EFFECT OF MIXING ON RHEOLOGICAL AND STRUCTURAL CHANGES IN SLUDGE DURING THERMAL HYDROLYSIS

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Thermal hydrolysis (THP) is an established pretreatment method to enhance sludge biodegradability and dewaterability, yet the specific impact of mixing during THP on its efficiency remains unclear. This study systematically compared THP of waste activated sludge with and without mechanical mixing under identical thermal conditions (160 °C, 30 min) to evaluate sludge solubilization (dissolved solids and soluble COD release), disintegration degree, extracellular polymeric substance (EPS) degradation, sludge rheology, and dewaterability. The results showed that mixing enhanced thermal disruption, increasing soluble COD by ~60% and disintegration degree from 12.4% to 20.0%. Combined mixing and heating reduced viscosity and particle size, indicating enhanced floc fragmentation and improved fluidity. Surprisingly, however, this enhanced fragmentation did not translate into improved dewaterability. The centrifuged cake solids content was 12.7% for mixing and heating, which was slightly lower than the 13.7% for the heating-only treatment. These results demonstrate that mechanical shear synergistically enhances sludge disintegration during THP, yet mixing may hinder solid–liquid separation in the downstream treatment. Further evaluation under realistic THP operating conditions (≈16 % solids, including flash phase) is recommended to identify the optimal balance between disintegration and dewaterability.

Key words: Degree of disintegration, dewaterability, extracellular polymeric substances, mechanical mixing, particle size distribution, thermal hydrolysis pretreatment, viscosity

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1. Introduction

Biological wastewater treatment generates large volumes of sludge with low total solids (TS) content, usually around 1 %. To improve the energy efficiency of downstream processes such as anaerobic digestion (AD) and incineration, the sludge volume must be reduced through effective thickening or dewatering. Therefore, improving sludge dewaterability remains a key objective in sustainable sludge management. Various conditioning methods have been explored to achieve this, including thermal treatment, freeze–thaw cycles, coagulation–flocculation, and advanced oxidation processes [1].

Among these, the thermal hydrolysis process (THP) is widely used to break down the sludge structure and change its rheological properties. Sewage sludge consists of microbial cells embedded in a network of biopolymers known as extracellular polymeric substances (EPS) [2–4].

At the floc level, EPS governs the aggregation and stability of microbial flocs through entanglement, bridging, and hydrophobic interactions [1]. The macromolecular and hydrophilic components in EPS form cross-linked, three-dimensional networks within and between the sludge flocs [2,3]. These networks impart gel-like characteristics to sludge [4], where two structural levels can be distinguished: (i) the supra-floc network connecting multiple flocs, and (ii) individual flocs enclosed by polymeric envelopes. This so-called binary gel-like structure plays an important role in sludge water retention and sludge dewaterability [5], as it retains both free and bound water [3,4].

During THP, temperatures of 160–180 °C and retention times of 20–60 min disrupt the EPS network, rupture microbial cell walls, and release intracellular material and tightly bound water from the floc structure [2,3]. After the thermal treatment, rapid depressurization applies intense shear force, further breaking down cellular structures and improving sludge disintegration [2–6]. The flash phase takes place in the flash tank following the THP reactor in a full-scale process, e.g., CambiTHP™ [6]. This step results in better dewaterability [7,8] and a significant reduction in viscosity [4,9]. Heating to about 160 °C changes sludge from a non-Newtonian, shear-thinning fluid close to a Newtonian fluid, making pumping and handling easier [9]. Disintegration of sludge shifts the particle size distribution (PSD) toward smaller fractions, with the mean particle diameter of thickened waste activated sludge up to 50% after hydrothermal treatment [7,8].

The objective of THP is to process thickened sludge, typically dewatered to above 16% TS [8,9]. Thickening reduces water, which reduces the energy required for heating the THP reactor. Additionally, high solids content allows for maximizing the subsequent digester loading. However, handling high solids content presents significant challenges toward efficient mass and heat transfer [10]. THP reactors vary in design, with some

