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Ambient temperature alkaline pretreatment for enhanced chemical oxygen demand solubilization and extracellular polymeric substances disruption in waste activated sludge

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ABSTRACT

This study investigated the efficacy of alkaline pretreatment using potassium hydroxide (KOH) as an agent for waste activated sludge (WAS) under ambient conditions to enhance subsequent anaerobic digestion. Gravity-thickened WAS was treated with KOH dosages ranging from 0 to 0.15 g/g volatile solids (VS) for 3 hour at 25 ± 2 °C. Pretreatment rapidly elevated and stabilized sludge pH at approximately 12, inducing extensive disruption of extracellular polymeric substances (EPS) while essentially preserving microbial cell integrity. Soluble chemical oxygen demand (sCOD) increased from 1.42÷1.68 g/L to a maximum of 6.65 g/L, raising the sCOD/TCOD ratio from 14÷16% to 44÷50%. The degree of COD solubilization (DS) reached nearly 50% at the highest dosage, with a strong linear correlation to KOH dose ($R^2 = 0.8$). Concurrently, mixed liquor suspended solids (MLSS) decreased by up to 18.5%, whereas the sludge volume index (SVI_{30}) rose from 88 to 187 mL/g, reflecting significant deflocculation and impaired settleability. Scanning electron microscopy confirmed severe degradation of the EPS matrix and exposure of microbial aggregates. These results demonstrate that KOH is a highly effective, low-energy pretreatment reagent capable of achieving comparable or superior organic solubilization to NaOH, while offering potential advantages in ionic mobility and reduced methanogenic inhibition. Optimal performance was observed at 0.09÷0.12 g KOH/gVS, providing a practical basis for enhancing biogas recovery from municipal WAS.

Keywords: waste activated sludge, alkaline pretreatment, potassium hydroxide, COD solubilization, EPS disruption, anaerobic digestion.

INTRODUCTION

Rapid urbanization and increasingly stringent effluent discharge standards have driven a substantial increase in waste activated sludge (WAS) production from municipal wastewater treatment plants over recent decades. As an unavoidable byproduct of activated sludge processes, WAS poses significant operational, economic, and environmental burdens, accounting for 30÷60% of total plant operating costs while exhibiting high moisture content, limited biodegradability, and pronounced biological stability (Appels *et al.*, 2008; Gherghel *et al.*, 2019). Effective sludge management, therefore, requires advanced pretreatment strategies to disrupt the EPS and microbial cell walls prior to downstream biological treatment, particularly anaerobic digestion. Pretreatment accelerates the rate-limiting hydrolysis step by transferring complex particulate organics – primarily proteins, polysaccharides, and lipids into the soluble phase, thereby (i) enhancing biodegradability, (ii) reducing hydraulic retention time, (iii) minimizing residual solids volume, and (iv) increasing methane yield (Chen *et al.*, 2020; Mitraka *et al.*, 2022).

Previous studies have shown that some pretreatment technologies have been explored, encompassing mechanical and physical approaches (e.g., ultrasound, hydrodynamic cavitation), thermal or microwave processes, chemical oxidation, biological enzymatic methods, and alkaline chemical pretreatment. Among these, alkaline pretreatment has garnered considerable attention due to its robust cell disruption efficacy, markedly enhancing the soluble chemical oxygen demand (sCOD), and favorable cost effectiveness and operational simplicity compared with energy-intensive alternatives. Hybrid configurations, such as thermal alkaline, microwave alkaline, or mechanical alkaline systems, further amplify COD solubilization and biogas production through synergistic effects (EPA, 2001; Muhammad *et al.*, 2016; Li *et al.*, 2017). At pH more than 11, alkaline agents elevate osmotic pressure and induce microbial cell lysis via saponification, deprotonation of functional groups, and neutralization of volatile fatty acids, thereby releasing intracellular proteins and carbohydrates into the aqueous phase (EPA, 2001; Sadia *et al.*, 2025).

Despite extensive research, alkaline pretreatment studies have predominantly focused on NaOH and $Ca(OH)_2$. Comparative analyses reveal that NaOH typically outperforms $Ca(OH)_2$ in terms of hydrolysis efficiency and COD solubilization; however, its higher cost and residual sodium accumulation can inhibit methanogenic archaea during subsequent anaerobic digestion (Sadia *et al.*, 2025). Potassium hydroxide (KOH), another strong monovalent base, offers theoretical advantages - including superior ionic mobility, enhanced cation exchange capacity with EPS bound divalent metals, and reduced risk of sodium induced toxicity yet remains underexplored (Appels *et al.*, 2008; Yongzheng *et al.*, 2024). To date, no systematic investigation has quantitatively delineated the dose response and temporal dynamics of KOH pretreatment on key operational parameters (e.g., mixed liquor suspended solids (MLSS), sCOD, sCOD/TCOD ratio, and sludge volume index (SVI) under ambient conditions, nor has its mechanistic interplay with floc disintegration and solubilization kinetics been rigorously established.

This study addresses these critical knowledge gaps by conducting controlled batch experiments with KOH dosages of 0÷0.15 g/g volatile solids (VS) over 3 hours at ambient temperature (25 ± 2 °C). Through comprehensive monitoring of physicochemical indicators and scanning electron microscopy (SEM), we elucidate the dose dependent relationships governing pH stabilization, EPS degradation, particulate to soluble organic transfer, and settleability deterioration. These novel findings expand the experimental evidence base for KOH mediated pretreatment, clarify its mechanistic superiority over conventional alkalis in select contexts, and provide actionable design parameters to optimize energy recovery and biogas yield from urban WAS in anaerobic digestion systems.

