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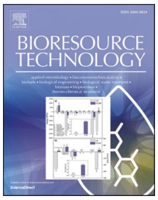

# Potential of 2,3-butanediol, volatile fatty acid and methane production from medium and low-value forest industry side streams

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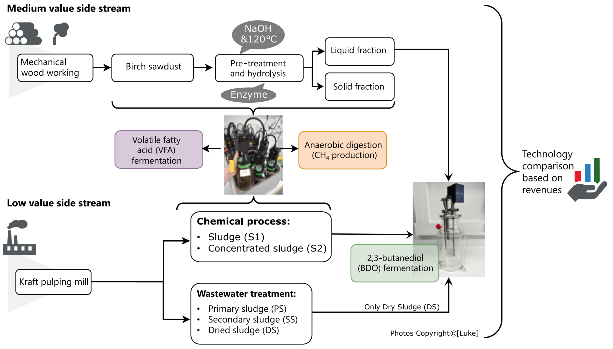
## HIGHLIGHTS

- Forest industry side streams can yield high-value products via fermentation.
- Birch sawdust produced more fatty acids than 2,3-butanediol after pre-treatment.
- Kraft mill sludges also produced 2,3-butanediol and methane without pretreatment.
- Integrating biochemical production boosts biorefinery revenue and efficiency.
- Anaerobic digestion adds value to low-grade forest industry streams.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Medium- and low-value side streams from the forest industry, such as sawdust and biosludge, are typically used for energy production, yet they offer potential for higher-value products via biological conversion. This study explores the combined production of 2,3-butanediol (BDO) and volatile fatty acids (VFAs) from birch sawdust and sludges from kraft mill pulping and wastewater treatment, alongside anaerobic digestion for methane. Integration of BDO and VFA production is evaluated by valorising BDO process residues. Pretreated birch sawdust yielded BDO 0.12 g/g of total solids (TS), and VFA up to 0.68 g/gTS, indicating economic feasibility. Combining both processes in a biorefinery could enhance revenues. Kraft mill sludges produced BDO up to 0.88 g/gTS, VFAs between 0.45–0.64 g/gTS, and methane 430 $Nm^3$/t volatile solids (VS) without pretreatment. These findings demonstrate the potential of forest industry side streams for producing bio-based chemicals and methane, supporting integrated biorefineries for improved resource efficiency and value creation.

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## 1. Introduction

Globally, the estimated production of wood biomass is around 4 billion $m^3$ (FAO, 2023). Side streams of the forest industry are an abundantly available and sustainable resource. They can be divided into three categories based on their value: i) high-value streams, such as tall oil, mostly used as biochemicals and for renewable diesel production and to produce energy (Aryan and Kraft, 2021), ii) medium-value solid streams, such as sawdust and bark, and iii) low-value liquid streams, such as fibre sludge and biosludge, used for energy production via incineration or anaerobic digestion (Hassan et al., 2019). However, research related to the utilisation of the medium- and low-value side streams has raised interest in recent years (Miettinen and Ollikainen, 2022), and there is a strong potential for their use in biological processes such as fermentation, to obtain products of higher value. Furthermore, as the forest industry shifts to carbon–neutral electricity sources (Lipiäinen et al., 2023), a portion of the side streams previously used for energy generation may become available for alternative, higher-value applications.

A variety of end-products can be produced from fermentation of forest industry side streams, including 2,3-butanediol (BDO) and volatile fatty acids (VFAs). For example, BDO can be utilised as a precursor for diacetyl and 1,3-butadiene syntheses, as well as in various other manufacturing processes, such as the production of cosmetics and artificial rubber (Ebrahimian and Mohammadi, 2024). Similarly, VFAs have applications as precursors in the chemical, textile, and pharmaceutical industries (Giduthuri and Ahring, 2023), or as building blocks for polyhydroxyalkanoate (PHA) synthesis, a sustainable alternative to conventional plastic materials (Li et al., 2020). Moreover, the biological production of BDO and VFA improves sustainability since these precursors are traditionally obtained from fossil-based processes.

The challenge in the utilisation of lignocellulosic side streams is strongly related to their composition and structure. The structure of wood is composed of cellulose, hemicelluloses, and lignin, which form a complex network. Thereby, various mechanical, physicochemical, thermal, and chemical pretreatment methods have been studied to break down the structure and obtain the monosaccharides, sugars such as glucose and xylose, which can then be further biologically converted into other value-added products (Dusselier et al., 2014).

In BDO fermentation of medium-value wood waste such as wood chips, pretreatments often consist of several steps, including mechanical grinding, alkaline/acidic treatment, heat, filtration, or enzymatic treatments, both to ensure the absence of inhibitors and improve digestibility, thus obtaining higher yields (Ebrahimian and Mohammadi, 2024; Hazeena et al., 2022). Dry wood materials, such as sawdust, have previously resulted in low VFA production despite pretreatments being used, at least in comparison with other lignocellulosic materials, such as straw (Gladchenko et al., 2014). On the other hand, woody low-value liquid waste streams, such as paper and fibre mill sludges, have been utilised in VFA fermentation processes without pretreatments (Ebrahimian and Mohammadi, 2024; Garcia-Aguirre et al., 2017). However, the fermentation processes in these cases were rather inefficient (Mato et al., 2010).

Overall, the utilisation of the woody side streams in VFA and BDO production requires more investigation, in terms of optimal pretreatments and suitable process conditions. In addition, the potential of the two processes has not been compared side-by-side. While BDO fermentation is selective in its use of sugars, VFA fermentation can convert a variety of sugars and other organic matter components, such as proteins (Giduthuri and Ahring, 2023). This could allow the two processes to be effectively combined in a biorefinery concept.

This study aims to compare and explore ways to combine the production of both BDO and VFAs from medium- and low-value forest industry side streams, i.e. sawdust and sludges from the chemical process and wastewater treatment of a kraft pulping mill. Methane production via anaerobic digestion is also assessed using low value side streams. Robust pretreatment methods for saw dust are tested to release sugars for BDO fermentation, and the suitability of untreated sludges for BDO production is evaluated. VFA production potential is analysed for all materials, and possibilities for process integration are examined by valorising residues from BDO production.

