, J.J. Liu, Impacts of sterilization, microwave and ultrasonication pretreatment on hydrogen producing using waste sludge, Bioresour. Technol. (2008) 3651–3658, https://doi.org/10.1016/j.biortech.2007.07.026.
[26] S.M. Mirsoleimani Azizi, N. Haffiez, A. Mostafa, A. Hussain, M. Abdallah, A. Al-Mamun, A. Bhatnagar, B.R. Dhar, Low- and high-temperature thermal hydrolysis pretreatment for anaerobic digestion of sludge: process evaluation and fate of emerging pollutants, Renew. Sust. Energ. Rev. 200 (2024) 114453, https://doi.org/10.1016/j.rser.2024.114453.
[27] X. Shi, L. Zhu, B. Li, J. Liang, X. Li, Surfactant-assisted thermal hydrolysis off waste activated sludge for improved dewaterability, organic release, and volatile fatty acid production, Waste Manag. 124 (2021) 339–347, https://doi.org/10.1016/j.wasman.2021.02.024.
[28] L.-F. Wang, B.-C. Huang, L.-L. Wang, Y. Min, H.-Q. Yu, Experimental and theoretical analyses on the impacts of ionic surfactants on sludge properties, Sci. Total Environ. 633 (2018) 198–205, https://doi.org/10.1016/j.scitotenv.2018.03.187.
[29] K. Dopierala, K. Prochaska, The effect of molecular structure on the surface properties of selected quaternary ammonium salts, J. Colloid Interface Sci. 321 (2008) 220–226, https://doi.org/10.1016/j.jcis.2008.01.049.
[30] L. Romero, S. Weng, P. Oulego, S. Collado, I. Marcet, M. Díaz, Hydrolyzed sewage sludge as raw bio-based material for hermetic bag production, Waste Manag. 174 (2024) 31–43, https://doi.org/10.1016/j.wasman.2023.11.022.
[31] OliverH. Lowry, NiraJ. Rosebrough, A.L. Farr, RoseJ. Randall, Protein measurement with the Folin phenol reagent, J. Biol. Chem. 193 (1951) 265–275, https://doi.org/10.1016/S0021-9258(19)52451-6.
[32] Michel DuBois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, Fred Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem. 28 (1956) 350–356, https://doi.org/10.1021/ac60111a017.
[33] K. Burton, A study of the conditions and mechanism of the diphenylamine reaction for the colorimetric estimation of deoxyribonucleic acid, Biochem J. 62 (1956) 315–323, https://doi.org/10.1042/bj0620315.
[34] APHA, Standard Methods for Examination of Water and Wastewater, American Public Health Association, Washington, 2012.
[35] L. Pola, S. Collado, P. Oulego, M. Díaz, A proposal for the classification of sludge products throughout hydrothermal treatment, Chem. Eng. J. 430 (2022) 132746, https://doi.org/10.1016/j.cej.2021.132746.
[36] F.J. Romero, L.A. García, J.A. Salas, M. Díaz, L.M. Quirös, Production, purification and partial characterization of two extracellular proteases from Serratia marcescens grown in whey, Process Biochem 36 (2001) 507–515, https://doi.org/10.1016/S0032-9592(00)00221-1.
[37] R. Srivastava, Md.S. Alam, Influence of micelles on protein's denaturation, Int J. Biol. Macromol. 145 (2020) 252–261, https://doi.org/10.1016/j.ijbiomac.2019.12.154.
[38] K.K. Andersen, C.L. Oliveira, K.L. Larsen, F.M. Poulsen, T.H. Callisen, P. Westh, J. S. Pedersen, D. Otzen, The role of decorated SDS Micelles in Sub-CMC protein denaturation and association, J. Mol. Biol. 391 (2009) 207–226, https://doi.org/10.1016/j.jmb.2009.06.019.
[39] X. Li, Q. Fu, W. Wang, X. Liu, D. He, X. Jiang, Q. Yang, D. Wang, Surfactant enhances anaerobic fermentative hydrogen sulfide production: changes in sulfur-containing organics structure and microbial community, Sci. Total Environ. 880 (2023) 163025, https://doi.org/10.1016/j.scitotenv.2023.163025.
[40] M. Tabak, D. de Sousa Neto, C.E.G. Salmon, On the interaction of Bovine Serum Albumin (BSA) with cethyltrimethyl ammonium chloride surfactant: electron paramagnetic resonance (EPR) study, Braz. J. Phys. 36 (2006), https://doi.org/10.1590/S0103-97332006000100014.
[41] M.M. Yee, T. Miyajima, N. Takisawa, Study of ionic surfactants binding to humic acid and fulvic acid by potentiometric titration and dynamic light scattering, Colloids Surf. A Physicochem Eng. Asp. 347 (2009) 128–132, https://doi.org/10.1016/j.colsurfa.2009.02.010.