MATERIALS AND METHODS

Source of waste activated sludge

The waste activated sludge (WAS) samples used in this experiment were collected from a municipal wastewater treatment plant in Binh Nguyen commune, Phu Tho province, Vietnam, at coordinates 21°16’50.8’’N and 105°37’55.7’’E. The plant employs a conventional activated sludge process with a nominal design capacity of 5,000 m^3/day, including primary settling, aeration, and secondary clarification units. The WAS were collected directly from the secondary settling tank using specialized sampling buckets at different depths and points to ensure representative data, characterized by a high moisture content (approximately 98÷99 % w/w) and a moderate volatile solids fraction. Subsequently, the raw sludge mixture was gravity-thickened by natural settling for 2÷4 hours, after which the supernatant was carefully decanted to increase the total solids (TS) concentration and minimize the influence of high-water content in the following procedures.

All analyses were performed immediately after sampling whenever possible. When immediate analysis was not feasible, samples were stored at 4 ± 1 °C in sealed containers for up to 72 hours to suppress microbial activity and limit undesirable biochemical transformations. This short-term refrigeration protocol is consistent with established guidelines (Tong *et al.*, 2009; Zhen *et al.*, 2017) and has been demonstrated not to significantly affect key physicochemical properties of WAS, including TS, volatile solids (VS), pH and organic matter composition, for storage durations of up to one week.

Alkaline sludge pretreatment

Alkaline pretreatment was conducted using potassium hydroxide (KOH) under controlled laboratory conditions. Batch experiments were performed in 1-L Erlenmeyer flasks containing 500 mL of gravity thickened waste activated sludge. KOH (analytical grade, more than 85%

purity) was added at dosages of 0 (control), 0.03, 0.06, 0.09, 0.12, and 0.15 g KOH/g VS, corresponding to alkali concentrations of 0÷1.8 M depending on the initial VS content of the sludge (typically 12÷18 g VS/L). Among these, the sludge sample with a KOH dosage of 0 was the blank sample used as a control for comparison with the other samples.

After KOH addition, the pH typically rose to 10.5÷13.5, depending on the dosage. The suspensions were maintained at room temperature (25 ± 2 °C) and continuously mixed using a magnetic stirrer at 40÷50 rpm to ensure homogeneous alkali distribution while minimizing mechanical disruption of sludge flocs and avoiding excessive shear that could independently enhance solubilization.

The pretreatment duration was fixed at 3 hours. To monitor the kinetics of sludge disintegration and organic matter solubilization, 10 mL aliquots were withdrawn at predetermined intervals (0, 0.5, 1.0, 1.5, 2.0, 2.5, and 3.0 hour), immediately centrifuged at 10,000 round per min for 10 min and kept at 4 °C, and the supernatant filtered through 0.45 µm membrane filters prior to analysis of key physicochemical parameters (soluble chemical oxygen demand (SCOD), soluble proteins, carbohydrates, volatile fatty acids, pH, and ammonia nitrogen).

These experimental conditions were specifically selected to systematically investigate the influence of alkali dosage and contact time on the extent of sludge solubilization, hydrolysis efficiency, and subsequent transformation of particulate organic matter, thereby providing quantitative data for performance evaluation and mechanistic understanding of KOH-based pretreatment of waste activated sludge. All experiments were carried out in triplicate.

Analytical methods

Morphological changes in waste activated sludge before and after KOH pretreatment were viewed via scanning electron microscopy - SEM (JSM-6500) (JEOL, Tokyo, Japan). pH was measured immediately after sampling using a calibrated benchtop pH meter (Hanna HI-2211).

The physicochemical parameters included total solids (TS), volatile solids (VS), mixed liquor suspended solids (MLSS), 30 min sludge volume index (SVI30), total COD (tCOD), soluble COD (sCOD), ammonia nitrogen (NH_4^+-N), total nitrogen (TN), and total phosphorus (TP). All of these parameter analyses were performed in triplicate according to Standard Methods for the Examination of Water and Wastewater, 24th edition (American Public Health Association – APHA, 2023) unless otherwise stated.

TS and VS were determined gravimetrically following Methods 2540B and 2540E, respectively, whereas MLSS was quantified according to Method 2540D. The SVI30 was calculated from the settled sludge volume in a 1-L graduated cylinder after 30 min of quiescent settling (Method 2710D) using equation (1):

$$SVI_x\left(\frac{mL}{g}\right) = \frac{settled\ sludge\ volume\ (mL.L^{-1}) \times 1000}{mixed\ liquor\ suspended\ solid\ (mg.L^{-1})} \quad (1)$$

where: x is the corresponding settling time (min), hereby $x = 30$

COD was analyzed by the closed reflux titrimetric method (Method 5220C), whereby tCOD was determined by analyzing the raw sample. The measurements for sCOD, nitrogen, and phosphorus were performed on filtered samples obtained by filtering the raw sample through 0.45 µm cellulose acetate membrane filters. Ammonia nitrogen (NH_4^+-N) was determined by the salicylate hypochlorite method (Method 4500-NH3 F). TN and TP were measured after persulfate digestion, followed by spectrophotometric detection (Methods 4500-N C and 4500-P B.5).

Selected heavy metals (Cu, Zn, Pb, Cd, Cr, Ni) concentrations were quantified by inductively coupled plasma optical emission spectrometry (ICP-OES, PerkinElmer Optima 8300) after microwave-assisted acid digestion (HNO_3/HCl, EPA Method 3015A).

Statistical analysis

The statistical analysis of the variables was conducted in R (R Core Team, 2022), specifically using linear regression to model the influence of alkali dosage on the measured parameters. To ensure data integrity, quality assurance, and quality control protocols, blanks, duplicates, and certified reference materials were incorporated into each analytical batch. All physicochemical parameter results were expressed as mean ± standard deviation. The relative standard deviations were consistently below 5% for SCOD, TS, VS, and heavy metals, and below 2% for pH and SVI_{30}.