## 2. Materials and methods

### 2.1. Forest industry side streams

The study was conducted for both medium and low-value side stream samples originating from the forest industry. Birch (*Betula pendula*) sawdust represented the medium-value side stream generated during birch sawing and contained both bark and wood (Fig. 1). The sample consisted of manually prepared, fresh wood cuttings originating from the Northern Savo region, Kuopio, Finland, which were stored at room temperature and pretreated and processed into fractions as described in 2.2.1 and 2.2.2. Birch was selected as the main medium-value side stream based on a pre-test with several birch and spruce (*Picea abies*) side streams. Based on the pre-test analysis results (see Supplementary Material), birch-based side streams, especially birch sawdust, were found to be the most suitable substrate for microbial fermentation due to its low content of lignin, which typically is rich in antimicrobial agents (Li et al., 2023) and could inhibit the fermentation process.

Low-value side streams originated from the wastewater treatment of a kraft pulping mill utilising birch. The samples were sludge (S1) and concentrated sludge (S2) from the chemical process, as well as primary sludge (PS), secondary sludge (SS), and dried sludge (DS) from the plant's wastewater treatment. No further treatments were performed on these materials.

### 2.2. BDO production

#### 2.2.1. Strain selection

*Bacillus licheniformis* DSM 8785 was obtained from the German Collection of Microorganisms and Cell Cultures (Leibniz Institute DSMZ, Braunschweig, Germany). To revive the culture, a cryovial was inoculated into nutrient broth. From this, a stock culture was prepared by mixing 0.5 mL of the revived culture with 0.5 mL of 30% (v/v) sterilised glycerol in 2 mL tubes, which were stored at −80°C for subsequent use in inoculum preparation. In addition, *B. licheniformis* G4 strain, isolated by the Natural Resources Institute Finland (Luke) from soil, was tested.

For seed culture preparation, the stocks were plated into nutrient agar and incubated at 30°C for 24 h. A single colony was then transferred to a 50 mL falcon tube containing 15 mL of nutrient broth. The next day, 0.5 mL of that culture was transferred into 250 mL shake flasks containing 50 mL of nutrient broth. All liquid incubations were performed at 30°C with agitation at 180 rpm for 24 h.

#### 2.2.2. Pretreatment of sawdust

The sawdust sample (0.5–2 mm) from birch (10% w/w) was pretreated in 5 000 mL beakers with 2% NaOH (w/w) at 121°C, 1 atm for 20 min. After cooling, excess alkali was washed thoroughly with water and dried at 65°C for 12–24 h then subjected to enzymatic hydrolysis using MetZyme®SUNO™400 enzyme for hydrolysis (MetGen Oy, Finland).

Before hydrolysis, several buffers, often standards in wood hydrolysis protocols (0.1 M citrate buffer, 0.1 M succinate buffer and 0.05 M acetate), were tested to see how they affected bacterial growth. This allowed an assessment of whether the buffers themselves, rather than hydrolysis products, could influence bacterial growth. The least inhibiting was acetate, which was selected for further use.

The alkali pretreated mass (10% w/w) was mixed with 0.05 M acetate buffer and 2% of enzyme solution at pH 5, and incubated 72 h at 50°C with shaking at 180 rpm. The reaction was stopped by autoclaving, to ensure the biomass sample was sterile before inoculation for

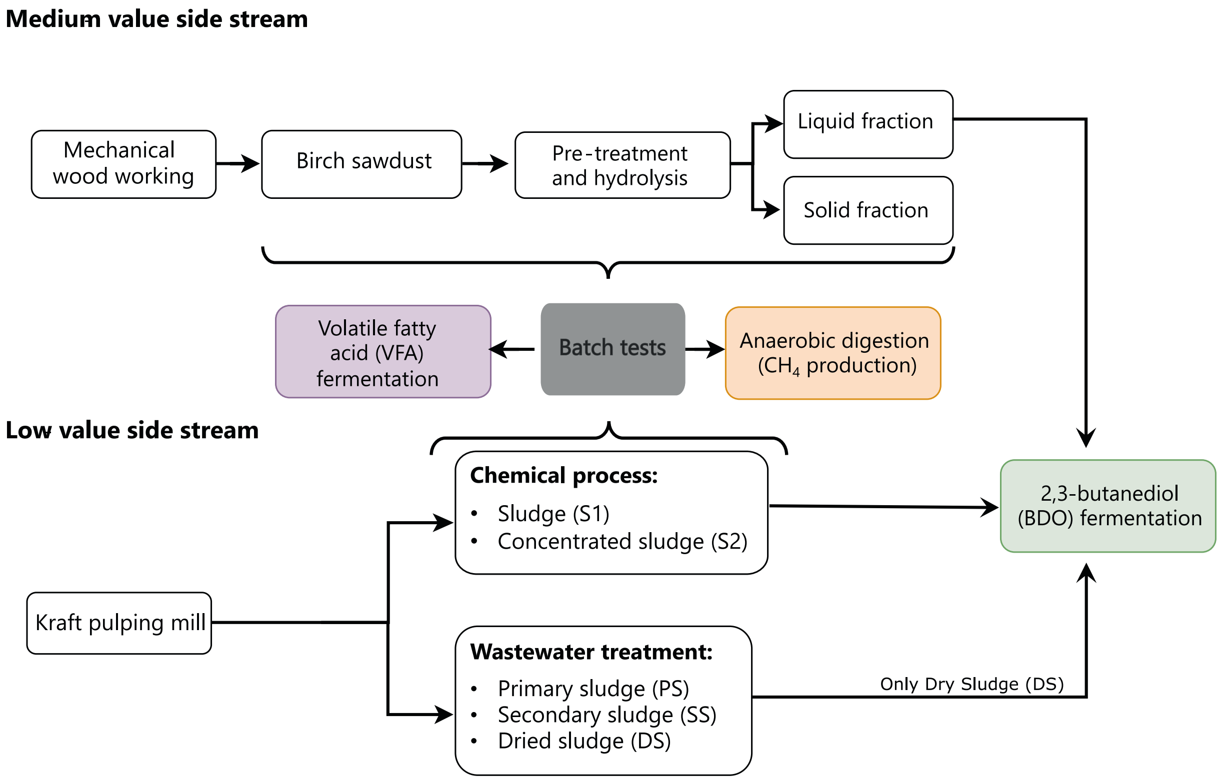

**Fig. 1.** Tested medium- and low value side streams from the forest industry and applied processing technologies.

saccharification. After hydrolysis, the hydrolysate was centrifuged (8000 g, 10 min at 10°C) to separate liquid and solid fractions. The liquid fraction was directed to the saccharification (BDO fermentation), while the solid fraction was used as a substrate in the VFA production experiment. The liquid fraction was autoclaved before fermentation to enable sterile conditions for BDO fermentation.

