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Staged modulation using synergistic alkaline biochar-ferrate enhances medium-chain fatty acid production from waste activated sludge

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Abstract

Medium-chain fatty acids (MCFAs) are carbon-neutral alternative to petroleum-derived chemicals, offering sustainable valorization for waste activated sludge. Current bioproduction systems, however, face a critical dual bottleneck stemming from complex sludge matrices and inefficient carbon flux regulation. Here, we develop a stage-optimized modulation strategy employing alkaline biochar (AlkBC)-ferrate to sequentially enhance sludge solubilization and targeted MCFA-producing fermentation. Maximum MCFA production reaches 10495.0 mg chemical oxygen demand L^{-1}, 20.6-, 15.2- and 2.3-fold higher than the control, AlkBC-alone and ferrate-alone groups, respectively. Mechanistically, AlkBC initiates ferrate activation to produce metastable Fe(IV)/Fe(V) intermediates via physical adsorption, electron donation, and oxygen-functionalized coordination. High-valent Fe species oxidation coupled with AlkBC-elevated alkalinity efficiently disrupts sludge polymerized structure and drives bioconversion of released organics. Simultaneously, AlkBC is structurally reconfigured due to ferrate oxidation with surface-loaded Fe_2O_3 as active component, substantially enhancing chain elongation. Furthermore, AlkBC-ferrate enriches key functional bacteria while suppressing methanogens, steering carbon flux towards MCFA production.

Keywords

Medium-chain fatty acids (MCFAs); Ferrate; Alkaline biochar (AlkBC); Chain elongation; Fe(IV)/Fe(V)

Introduction

The inevitable generation of waste activated sludge (WAS) from wastewater treatment imposes escalating environmental burdens[1,2]. To address this challenge, an emerging strategy integrating anaerobic fermentation with chain elongation has been proposed, efficiently converting sludge organics into versatile, high-value medium-chain fatty acids (MCFAs, C6–C12 monocarboxylic acids) for sustainable waste valorization[3,4]. Crucially, MCFA biosynthesis contributes to CO_2 mitigation, as approximately 30% of the carbon in chain-elongating biomass derives from CO_2 fixation[5,6]. Nevertheless, WAS-derived MCFA production remains constrained by inefficient sludge degradation, suboptimal electron transfer, and competing metabolic pathways[7].

Ferrate ($Fe^{VI}O_4^{2-}$), a multifunctional strong oxidant, combines chemical oxidation, coagulation, and disinfection capabilities[8]. It plays an important role in organic pollutant removal, pathogen inactivation, and sludge treatment[9,10]. The reactive Fe species in ferrate-based systems include Fe(VI), Fe(IV), and Fe(V)[11]. Among these, Fe(IV) (E^0=1.0−1.4 V) and Fe(V) (E^0=1.7 V) exhibit substantially higher reactivity than Fe(VI) (E^0=0.7 V) under alkaline conditions[12]. Our prior work revealed that ferrate pretreatment substantially improved sludge solubilization and organic matter release, achieving maximum MCFA yield of 8106.3 mg chemical oxygen demand (COD) L^{-1}[13]. Notably, iron oxides (e.g., Fe_2O_3) from ferrate reduction could serve as effective electron conduits during anaerobic sludge fermentation[14-16]. However, rapid self-decay of ferrate in aqueous systems reduces high-valent Fe species availability, diminishing

its oxidative efficiency towards WAS organic components and increasing operational costs[17,18].

Biochar (BC) is a conductive carbon material produced through high-temperature carbonization of organic waste[19]. Its aromatic functional groups (e.g., quinone, phenolic and hydroquinone moieties) exhibit redox activity, enabling participation in advanced oxidation processes and mediation of extracellular electron transfer[20,21]. BC addition substantially enhances MCFA production from WAS alkaline fermentation liquor or raw sludge by improving electron transfer efficiency[22,23]. Notably, the pH of BC depends on feedstock origin, with some types like banana peel-derived BC exhibiting alkaline properties[24]. Although the inherent alkalinity of alkaline BC (AlkBC) benefits WAS solubilization and hydrolysis, it requires high doses (15−25 g L^{-1}) to attain considerable organic release during pretreatment[25]. To reduce dosage requirements of sole pretreatment and mitigate high-valent Fe species self-decomposition, we propose integrating AlkBC with ferrate for WAS pretreatment. This integrated strategy shows potential for enabling stage-optimized modulation of sludge-to-MCFA conversion. Specifically, AlkBC would enhance ferrate pretreatment for sludge disintegration, with the resulting AlkBC-Fe(III) composites serving as highly efficient electron conduits during fermentation.

This study systematically evaluated the efficacy of AlkBC-ferrate in enhancing MCFA production and suppressing competitive pathways through comprehensive analysis of gas and liquid phase products. Mechanistic investigations were conducted on the two-stage process, namely sludge pretreatment and targeted MCFA-producing

fermentation. First, the effects of AlkBC-ferrate on sludge polymerization structures and organics release were elucidated, identifying the key drivers behind its role in promoting chemical decomposition and bioconversion of macromolecular organics. Second, ferrate modification of AlkBC was confirmed by characterizing functional group alterations, surface iron oxide composition, and Fe(III)-AlkBC composite microstructure, with subsequent assessment of the modified material's impact on chain elongation. Furthermore, microbial communities were analyzed using 16S rRNA high-throughput sequencing to elucidate modulation of microbial composition and abundances by AlkBC-ferrate.

Methods

Sources of WAS, inoculum and AlkBC

The WAS was sourced from a secondary sedimentation tank in Tianjin, with its main properties listed in Supplementary Table 1. The inoculum was cultured using WAS as substrate and ethanol as electron donor (Supplementary Table 1). AlkBC was prepared by carbonizing banana peel at high temperature, with the steps detailed in Supplementary Text 1. The recovery efficiency of AlkBC was 33.7%. The pH of its aqueous suspension (5 g L^{-1}) was 10.22 ± 0.13, determined using a calibrated pH meter.

AlkBC-ferrate pretreatment and MCFA production

Batch experiments were conducted in 300 mL bioreactors (V=300 mL), designated as control, AlkBC, ferrate or AlkBC-ferrate, and each was loaded with 150 mL WAS. The experimental groups were supplemented with 5 g L^{-1} AlkBC, 0.15 g potassium ferrate per g total suspended solids (TSS), or a combination of both (5 g L^{-1} AlkBC +

0.15 g potassium ferrate per g TSS). These dosages were notably lower than those reported in previous studies[13,18,22]. All reactors were incubated at medium temperature with reciprocal shaking (120 rpm) for 2 days to facilitate sludge solubilization and biochemical conversion of organic matter. Following pretreatment, the sludge pH was adjusted to 6.5 ± 0.1 before sequential addition of 15 mL inoculum and 160 mM ethanol. Prior to ethanol supplementation, the reactors were purged with N_2 to maintain anaerobic conditions. Finally, the sealed reactors were transferred to a temperature-controlled incubator (35 °C, 120 rpm) for MCFA production via anaerobic fermentation. All experiments were conducted in triplicate.

Impact identification of AlkBC-ferrate on WAS dissolution and organics conversion

To elucidate the enhancement effect of AlkBC in ferrate pretreatment, the dissolution and conversion of solid organic matter in sludge was firstly investigated. At 2, 6, 12, 24 and 48 hours during the pretreatment, liquid samples were collected from the control, AlkBC, ferrate and AlkBC-ferrate reactors, and concentrations of dissolved substrates and hydrolysis-acidification products were detected. To recognize the influence of AlkBC-ferrate on sludge polymerization structures, the bound extracellular polymeric substances (EPS), i.e., tightly-bound EPS and loosely-bound EPS, were obtained by a heat-extracted method[26]. The release and decomposition of extracellular macromolecular organic matter were evaluated by analyzing changes in functional group structures and proteinic secondary structures in the WAS supernatant[27]. The ability of hydrolytic bacteria to degrade released organics was examined by hydrolase

activities[28]. Further, the potential action of AlkBC on biotransformation of dissolved macromolecular organics in the AlkBC-ferrate system was assessed by synthetic wastewater experiments[4,29], as detailed in Supplementary Text 2.