incorporating continuous mixing and feeding to improve the homogeneous heat distribution [11]. Mixing strongly influences the thermal behaviour by promoting uniform heat transfer and reducing dead zones [12]. Effective mixing of AD reactor increases the available sludge surface area, increasing anaerobic digestion rate [13] and biogas yield [14]. Im et al. [15] studied various physical properties of the THP reactor, including the amount of sludge fed, the reactor's slope degree, and the stirring speed [15]. They confirmed that mixing improved both heat distribution and sludge disintegration [14]. Furthermore, they proposed an optimal stirring speed of 150 rpm for THP at 200°C for 1 hour applied on dewatered sludge. However, such conditions (200°C, 1 h) do not represent full-scale THP operation [15], where a 140-160°C range has been identified as the optimal trade-off between AD performance, dewaterability, and filtrate quality (measured in terms of refractory soluble COD (sCOD), recalcitrant dissolved organics, and colour) [16]. Evidently, to assess the synergy between mixing and heating, it is of primary importance that the mixing itself does not cause a mechanical disintegration of flocs.

Thermally hydrolysed sludge is a highly valuable resource for renewable energy in wastewater treatment, contributing to increased biogas production during anaerobic digestion through sludge disintegration and to lower sludge heating requirements through higher dewaterability. Despite the recognized importance of mixing for an optimal heat transfer, its specific effect during THP of waste activated sludge remains insufficiently understood. Therefore, this study systematically compared THP performance with and without mixing under identical conditions. Understanding how mixing during THP affects sludge disintegration degree, EPS release, viscosity, PSD, and dewaterability is therefore directly relevant to the energy-recovery perspective, as it elucidates the importance of mixing during THP.

2. Experimental part

2.1. Experimental design

Four experimental conditions were established to investigate the combined effects of heating and mixing on sludge solubilization and structural properties. Gravity-thickened waste activated sludge was sampled from the Mechelen-Noord municipal wastewater treatment plant (Belgium), which operates without primary sedimentation. The samples were labelled according to treatment conditions: Control (untreated sludge), Mixed (mechanically mixed at ambient temperature), Heated (thermally treated without mixing), and Mix+Heat (simultaneous thermal treatment and mixing). The experimental matrix is summarized in Table 1. All experiments were conducted in triplicate. The mixed and control samples were maintained at ambient temperature (23 ± 1 °C) for a residence time comparable to the duration of THP reaction (30 min). A custom-designed laboratory batch reactor (Figure 1), equipped with outer wall heating and a mechanical stirrer, was used for the THP experiments.

Table 1 Breakdown of the experimental design for heating and mixing of samples.

Sample	Heating [°C]	Mixing [rpm]
Control	–	–
Mixed	– (23.2)	41
Heated	161.0 ± 1.2	–
Mix+Heat	162.0 ± 3.4	41

The reactor (with a working volume of approximately 1.0 L) was made of stainless steel and designed to operate at up to 200 °C and 10 bar. Mixing was conducted at 41 rpm, identified as the optimal speed to prevent the mechanical disintegration of sludge flocs under non-heated conditions (16). The temperature was raised to 160 ± 5 °C and maintained throughout the test.

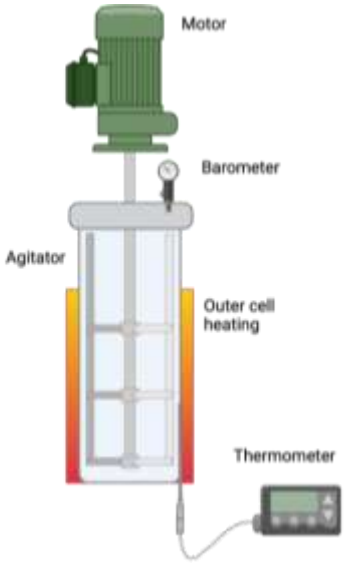

Fig 1 Scheme of a custom-designed laboratory THP reactor used during this experiment.

The temperature profile and process stability (Figure 2) were recorded for both mixed and non-mixed experiments. Rapid depressurization was not part of the procedure since the experiment was conducted on a laboratory scale. Instead, the sludge was cooled slowly, as shown in Figure 2.

2.2. Solids and COD analysis

Total solids (TS), volatile solids (VS), and dissolved solids (DS) were determined according to Standard Methods for the Examination of Water and Wastewater (17). The total chemical oxygen demand (tCOD) and sCOD were measured using HACH Lange LCK514 test kits. The disintegration degree (DD) was calculated following (18):

$$DD\ (\%) = \frac{sCOD_H - sCOD_0}{tCOD_0 - sCOD_0} \times 100$$

where $sCOD_0$ and $tCOD_0$ are the soluble and total COD of the control ($g.L^{-1}$), and $sCOD_H$ is the sCOD of the treated sludge (i.e., Mixed, Heated, Mix+Heat).