### 2.2.3. *Saccharification*

The growth of the *B. licheniformis* strains DSM 8785 and G4 was tested in different glucose concentrations (5, 8, 10 and 16%) at different incubation times (24, 48, and 72 h), from which the best performing conditions were selected.

Bacterial strains were cultured in birch sawdust hydrolysate diluted with tryptic soy broth (TSB) to assess the material's inhibitory threshold. Hydrolysate concentrations of 20%, 30%, and 50% (v/v) were tested. For example, the 80% condition consisted of 80% TSB (17 g/L tryptone, 3 g/L soy peptone, 5 g/L NaCl, 2.5 g/L $K_2HPO_4$, 2.5 g/L glucose, and distilled water to 1 L) and 20% hydrolysate. Based on growth performance, the 80% hydrolysate dilution was selected for further experiments. No additional glucose was added beyond the 2% glucose inherently present in the TSB.

The selected strain was subsequently used to ferment the 20:80 (hydrolysate:TSB) mixture. Additionally, a medium prepared by diluting hydrolysate with nutrient broth in a 30:70 ratio was employed. Fermentations were conducted in 20 mL volumes at pH 6.0, 37°C, and 180 rpm for 24 h in triplicate.

Bacterial strains were also grown in S1, S2, and DS without any pretreatment. Due to the high acetic acid content, the samples S1 and S2 were diluted 1:10 with TSB, without the addition of external sugars; sample DS was diluted 50:50. Incubation conditions were identical to those used for other lignocellulosic substrates.

## 2.3. *VFA fermentation*

VFA fermentation experiments were conducted in 500 mL glass bottles at 37°C using Automatic Methane Potential Test Systems (AMPTS, BPC Instruments, Sweden). Each sample was experimented in triplicate. In addition, each sample had 12 identical bottles of which three were destructively sampled on days 2, 6, 10 and 15 days after the experiment was started. Bottle headspaces were flushed with pure $N_2$ before connecting to the gas measurement system. Carbon dioxide from the produced gas was captured by a 3 M NaOH solution bottle before the gas measurement system. Each bottle was mixed for 60 s every 60 min.

In Experiment 1, the birch sawdust and its pretreated and BDO-fermented fractions were tested. Each bottle was filled with 280 g of inoculum. Samples were added in $VS_{substrate}/VS_{inoculum}$ ratio of 3, after which the bottles were filled to 300 g with MilliQ water. Inoculum for Experiment 1 was sourced from a bioreactor using garden waste as feedstock.

In the Experiment 2, low-value side streams of kraft pulping process were tested. Each bottle was filled with 340 g of inoculum and a set amount of VS with samples to achieve inoculum (S/I) ratios of 1.0 with samples S1, S2 and DS, 0.6 with sample SS, and 0.15 with sample PS. Finally, the bottles were filled to 400 g with MilliQ water. The S/I ratio with samples SS and PS is due to them being very dilute feedstocks and only being available in limited amounts, thus requiring the S/I ratio to be decreased. For the Experiment 2, inoculum was sourced from an anaerobic digestion plant that uses local municipal and industrial organic wastes as feedstock. For VFA experiments, both inoculums (in Experiments 1 and 2) were thermally treated by heating them to 90°C for 10 min to deactivate methanogens (Magrini et al., 2021).

The volumes of both inoculum and substrate, and the total liquid volume of the sample bottle (400 mL), were considered when calculating the initial sample concentrations. Gas productions were converted to standard conditions (0°C, 1 atm).

## 2.4. *Biomethane potential*

Biochemical methane potential tests were conducted only for the low-value forest industry side streams from a kraft pulping process, i.e.

samples S1, S2, PS, SS, DS. The experiment was executed in the same system and similar bottles as the VFA experiment, except that the inoculum used was not thermally treated. Each sample was tested in triplicate. The experiment was stopped when the bottles produced less than 1 mL of gas over a single day. The methane production of the inoculum was subtracted from the production of inoculum plus sample.

### 2.5. Chemical analyses

The amounts of total solids (TS) and volatile solids (VS) were analysed according to SFS 3008 (SFS, 1990). The content of carbon (C), hydrogen (H), nitrogen (N), and sulphur (S) was determined using a LECO CHN628 elemental analyser (LECO Corporation, USA), and oxygen (O) was calculated based on mass balance. Chemical composition of the samples, hemicellulose, cellulose and lignin was analysed according to (Raitanen et al., 2020). Briefly, the hemicellulose content was determined by acid methanolysis (Sundheq et al., 1996). Extractives were obtained from samples using an accelerated solvent extractor (ASE-350) by extracting with hexane, acetone/water (95/5, v/v) and ethanol. Lignin content was subsequently determined from extractive-free samples. The cellulose content was calculated as the difference between the amount of glucose obtained from acid hydrolysis and that obtained from acid methanolysis. VFAs, including acetic, propionic, isobutyric, *n*-butyric, isovaleric, valeric and caproic acids, were analysed as in Tampio et al. (2019). pH was measured with Orion 4 Star pH meter (Thermo Electron Corporation).

For the analysis of BDO, the fermentation broth samples 50 μL aliquots were mixed with 475 μL of deuterated methanol (MeOD, Methanol$_4$ 99.80% D, Eurisotop SAS, Saint-Aubin, France) containing 1.09 mM 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid sodium salt (TSP; Thermo Scientific, Waltham, MA, USA). The 1H NMR spectra were measured by Bruker AVANCE III HD 600 MHz NMR spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). The TSP signal (1.09 mM) was used as an internal quantification standard. Furthermore, high purity BDO standards were used to confirm the identification and correct quantification. The concentration of TSP in the MeOD was validated using trimethoxybenzene (Reagent plus, Sigma-Aldrich,138827) and dimethylsulfone (DMS quantitative NMR TraceCERT, Sigma-aldrich 41867-1G) as internal standards. In addition to BDO, ethanol and acetic acid concentrations were determined.