[42] Y. Li, D. Wang, Q. Xu, X. Liu, Y. Wang, Y. Wu, G. Yang, X. Yuan, Z. Wu, R. Guan, T. Xiong, D. He, Q. Fu, New insight into modification of extracellular polymeric substances extracted from waste activated sludge by homogeneous Fe(II)/persulfate process, Chemosphere 247 (2020) 125804, https://doi.org/10.1016/j.chemosphere.2019.125804.
[43] L.K. Koopal, T.P. Goloub, T.A. Davis, Binding of ionic surfactants to purified humic acid, J. Colloid Interface Sci. 275 (2004) 360–367, https://doi.org/10.1016/j.jcis.2004.02.061.
[44] N. Deo, S. Jockusch, N.J. Turro, P. Somasundaran, Surfactant interactions with zein protein, Langmuir 19 (2003) 5083–5088, https://doi.org/10.1021/la020854s.
[45] J. Yan, Z. Fan, L. Zhi, Functionalized Carbon Nanotubes and Their Enhanced Polymers. in: Polymer Science: A Comprehensive Reference, Elsevier, 2012, pp. 439–478, https://doi.org/10.1016/B978-0-444-53349-4.00217-X.
[46] M. García, S. Collado, P. Oulego, M. Díaz, The wet oxidation of aqueous humic acids, J. Hazard Mater. 396 (2020) 122402, https://doi.org/10.1016/j.jhazmat.2020.122402.
[47] E. Grządka, J. Matusiak, M. Stankevič, Interactions between fluorocarbon surfactants and polysaccharides, J. Mol. Liq. 283 (2019) 81–90, https://doi.org/10.1016/j.molliq.2019.03.059.
[48] X. Zhang, R. Bai, Mechanisms and kinetics of humic acid adsorption onto chitosan-coated granules, J. Colloid Interface Sci. 264 (2003) 30–38, https://doi.org/10.1016/S0021-9797(03)00393-X.
[49] M. Aguirre-Ramírez, H. Silva-Jiménez, I.M. Banat, M.A. Díaz De Rienzo, Surfactants: physicochemical interactions with biological macromolecules, Biotechnol. Lett. 43 (2021) 523–535, https://doi.org/10.1007/s10529-020-03054-1.
[50] M. Grube, J.G. Lin, P.H. Lee, S. Kokorevicha, Evaluation of sewage sludge-based compost by FT-IR spectroscopy, Geoderma 130 (2006) 324–333, https://doi.org/10.1016/j.geoderma.2005.02.005.
[51] J. Coates, Interpretation of Infrared Spectra, A Practical Approach. in: Encyclopedia of Analytical Chemistry, John Wiley & Sons, Ltd, Chichester, UK, 2006, https://doi.org/10.1002/9780470027318.a5606.
[52] E. Smidt, P. Lechner, M. Schwanninger, G. Haberhauer, M.H. Gerzabek, Characterization of waste organic matter by FT-IR spectroscopy: application in waste science, Appl. Spectrosc. 56 (2002) 1170–1175, https://doi.org/10.1366/000370202760295412.
[53] M.K. Singh, A. Agarwal, R. Gopal, R.K. Swarnkar, R.K. Kotnala, Dumbbell shaped nickel nanocrystals synthesized by a laser induced fragmentation method, J. Mater. Chem. 21 (2011) 11074, https://doi.org/10.1039/c1jm12320c.
[54] G. Socrates, Infrared and Raman Characteristic Group Frequencies: Tables and Charts, John Wiley & Sons, Ltd, 2003.
[55] R.P. Sperline, Y. Song, H. Freiser, FTIR-ATR study of the co-adsorption of benzophenone with sodium dodecyl sulfate and sodium dodecylbenzenesulfonate using Al2O3-coated optics, Colloid Surf. A 93 (1994) 111–126, https://doi.org/10.1016/0927-7757(94)02894-X.
[56] A. Maurya, R. Singh, S. Rastogi, Study of vibrational spectra of polycyclic aromatic hydrocarbons with phenyl side group, J. Mol. Spectrosc. 391 (2023) 111720, https://doi.org/10.1016/j.jms.2022.111720.
[57] R.A. Campbell, S.R.W. Parker, J.P.R. Day, C.D. Bain, External reflection FTIR spectroscopy of the cationic surfactant hexadecyltrimethylammonium bromide (CTAB) on an overflowing cylinder, Langmuir 20 (2004) 8740–8753, https://doi.org/10.1021/la048680x.