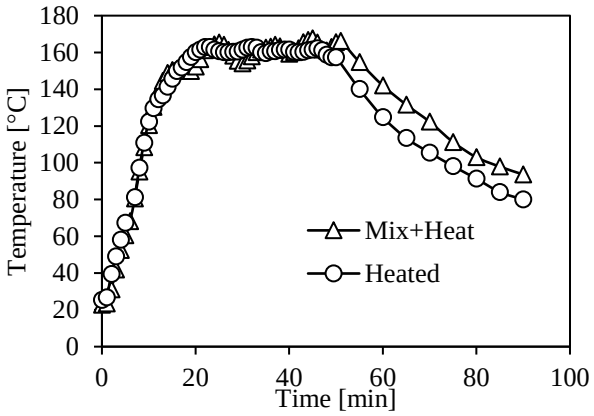

Fig 2 Temperature ramp for heating the sludge and temperature stability with and without mixing.

2.3. EPS extraction and analysis

For EPS determination, the sludge samples were first diluted with deionized water and centrifuged at 5,000 × g for 10 min at 4 °C (Eppendorf 5810R). The resulting supernatant (i.e., fugate) was filtered through a pre-rinsed Whatman Grade 1 filter using vacuum filtration (Figure 3). The filtrate (SMP) represented the soluble EPS fraction in all the samples. In the thermally treated samples, this fraction also contained soluble organic material released from floc and cellular disruption during heating, corresponding to bound EPS components freed into the liquid phase.

The remaining sludge cake was buffered with phosphate-buffered saline (PBS, Thermo Scientific™) and used for extraction of bound EPS. Two extraction procedures were applied in parallel:

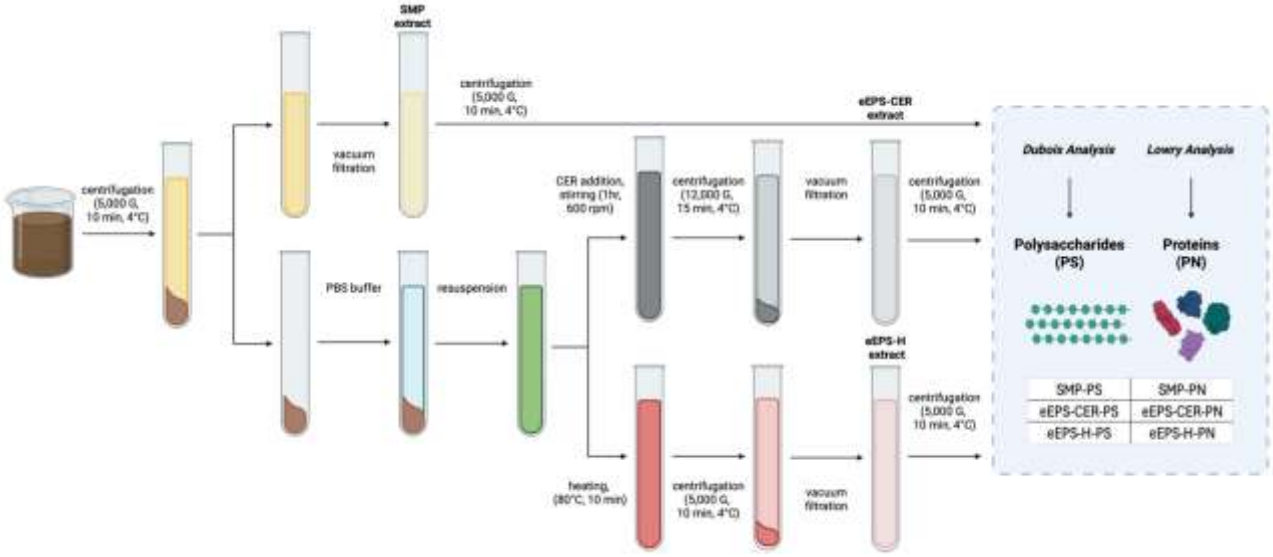

Fig. 3 Schematic of methodology used for EPS extraction

Cation exchange resin method (eEPS-CER), adapted from Frølund et al. [19]. CER (Dowex® Marathon C, Na^+ form) was added at a dosage of 70 g g^{-1} VSS, and the mixture was agitated at 4 °C for 1 h.