### 2.6. Statistical analyses

All statistical analyses were performed with R software (R Core Team, 2025). VFA concentrations were compared among samples using non-parametric Kruskal–Wallis tests, followed by Dunn's post hoc comparisons with adjusted p-values, using the Bonferroni method reported. Cumulative methane production curves were modelled using the modified Gompertz equation to estimate maximum yield, production rate, and lag phase. Replicate parameter values were compared across samples using the same non-parametric tests applied to VFA data.

## 3. Results and discussion

### 3.1. Side stream characteristics

The tested side streams were characterised for their total TS and VS, VFA (Supplementary Material, Table S1) and sugar (details in Fig. 2) content. The TS content varied greatly depending on the matrix of the sample, which varied from dry solid material (birch sawdust TS 97%) to sludge and liquid materials originating from the treatment of the sawdust (TS 9–16%), as well as from the kraft pulping mill (0.7–57%, Table S1). The birch sawdust had a CHNSO content of 50.9%, 6.2%, 0.3%, 0.05% and 42.2%, respectively.

The content of sugars increased after the thermal-alkali pretreatment and hydrolysis of the birch sawdust (Fig. 2). The sugar content for the low value side-streams (S1, S2, PS, SS, DS) was not available, but the initial VFA content of these samples indicated the highest organic matter solubilisation in the samples S1 and S2, where VFA content was 78 and 12 mg/L, respectively (Table S1). This was a consequence of the kraft

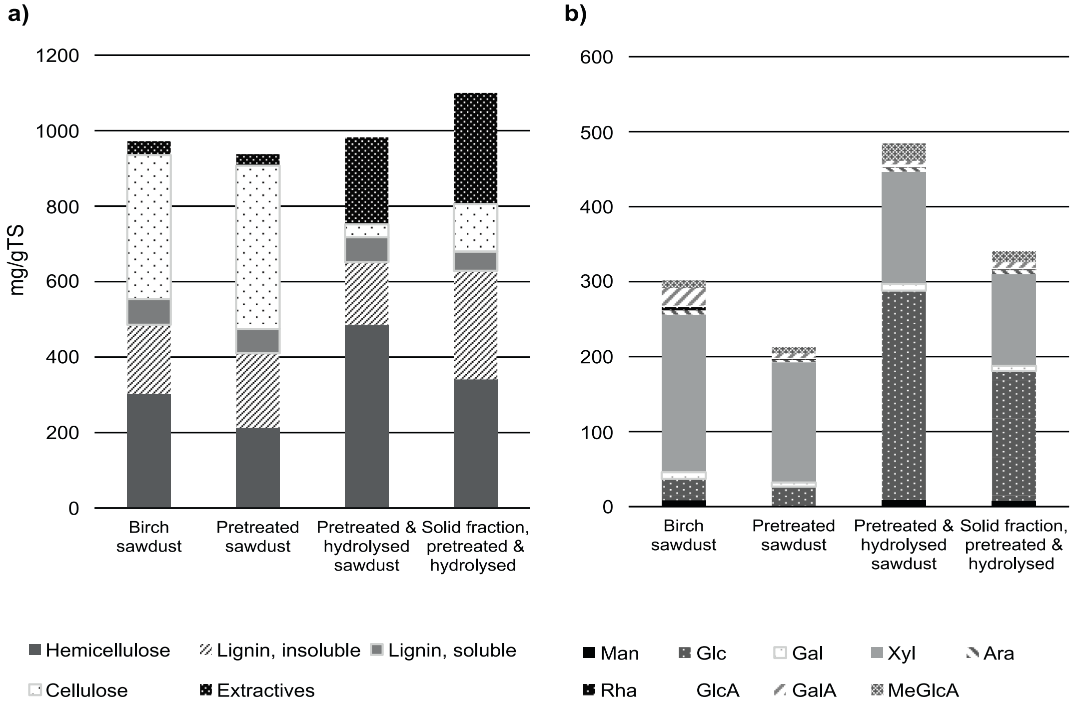

**Fig. 2.** a) Chemical composition and b) non-cellulosic carbohydrates in the initial birch sawdust and in the treated fractions after pre-treatment, hydrolysis and centrifugation. Values are averages of triplicate samples; see Supplementary Material for standard deviations. Man = Mannose, Glc = Glucose, Gal = Galactose, Xyl = Xylose, Ara = Arabinose, Rha = Rhamnose, GlcA = Glucuronic acid, GalA = Galacturonic acid, MeGlcA = 4-O-methyl-glucuronic acid.

pulping process, which acidified the material. The low VFA concentrations in the sludge samples from the wastewater treatment were not in line with a study by Veluchamy and Kalamdhad (2017), who previously indicated pulp mill sludges to contain higher VFA concentrations (~470 mg/L). However, the variation within the mills and their processes, as well as the storage time of the samples, explains the differences.

## 3.2. BDO fermentation

### 3.2.1. Birch sawdust hydrolysis efficiency

The liquid fraction of the pretreated and hydrolysed birch sawdust were subjected to BDO fermentations, while the solid fraction, as well as the raw sawdust and the intermediate fractions, were tested for VFA production (see 3.3). The amounts of extractives (37 mg/gTS vs. 30 mg/gTS), cellulose (381 mg/gTS vs. 390 mg/gTS) and hemicellulose (302 mg/gTS vs. 300 mg/gTS) in birch sawdust (Fig. 2) were similar to those in previous research (Kilpeläinen et al., 2014).

The pretreatment steps changed the shares of the different lignocellulose components. Lignin and hemicellulose were degraded in alkali pretreatment, and the share of cellulose decreased due to pretreatment and hydrolysis (Fig. 2). There was only a small amount of ordered (crystalline) cellulose left in pretreated and hydrolysed samples, as acid methanolysis could detect a larger proportion of glucose in samples that was present in the original sawdust sample. The amount of extractives increased substantially due to the hydrolysis of structural cellulose and hemicellulose polymers to oligo- and monosaccharides that could be extracted with acetone and ethanol.

The majority of soluble sugars were recovered in the liquid fraction and utilised in the BDO fermentation, but also some fermentable compounds were left in the solid cake and other intermediate fractions for the VFA production.