Elucidating the mechanism of AlkBC enhancing ferrate pretreatment

After ferrate and AlkBC addition, the pH value of WAS increased immediately, and then gradually decreased (Supplementary Fig. 4a). The maximum pH values reached 9.35 in the ferrate reactor and 9.75 in the AlkBC-ferrate reactor. To elucidate the mechanism of AlkBC-enhanced ferrate pretreatment, the individual contributions of alkaline condition and oxidation to sludge dissolution were quantified. Specifically, eight groups were set up in this experiment, i.e., control, pH=9.35, pH=9.75, AlkBC, ferrate, AlkBC-ferrate, ferrate (60 min) and AlkBC-ferrate (60 min). Among them, pH=9.35 or pH=9.75 were respectively aimed to describe the alkaline effect of ferrate or AlkBC-ferrate. While ferrate (60 min) or AlkBC-ferrate (60 min) indicated that ferrate or AlkBC-ferrate oxidized WAS for 60 min, and then the pH value was adjusted to neutral. Accordingly, the activation of AlkBC on ferrate reaction could be assessed by organics release from WAS after pretreatment. Additionally, Fe(VI), Fe(V) and Fe(IV) were measured to further clarify the role of AlkBC in ferrate oxidation.

Revealing the variations in physicochemical properties of AlkBC and potential effect of ferrate-modified AlkBC on chain elongation biostep

Ferrate oxidation may alter the surface functional groups and characteristics of AlkBC, such as redox capacity and adsorption property. Besides, ferrate-reduced particles (i.e., iron (hydr)oxide) would be loaded onto the AlkBC surface to reduce the

specific surface area and increase electron transfer activity. After AlkBC-ferrate pretreatment, ferrate-modified AlkBC will enter the sludge fermentation system, posing a potential impact on chain elongation (i.e., the essential bioprocess for MCFA generation). Consequently, the physicochemical properties of raw AlkBC and Fe(VI)-modified AlkBC were investigated by scanning electron microscopy, X-ray photoelectron spectroscopy and Fourier-transform infrared spectroscopy. The chain elongation experiment was conducted in six bioreactors, including control, raw AlkBC, ferrate-reduced particles, ferrate-modified AlkBC, $Fe(OH)_3$, raw AlkBC-$Fe(OH)_3$. The preparation of ferrate-reduced particles and ferrate-modified AlkBC, and details of chain elongation experiment were described in Supplementary Text 3.

16S rRNA high-throughput sequencing

The alterations in bacterial and archaeal communities induced by AlkBC-ferrate were elucidated through 16S rRNA high-throughput sequencing. At the end of anaerobic fermentation (Section of *AlkBC-ferrate pretreatment and MCFA production*), 10 mL sludge mixture was separately extracted from the control, AlkBC, ferrate and AlkBC-ferrate reactors. DNA was extracted from the sludge samples using the FastPure Soil DNA Isolation Kit (MJYH, shanghai, China), and qualified by 1.0% agarose gel electrophoresis. The hypervariable region V4 of microbial 16S rRNA gene were amplified with forward primer 515FmodF (GTGYCAGCMGCCGCGGTAA) and reverse primer 806RmodR (GGACTACNVGGGTWTCTAAT) by Applied Biosystems GeneAmp® 9700 PCR system. After purification, the amplification products were used for library construction and sequenced on the Illumina Nextseq 2000 platform at Major

Bio. (Shanghai, China). Operational taxonomic units were clustered from the optimized sequences using UPARSE (version 7.1) at a 97% similarity threshold. The representative sequences of operational taxonomic units were taxonomically classified using the RDP classifier (version 2.11) against the 16S rRNA gene database (SILVA, version 138) with a confidence threshold of 70%. The raw sequencing data have been deposited in the Sequence Read Archive database at NCBI under accession number PRJNA1346931.

Analytical methods

Volatile/total (suspended) solids and ammonia nitrogen were measured by the standard methods[30]. Proteins, carbohydrates and COD were respectively quantified using the Lowry-Folin method, Anthrone-H_2SO_4 method and HACH test kits[31,32]. The functional groups of AlkBC and released organics were characterized by Fourier-transform infrared spectrometer (Nicolet iS5)[22]. The chemical nature of C and Fe on AlkBC was examined by X-ray photoelectron spectroscopy (Thermo Scientific Escalab 250Xi)[33]. The microscopic morphology of raw AlkBC and ferrate-modified AlkBC, and interactions of microorganisms-iron oxides-biochar were analyzed by scanning electron microscopy[34]. Electron transport system activity was assessed following previous methodology[35]. Fe(VI), Fe(V) and Fe(IV) in the AlkBC-ferrate oxidation process were measured by ultraviolet-visible spectroscopy[36,37]. MCFAs, short-chain fatty acids (SCFAs) and alcohols were detected by a gas chromatograph (GC-2014, SHIMADZU). The volumes and contents of gas phase products (i.e., hydrogen and methane) were measured using the drainage method and a gas chromatography (SP-7890 Plus, Lunan),

respectively.

Results

Performance of MCFA biosynthesis from WAS fermentation driven by AlkBC-ferrate

The MCFA concentration gradually increases with fermentation time and almost reaches a stable state after 12 days (Fig. 1a). The maximum MCFA production in the control and AlkBC reactors is only 486.3 and 647.7 mg COD L^{-1}, respectively. This contrasts with a previous study reporting 4800−5700 mg COD L^{-1} of MCFA production from WAS alkaline fermentation liquor with BC addition[22]. The low MCFA output in the control and AlkBC groups may be attributed to insufficient electron acceptors directly available for chain elongation reactions, as evidenced by limited increase in soluble chemical oxygen demand (SCOD) after fermentation (Supplementary Fig. 1a). Also, potentially competing microorganisms may occupy higher ecological niches in these two reactors, rendering chain elongation bacteria at a competitive disadvantage. Methanogens are the main competitor for MCFA production via open-culture fermentation, and would cause electron acceptor (i.e., SCFA) depletion or hydrogen partial pressure reduction, posing adverse impacts on chain elongation step[7]. Accordingly, the roles of AlkBC-ferrate in controlling methanogenesis and improving elongating bacterial activity could be respectively reflected by methane and hydrogen production in Fig. 1b and Supplementary Fig. 1b. After ferrate pretreatment, MCFA production increases to 3212.2 mg COD L^{-1}, similar to our previous study[13]. However, AlkBC-ferrate pretreatment results in a dramatic increase in MCFA production, which

212 eventually reaches 10495.0 mg COD/L, a 20.6-, 15.2- and 2.3-fold enhancement over
213 the control, AlkBC alone and ferrate alone, respectively.

214 As shown in Fig. 1c, butyric acid is the primary product in the control or single-
215 treatment groups, while caproic acid dominates the liquid-phase products in the AlkBC-
216 ferrate group. Specifically, MCFA proportion for the control, AlkBC and ferrate is
217 respectively 3.7%, 4.7% and 19.7%, whereas it increases markedly to 62.8% by AlkBC-
218 ferrate. It indicates that AlkBC-ferrate can lead to a higher conversion preference
219 towards MCFAs, consistent with the increased conversion efficiency in Supplementary
220 Fig. 1a. Long-chain alcohols (i.e., butanol and hexanol) are major byproducts of chain
221 elongation reactions. They may originate from the reduction of chain elongation
222 products or intermediates, such as butyryl/caproyl coenzyme A and butyric/caproic acid,
223 leading to waste of electron donors[38]. In Fig. 1d, maximum concentration of long-chain
224 alcohols for the control reaches 5229.6 mg COD L^{-1}, while it reduces to 4785.5, 3485.7
225 and 1160.6 mg COD L^{-1} at AlkBC, ferrate and AlkBC-ferrate, respectively, implying
226 increased inhibitions of the microorganisms producing these alcohols with pretreatment
227 intensity. These findings collectively enclose that AlkBC-ferrate efficiently suppresses
228 the competitive metabolisms against chain elongation process, facilitates conversion of
229 more substrates to MCFAs, and saves the cost for methanogen inhibitor dose.