The heat extraction method (eEPS-H) was modified from Tay et al. [20]. Briefly, the sludge suspension was heated to 80 °C for 10 min in a thermostatic bath and immediately cooled on ice to prevent further hydrolysis.

After extraction, all samples were centrifuged (5,000 × g, 10 min, 4 °C) and filtered through pre-rinsed Whatman Grade 1 filters. The resulting extracts, eEPS-CER and eEPS-H, and the soluble fraction, SMP, were analyzed for polysaccharides and protein content using the Dubois method [21] and the Lowry method [22], respectively, as described by D'Abzac et al. [23].

2.4. Viscosity

Apparent dynamic viscosity was measured using a rotational viscometer (Viscometer ViscoQC 300 R, Anton Paar, Austria) equipped with an RH2 spindle to measure medium-viscosity samples. The viscometer producer recommends the RH2 spindle for measuring the apparent viscosity of a broad range of liquids. The measurements were carried out at 23°C ± 1°C and 250 rpm, after gentle homogenization of the sludge.

2.5. Particle size distribution (PSD)

Particle size distribution analysis was conducted according to ISO 13320:2020 using both undersize (cumulative) and size distribution data obtained from the PSA 1190 particle size analyzer (Anton Paar). The undersize data, representing cumulative percentages, was used to determine D_{10}, D_{50}, and D_{90} values through linear interpolation between the closest data points. The span was calculated as (D_{90} - D_{10}) / D_{50}. For the size distribution analysis, particle sizes were grouped into ranges (< 1, 1-25, 25-130, 130-250, 250-1000, >1000 μm) by summing the percentage values within each range. This grouping allowed for a comparison between treatments while maintaining resolution in key size ranges.

2.6. Dewaterability

Sludge dewaterability was determined using a centrifugation-based method (24). Briefly, samples were centrifuged for 10 min at 14,926 × g without polymer addition, and the dry solids content of the cake was measured to evaluate water removal efficiency.

2.7. Statistical analysis

All measurements were performed in triplicate. Data are reported as mean ± standard deviation. Statistical comparisons between treatments were performed using one-way analysis of variance (ANOVA) at a significance level of $p < 0.05$ unless stated otherwise. Stronger significance levels ($p < 0.01$, $p < 0.001$) were reported where relevant. When ANOVA indicated significant differences, pairwise comparisons were conducted using Tukey's post hoc test to identify statistically distinct treatment groups.

3. Results and discussion

3.1. Sludge characteristics and solubilization

The physicochemical characteristics of the sludge under different operating conditions are summarized in Table 2. Heating primarily affected the soluble fraction. While TS and tCOD remained statistically unchanged ($p > 0.05$) across treatments, DS and sCOD increased significantly after THP ($p < 0.001$).

Table 2 Sludge characteristics and disintegration degree (DD), composition of EPS fractions (SMP, eEPS) and apparent viscosity under different treatment conditions.

Parameter	Unit	Control	Mixed	Heated	Mix+Heat
TS	[g·L^{-1}]	32.16 ± 0.84	27.44 ± 0.27	29.74 ± 1.87	32.81 ± 0.81
VS	[g·L^{-1}]	22.24 ± 0.41	18.42 ± 0.25	20.55 ± 1.14	23.03 ± 0.60
DS	[g·L^{-1}]	1.26 ± 0.06	1.30 ± 0.01	4.73 ±0.02	6.75 ± 0.05
sCOD	[g·L^{-1}]	0.20 ± 0.01	0.30 ± 0.02	4.27 ±0.37	6.80 ± 0.42
tCOD	[g·L^{-1}]	33.13 ± 1.13	40.80 ± 1.06	31.73 ± 3.74	38.35 ± 2.19
DD	[%]	–	0.31 ± 0.06	12.36 ± 1.21	20.04 ± 1.44
Viscosity	[Pa·s]	111.4 ± 0.25	98.2 ± 0.09	87.9 ± 0.24	97.2 ± 0.00
SMP - Proteins	[mg·L^{-1}]	15.6 ± 0.9	7.9 ± 0.4	77.3 ± 2.7	96.1 ± 4.2
SMP - Polysaccharides	[mg·L^{-1}]	15.0 ± 4.2	7.5 ± 1.2	38.1 ± 4.8	65.8 ± 1.9
eEPS (Heat) - Proteins	[mg·L^{-1}]	59.2 ± 2.4	75.0 ± 5.4	20.2 ± 0.7	26.5 ± 0.4
eEPS (Heat) - Polysaccharides	[mg·L^{-1}]	32.3 ± 4.9	38.6 ± 9.3	25.0 ± 1.7	22.6 ± 0.7
eEPS (CER) - Proteins	[mg·L^{-1}]	30.5 ± 1.1	42.8 ± 1.4	21.4 ± 2.1	20.0 ± 2.0
eEPS (CER) - Polysaccharides	[mg·L^{-1}]	18.5 ± 1.2	25.1 ± 0.3	21.9 ± 0.8	19.4 ± 0.6