RESULTS AND DISCUSSION

Characteristic of the raw activated sludge

The initial physicochemical properties of the waste activated sludge (WAS) are summarized in Table 1. The sludge exhibited a moisture content of 98.1 ± 0.3%, corresponding to a total solids (TS) concentration of 25.63 ± 0.42 g/L. The volatile solids (VS) content was 19.32 ± 0.31 g/L, yielding a VS/TS ratio of 0.75 ± 0.02. This elevated organic fraction is characteristic of biologically active municipal WAS and reflects a predominantly unstable particulate organic matrix (Chen *et al.*, 2020; Yakamercan *et al.*, 2021).

The pH of the raw sludge was 7.2 ± 0.05, within the neutral range that supports microbial viability while minimizing spontaneous anaerobic fermentation during short term refrigerated storage. Mixed liquor suspended solids (MLSS) reached 21.55 ± 0.58 g/L, indicative of high biomass density. The 30 min sludge volume index (SVI_{30}) was 88.12 ± 4.35 mL/g, falling within the desirable operational range of 50–150 mL/g and confirming compact floc morphology with excellent settling properties (Wang *et al.*, 2018).

Total chemical oxygen demand (TCOD) was 10.05 ± 0.38 g/L, whereas soluble COD (SCOD) was only 1.42 ± 0.11 g/L, representing approximately 14.1% of TCOD. The low initial SCOD/TCOD ratio demonstrates that the majority of organic matter (>85%) existed in particulate or intracellular form, rendering it poorly bioavailable under conventional anaerobic conditions - a feature consistently reported for untreated WAS (Bize *et al.*, 2015; Yakamercan *et al.*, 2021). This substantial, recalcitrant organic fraction provides a strong rationale for applying alkaline pretreatment to disrupt microbial cell walls, solubilise high molecular weight biopolymers and significantly enhance subsequent anaerobic biodegradability, and methane yield.

Nutrient profiling revealed that total phosphorus (TP) constituted 3.12 ± 0.18% of TS (equivalent to 799 ± 26 mg/kg TS), while total nitrogen (TN) accounted for 2.74 ± 0.21% of TS (701 ± 14 mg/kg TS). These values are consistent with substantial accumulation of nitrogen and phosphorus within extracellular polymeric substances (EPS) and microbial biomass during biological nutrient removal processes commonly employed in municipal wastewater treatment plants treating predominantly domestic sewage (Siddiqui *et al.*, 2012; Lu *et al.*, 2018). The observed N/P mass ratio of approximately 8.9/1 falls within the typical range reported for activated sludge from urban sources (6/1÷12/1), further confirming the predominantly domestic origin of the influent and limited contribution from industrial discharges.

Heavy metal analysis demonstrated notably low concentrations: Cd 0.12 ± 0.03 mg/L (equivalent to 4.8 mg/kg TS), Zn 0.23 ± 0.05 mg/L (equivalent to 9.2 mg/kg TS), Pb 0.22 ± 0.04 mg/L (equivalent to 8.6 mg/kg TS), Mn 0.14 ± 0.03 mg/L (equivalent to 5.5 mg/kg TS), Cr 0.09 ± 0.02 mg/L and Ni below the detection limit (<0.05 mg/L). Copper was not detected (<0.1 mg/L). All measured concentrations were significantly below the upper limits established by international regulations for agricultural reuse of sewage sludge (e.g., U.S. EPA Part 503, EU Directive 86/278/EEC), and are characteristic of sludge generated from residential catchments with negligible industrial inputs (Lu *et al.*, 2018; Garg *et al.*, 2020).

The characterization of raw waste activated sludge (WAS) reveals a complex interplay of organic and inorganic materials, positioning it as a valuable resource within wastewater management systems. The observed organic content, as indicated by a volatile solid to total solids (VS/TS) ratio of 0.75, indicates significant levels of biodegradable material. High nutrient levels further characterize this WAS, making it conducive for processes such as anaerobic digestion, which is recognized for its capacity to recover energy and nutrients through the generation of biogas and enriched digestate, respectively (Smith *et al.*, 2009; Solé-Bundó *et al.*, 2017). The settling properties inherent to activated sludge contribute to operational efficiencies in wastewater treatment facilities, while the reported low initial degree of solubilization (SCOD/TCOD ≈ 14%) suggests that while the sludge is rich in organic matter, it requires targeted strategies such as pretreatment to optimize methane yield during anaerobic digestion (Moško *et al.*, 2018; Borowski *et al.*, 2023).

The dual potential of WAS is particularly notable; the substantial presence of recalcitrant organic matter, along with both macro and micronutrients, positions it as an ideal candidate for energy recovery and nutrient recovery strategies, including struvite precipitation and soil amendments (Lin *et al.*, 2009; Zhang *et al.*, 2025). However, the challenge remains to increase the solubilization of organic matter and the bioavailability of nutrients, which may be achieved through various pretreatment methods. Importantly, addressing the contamination profile regarding heavy metals in the WAS is essential for its suitability for agricultural applications or as a feedstock for the development of bio based materials, contingent upon the application of effective disinfection and stabilization practices (Smith *et al.*, 2009; Moško *et al.*, 2018).

The findings substantiate the rationale for the investigative focus on alkaline pretreatment as a method to maximize both organic matter solubilization and nutrient release from WAS, while simultaneously ensuring the integrity of its contaminant profile. These findings align with the ongoing shift towards recognizing wastewater treatment facilities as resource recovery centers rather than mere waste disposal sites. Hence, strategic interventions in sludge processing not only enhance energy recovery but also align with sustainable practice methodologies that are essential to modern wastewater management systems.