### 3.2.2. BDO fermentation performance

The BDO fermentation was done using both medium (birch sawdust) and low value side streams (sludges from the kraft pulping mill). Based on the pre-testing, the *B. licheniformis* strain DSM8785 was selected to be used for further BDO experiments. While it exhibits slightly higher sensitivity compared to the G4 isolate (Fig. S3), its extensive documentation in the literature was a key factor in its selection.

For BDO production from birch sawdust, a total of 700 g of raw material was treated with 6300 mL of 2% (w/v) NaOH. Following incubation, washing, and drying, 506 g of alkali-treated biomass was recovered, corresponding to 72.3% of the initial dry weight. Of this, 385 g (55.0% of the original sawdust) was subjected to enzymatic hydrolysis in 2950 mL of acetate buffer. Centrifugation of the hydrolysate yielded 2744 mL of supernatant and 505.7 g of wet solid residue. A 20 mL aliquot of the liquid phase (0.73% of the total hydrolysate) was combined with 80 mL of TSB and fermented, producing 5 g/L of BDO. This corresponds to a BDO yield of 0.13 g per gram of raw sawdust (0.12 g/gTS), equivalent to 12.8% of the theoretical maximum yield based on raw biomass input.

BDO production was also evaluated using kraft pulping mill samples S1, S2, and DS, without any pretreatment or hydrolysis. The resulting BDO yields, expressed per gram of raw sample, were 0.19 g/g for S1, 0.74 g/g for S2, and 4.6 g/g for DS, respectively. It should be noted that the TS of the samples varied, and thus, the TS-based BDO concentrations were 0.01, 0.42, and 0.88 g/gTS. BDO yields of both S2 and DS were higher than the BDO production achieved with the birch sawdust (0.12 g/gTS). This confirms that the materials degraded and released fermentable sugars during the kraft pulping mill processes. As low value side streams, these materials provide interesting feedstock for fermentation processes, such as BDO. To the authors knowledge, this is the first study to report BDO production from kraft pulping mill sludges.

The BDO production from the birch sawdust was very moderate (0.13 g/g) compared to BDO yields that have been reported with engineered *B. licheniformis* strains and pure glucose substrates (0.4–0.49 g/g glucose, Jurchescu et al., 2013; Qiu et al., 2016; Tsigoriyna et al., 2021). However, it is widely known that the fermentation with mixed sugars results in lower yields (Guragain et al., 2017). In addition, studies generally use pretreated and enzymatically hydrolysed biomass, not raw sawdust, allowing microbial strains to achieve $\geq 0.3$ g per g sugar or hydrolysate, translating in practice to $\sim 0.2$–$0.4$ g per g of biomass when taking into account hydrolysis efficiency.

Overall, there is limited literature regarding the BDO yields from forest industry side streams with *B. licheniformis* strains. High BDO production (up to 0.45 g/g) has been reported with *Klebsiella* sp. using rice straw hydrolysate (Huang et al., 2013). Hazeena et al. (2022) have reported BDO production from oat hull hydrolysate using *Enterobacter cloacae* sp.SG1, which yielded 37.59 g/L BDO when supplemented with 20 g/L of glucose. In the present study, the objective was to assess the potential of wood residues as a standalone carbon source for BDO production, thus adding external sugars would confound the results, making it difficult to evaluate the efficiency of biomass hydrolysis and microbial sugar uptake. In an industrial setting, supplementing sugar would increase feedstock costs and reduce the economic viability of the process (Ebrahimian and Mohammadi, 2024).

Low BDO production from birch sawdust was not attributed to a lack of fermentable sugars, as both birch and its hydrolysate were confirmed to contain glucose (Fig. 2). However, it remains possible that glucose was depleted prior to the fermentation experiment. In addition, the low BDO production in the fermentation process involving *Bacillus licheniformis* strain can be explained by considering three primary factors: aeration issues and inhibitory compounds in wood residues, and the sugar composition of the sawdust hydrolysate. *B. licheniformis* requires a balance of aerobic and anaerobic conditions to efficiently shift metabolism toward BDO production, where too little oxygen can lead to excessive acid formation (e.g., lactate, acetate) instead of BDO (Celińska and Grajek, 2009), although no excessive amounts of acetate were detected. Furthermore, the relatively high proportion of xylose (C5) compared to glucose (C6) in sawdust hydrolysates may have constrained BDO yields, since *B. licheniformis* DSM 8785 and related strains preferentially utilise glucose, while xylose uptake and metabolism are limited and often subject to catabolite repression if not fed as a sole sugar (Guragain, 2015; Song et al., 2018). The test material could have also contained inhibitory compounds, such as phenolic compounds, which act as microbial growth inhibitors (Fernández-Sandoval et al., 2024). Nonetheless, the risk of inhibition by lignin-derived compounds was minimised by selecting birch with low lignin content (see Supplementary Material) as the test material. The combination of poor aeration, leading to redox imbalance and impaired metabolic flux, and toxic inhibitors from wood residues, interfering with enzymatic activity, explains the observed lack of BDO production. Further optimisation, such as controlled oxygenation strategies and detoxification of lignocellulosic hydrolysates (e.g., activated carbon treatment, enzymatic detoxification) (Guo et al., 2022) together with dilution of the material, as in our approach, improve fermentation efficiency.

## 3.3. VFA fermentation

### 3.3.1. VFAs from birch sawdust

VFA fermentation with a medium-value side stream (birch sawdust) was tested, using both raw sawdust and the pretreated, hydrolysed, and centrifuged solid fraction. The objective was to evaluate the combined and separate efficiencies of BDO and VFA production. Overall, the produced VFA concentrations were low. During the 15-day experiment, birch sawdust and pretreated sawdust produced VFAs 0.2 and 0.3 g/gTS (Fig. 3a), which corresponds to 1.0 g/L and 1.6 g/L, respectively. Production from solid fraction was higher, yielding approximately 0.5 g/gTS (2.2 g/L). Although the concentration peaked on day 6, the difference was statistically significant only on day 15 against the sawdust sample ($p < 0.05$; Table S5b). By utilising solid fraction in addition to the liquid fraction as a substrate for BDO production, a biorefinery