230 **Fig. 1**

231 **Phase transfer and bioavailability evolution of particulate organic matter**
232 **triggered by AlkBC-ferrate pretreatment**

233 EPS, formed by microbial growth, maintains the mechanical stability of sludge

234 flocs and impede the leaching of intracellular organic matter[39]. Thereby, disruption of
235 sludge polymeric structure is an indispensable step to promote solid-phase organics
236 release, sludge degradation and electron acceptor generation[40]. As shown in Fig. 2a,
237 both ferrate and AlkBC-ferrate increase the proteins and polysaccharides contents in
238 loosely-bound EPS, whereas reduce these organics in tightly-bound EPS compared to
239 control ($p<0.05$). For example, the protein concentration of tightly-bound EPS fraction
240 is 326.6, 353.3, 254.1 and 267.1 mg COD L^{-1} in the control, AlkBC, ferrate and AlkBC-
241 ferrate groups, respectively. The concentrations of proteins and polysaccharides in the
242 tightly-bound EPS fraction could not be further reduced by AlkBC-ferrate, compared
243 to the ferrate-only group. This may be explained by AlkBC promoting EPS secretion
244 though functioning as an electron conduit in anaerobic environments.

245 Accordingly, the concentrations of proteins, carbohydrates and SCOD in the
246 sludge supernatant increase notably with pretreatment intensity (namely, AlkBC-
247 ferrate > ferrate > AlkBC > control) within the initial 12 hours, and then tend to stabilize
248 or decrease (Fig. 2b-2d). This indicates that the extension of pretreatment time to 48
249 hours benefits hydrolysis and acidification of released organics. Specially, SCOD
250 content attains 5450 mg COD L^{-1} after AlkBC-ferrate pretreatment, elevating by
251 564.6%, 249.4%, 36.3% than the control, AlkBC and ferrate, respectively. Additionally,
252 the total SCOD of extracellular organics in the AlkBC-ferrate group is considerably
253 higher than that of the control, AlkBC or ferrate (Fig. 2d), implying more severe cell
254 leakage induced by the combination technique. These results prove that AlkBC-ferrate
255 further accelerates sludge floc disruption and particulate organic matter release,

256 providing abundant soluble organic components for subsequent hydrolysis,
257 acidification and chain elongation processes.

258 **Fig. 2.**

259 Proteins are the main organic components in sludge and their biodegradability is
260 affected by the spatial structure[41]. To elucidate the structural characteristics of released
261 proteins under different pretreatment conditions, the amide I region (1600-1700 cm^{-1})
262 in Fourier-transform infrared spectra (Supplementary Fig. 2a) was subjected to
263 deconvolution and second-order derivative fitting[42]. It could be divided into 6 peaks,
264 including aggregated strands (peak 1), β-sheet (peak 2), random coils (peak 3), α-helix
265 (peak 4), 3-turn helix (peak 5) and antiparallel β-sheet/aggregated strands (peak 6) (Fig.
266 2e). It has been indicated that α-helix and β-sheet favor sludge flocculation[43], and a low
267 ratio of α-helix/(β-sheet + random coil) could be responsible for looser proteinic
268 structure[44]. As shown in Supplementary Fig. 2b, the proportion of proteinic secondary
269 structure obviously changes with pretreatment conditions. The ratios of α-helix and β-
270 sheet are 34.2%, 33.2%, 31.4% and 27.6% for the control, AlkBC, ferrate and AlkBC-
271 ferrate, respectively, presenting a decreasing trend. Likewise, the proportion of α-
272 helix/(β-sheet + random coil) is reduced from 69.7% (control) to 57.8% (AlkBC), 51.4%
273 (ferrate) or 49.2% (AlkBC-ferrate). The structure of random coil may be also destructed
274 by AlkBC-ferrate, resulting in no further obvious reduction of this ratio. Collectively,
275 AlkBC-ferrate pretreatment predominantly disrupts hydrogen bonding networks
276 critical for α-helix and β-sheet integrity, thereby exposing proteolytic cleavage sites and
277 enhancing substrate bioavailability for fermentative microbial consortia. The

fluorescence response values of released organics in the AlkBC-ferrate system demonstrate parity with or marginal reduction compared to the ferrate-only system during pretreatment (Supplementary Fig. 3). This phenomenon is likely due to the intensified oxidative dismantling of fluorescent moieties through synergistic AlkBC-ferrate interactions. This mechanistic behavior substantiates its superior capability in chemically decomposing macromolecular organics, as evidenced by the structural disintegration of α-helix and β-sheet conformations integral to sludge stability.

Degradable components in the supernatant of pretreated sludge include organic macromolecules, hydrolysis products, other intermediates and SCFAs. Thus, the biotransformation of macromolecular substrates in WAS can be readily reflected by hydrolase activity and degradation product content. After pretreatment, protease, α-glucosidase, ammonia nitrogen and SCFAs are obviously elevated by AlkBC-ferrate regarding their activity or concentration (Fig. 2f). For example, AlkBC-ferrate improves protease activity and SCFA production by 23.1% and 32.7%, respectively, in comparison with ferrate alone. These results demonstrate that AlkBC-ferrate accelerates SCFA generation by driving the chemical decomposition and biotransformation of soluble macromolecular organics, thereby increasing electron acceptor availability for chain elongation.

Elucidating synergistic mechanisms of AlkBC and ferrate in particulate organics destabilization and bioavailability enhancement during WAS pretreatment

The pH value of WAS largely varies with pretreatment conditions and reaction time (Supplementary Fig. 4a). After 30 minutes, the AlkBC, ferrate and AlkBC-ferrate

reactors reach their respective maximum pH, i.e., 8.09, 9.35 and 9.75. Notably, AlkBC addition results in an increase of pH, which may further facilitate WAS dissolution, and assure effective sludge oxidation via weakening ferrate self-decomposition.

To elucidate the mechanism of AlkBC enhancing ferrate pretreatment, the respective contributions of alkalinity and oxidation to sludge dissolution were distinguished (Fig. 3a-3b). The pH9.35 and pH9.75 series represent the alkalinity induced by ferrate and AlkBC-ferrate, while [ferrate(1h)] and [AlkBC-ferrate(1h)] refer to their oxidizing effects, respectively. The variations in soluble protein concentrations with pretreatment conditions demonstrate that both alkalinity and oxidation critically drive sludge disintegration in the ferrate and AlkBC-ferrate systems (Fig. 3a). After 12 hours of pretreatment, the oxidation effects of ferrate and AlkBC-ferrate increase SCOD concentration by 1080 mg L^{-1} and 2360 mg L^{-1}, respectively, as calculated by subtracting the alkaline contributions (Fig. 3b). Such increment (i.e., 2360-1080=1280 mg L^{-1}) is close to the difference (i.e., 1190 mg L^{-1} of SCOD) between ferrate(1h)] and [AlkBC-ferrate(1h)], demonstrating that AlkBC enhances ferrate oxidation.