Table 1. Characteristics of raw WAS samples

Parameters	Unit	Values
pH		7.2
Moisture	%	98.1
TS	g/L	25.63
MLSS	g/L	21.55
SVI_{30}	mL/g	88.12
VS	g/L	19.32
tCOD	g/L	10.05
sCOD	g/L	1.42
TP	%TS	3.12
TN	%TS	2.74
Cd	mg/L	0.12
Zn	mg/L	0.23
Pb	mg/L	0.22
Cu	mg/L	ND
Mn	mg/L	0.14

Evolution of pH during alkaline pretreatment

The addition of KOH resulted in an immediate, dose dependent increase in sludge pH (Figure1). In the control series (0 g KOH/g VS), pH remained stable at 7.18 ± 0.06 throughout the 3-hour reaction period, consistent with the neutral character of the raw WAS. In contrast, KOH amended samples exhibited a rapid rise in pH within the first 5÷10 min, followed by stabilization for the remainder of the pretreatment. At the lowest dosage (0.03 g KOH/g VS), pH reached 11.48 ± 0.11 and showed negligible further variation. Higher dosages of 0.06, 0.09, 0.12, and 0.15 g KOH/g VS elevated the pH to 12.02 ± 0.09, 12.31 ± 0.07, 12.42 ± 0.06, and 12.51 ± 0.05, respectively, with plateauing observed after approximately 15÷20 min of reaction. From 30 min onward, pH fluctuations were minimal (less than 0.1 units), indicating effective establishment of a sustained alkaline environment.

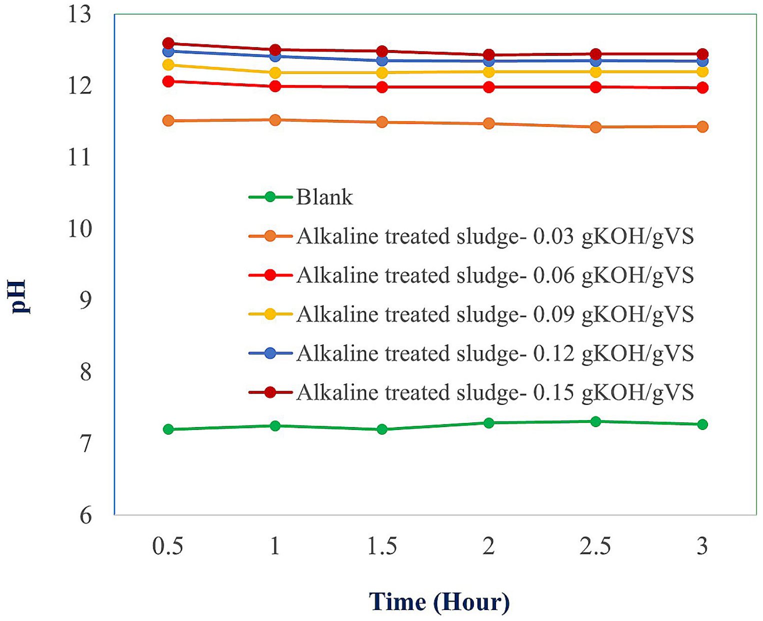

Figure 1. Change in pH during sludge pretreatment with KOH

The pH stabilization observed during stirring at elevated alkalinity levels in WAS indicates the sludge matrix's substantial acid buffering capacity. This phenomenon arises from the interactions among various equilibria, particularly the bicarbonate and carbonate systems, which are crucial for maintaining pH in aqueous environments. The presence of EPS, which can undergo protonation and deprotonation, significantly contributes to this buffering mechanism (Zhang *et al.*, 2010; Wang s *et al.*, 2018). Additionally, the dissolution and precipitation dynamics involving alkaline earth metals such as calcium and magnesium, along with phosphate species present in the sludge, further enhance the buffering capacity (Zhang *et al.*, 2010; Şahinkaya *et al.*, 2013).

The observed rapid consumption of hydroxide ions (OH^-) during the initial stages of alkalization suggests that a portion of these ions is used to neutralize the buffering agents present, leading to a non linear relationship between KOH dosage and the final pH achieved (Lin *et al.*, 2009). This dynamic is essential for establishing a quasi steady state alkaline environment, which is fundamentally critical for effective saponification processes and the solubilization of complex lipidic and proteinaceous materials.

Maintaining a pH more than 12 by applying adequate dosages ($\geq$ 0.09 g KOH/g VS) over a sustained period is crucial. This ensures optimal conditions for the degradation of particulate organic matter while simultaneously reducing the risk of reflocculation or reprecipitation of previously solubilized substances. Such an approach is fundamental for maximizing the efficiency of the pretreatment process (Muhammad *et al.*, 2016). The findings suggest a robust framework for leveraging alkalinity enhanced pretreatment methods to optimize waste sludge management and biogas production.

Morphological changes in waste activated sludge following KOH pretreatment

Structural alterations induced by alkaline pretreatment were evaluated by field emission scanning electron microscopy at 3KV after fixation, dehydration, and gold sputter coating. The untreated sludge (Figure 2a) exhibited a highly compact, integrated floc architecture, characterized by densely entangled fibrous networks of extracellular polymeric substances (EPS) that enmeshed microbial aggregates. Representative micrographs of raw and KOH pretreated sludge (0.12 g KOH/gVS, 3 hours) are presented in Figure 2b. The surface appeared smooth and continuous, with individual bacterial cells largely obscured within the protective polysaccharide protein matrix – a typical morphology of well-settled activated sludge flocs. In stark contrast, KOH pretreated sludge showed extensive disintegration of the floc matrix. The originally continuous EPS layer was severely degraded and fragmented, exposing underlying microbial cells and resulting in a loose, porous, and disintegrated structure. Numerous voids, fissures, and detached fibrous fragments were evident, indicating substantial solubilization and removal of bridging polymeric material. However, the majority of individual microbial cells retained their morphological integrity (rod- and coccus-shaped outlines remained discernible), suggesting that cell lysis was limited under the applied condition.