This shift toward the soluble phase demonstrated extensive disintegration of the sludge matrix. The solubilization effect was corroborated by the significant increase in sCOD, which increased from 0.20 g·L^{-1} in the control to 6.80 g·L^{-1} in the Mix+Heat treatment. This represents a 34-fold increase, confirming that the treatment effect was dominant over experimental variance, although the SD increased in the treated samples. Correspondingly, the DD followed the same trend, increasing from 0.3 % (mixed) to 12.4 % (heated) and 20.0 % (Mix+Heat). This is a 40-fold and 65-fold increase, respectively. The enhanced solubilization under combined treatment appears to demonstrate a synergistic interaction between mechanical shear and thermal stress, which promotes uniform heat distribution and rupturing of microbial flocs.

Microscopic observations confirmed the quantitative data (Figure 4). The untreated sludge consisted of compact flocs with discernible microbial cells, while THP clearly led to fragmentation and dispersion of smaller aggregates. The Mix+Heat treatment caused the most pronounced morphological disruption. As shown in Figure 4, a dispersed suspension of fragmented aggregates and cellular debris replaced the original compact floc structure. This visual disintegration aligns with the quantitative increase in soluble organics. While microscopy provides valuable qualitative support, further quantitative image analysis or high-resolution methods such as scanning electron microscopy (SEM) or confocal laser scanning microscopy (CLSM) could provide additional insights into floc architecture and cell damage.

3.2. Extracellular polymeric substances (EPS)

Thermal treatment markedly altered the distribution and composition of EPS ($p < 0.001$ for both polysaccharides and proteins). Tukey analysis showed that both SMP-polysaccharides and SMP-protein concentrations increased significantly in all thermally treated samples compared to the control samples ($p < 0.001$). As summarized in Table 2, SMP-polysaccharides increased fourfold in the Mix+Heat samples compared to control. Similarly, SMP-proteins rose sixfold, reaching the highest concentration among all treatments. The bound EPS fractions (eEPS-H and eEPS-CER) exhibited the inverse pattern, with protein and polysaccharides concentrations significantly decreasing after heating ($p < 0.01$).

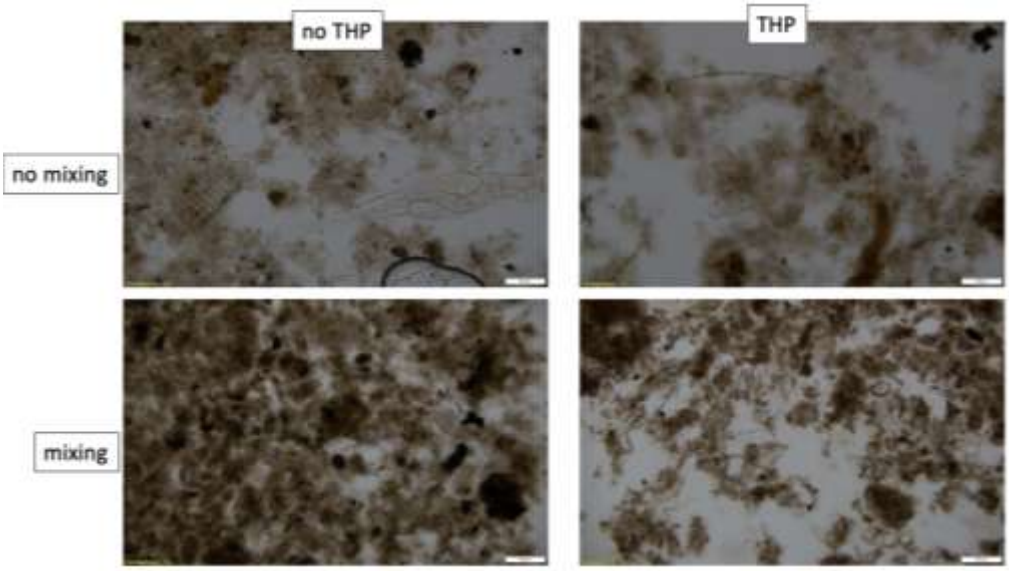

Fig. 4 Micrographs of untreated sludge, treated with THP, mixed, and mixed and treated with THP. 20x, 50 μm (upper two)/ 10x 100 μm (lower two).