Ferrate (Fe(VI)) is unstable in water and will self-decay into Fe(IV), Fe(V) and H_2O_2, with Fe(III) and O_2 as the final forms[45]. During the ferrate self-decomposition process, Fe(V) and Fe(IV) may self-decay to Fe(III), while H_2O_2 also converts Fe(IV) and Fe(V) to Fe(II) and Fe(III), respectively, as shown in Eqs. (1)-(11)[11,46]. Apparently, the excessive self-decomposition of high-valent Fe intermediates would decrease the oxidation capacity of ferrate[17]. Thereby, ferrate activation and extension of Fe(V)/Fe(IV) lifetime can boost the efficiency of sludge treatment by ferrate. The ultraviolet-visible

absorbance of dissolved ferrate is rapidly reduced by AlkBC (Supplementary Fig. 4b). After 1 hour, Fe(VI) (λ_{max}=510 nm) decreases by 20.3% in the ferrate group, but it decreases by 90.7% in the AlkBC-ferrate group (Fig. 3c). Fe(V) and Fe(IV) have been reported to have the maximum absorbance at 380 nm and 420 nm, respectively[36,37]. Soluble Fe(III) is present as $Fe(OH)_2^+$ and its maximum absorbance in the ultraviolet-visible spectrum occurs at ca. 305 nm when pH is greater than 5[47]. Thereby, the strong absorption peaks at near 300 nm, 370 nm and 435 nm in the AlkBC-ferrate system are related to produced Fe(III), Fe(V) and Fe(IV), respectively (Fig. 3c and Supplementary Fig. 4b). The intensity of absorption peaks reflects that AlkBC-ferrate produces more Fe(V)/Fe(IV) species than ferrate alone. The mechanism of AlkBC activating ferrate oxidation may involve two steps. First, Fe(VI) is physically adsorbed onto the surface of biochar, as supported by its rapid decrease (by 60%) after 10 min of reaction. Then, Fe(VI) reacts with electron-rich aromatic moieties on AlkBC to transfer into metastable biochar-complexes or free Fe(V)/Fe(IV)[48]. Accordingly, these high-valent Fe intermediates in the AlkBC-ferrate system are more efficient for WAS disintegration due to the higher pH value (i.e., alleviation of self-decay) and the presence of ligand (i.e., AlkBC surfaces)[46].

$$Fe(VI) + H_2O \rightarrow Fe(IV) + H_2O_2 \quad (1)$$

$$Fe(V) + H_2O \rightarrow Fe(III) + H_2O_2 \quad (2)$$

$$Fe(IV) + H_2O \rightarrow Fe(III) + H_2O_2 \quad (3)$$

$$Fe(VI) + 2Fe(III) \rightarrow 3Fe(IV) \quad (4)$$

$$Fe(VI) + Fe(II) \rightarrow Fe(V) + Fe(III) \quad (5)$$

$$2Fe(VI) \rightarrow 2Fe(IV) + H_2O_2 \quad (6)$$

$$2Fe(V) \rightarrow 2Fe(III) + H_2O_2 \quad (7)$$

$$Fe(IV) + Fe(II) \rightarrow 2Fe(III) \quad (8)$$

$$Fe(VI) + H_2O_2 \rightarrow Fe(IV) + O_2 \quad (9)$$

$$Fe(V) + H_2O_2 \rightarrow Fe(III) + O_2 \quad (10)$$

$$Fe(IV) + H_2O_2 \rightarrow Fe(II) + O_2 \quad (11)$$

Fig. 3

Beyond chemical mechanisms, AlkBC-ferrate reaction products likely act as driving forces to stimulate the metabolic activity of functional bacteria and promote biodegradation of released organic matter. Fig. 3d shows the changes in the degradation extent of bovine serum albumin and acidification efficiency of small organic molecules (i.e., amino acids and glucose) with/without AlkBC addition. Due to the presence of AlkBC, the degradation efficiency of bovine serum albumin increases from 57.8% to 70.0% after 72 hours, while SCFA production rises from 1795.5 to 3319.8 mg COD L^{-1} after 48 hours. Additionally, Fe(III) produced by ferrate reduction could also promote the hydrolyzing and acidifying of organic matter through dissimilatory Fe reduction process[16]. Therefore, the increased alkalinity and AlkBC-activated ferrate oxidation are responsible for sludge dissolution and structural decomposition of released organics, while the AlkBC-ferrate reaction products trigger biological effects to enhance biodegradation of soluble organic matter.

Ferrate-induced AlkBC modification and its catalytic role in chain elongation

The ability of biochar facilitating electron transfer is closely related to its surface functional group structure and supported metal oxides. Previous research showed that

chemical oxidation treatment (e.g., with H_2O_2) could modify biochar, increasing oxygen-containing functional groups on its surface and enhancing redox properties and electron transport capacity[49]. It was suggested that ferrate decomposition in water resulted in the formation of nanoparticles that had core-shell nanoarchitecture with γ-Fe_2O_3 as core and γ-FeOOH as shell[14]. Thereby, the physicochemical property of AlkBC may be modified due to ferrate oxidation and formation of Fe(III) particles, posing potential impacts on the subsequent fermentation process, especially chain elongation.

The Fourier-transform infrared spectra in Fig. 4a reveal changes in major functional groups of AlkBC before and after ferrate oxidation. The characteristic peaks at 1110 cm^{-1}, 1570 cm^{-1} and 3430 cm^{-1} are separately related with C-O, C=O and C-OH functional groups[22], and their intensities are greatly enhanced under ferrate oxidation, implying an increase of C-O and C=O on the AlkBC surface. The bonding state of carbon elements is also verified by C1s spectra obtained from X-ray photoelectron spectroscopy (Fig. 4b-4c). It could be divided to three peaks with binding energies of 284.8, 286 and 288.5 eV, individually representing C-C, C-O and C=O groups[22]. After ferrate oxidation, C-C decreases from 77.4% to 70.2%, while C-O increases from 9.3% to 17.7%. As exhibited by scanning electron microscopy micrographs in Supplementary Fig. 5, the morphology of AlkBC becomes blurred and iron oxides are loaded onto its surface after ferrate oxidation. Obviously, ferrate oxidation alters the microstructure of AlkBC and increases oxygen-containing functional groups, which enhances adsorption-binding effect with iron oxides to further improve the reactivity and electron

transfer capacity of AlkBC. In the Fe $2p_{3/2}$ and Fe $2p_{1/2}$ regions, the characteristic peaks at 711.3 eV/724.9 eV, 719.46 eV/733.6 eV and 713.19 eV/726.79 eV correspond to Fe_2O_3, Fe(III) satellite peak and $Fe(OH)_3$ (Fig. 4d), respectively[15,50], suggesting ferrate-induced formation of Fe_2O_3. Based on the peaks in Fe $2p_{3/2}$, it can be calculated that Fe_2O_3 predominates in the Fe(III) particles on AlkBC surface, accounting for 65.9%. Additionally, the absorption peaks near 561 cm^{-1} and 462 cm^{-1} (Fig. 4a) of ferrate-modified AlkBC could also be attributed to F-O bonds in Fe_2O_3[15]. Our previous studies demonstrated that Fe_2O_3 addition substantially increased MCFA production mainly through facilitating electron transfer of chain elongation step[16]. It was revealed that Fe_2O_3-modified digestate BC prepared via co-precipitation or impregnation exhibited superior performance to Fe_2O_3 or raw BC in facilitating electron transfer and methane production during anaerobic digestion of kitchen waste[51].