The observations presented can be understood as a consequence of the selective disruption of the EPS matrix and outer cell membranes under moderate to high pH alkaline pretreatment. Under these conditions, the alkaline environment promotes saponification of lipids, deprotonation of functional groups, and disruption of both hydrogen bonding and electrostatic interactions within the EPS framework. Such a mechanism is consistent with previous findings that underscore the central role of the EPS matrix in maintaining floc structure and integrity, where its disruption leads to deflocculation and enhanced biodegradability (Gayathri *et al.*, 2015).

Furthermore, the rapid increase in pH upon the addition of KOH enhances the OH^- mediated hydrolysis of glycosidic and peptide bonds within the EPS. This promotes the cleavage of structural polysaccharides and proteins, a process supported by studies demonstrating that alkaline conditions can lower the activation energy for bond hydrolysis in similar chemical systems (Ivleva *et al.*, 2010). Concurrently, the substitution of K^+ ions for bridging divalent cations (such as Ca^{2+} and Mg^{2+}) further destabilizes the tertiary network of the flocs by disrupting the ionic bridges that reinforce the EPS structure (Li *et al.*, 2017). Evidence from studies on EPS interactions within microbial biofilms suggests that cation exchange can critically modify the physical cohesion of these matrices, although the specific impacts can vary depending on the nature of the microorganisms and environmental conditions (Gayathri *et al.*, 2015).

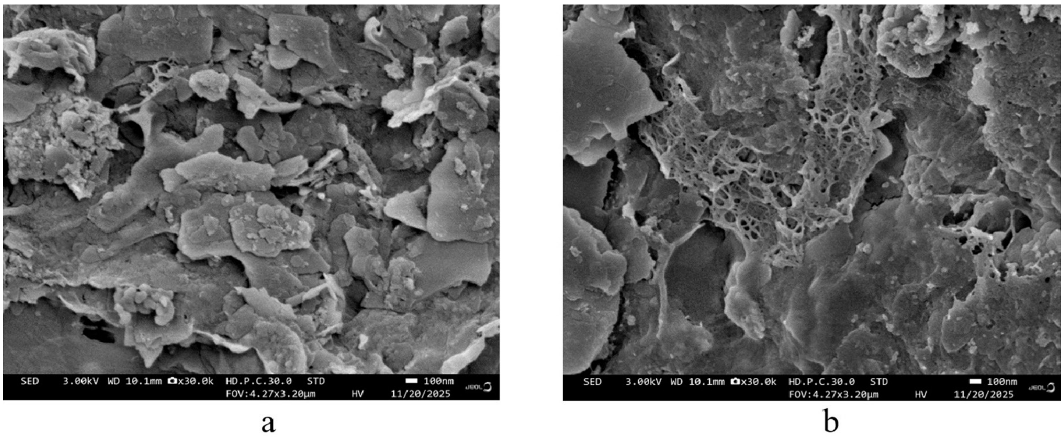

Figure 2. SEM image of raw sludge (a) and SEM image of alkaline treated sludge (b)

The preferential degradation of the EPS scaffold, rather than widespread cell lysis, explains the marked increase in soluble organic fractions, as measured by SCOD/TCOD ratios exceeding 80%, while avoiding the release of intracellular recalcitrant substances or toxic ammonia levels. This targeted pretreatment approach not only dismantles the physical and biochemical barriers that limit hydrolytic access in raw waste-activated sludge (WAS) but also preserves overall microbial viability – an outcome crucial for sustaining subsequent anaerobic digestion processes (Appels *et al.*, 2008; Tyagi *et al.*, 2013; Zhen *et al.*, 2017). The morphological and physicochemical evidence collectively corroborate that KOH pretreatment effectively enhances substrate bioavailability by facilitating the breakdown of structural EPS components without compromising microbial cell integrity.

Changes in MLSS and SVI during alkaline pretreatment

The effects of KOH dosage on mixed liquor suspended solids (MLSS) and 30 min sludge volume index (SVI_{30}) are presented in Figure 3. An inverse relationship between these two parameters was observed as alkali dosage increased. MLSS decreased progressively from 21.55 ± 0.58 g/L in the untreated control to 20.41, 19.67, 18.73, 17.92, and 17.56 ± 0.61 g/L at KOH dosages of 0.03, 0.06, 0.09, 0.12, and 0.15 g/g VS, respectively. This corresponds to a maximum reduction of 18.5% at the highest dosage. The decline in MLSS reflects partial solubilization of particulate organic matter and disintegration of the floc matrix, whereby EPS bound and loosely associated solids are transferred into the soluble phase through alkaline hydrolysis and deflocculation (Cassini *et al.*, 2006).

Conversely, SVI_{30} increased markedly from 90.2 ± 4.1 mL/g (raw sludge) to 81.1, 107.3, 150.4, 179.2, and 187.4 ± 6.8 mL/g at the exact incremental KOH dosages. Values exceeding 150 mL/g at dosages ≥ 0.09 g/g VS indicate a transition from good settling characteristics to moderately poor, bulky sludge behavior. The sharp deterioration in settleability is attributed primarily to the disruption of divalent cation bridging (Ca^{2+}, Mg^{2+}) within the EPS network and ionization of functional groups (carboxyl, hydroxyl, and amino groups) at high pH, resulting in electrostatic repulsion among negatively charged floc fragments and dispersion into finer, less dense colloidal particles (Wilén *et al.*, 2003).