This redistribution confirms that thermal disruption converts bound EPS into SMP, transforming the sludge matrix from gel-like to dispersed suspension. Mixing amplified this transition. The resulting protein-to-polysaccharides ratio increased from 1.0 in the control to 1.5 in heated samples, signifying preferential protein solubilization, consistent with previous observations by Li et al. [25] and Neyens et al., [26].

Additionally, mixing appears to amplify the effects of thermal treatment, yielding higher SMP and lower bound EPS than heating alone. Improved heat distribution and mechanical shear likely intensified EPS degradation, accelerating solute release. The reduced SMP/sCOD ratio from 0.15 in control to 0.02 in Mix+Heat samples suggests that a larger fraction of solubilized organics contributes directly to the COD pool rather than forming colloids.

3.3. Viscosity

The apparent viscosity (250 rpm) of the sludge decreased significantly across all treatments compared to the control ($p<0.001$). Additionally, all pairwise sample comparisons were significant ($p<0.05$). Mixing alone decreased viscosity (Table 2) by approximately 12 %, while solely thermal treatment caused the strongest reduction of 21%. Reflecting the breakdown of EPS and release of bound water. The combined heating and mixing treatment (Mix+Heat) produced a smaller overall reduction by 13%, suggesting non-linear interactions between thermal degradation and shear-induced restructuring. Statistically, these results confirm that both thermal and mechanical forces significantly enhance sludge fluidity ($p < 0.001$), with implications for energy-efficient pumping and digester mixing in full-scale applications [27]. The observed results are consistent with findings by Feng et al., who observed viscosity reduction, attributed to the breakdown of EPS and disruption of floc structure [28]. Their results confirmed that treated sludge exhibited near-Newtonian behavior and significantly enhanced fluidity, supporting the observed statistical differences in our treatment comparisons.

It should be noted that the ViscoQC viscometer provides a single-point apparent viscosity at a fixed shear rate, which is suitable for viscosity comparison of sludge obtained from different stages in sludge treatment [29]. Yet for a comprehensive rheological assessment, a relationship between shear rate and shear stress (rheogram) is recommended, and the potential for wall slip should be considered

3.4. Particle size distribution (PSD)

The reduction in PSD (Table 3) was consistent with microscopic and viscosity observations.

Table 3 Sludge particle size distribution – D_{10}, D_{50}, D_{90}, span.

Sample	D_{10} [μm]	D_{50} [μm]	D_{90} [μm]	Span [μm]
Control	26.70	113.30	286.82	2.30
Mixed	24.59	109.71	286.82	2.39
Heated	25.22	106.22	268.36	2.29
Mix+Heat	24.06	101.15	253.21	2.27

The median particle size (D_{50}) decreased by 11% from 113 μm (control) to 101 μm (Mix+Heat), while D_{10} and D_{90} values declined by 10% and 12% respectively. The constant span (2.27–2.39) indicates a uniform reduction in size without broadening of the distribution. However, this shift was lower than reported by Zhang et al., where the average particle size dropped from 56 to 22 μm after THP [30]. Neyens et al. and Duan et al. also reported more pronounced reductions, from 107 μm to 66 μm and

107 μm to 61 μm, respectively [31,32]. These discrepancies can be attributed to the longer retention times (60-120 min) and higher THP temperatures (120-180°C) applied in those two studies, while Zhang et al. (2018) analyzed hydrolyzed sludge from a full-scale THP with a smaller D_{50} of raw sludge [30–32].

Mixing alone caused minimal changes, confirming that the temperature effect dominates particle disintegration. The combination of heating and mixing enhanced fragmentation (Figure 5), increasing the fraction of particles in the 25–130 μm range from 47.2 % in the control to 51.4 % in Mix+Heat. Although PSD measurements were not replicated, the observed monotonic trend across treatments supports statistically validated disintegration parameters (DD and viscosity). This reduction in particle size presumably increased the specific surface area available for enzymatic hydrolysis during anaerobic digestion, leading to an accelerating biodegradation [33].