After pretreatment, ferrate-modified AlkBC enters WAS fermentation reactor, exerting potential impact on electron transfer capacity and chain elongation performance. As shown Fig. 4e, AlkBC, ferrate and AlkBC-ferrate increase the electron transfer system activity of WAS fermentation system by 26.6%, 75.5% and 237.8%, respectively, compared to the control. The intensity of redox peaks in cyclic voltammetry curves (Supplementary Fig. 4c) is increased by both AlkBC and ferrate, but it is higher in the AlkBC-ferrate group, indicating that ferrate-modified AlkBC accelerates the redox reaction and triggers faster extracellular electron transfer during sludge fermentation[52]. The semi-conductive Fe_2O_3 loaded on AlkBC can further promote microbial aggregation and increase their electrical connectivity by interacting

400 with electronegative EPS, establishing a microenvironment conducive to functional
401 microorganism enrichment and extracellular electron transfer (Fig. 4g). Furthermore,
402 Fig. 4f presents the MCFA production during 9 days of chain elongation with different
403 substance additions, i.e., raw AlkBC, ferrate-reduced particles, ferrate-modified AlkBC,
404 $Fe(OH)_3$ and AlkBC-$Fe(OH)_3$. MCFA concentration is 4857.9 mg COD L^{-1} in the
405 control group on day 9, and it largely increases to 7852.5 and 9089.8 mg COD L^{-1} by
406 raw AlkBC and ferrate-reduced particles, respectively. Expectedly, ferrate-modified
407 AlkBC further enhances MCFA concentration to 10200.4 mg COD L^{-1}, significantly
408 higher than the control ($p<0.001$), raw AlkBC ($p=0.002$) and ferrate-reduced particles
409 ($p=0.028$). Interestingly, MCFA generation is inhibited to some degree when ferrate-
410 reduced particles are fully replaced by $Fe(OH)_3$. Thus, Fe_2O_3 is the active Fe component
411 on the surface of AlkBC in accelerating electron transport and improving chain
412 elongation. These results indicate that ferrate-modified biochar more effectively
413 promotes chain elongation compared to unmodified biochar, thereby enhancing the
414 conversion of sludge-derived SCFAs to MCFAs.

415 **Fig. 4**

416 **Restructuring of microbial communities and functional taxa induced by AlkBC-**
417 **ferrate pretreatment**

418 To further reveal the mechanisms of MCFA production improvement by AlkBC-
419 ferrate, 16S rRNA high-throughput sequencing was used to investigate the alteration of
420 bacteria and archaea communities. The diversity indexes show that AlkBC-ferrate
421 reduces community richness and increases microbial diversity compared to the control

(Supplementary Fig. 6a). Principal coordinate analysis, based on inter-group distances, reveals a distinct microbial composition in the AlkBC-ferrate group compared to the other groups (Supplementary Fig. 6b). These observations suggest the presence of specific microbial proliferation and non-functional microbe removal in the combined group.

The microbial composition at the phylum level is displayed in Fig. 5a. Firmicutes is a typical phylum existing in sludge fermentation system, which is closely associated with sludge hydrolase secretion, SCFA production and chain elongation[4,18]. Its abundances in the control, AlkBC, ferrate and AlkBC-ferrate groups are 11.45%, 19.77%, 17.98% and 20.33%, respectively, indicating an apparent upward trend. Euryarchaeota, a phylum of archaea containing methanogens, displays reduced abundance in the ferrate and AlkBC-ferrate groups. These results demonstrate that AlkBC-ferrate further promotes enrichment of functional bacteria and removal of competitive archaea.

Fig. 5b depicts the correlation between the abundances of specific functional microorganisms and pretreatment conditions at the genus level. The functional microbes include hydrolyzing, acidifying and chain-elongating bacteria, such as *Macellibacteroides sp.*[53], *Proteiniclasticum sp.*[4], *Trichococcus sp.*[54], *Romboutsia sp.*[55], *Acetoanaerobium sp.*[40]. Generally, the total abundance of these microorganisms is 9.5% for control, whereas it is enriched to 12.7%, 18.8% and 24.0% by AlkBC, ferrate and AlkBC-ferrate, respectively. Notably, their metabolic functions (*i.e.*, hydrolysis, acidification, chain elongation) may not be completely differentiated in the WAS

444 fermentation system. For example, *Macellibacteroides* is a typical hydrolyzing and
445 acid-producing genus, capable of degrading macromolecules (such as peptone and
446 cellobiose) or glucose to SCFAs and lactic acid[53]. *Petrimonas sp.* can act as chain
447 elongation microbe[56], while its abundance is inconsistent with MCFA production,
448 implying other typical acidogens may participate in chain elongation reactions, such as
449 *Macellibacteroides sp*. and *Trichococcus sp*. Additionally, *Methanobacterium sp*.
450 belongs to hydrogenotrophic methanogens[57], which is able to decrease hydrogen partial
451 pressure and inhibit chain elongation. It accounts for 1.38% and 2.75% in the control
452 and AlkBC groups, whereas it is reduced to 0.65% and 0.04% by ferrate and AlkBC-
453 ferrate, respectively. This pronounced methanogen suppression directly correlates with
454 the >95% reduction in methane production (Fig. 1b). It is attributed to the synergistic
455 effects of elevated alkalinity and enhanced oxidizing capacity from ferrate activation,
456 both of which disrupt methanogen metabolic pathways. Thereby, AlkBC-ferrate
457 efficiently eliminates hydrogenotrophic methanogen-induced competitive inhibition on
458 chain elongation, redirecting electron flux from electron donors and acceptors towards
459 chain elongation pathways instead of methanogenesis.

460 **Fig. 5**

461 **Discussion**

462 **Conversion of sludge organics into high-value MCFAs based on a staged**
463 **enhancement strategy**

464 MCFA biosynthesis from WAS faces two critical limitations: restricted substrate
465 bioavailability and inefficient chain elongation process[22,29]. To address these challenges,

this study proposes a synergistic AlkBC-ferrate pretreatment technique. Mechanism investigations reveal that this strategy achieves the staged optimization of WAS-to-MCFA conversion, as depicted in the schematic diagram (Fig. 6). Overall, AlkBC-ferrate accelerates the release and biochemical transformation of sludge particulate organics, while inhibits potential competitive microorganisms during the pretreatment stage. The resulting ferrate-modified AlkBC then enters the targeted MCFA-producing fermentation system to efficiently facilitate chain elongation.

Fig. 6

The disruption of WAS anti-hydrolysis barrier is the precondition for sludge solubilization[1]. After AlkBC-ferrate pretreatment, EPS structures and cell walls are largely disintegrated, providing more soluble organics for functional microorganisms than ferrate alone (Fig. 2a-d). With ferrate oxidation and bio-stimulation effect of AlkBC, the chemical decomposition and bio-conversion of sludge particulate organics are also accelerated, increasing the production of electron acceptors (i.e., SCFAs) (Fig. 2e-f). Further investigations reveal that the mechanisms of AlkBC enhancing ferrate pretreatment include releasing alkali and activating ferrate reaction (Fig. 3b and Supplementary Fig. 4a). We find that more Fe(IV) and Fe(V) are produced in AlkBC-ferrate system (Supplementary Fig. 4b), suggesting the activation of ferrate reaction by AlkBC[58]. The activation mechanism can be interpreted as the two steps of physical adsorption and electron transfer (Fig. 3c)[48], and the electron-rich groups on AlkBC surface provide electron donors for ferrate reduction to high-valent Fe intermediates (i.e., Fe(IV) and Fe(V)) [21]. Meanwhile, the oxygen-containing functional groups on

AlkBC may act as ligands to increase the stability of Fe(IV)/Fe(V)[46].