A notable rheological change was also observed: at KOH dosages ≥ 0.12 g/g VS, the sludge suspension's apparent viscosity increased substantially, with mixtures exhibiting a gel like consistency and significantly reduced fluidity. This phenomenon is ascribed to the massive release of high molecular weight biopolymers predominantly proteins and polysaccharides from the disintegrated EPS and partially lysed cells into the aqueous phase. The resulting polymer-rich liquid phase imparts pronounced non-Newtonian behavior and hinders gravitational settling of residual particulates (Sheng *et al.*, 2010).

The combined effects of reduced MLSS, elevated SVI_{30}, and increased viscosity highlight a classic trade off in alkaline pretreatment. While high KOH dosages maximize organic matter solubilization and subsequent methane potential, they simultaneously impair sludge dewaterability and

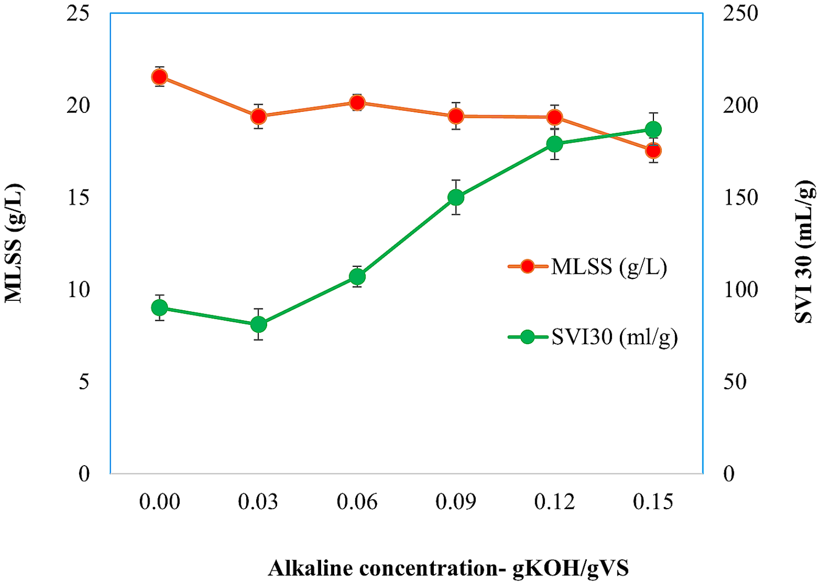

Figure 3. Changes of MLSS and SVI during sludge pretreatment with KOH

settleability. These findings are consistent with previous reports using NaOH or KOH, where SVI increases from 70÷100 mL/g to 150÷250 mL/g are commonly observed at pH > 12 (Tyagi *et al.*, 2013). Consequently, operational strategies for full scale implementation may require downstream solid liquid separation aids (e.g., polymers, centrifugation, or combined thermal alkaline processes) to mitigate the adverse impacts of high dose alkaline pretreatment on settling and dewatering performance.

COD solubilization

The results illustrated in Figures 4 and 5 demonstrate a pronounced increase in soluble chemical oxygen demand (sCOD) and the sCOD/tCOD ratio as functions of both pretreatment duration and KOH dosage, thereby confirming the substantial solubilization efficacy of alkaline pretreatment on waste activated sludge. In the control series (0 g KOH/gVS), sCOD exhibited only marginal variation, rising from 1.68 g/L to 1.81 g/L over the initial 3 hours, corresponding to sCOD/tCOD ratios of 15.6÷17.0%. This limited release indicates that the majority of organic matter remained entrapped within particulate fractions or intracellular compartments, rendering it inaccessible primarily to hydrolytic microorganisms. In marked contrast, KOH doses ≥ 0.09 g/gVS induced rapid solubilization, with sCOD increasing sharply within the first 30 min and attaining 5.75÷6.65 g/L after 3 hours – a 3÷4-fold augmentation relative to the initial value. The sCOD/tCOD ratio reached a maximum of 42.2%, with solubilization kinetics revealing near-saturation after 2.5÷3 hours of contact time.

The underlying mechanism is primarily attributable to the extreme alkaline environment (pH ≈ 12), which disrupts the structural integrity of EPS and microbial cell membranes, thereby liberating high molecular weight biopolymers (proteins, polysaccharides, and humic substances) from the solid to the aqueous phase (Muhammad *et al.*, 2016). Beyond the direct action of hydroxide ions, the monovalent K^+ cation contributes significantly through its high diffusivity and favorable ion exchange interactions with negatively charged functional groups on the sludge floc surface, accelerating deflocculation (Faris *et al.*, 2024). Compared with NaOH and $Ca(OH)_2$, KOH offers comparable or superior solubilization performance while maintaining greater pH stability and potentially lower alkali requirements owing to the enhanced ionic mobility of potassium (Li *et al.*, 2017; Zhen *et al.*, 2017).

The concomitant decline in mixed liquor suspended solids (MLSS) and elevation of sludge volume index (SVI) reported in preceding sections corroborate structural disintegration and the transfer of particulate organics into the soluble phase. This integrated response underscores the mechanistic linkage: intensified solubilization corresponds directly to deteriorated settleability, yet is accompanied by markedly enhanced hydrolytic accessibility. An sCOD/tCOD ratio exceeding 40% is widely recognized in the literature as