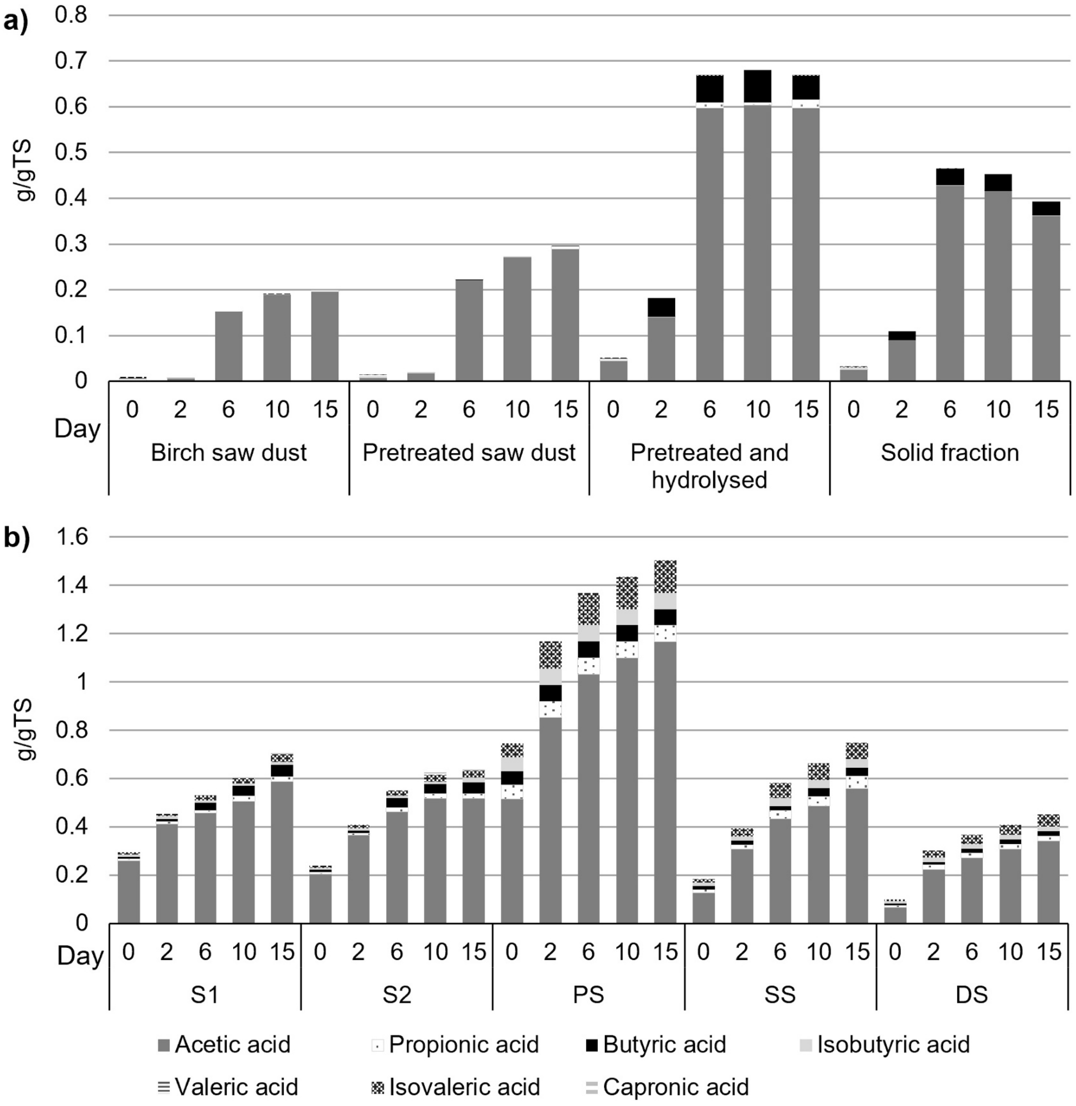

**Fig. 3.** a) VFA production from the birch sawdust and its pretreated fractions, b) VFA production from kraft pulping mill side streams. Sludge (S1) and concentrated sludge (S2) from the chemical process, primary sludge (PS), secondary sludge (SS) and dried sludge (DS) from the mill's wastewater treatment. Values are averages of triplicate samples; see Supplementary Material for standard deviations.

focusing on forest industry side streams could produce both BDO and VFAs from the same raw material. Thus, the overall highest VFA concentration was reached with combined pretreatment and hydrolysis of sawdust, which yielded VFAs up to 0.68 g/gTS (3.9 g/L), as the pretreatment and hydrolysis released sugars suitable for VFA fermentation.

For all samples, VFA concentrations multiplied from day 2 to day 6, which can be seen as a steep increase in the relative concentrations (Fig. 3a). However, the VFA concentration in solid fraction samples reached its peak on day 6, and decreased by 0.07 g/gTS (0.4 g/L) towards the end of the experiment, whereas the concentrations remained approximately the same or increased in other samples during the later measurement days.

The main acids found in this VFA fermentation were acetic, butyric, and propionic acids, with acetic acid being the dominant (75–100% of total VFAs) acid in all samples. Similar results have been reported for, e. g., sugarcane vinasse fermentation at neutral pH (Magrini et al., 2021). However, the butyric acid fraction was significant in the samples which were both pretreated and hydrolysed, as well as in the solid fraction samples.

### 3.3.2. *VFAs from kraft pulping mill side streams*

From the studied low value forest industry side-streams, higher VFA yields were achieved compared to the birch sawdust, even though no pretreatment was applied to the kraft mill materials. As sludges from the mill have undergone treatment and degradation steps, no additional pretreatment procedures were necessary. This makes the production of valuable biochemicals more economical. The highest VFA concentration (1.5 g/gTS) was reached with the primary sludge from wastewater treatment (PS), thus, as concentration, the VFA yield was low (2.2 g/L) due to the dilute sample (Fig. 3b, statistical analyses in Table S6b). The sludges S1 and S2, both originating from the chemical process, as well as the secondary sludge (SS), all yielded up to ~ 0.7 g/gTS on day 15 (respective concentrations ~ 6.5 and ~ 4.3 g/L). In all samples, the VFA concentrations increased steadily as the experiment progressed, and the highest concentrations were obtained on day 15 (last measuring day).

Experiment 2 produced mainly acetic, butyric, propionic, isobutyric, and isovaleric acids. Acetic acid dominated, accounting for 70–90% of total VFAs. Compared to Experiment 1 with birch sawdust, the acid profile was more diverse, with higher fractions of minor acids like isovaleric, reflecting the complex composition of kraft pulping mill sludges, especially wastewater samples rich in microbial biomass (Veluchamy &

Kalamdhad, 2017).