Beyond substrate bio-availability, the potential competitive microbes can alter the conversion pathways of electron donors/acceptors, causing electrons flowing to byproducts such as methane, butanol and hexanol[7]. Methanogens are prevalent in sludge fermentation systems, and they could cause competitive inhibition of chain elongation bacteria by lowering hydrogen partial pressure and consuming ethanol/acetic acid[59]. Thus, substantial amounts of inhibitors are usually required to eliminate methanogenic activity for efficient chain elongation reactions[4]. Long-chain alcohol producers are also potentially competitors, and their enrichment leads to over-conversion of substrates to by-products such as butanol and hexanol, resulting in a shortage of electron donors[7]. In this study, the cumulative methane production and maximum content of long-chain alcohols are substantially reduced by AlkBC-ferrate, in comparison with the control, AlkBC or ferrate (Fig. 1b and Fig. 1d). Microbial community analysis further confirms the enrichment of hydrolytic, acidogenic, and chain-elongating bacteria (e.g., *Macellibacteroides sp*., *Trichococcus sp*.) alongside the suppression of methanogens (i.e., *Methanobacterium sp*.) (Fig. 5). Hence, AlkBC-ferrate effectively modulates microbial competition, selectively inhibiting undesirable microbes while preserving functional populations.

Ferrate oxidation increases the number of oxygen-containing functional groups on AlkBC, improving its redox properties and adsorption capacity for metal ions (Fig. 4a-4c). This alteration strengthens interactions between AlkBC and ferrate-derived particles (mainly Fe_2O_3) (Fig. 4d). Previous studies reported that Fe_2O_3 dosing elevated

MCFA production from 3793 to 9162 mg COD L^{-1}[116], while Fe_2O_3 loading enhanced the electron transfer capacity of digestate BC[51]. Hence, AlkBC is synergistically modified by ferrate oxidation and in-situ Fe_2O_3 deposition in this work. Electron transfer system activity and cyclic voltammetry curves confirm that AlkBC-ferrate triggers more rapid extracellular electron transport (Fig. 4e and Supplementary Fig. 4c). Additionally, AlkBC provides microbial attachment sites, while iron oxides on AlkBC interact with electronegative EPS, promoting microbial aggregation in chain elongation system (Fig. 4g). The enhanced microbial aggregation can facilitate the establishment of electrical connections and electron transfer between microorganisms[60]. Consequently, ferrate-modified AlkBC improves the chain elongation bioprocess with Fe_2O_3 as the active iron component on its surface, resulting in significantly higher MCFA production than control, raw AlkBC and ferrate-reduced particles (Fig. 4f).

Implications

This study presents an innovative and sustainable technology for in-situ upgrading of WAS-derived SCFAs into higher-value MCFAs. The AlkBC-ferrate system reaches 10495.0 mg COD L^{-1} MCFAs, surpassing prior records achieved by free ammonia (i.e., 8300 mg COD L^{-1})[29], ferrate (i.e., 8106.3 mg COD L^{-1})[13], electro-fermentation (i.e., 1320 mg COD L^{-1})[61] and Fe_3O_4 (i.e., 7953.6 mg COD L^{-1})[35]. A preliminary economic assessment was performed based on sludge reduction and yield of liquid products (Supplementary Table 2). The revenues of fermentation products in the control, AlkBC, ferrate and AlkBC-ferrate groups can offset the costs of chemical dosing, AlkBC preparation and sludge disposal, with the net economic benefit reaching $29, $58, $210

and $680 per tonne of dry sludge, respectively. Notably, the cost input of AlkBC-ferrate technology is mainly derived from potassium ferrate, which is much higher than AlkBC preparation. The combination not only reduces the ferrate dosage, but also achieves higher MCFA production and sludge reduction. However, the potential challenges for scaling up the AlkBC-ferrate technology remain the cost of ferrate and source of electron donor. Future research should prioritize developing cost-effective methods for in-situ ferrate generation and confirming the efficiency of endogenous ethanol production from pretreated WAS, to reduce reliance on commercial chemicals (e.g., potassium ferrate and ethanol) and avoid complex processes of utilizing other biowaste-derived electron donors. Besides, integrating this pretreatment into a broader biorefinery framework (e.g., co-producing MCFAs and biogas) will further enhance overall economic viability by maximizing resource valorization[62,63]. Following MCFA extraction, the fermentation residue undergoes anaerobic digestion for methane-rich biogas production, where the retained ferrate-modified AlkBC continuously promotes electron transfer to boost methanogenesis[64]. Ultimately, iron (hydr)oxides derived from ferrate reduction accumulate in the digestate, allowing magnetic recovery of vivianite ($Fe_3(PO_4)_2 \cdot 8H_2O$)[65]. It is a high-value phosphate product, thus closing the iron loop. This synergistic integration maximizes resource recovery (namely, MCFAs, biogas, phosphorus) from WAS, while incorporating biochar functionality and iron components within a circular economy framework.

553 **Data Availability Statement**

554 The data supporting the findings of this study are available in the main text and
555 the Supplementary Information. The raw sequencing data generated in this study are
556 available in the NCBI Sequence Read Archive database under accession number
557 PRJNA1346931.

References

1. K, A. & Selvasembian, R. Insights into the recent advances of chemical pretreatment of waste activated sludge to enhance biomethane production. *Journal of Environmental Chemical Engineering* **12**, 113999 (2024).
2. Zhao, H. *et al.* Solar-driven sewage sludge electroreforming coupled with biological funnelling to cogenerate green food and hydrogen. *Nature Water* **2**, 1102-1115 (2024).
3. Battista, F. *et al.* Treatment of food processing wastes for the production of medium chain fatty acids via chain elongation. *Environmental Technology & Innovation* **33**, 103453 (2024).
4. Wang, Y., Wei, W., Wu, S.-L. & Ni, B.-J. Zerovalent Iron Effectively Enhances Medium-Chain Fatty Acids Production from Waste Activated Sludge through Improving Sludge Biodegradability and Electron Transfer Efficiency. *Environmental Science & Technology* **54**, 10904-10915 (2020).
5. Chaitanya, N. K. & Chatterjee, P. Medium chain fatty acid production from CO_2 in integrated dark fermentation-microbial electrosynthesis reactor. *Bioresource Technology* **426**, 132371 (2025).
6. San-Valero, P., Abubackar, H. N., Veiga, M. C. & Kennes, C. Effect of pH, yeast extract and inorganic carbon on chain elongation for hexanoic acid production. *Bioresource Technology* **300**, 122659 (2020).
7. Wang, Y. *et al.* Medium-chain fatty acids production from sewage sludge through anaerobic fermentation: A critical review. *Chemical Engineering Journal* **477**, 147138 (2023).
8. Gunawan, G., Basid Adiwibawa Prasetya, N. & Adi Wijaya, R. Electrosynthesis of ferrate from iron waste and seawater salts as Antibacterials for water pollutant treatment. *Chemical Engineering Journal* **498**, 155422 (2024).
9. Cao, J.-Y. *et al.* Ferrate(VI)-based synergistic oxidation processes (Fe(VI)-SOPs): Promoted reactive species production, micropollutant/microorganism elimination, and toxicity reduction. *Chemical Engineering Journal* **489**, 151180 (2024).
10. Jiang, J.-Q., Panagoulopoulos, A., Bauer, M. & Pearce, P. The application of potassium ferrate for sewage treatment. *Journal of Environmental Management* **79**, 215-220 (2006).
11. Luo, M. *et al.* Insights into the role of in-situ and ex-situ hydrogen peroxide for enhanced ferrate(VI) towards oxidation of organic contaminants. *Water Research* **203**, 117548 (2021).
12. Mao, Y. *et al.* Removal of micro organic pollutants in high salinity wastewater by comproportionation system of Fe(VI)/Fe(III): Enhancement of chloride and bicarbonate. *Water Research* **214**, 118182 (2022).
13. Wang, Y. *et al.* Ferrate pretreatment-anaerobic fermentation enhances medium-chain fatty acids production from waste activated sludge: Performance and mechanisms. *Water Research* **229**, 119457 (2023).
14. Robert *et al.* Ferrate(VI)-lnduced Arsenite and Arsenate Removal by In Situ Structural Incorporation into Magnetic Iron(III) Oxide Nanoparticles.