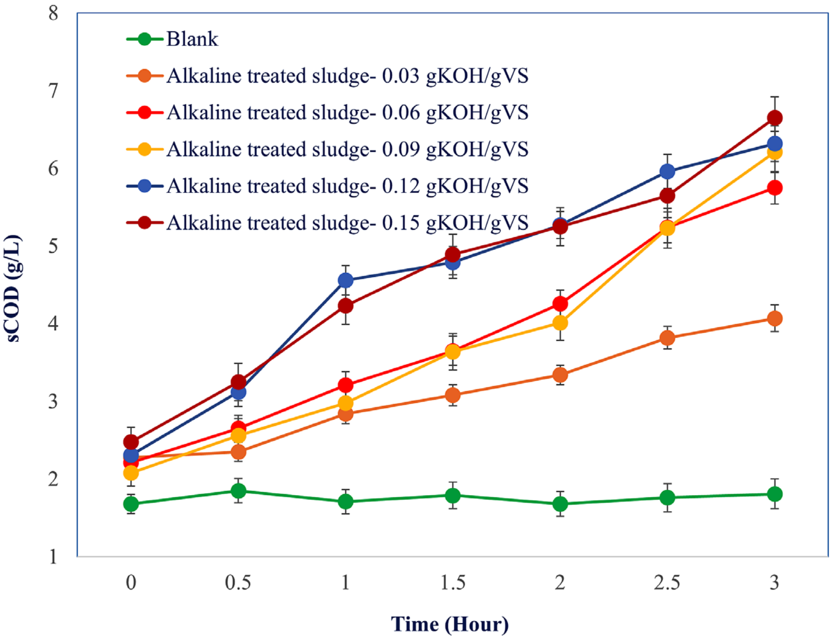

Figure 4. Change of sCOD during sludge pretreatment with KOH

a critical threshold for substantially accelerating the rate limiting hydrolysis step and maximizing methanogenic potential during subsequent anaerobic digestion (Appels *et al.*, 2008; Zhen *et al.*, 2017). Consequently, the present findings substantiate the technical viability of KOH as a highly effective pretreatment reagent for waste activated sludge, particularly in process configurations targeting optimized energy recovery and volumetric methane productivity.

Soluble COD (sCOD) and its relationship with KOH dosage

The degree of solubilization (DS) denotes the fraction of solid COD that is transformed into its soluble form during the pretreatment stage. It serves as an indicator of sludge structure breakdown and the conversion of insoluble organic matter to a dissolved state (Hu *et al.*, 2025). Unlike the simple ratio of soluble COD (sCOD) to total COD (tCOD), DS provides a more direct measure of the effectiveness in converting polymeric and cell bound organic constituents into forms that can be readily hydrolysed (Penghe *et al.*, 2020). Prior research has suggested that an alkaline pretreatment lasting 2÷3 hours can increase the sCOD/tCOD ratio by approximately 40%, thereby enhancing hydrolysis during anaerobic degradation (Takashima *et al.*, 2008).

Experimental results indicate that DS increases significantly with increasing KOH dosage. In the absence of KOH (0 g KOH/g VS), DS registered only about 1.4%, indicating most of the COD remains in the solid phase. With an application of 0.03 g KOH/g VS, DS increased to 21.57%, and further increasing the dosage to 0.06 g KOH/g VS resulted in a DS of 42.29% (Table 2). At dosages between 0.09÷0.15 g KOH/g VS, DS reached approximately 49÷50%, demonstrating that nearly half of the solid COD was solubilized after three hours of pretreatment. Such an increase underscores the influence of KOH concentration on the solubilization process (Ahn *et al.*, 2022).

The linear equation modelled the relationship between DS and KOH dosage: DS (%) = 319.4 × (KOH g/g VS) + 11.77, with an R^2 of 0.8 (Figure 6). This suggests that around 80% of the variation in solubilization efficiency can be attributed to changes in the alkaline concentration. Given the inherent heterogeneity of activated sludge and the influence of multiple physicochemical and biological factors (such as the EPS degradation and cell wall disruption), an R^2 value exceeding 0.8 is considered acceptable in investigations of sludge pretreatment. Furthermore, the 95% confidence band around the regression line indicates certainty in the mean estimate. In comparison, the broader 95% prediction interval confirms that all experimental points fall within the predicted range, validating the linear model within the experimental domain. Notably, the observed saturation in DS at dosages above 0.09 g KOH/g VS suggests that increasing the alkaline dosage

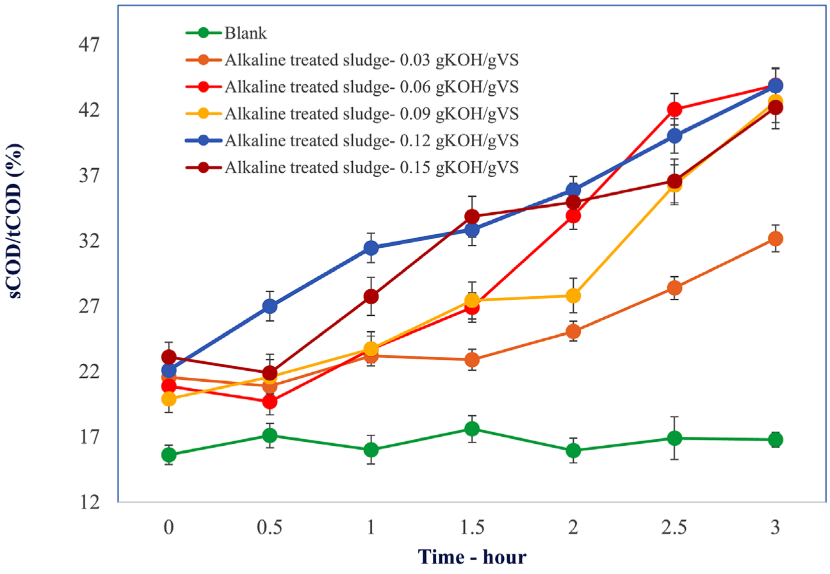

Figure 5. Comparison of sCOD to tCOD

Table 2. Relationship between KOH dosage and the resulting soluble sCOD

KOH (g KOH/g VS)	$sCOD_0$ (g/L)	$sCOD_3h$ (g/L)	$tCOD_0$ (g/L)	DS (%)
0	1.68	1.81	10.75	1.43
0.03	2.28	4.07	10.58	21.57
0.06	2.21	5.75	10.58	42.29
0.09	2.08	6.21	10.45	49.34
0.12	2.31	6.32	10.45	49.26
0.15	2.48	6.65	10.74	50.48