[58] S.A. Elfeky, S.E. Mahmoud, A.F. Youssef, Applications of CTAB modified magnetic nanoparticles for removal of chromium (VI) from contaminated water, J. Adv. Res. 8 (2017) 435–443, https://doi.org/10.1016/j.jare.2017.06.002.
[59] T. He, T. Zhou, Y. Wan, T. Tan, A simple strategy based on deep eutectic solvent for determination of aflatoxins in rice samples, Food Anal. Method 13 (2020) 542–550, https://doi.org/10.1007/s12161-019-01665-7.
[60] Y.-Y. Choi, S.-R. Baek, J.-I. Kim, J.-W. Choi, J. Hur, T.-U. Lee, C.-J. Park, B. Lee, Characteristics and biodegradability of wastewater organic matter in municipal wastewater treatment plants collecting domestic wastewater and industrial discharge, Water Sui 9 (2017) 409, https://doi.org/10.3390/w9060409.
[61] E. Płuciennik-Koropczuk, S. Myszograj, New approach in COD fractionation methods, Water Sui 11 (2019) 1484, https://doi.org/10.3390/w11071484.
[62] I. Pasztor, P. Thury, J. Pulai, Chemical oxygen demand fractions of municipal wastewater for modeling of wastewater treatment, Int J. Environ. Sci. Te 6 (2009) 51–56, https://doi.org/10.1007/BF03326059.
[63] A.A. Najim, Z.Z. Ismail, K.K. Hummadi, Biodegradation potential of sodium dodecyl sulphate (SDS) by mixed cells in domestic and non-domestic actual wastewaters: experimental and kinetic studies, Biochem. Eng. J. 180 (2022) 108374, https://doi.org/10.1016/j.bej.2022.108374.
[64] G.-G. Ying, Fate, behavior and effects of surfactants and their degradation products in the environment, Environ. Int. 32 (2006) 417–431, https://doi.org/10.1016/j.envint.2005.07.004.
[65] W.H. Danial, Surfactant Biodegradation. Handbook of Biodegradable Materials, Springer International Publishing, Cham, 2022, pp. 1–29, https://doi.org/10.1007/978-3-030-83783-9_26-1.

[66] M.J. Scott, M.N. Jones, The biodegradation of surfactants in the environment, BBA Biomembr. 1508 (2000) 235–251, https://doi.org/10.1016/S0304-4157(00)00013-7.
[67] S. Chowdhury, A. Rakshit, A. Acharjee, B. Saha, Biodegradability and biocompatibility: Advancements in synthetic surfactants, J. Mol. Liq. 324 (2021) 115105, https://doi.org/10.1016/j.molliq.2020.115105.
[68] European chemicals agency, Classication of Tetraethylammonium chloride for effects in the environment, n.d. ⟨https://echa.europa.eu/registration-dossier/-/registered-dossier/20036/2/3#⟩ (accessed May 11, 2023).
[69] I. Johansson, P. Somasundaran, Handbook for Cleaning/decontamination of Surfaces, Elsevier Science, 2007 https://books.google.es/books?id=5-tINc5v254C.
[70] H. Schott, C.Y. Young, Electrokinetic studies of bacteria II: effect of cetyltrimethylammoniurn bromide on streptococcus faecalis, J. Pharm. Sci. 61 (1972) 762–765, https://doi.org/10.1002/jps.2600610518.
[71] W.J. Payne, V.E. Feisal, Bacterial utilization of dodecyl sulfate and dodecyl benzene sulfonate, Appl. Microbiol. 11 (1963) 339–344, https://doi.org/10.1128/am.11.4.339-344.1963.
[72] A. Haddar, N. Hmidet, O. Ghorbel-Bellaaj, N. Fakhfakh-Zouari, A. Sellami-Kamoun, M. Nasri, Alkaline proteases produced by Bacillus licheniformis RP1 grown on shrimp wastes: application in chitin extraction, chicken feather-degradation and as a dehairing agent, Biotechnol. Bioproc E 16 (2011) 669–678, https://doi.org/10.1007/s12257-010-0410-7.
[73] H. He, Q. Yu, Z. Ding, L. Zhang, G. Shi, Y. Li, Biotechnological and food synthetic biology potential of platform strain: Bacillus licheniformis, Synth. Syst. Biotechnol. 8 (2023) 281–291, https://doi.org/10.1016/j.synbio.2023.03.008.
[74] N. Annamalai, M.V. Rajeswari, S.K. Sahu, T. Balasubramanian, Purification and characterization of solvent stable, alkaline protease from Bacillus firmus CAS 7 by microbial conversion of marine wastes and molecular mechanism underlying solvent stability, Process Biochem. 49 (2014) 1012–1019, https://doi.org/10.1016/j.procbio.2014.03.007.