Environmental Science & Technology Es & T (2013).
15. Goodwill, J. E., Jiang, Y., Reckhow, D. A., Gikonyo, J. & Tobiason, J. E. Characterization of Particles from Ferrate Preoxidation. *Environmental Science & Technology* **49**, 4955-4962 (2015).
16. Wang, Y. *et al.* Ferric oxide stimulates medium-chain carboxylic acids synthesis from waste activated sludge via ethanol-driven chain elongation: Mechanisms and implications. *Journal of Cleaner Production* **389**, 136044 (2023).
17. Dong, F. *et al.* Simultaneous generation of free radicals, Fe(IV) and Fe(V) by ferrate activation: A review. *Chemical Engineering Journal* **481**, 148669 (2024).
18. Li, L. *et al.* Efficient Volatile Fatty Acids Production from Waste Activated Sludge after Ferrate Pretreatment with Alkaline Environment and the Responding Microbial Community Shift. *ACS Sustainable Chemistry & Engineering* **6**, 16819-16827 (2018).
19. Fakhar, A., Canatoy, R. C., Galgo, S. J. C., Rafique, M. & Sarfraz, R. Advancements in modified biochar production techniques and soil application: a critical review. *Fuel* **400**, 135745 (2025).
20. Gao, Y. *et al.* Intrinsic properties of biochar for electron transfer. *Chemical Engineering Journal* **475**, 146356 (2023).
21. Tian, S.-Q., Wang, L., Liu, Y.-L. & Ma, J. Degradation of organic pollutants by ferrate/biochar: Enhanced formation of strong intermediate oxidative iron species. *Water Research* **183**, 116054 (2020).
22. Wu, S.-L. *et al.* Revealing the Mechanism of Biochar Enhancing the Production of Medium Chain Fatty Acids from Waste Activated Sludge Alkaline Fermentation Liquor. *Acs Es&T Water* **1**, 1014-1024 (2021).
23. Lou, T., Yin, Y. & Wang, J. Influence of adding strategy of biochar on medium-chain fatty acids production from sewage sludge. *Chemosphere* **354**, 141660 (2024).
24. Chen, L. *et al.* Functionalized biochars with highly-efficient malachite green adsorption property produced from banana peels via microwave-assisted pyrolysis. *Bioresource Technology* **376**, 128840 (2023).
25. Gao, X. *et al.* Performance and mechanism of biochar pretreatment on improving short-chain fatty acids production from anaerobic fermentation of waste activated sludge. *Journal of Environmental Chemical Engineering* **12**, 114828 (2024).
26. Li, X. Y. & Yang, S. F. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. *Water Research* **41**, 1022-1030 (2007).
27. Hu, T. *et al.* Micro-mechanism of rhamnolipid promoting acid production during anaerobic digestion: protein structures, metagenomics and molecular dynamics simulations. *Water Research* **283**, 123795 (2025).
28. Maleki, E., Yu, D. & Parker, W. Assessment of active heterotrophs and hydrolytic enzyme activities as indicators of hydrolysis rates in anaerobic digestion of mixed primary and waste activated sludges. *Journal of Environmental Chemical Engineering* **13**, 115302 (2025).
29. Wang, Y. *et al.* Improving Medium-Chain Fatty Acid Production from Anaerobic

Fermentation of Waste Activated Sludge Using Free Ammonia. *Acs Es&T Engineering* **1**, 478-489 (2021).
30. Miner, G. Standard Methods for the Examination of Water and Wastewater, 21st Edition. *Journal American Water Works Association*, 130 (2006).
31. Tantray, J. A., Mansoor, S., Wani, R. F. C. & Nissa, N. U. in *Basic Life Science Methods* (eds Javeed Ahmad Tantray, Sheikh Mansoor, Rasy Fayaz Choh Wani, & Nighat Un Nissa) 65-67 (Academic Press, 2023).
32. Prakash Jain, B., Pandey, S. & Goswami, S. K. in *Protocols in Biochemistry and Clinical Biochemistry (Second Edition)* (eds Buddhi Prakash Jain, Shweta Pandey, & Shyamal K. Goswami) 35-36 (Academic Press, 2025).
33. Chubar, N., Gerda, V., Szlachta, M. & Yablokova, G. Effect of Fe oxidation state (+2 versus +3) in precursor on the structure of Fe oxides/carbonates-based composites examined by XPS, FTIR and EXAFS. *Solid State Sciences* **121**, 106752 (2021).
34. Mishra, S. & Kumar Tiwari, M. Adsorptive removal of diclofenac on nanoporous anoxic sewage sludge biochar: Investigating the influence of carbonization temperature, residence time and specific Fe(III) infusion. *Separation and Purification Technology* **354**, 129322 (2025).
35. Wang, Y. *et al.* New insight into mechanisms of ferroferric oxide enhancing medium-chain fatty acids production from waste activated sludge through anaerobic fermentation. *Bioresource Technology* **360**, 127629 (2022).
36. Sharma, V. K. Ferrate(VI) and ferrate(V) oxidation of organic compounds: Kinetics and mechanism. *Coordination Chemistry Reviews* **257**, 495-510 (2013).
37. Rush, J. D. & Bielski, B. H. J. Pulse radiolysis studies of alkaline iron(III) and iron(VI) solutions. Observation of transient iron complexes with intermediate oxidation states. *Journal of the American Chemical Society* **108**, 523-525 (1986).
38. Antonicelli, G. *et al.* Harnessing an adapted strain of Clostridium carboxidivorans to unlock hexanol production from carbon dioxide and hydrogen in elevated-pressure stirred tank reactors. *Bioresource Technology* **418**, 131966 (2025).
39. Lai, J. *et al.* Promotion of aromatic amino acids of extracellular polymeric substance targeted transformation via sulfite mediated iron redox cycling in sludge solid-liquid separation. *Water Research* **266**, 122369 (2024).
40. Wu, S.-L. *et al.* Unveiling the mechanisms of medium-chain fatty acid production from waste activated sludge alkaline fermentation liquor through physiological, thermodynamic and metagenomic investigations. *Water Research* **169**, 115218 (2020).
41. Cao, W. *et al.* Choline chloride pretreatment on volatile fatty acids promotion from sludge anaerobic fermentation: In-situ deep eutectic solvents-like formation for EPS disintegration and associated microbial functional profiles upregulation. *Chemical Engineering Journal* **467**, 143556 (2023).
42. Wang, Q. *et al.* Study on short-chain fatty acids production from anaerobic fermentation of waste activated sludge pretreated by alkali-activated ammonium persulfate. *Bioresource Technology* **428**, 132461 (2025).
43. Chen, Y., Ping, Q., Li, D., Dai, X. & Li, Y. Comprehensive insights into the impact

of pretreatment on anaerobic digestion of waste active sludge from perspectives of organic matter composition, thermodynamics, and multi-omics. *Water Research* **226**, 119240 (2022).