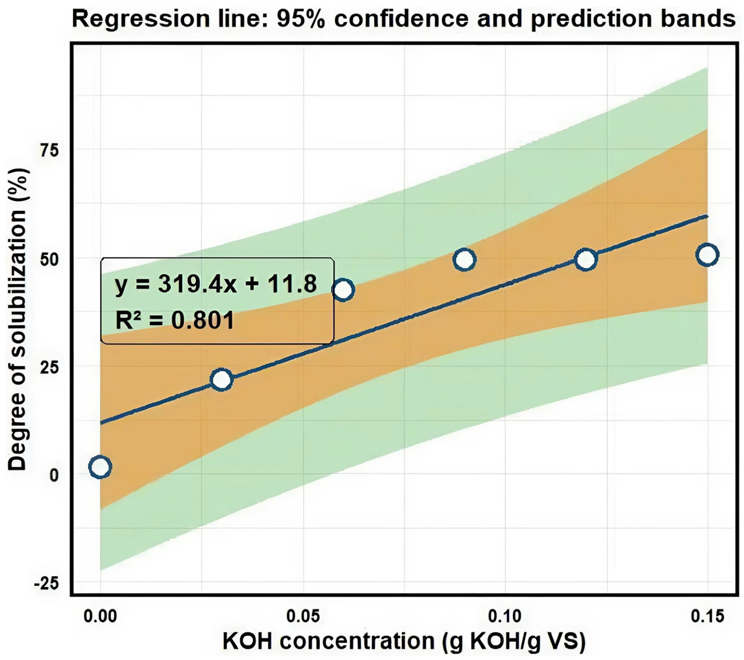

Figure 6. Relationship between KOH dosage (g KOH/g VS) and the degree of COD solubilization (DS%) after alkaline pretreatment

beyond this point yields diminishing returns in solubilization and may entail greater chemical costs and additional post-treatment neutralization requirements (Ahn *et al.,* 2022).

Therefore, these findings collectively support the conclusion that KOH dosing effectively enhances the solubilization of COD in activated sludge. The quantitatively defined relationship emphasises the primary role of alkaline concentration in the solubilization process, aiding in selecting an optimal dosage that balances treatment efficiency with cost considerations.

CONCLUSIONS

This study systematically demonstrates that potassium hydroxide (KOH) pretreatment at ambient temperature is a highly effective strategy for disintegrating waste activated sludge (WAS) and enhancing its biodegradability prior to anaerobic digestion. KOH dosages of 0.09÷0.15 g/gVS rapidly established a stable alkaline environment (pH ≈ 12), leading to pronounced EPS degradation and transfer of particulate organic matter into the soluble phase. Key outcomes included a 3÷4 fold increase in sCOD (up to 6.65 g/L), elevation of the sCOD/TCOD ratio to 44÷50%, and a degree of COD solubilization approaching 50%, with solubilization efficiency exhibiting a strong linear dependence on KOH dosage (DS (%) = 319.4 × KOH + 11.77, $R^2 = 0.8$). Morphological evidence from SEM revealed extensive fragmentation of the floc structure and exposure of microbial cells without widespread lysis, confirming that disintegration primarily targets the EPS matrix. Although high dosages markedly deteriorated settleability ($SVI_{30} > 180$ mL/g) and reduced MLSS by up to 18.5%, these changes are consistent with effective deflocculation. They can be reversed by subsequent neutralization or by using solid liquid separation aids.

Compared to the more commonly studied NaOH and $Ca(OH)_2$, KOH exhibited comparable or superior solubilization performance under ambient conditions, with potential benefits arising from its higher K^+ diffusivity and lower risk of sodium-related inhibition of methanogens. An optimal dosage range of 0.09÷0.12 g KOH/g VS is recommended to maximize organic release while limiting excessive chemical consumption and settleability impairment. These findings fill a critical knowledge gap regarding KOH based pretreatment and provide robust technical evidence for its implementation as a cost effectiveness, single step process to significantly improve methane yield and volumetric productivity in the anaerobic digestion of municipal WAS.

The use of KOH as a pretreatment method for WAS offers potential benefits for the quality of the anaerobic digestate as well. Specifically, the digestate contains a high potassium content – one of the three primary nutrients that improve agricultural soil health (N-P-K). Some studies show that K+ helps maintain the morphology and vitality of microorganisms under high salinity conditions, and its presence can antagonize and reduce the toxicity of other ions, such as ammonia.

If the use of KOH as a pretreatment ensures that the AD digestate is of sufficient quality to be used as a soil conditioner, the wastewater treatment plant will save on landfill costs. Thus, KOH offers an opportunity to transform sludge from a cost burden into a valuable agricultural resource. However, scaling up this process to industrial application requires the proper management mindset to consider wastewater treatment plants beyond just waste treatment facilities, but water resource recovery, and applied to specific cases such as:

- Plants located in areas with strict regulations on agricultural soil salinity or prohibitions on landfilling organic waste.
- Projects aiming for a circular economy model, where nutrient (potassium) recovery is prioritized alongside energy recovery.
- Systems that are already overloaded with sodium need to minimize the risk of biological inhibition.

The rate of dissolution of organic matter with KOH increases sharply with temperature, following the Arrhenius equation. Therefore, on an industrial scale, the combination of heat and alkali (thermo-alkaline) is a trend to reduce chemical dosage and retention time, while optimizing digestate quality to meet the requirements for direct soil conditioners for crops.

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