However, excessive fragmentation can also increase colloidal stability, hindering floc formation and affecting downstream solid–liquid separation [34]. Thus, smaller particles are favourable for biological conversion but may complicate mechanical dewatering [27].

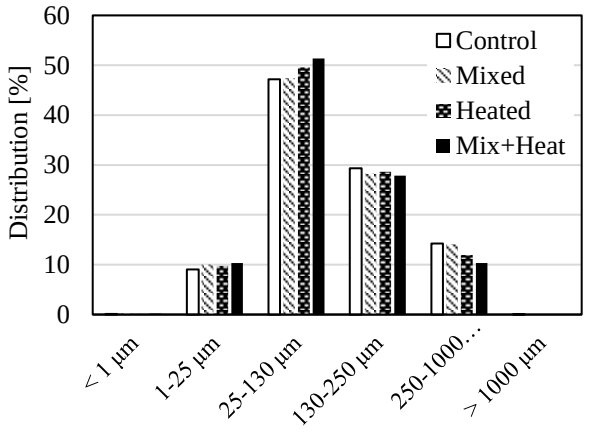

Fig 5 Sludge particle size distribution [%] divided into key size ranges.

3.5. Dewaterability

ANOVA revealed significant differences in dewaterability among treatments ($p = 6.45 \times 10^{-6}$). Tukey's test identified that heated and mixed samples exhibited higher solids contents in the dewatered cake (≈ 13.6–13.7 %) compared to both control and Mix+Heat samples (≈ 12.7–12.8 %). The Mix+Heat condition achieved a significantly lower dewaterability than heated samples ($p < 0.01$), confirming that the combined treatment unexpectedly reduced the dewaterability.

Although THP is generally reported to enhance dewaterability (17, 23), the current results indicate that the simultaneous application of heating and mixing introduces unexpected competing mechanisms. The strong solubilization observed with Mix+Heat conditions releases large amounts of EPS-derived proteins and polysaccharides, which can increase bound water retention and deteriorate dewatering performance. Neyens and Baeyens reported similar behaviour, attributing poorer dewatering to the formation of a viscous, colloidal phase rich in soluble biopolymers [20]. Likewise, Im et al. (2022) observed decreasing capillary suction time (CST) at 200°C as rotor speed increased [9].

Therefore, while heating alone improves dewaterability by weakening floc structure, combining it with mixing may over-disintegrate the sludge, increasing its water-binding capacity. Further assessment using complementary indices such as CST or specific resistance to filtration (SRF) or pilot-scale dewatering would offer more insight into sludge dewaterability as well as filtrate viscosity. Additional work should use highly thickened sludge (~16% TS) to better translate these laboratory findings to operational conditions, capturing realistic sludge rheology and heat transfer behaviour in full-scale THP application, including the flash phase. Moreover, future studies should quantify the thresholds for mixing intensity, such as varying rpm at operational temperatures between 160-180 °C, the typical temperature range for full-scale THP.

4. Conclusions

Mechanical mixing during THP greatly enhances sludge disintegration and solubilization, but it also presents trade-offs that need careful consideration. In this study, combining mixing and heating resulted in increased release of soluble organic matter and EPS,shown by higher sCOD and a ~1.6-fold increase in disintegration degree over solely heating. These changes led to lower apparent viscosity and smaller particle sizes, which benefited subsequent anaerobic digestion and sludge handling. However, the anticipated improvement in dewaterability was not achieved. In fact, the Mix+Heat treatment slightly reduced dewaterability compared to thermal treatment alone. This is likely due to excessive release of EPS-derived polymers, forming a colloidal phase that traps water. While these lab-scale results indicate a clear trade-off between disintegration and dewaterability, further evaluation at pilot-scale is recommended to validate these trends.

In summary, while mixing can improve THP uniformity, excessive mixing may counteract its benefits. Future research should focus on high-solids sludge (~16% TS) to verify these results under practical feed conditions and investigate different mixing speeds, impeller geometry, or post-THP conditioning (such as polymer dosing) to reduce any negative impacts on dewaterability, mainly after AD. By balancing thermal intensity and mixing energy, THP can be optimized to enhance sludge biodegradability and throughput without harming downstream dewatering.

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