44. Li, Y. *et al.* Fe(II) catalyzing sodium percarbonate facilitates the dewaterability of waste activated sludge: Performance, mechanism, and implication. *Water Research* **174**, 115626 (2020).
45. Sharma, V. K., Zboril, R. & Varma, R. S. Ferrates: Greener Oxidants with Multimodal Action in Water Treatment Technologies. *Accounts of Chemical Research* **48**, 182-191 (2015).
46. Sharma, V. K. *et al.* Reactive High-Valent Iron Intermediates in Enhancing Treatment of Water by Ferrate. *Environmental Science & Technology* **56**, 30-47 (2022).
47. Loures, C. *et al.* Advanced Oxidative Degradation Processes: Fundamentals and Applications. *2035-1755* **5** (2013).
48. Tian, S.-Q. *et al.* Enhanced Permanganate Oxidation of Sulfamethoxazole and Removal of Dissolved Organics with Biochar: Formation of Highly Oxidative Manganese Intermediate Species and in Situ Activation of Biochar. *Environmental Science & Technology* **53**, 5282-5291 (2019).
49. Jiang, Q. *et al.* Deciphering the effects of engineered biochar on methane production and the mechanisms during anaerobic digestion: Surface functional groups and electron exchange capacity. *Energy Conversion and Management* **258**, 115417 (2022).
50. Lv, X. *et al.* Nickel phosphide nanoarrays decorated on amorphous NiPOx/$Fe(OH)_3$: A stable core–shell electrocatalyst for efficient oxygen evolution at large current density. *Chemical Engineering Journal* **475**, 146128 (2023).
51. Wang, Y. *et al.* Preparation of iron oxide-modified digestate biochar and effect on anaerobic digestion of kitchen waste. *Bioresource Technology* **398**, 130515 (2024).
52. Yang, Y., Ma, P., Li, Y., Chen, Y. & Zhang, H. Sludge-derived biochar improves sludge electro-dewatering performance: Conductivity analysis. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **701**, 134838 (2024).
53. Linda *et al.* Macellibacteroides fermentans gen. nov., sp. nov., a member of the family Porphyromonadaceae isolated from an upflow anaerobic filter treating abattoir wastewaters. *International journal of systematic and evolutionary microbiology* **62**, 2522-2527 (2012).
54. Pikuta, E. V. *et al.* Trichococcus patagoniensis sp nov., a facultative anaerobe that grows at -5 degrees C, isolated from penguin guano in Chilean Patagonia. *International Journal of Systematic and Evolutionary Microbiology* **56**, 2055-2062 (2006).
55. He, Z.-W. *et al.* Freezing-low temperature treatment facilitates short-chain fatty acids production from waste activated sludge with short-term fermentation. *Bioresource Technology* **347** (2022).
56. He, J. *et al.* Phenol promoted caproate production via two-stage batch anaerobic fermentation of organic substance with ethanol as electron donor for chain elongation. *Water Research* **204**, 117601 (2021).

57. Zheng, S., Li, M., Liu, Y. & Liu, F. Desulfovibrio feeding Methanobacterium with electrons in conductive methanogenic aggregates from coastal zones. *Water Research* **202**, 117490 (2021).
58. Zhang, H. *et al.* Enhanced ferrate(VI)) oxidation of sulfamethoxazole in water by CaO_2: The role of Fe(IV) and Fe(V). *Journal of Hazardous Materials* **425**, 128045 (2022).
59. Wu, Q. *et al.* Medium chain carboxylic acids production from waste biomass: Current advances and perspectives. *Biotechnology Advances* **37**, 599-615 (2019).
60. Barua, S. & Dhar, B. R. Advances towards understanding and engineering direct interspecies electron transfer in anaerobic digestion. *Bioresource Technology* **244**, 698-707 (2017).
61. Su, W. *et al.* Boosting the biosynthesis of medium-chain fatty acids from waste activated sludge electro-fermentation: Roles of syngas and ethanol serving as co-electron donors. *Journal of Cleaner Production* **502**, 145383 (2025).
62. Battista, F. & Bolzonella, D. Beyond Anaerobic Digestion: New Perspectives for the Development of a Biorefinery Platform for the Simultaneous Production of Medium-Chain Fatty Acids by Chain Elongation and Biogas from Food Wastes. *ACS Sustainable Chemistry & Engineering* **12**, 15294-15306 (2024).
63. Battista, F. *et al.* Designing a flexible anaerobic biorefinery for the valorization of red grape marcs into biofuels and biochemicals. *Environmental Technology & Innovation* **39**, 104340 (2025).
64. Jin, H.-Y. *et al.* Deciphering the synergistic effects and mechanisms of biochar and magnetite contained in magnetic biochar for enhancing methane production in anaerobic digestion of waste activated sludge. *Water Research* **282**, 123734 (2025).
65. Cheng, Y. *et al.* Pyrochar relieved the negative effects of hydrothermal pretreatment of sludge on vivianite production in anaerobic digestion. *Chemical Engineering Journal* **516**, 164096 (2025).

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Ethics declarations

Competing interests

The authors declare no competing interests.

Figure captions

Fig. 1. Product profiles in the control, alkaline biochar (AlkBC), ferrate and AlkBC-ferrte systems during medium-chain fatty acid (MCFA) biosynthesis from anaerobic fermentation of waste activated sludge. a MCFA production, expressed as chemical oxygen demand (COD). **b** Cumulative CH_4 production. **c** Product distributions at the end of anaerobic fermentation. **d** Long-chain alcohol production, expressed as COD. Error bars denote the standard deviations of triplicate experiments.

Fig. 2. Dynamics of soluble organics and bound extracellular polymeric substances (EPS), protein secondary structure, and enzymatic/metabolic activities during 48-hour WAS pretreatment. a Concentration of proteins and polysaccharides in extracted bound EPS. **b-d** Variations in concentration of proteins, carbohydrates and chemical oxygen demand (COD) in the soluble fraction during pretreatment, with **d** showing the COD distributions in the soluble fraction and extracted bound EPS. **e** Fitting curve of the amide I region (1600−1700 cm^{-1}) in Fourier-transform infrared spectra of soluble organics. **f** Activity of protease and α-glucosidase, ammonia nitrogen release and short-chain fatty acid (SCFA) production. Error bars denote the standard deviations of triplicate experiments.

Fig. 3. Mechanisms of sludge dissolution and organic biotransformation in the alkaline biochar (AlkBC)-ferrate pretreatment system. a Soluble protein concentration during 48-hour pretreatment. **b** Concentration of soluble chemical

oxygen demand (COD) after 12-hour pretreatment. **c** Decay profiles of Fe(VI) in distilled water following addition of ferrate alone or AlkBC-ferrate, with inset showing enlarged ultraviolet-visible spectra for the AlkBC-ferrate group. **d** Degradation efficiency of bovine serum albumin, and short-chain fatty acid (SCFA) concentrations from acidification of amino acids and glucose in synthetic wastewater experiments. Error bars denote the standard deviations of triplicate experiments.

Fig. 4. Structural properties of ferrate-modified alkaline biochar (AlkBC) and its enhancement of chain elongation. a Fourier-transform infrared spectra of raw AlkBC and ferrate-modified AlkBC. **b, c** C1s spectra from X-ray photoelectron spectroscopy for raw AlkBC and ferrate-modified AlkBC. **d** Fe2p spectra from X-ray photoelectron spectroscopy for Fe element on the surface of ferrate-modified AlkBC. **e** Electron transport system activity of different groups during WAS fermentation. **f** Medium-chain fatty acid (MCFA) production, expressed as chemical oxygen demand (COD), in chain elongation biostep with the presence of different Fe and/or AlkBC, assessed by synthetic wastewater experiments. **g** Scanning electron microscopy images of sludge samples from the chain elongation experiment, including the control, raw AlkBC, ferrate-reduced particles, and ferrate-modified AlkBC groups. Error bars denote the standard deviations of triplicate experiments.

Fig. 5. Microbial community structure and associated functional taxa profiles of the control, alkaline biochar (AlkBC), ferrate and AlkBC-ferrate groups after

anaerobic fermentation. a Phylum-level taxonomic composition of microbial communities, where circle colors denote the different pretreatment conditions (i.e., Control, AlkBC, ferrate, and AlkBC-ferrate). **b** Heatmap of genus-level functional taxa involved in key bioprocesses.

Fig. 6. Schematic diagram of mechanisms. Stage-optimized modulation of sludge-to-MCFA conversion driven by the AlkBC-ferrate strategy.

Editor's Summary:
Yufen Wang and colleagues report a synergistic alkaline biochar-ferrate technique to enhance medium-chain fatty acid (MCFA) production from sludge by stage-optimized modulation. This strategy overcomes dual bottlenecks in sludge-to-MCFA conversion.

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