### 3.3.3. VFAs from medium and low value forest industry side streams

In general, the achieved VFA production with medium and low value side streams was in line with what was expected, as the highest achieved concentrations were 1.5 g/gTS with the sample from the wastewater treatment of a kraft pulping mill, and the lowest yield with untreated birch sawdust (around 0.2 g/gTS). Although the different S/I ratios used in Experiments 1 and 2 complicate direct comparison of the results, the low VFA yields from sawdust clearly originate from its lignocellulosic structure, which is difficult to degrade and utilise efficiently (Veluchamy & Kalamdhad, 2017). Kim et al., 2013) (as acetic and butyric acid) has been also previously reported for a lignocellulosic side stream, cassava pulp (Mulyawati et al., 2024).

As for concentrations in the fermentation broth, the VFA results appear to be lower than what has been previously reported for a variety of lignocellulosic materials and different experimental set-ups (up to 20 g/L, reviewed by Sun et al., 2021). A possible explanation for this was the methane production during the VFA experiment (Experiment 1 with birch sawdust), which was as high as 60 $Nm^3$/tVS, and indicated that the inactivation of the methanogens (i.e. methane producing archaea) in the inoculum had not been successful. Due to the production of methane, the acid-producing microbes may not have been in optimal conditions. In addition, pH remained around 7 with birch sawdust and most of its pretreated fractions (Table S4). However, the targeted acidification effect was observed in the pretreated and hydrolysed samples, where pH decreased to 6 during Experiment 1. In Experiment 2, methane production was monitored and remained low ($<$15 $Nm^3$/tVS), indicating that methanogens did not compete with acid-producing microbes. pH levels stayed between 8 and 9 due to the applied low S/I ratios (Table S4). Thus, the VFA concentrations for kraft pulping mill sludges in Experiment 2 reached approximately 6.5 g/L, which aligns with the yield of about 4.2 g/L previously reported for primary sludge from the pulp and paper industry (Li et al., 2022a).

The results showed that the VFA concentration for the alkali pretreated birch sawdust was 1.5 g/L and for pretreated and hydrolysed sawdust 3.5 g/L, whereas the concentration was limited to 1.0 g/L with untreated birch sawdust. In comparison, similar results with sawdust (originating from *Platanus*) were reached with heat and chemical pretreatments (Kim et al., 2013). When heat and $Ca(OH)_2$ treatments were applied, the highest VFA concentration was 2.5 g/L in total and 1.0 g/L as calculated from lignocellulosic biomass. Moreover, with combined heat, $Ca(OH)_2$, $NH_3$ and NaOH pretreatments, the VFA concentrations increased by 117% compared to utilising only heat treatment (Kim et al., 2013). As a conclusion, although the obtained concentrations in the present study were low in general, the impact of combined heat and chemical and/or enzymatic hydrolysis has been found to be important in improving the VFA concentrations produced from sawdust in both studies.

Moreover, the observation supports the combining BDO and VFA production processes. When lignocellulosic substrates are pretreated for the BDO production process, the pretreatment could be beneficial in increasing the concentrations obtained from VFA fermentation as well. It was evident that the pretreatment and hydrolysis improved VFA production. The solid fraction, which is a residue of birch sawdust prepared for the BDO fermentation, was further utilised to obtain increased concentrations of VFAs compared to the untreated sawdust. In conclusion, the solid fraction does not compete with the BDO production, yet provides additional value through the VFA production. In a biorefinery focusing on forest industry side streams, both BDO and VFAs could thereby be produced from the similarly treated sawdust.

### 3.4. Biochemical methane production potential of kraft pulping mill side streams

Methane production performance was analysed for the kraft pulping mill side streams, i.e. samples S1, S2, PS, SS, and DS. The highest production of methane was observed in the PS (430 $Nm^3$/tVS, Fig. S4), though this difference did not reach statistical significance ($p < 0.079$) due to substantial replicate variation (Tables S7a and S7b). The PS sludge was the most dilute sample, and it had only 0.65% TS content, which is why a lower S/I ratio (0.15) was used. Due to the low amount of VS added to the bottle, the results might be skewed in favour of the sample, as lower S/I ratios are known to yield higher methane potentials (Li et al., 2022b).

The other tested sludge samples produced methane in the range of 140–200 $Nm^3$/tVS, which is similar to what has been reported previously (reviewed by Veluchamy and Kalamdhad, 2017). A very small amount of $CH_4$ potential was lost during the concentration of the sludge from the mill's chemical process, as sample S1 consisted of untreated (production 200 $Nm^3$/tVS) and S2 of concentrated sludge (production 189 $Nm^3$/tVS).

### 3.5. Comparison and integration potential of BDO and VFA fermentation and anaerobic digestion

To enable the comparison between the tested BDO, VFA and methane production, the revenues of these products were compared, based on their studied yields and selling prices. Production costs (incl. costs for pretreatment, processing, and end-product recovery) were not included because the aim was a simplified, relative comparison of product value, and selling prices typically reflect average production costs and market conditions. The comparison was made for both the studied medium and low-value side streams. In addition to the comparison, the integration potential of BDO and VFA fermentation processes was estimated based on the assessed BDO production from pretreated and hydrolysed birch sawdust, and VFA production from the centrifuged solid fraction after hydrolysis. All results were calculated per g of TS of birch sawdust, or g of TS of the kraft pulping mill side stream. In the case of the birch sawdust, the dilution due to the pretreatment and hydrolysis was taken into consideration.

The technologies were compared through the market price of the products. For 2,3-butanediol, recent market prices were not available. However, Zang et al. (2020) suggest BDO market price to be close to the one of 1,4-butanediol, to which the BDO price was reflected in this study (2018–2025 EU market: 1.42–3.52 $/kg, see Table 1). For the market price of VFAs, the price of acetic acid in EU (0.39–0.79 $/kg, 2018–2025) was used (Table 1), as the share of other produced VFAs, such as propionic and butyric acids, in the obtained VFA mixture was low (Fig. 3). However, the price for longer-chain VFAs would be higher, even double or triple the price of acetic acid, as reported by Chen et al. (2022). For methane, the market prices depend on the gas upgrading, as well as possible subsidies. The price of natural gas for non-household consumers in EU ranged in 2008–2024 from 0.15 to 0.64 €/$m^3$ (Eurostat, 2024).

As a result, when comparing the revenues of BDO and VFA production from pretreated and hydrolysed birch sawdust fermentation, VFA production could be slightly more feasible product with revenues of 250–506 $/tTS vs. 176–436 $/tTS from BDO production (Table 1). However, the revenues are highly dependent on the price fluctuations. Without pretreatment and hydrolysis, the VFA production revenues were lower than those of BDO, 78–158 $/tTS. Thus, the pretreatment and hydrolysis of birch sawdust are essential, and, due to the moderate BDO production achieved in the present study, VFA production was deemed more economically feasible. However, by combining BDO and VFA fermentation processes in a birch sawdust biorefinery concept, even larger revenues (328–745 $/tTS) could be possible indicating that the process integration is highly beneficial. In the combined BDO and VFA production scenario birch sawdust was pretreated and hydrolysed, while liquid fraction was directed to the BDO production and solid fraction to the VFA fermentation.

Compared to the birch sawdust fermentation, the processing of kraft

**Table 1**
Comparison of the studied individual or combined process technologies in terms of production and potential revenues of BDO (2,3-butanediol), VFAs (volatile fatty acids) and $CH_4$ (methane). UT = untreated, PT + H = pre-treated and hydrolysed, S2 = concentrated sludge from the kraft pulping mill's chemical process, DS = dried sludge from the kraft pulping mill's wastewater treatment.

| Process according to end-product | | BDO | VFA | BDO and VFA combined | $CH_4$ |
|---|---|---|---|---|---|
| Market price ($/kg) | | 1.42–3.52[1] | 0.39–0.79[2] | n.d. | 0.15–0.64[3] |
| ***Average production (kg/tTS)*** | | | | | |
| Birch sawdust | UT | n.d. | 200 | n.d. | n.d. |
| | PT + H | 124 | 640 | 124 (BDO)+ 390 (VFA) | n.d. |
| Kraft pulping mill sludge | S2 | 420 | 640 | n.d. | 174[4] |
| | DS | 879 | 450 | n.d. | 180[4] |
| ***Revenue ($/tTS)*** | | | | | |
| Birch sawdust | UT | n.d. | 78–158 | n.d. | n.d. |
| | PT + H | 176–436 | 250–506 | 328–745 | n.d. |
| Kraft pulping mill sludge | S2 | 597–1480 | 251–508 | n.d. | 26–111 |
| | DS | 1248–3093 | 176–356 | n.d | 27–115 |

n.d., not determined.

[1] 1,4BDO: 2018–2025, BusinessAnalytic.com (2025a).

[2] Acetic acid: 2018–2025, BusinessAnalytic.com (2025b).

[3] as $\$/\mathrm{m}^3$; Gas prices for non-household consumers 2018–2024, Eurostat (2024).

[4] as $\mathrm{m}^3$/tTS.

pulping mill sludges was shown to be more feasible, in terms of potential revenues from BDO, VFA or methane production (Table 1). Due to the higher BDO production per kg of treated solids, the use of the studied low-value side stream sludges could lead to three to seven times higher revenues. In addition, as the sludge materials do not need any pretreatment, the economy of the whole processing could be much more profitable, as the pretreatment is known to play major part in the economy of BDO production (Ebrahimian and Mohammadi, 2024; Gadkari et al., 2023). The production of VFAs from kraft pulping sludges was comparable to the VFA production from pretreated and hydrolysed birch sawdust. Methane production through anaerobic digestion was not shown to be as profitable due to the moderate price of the methane gas. In addition, it should be noted that all processing options were evaluated individually, while BDO and VFA production was also assessed and combined treatment utilising BDO residues for VFA production. Likewise, VFA and methane production can be integrated by using residues from VFA processing as feedstock for methane generation.

The results of the integration potential assessment regarding birch sawdust utilisation are in line with Hakeem et al. (2023), who also reported in their review on increasing the economic performance in coproduction of biochemicals and biofuels in biorefinery solutions. As a comparative assessment, the VFA production was shown to be slightly more feasible than BDO and methane. Currently, the bio-based production of both BDO and VFA is in development stage, while anaerobic digestion can be regarded as a mature technology. The development stage of BDO and VFA production has been considered to be similar in a study by Ewing et al. (2022), who ranked VFAs and BDO in stages 17 and 19 regarding their potential to be in bulk production in 2050. Thus, if not combined, BDO and VFA production processes could be seen as competitive processes in the production of bio-based chemicals from forest industry side streams.

The recovery and purification of BDO and VFAs was not assessed in this study, but it has been proven technically feasible, while challenges remain due to their water solubility and low concentrations. Advanced methods, such as aqueous two-phase extraction combined with distillation for BDO, can achieve > 98% recovery (Gawal and Lataye, 2025), while various technologies have been reviewed to recover 70–90% VFAs with high purity (Chen et al., 2022).

## 4. Conclusions

This study demonstrates the promising potential of forest industry side streams as raw materials for producing bio-based and renewable chemicals. Birch sawdust, a medium-value side stream rich in lignocellulosic structures, requires effective pretreatment to enable microbial fermentation. In contrast, low-value side streams from kraft pulping mills can be utilised without extensive pretreatment, offering a more sustainable and cost-efficient alternative with a reduced carbon footprint. By comparing production yields and market values, kraft mill sludges showed high potential for BDO production, while birch sawdust benefited from integrated processing for BDO and VFAs. Process integration in sawdust-based biorefineries is recommended to enhance economic viability. Overall, integrating these processes into biorefinery concepts supports circular economy goals and adds value to underutilised forest industry residues.

## CRediT authorship contribution statement

**Elina Tampio:** Writing – review & editing, Writing – original draft, Visualization, Supervision, Resources, Methodology, Funding acquisition, Formal analysis, Conceptualization. **Lucia Blasco:** Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis. **Milla Tynkkynen:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Ilmari Laaksonen:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis. **Summaira Saghir:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Petri Kilpeläinen:** Writing – review & editing, Methodology, Formal analysis. **Reijo Lappalainen:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization.

## Declaration of competing interest

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

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biortech.2026.134089.

## Data availability

Data will be made available on request.

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