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Advancing psychrophilic fermentation: strategies for enhancing volatile fatty acid production

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Volatile fatty acids (VFAs) are valuable bio-based intermediates with applications in biofuels, bioplastics, and other industrial processes. As products of the carboxylate platform, VFAs serve as versatile precursors for various chemicals, contributing to a sustainable bioeconomy. Acidogenic fermentation under mesophilic and thermophilic conditions has been widely studied. However, these systems require significant energy inputs for heating, especially in colder climates. Psychrophilic fermentation offers a sustainable alternative with benefits such as lower energy inputs, enhanced carbon conversion efficiency, and reduced emissions. This review explores the strategies for enhancing VFA production under psychrophilic conditions to unlock the potential of low-temperature fermentation. A comprehensive discussion of the challenges associated with conventional fermentation systems highlights the unique advantages of psychrophilic fermentation. Key microbial adaptations and metabolic pathways in low-temperature environments are discussed, along with the influence of temperature on reaction kinetics and substrate utilization. Strategies for improving VFA yields include optimizing operational parameters, designing low-temperature reactors, applying pretreatment methods for substrates, and leveraging bioaugmentation with psychrophilic strains. Co-digestion of substrates and integration of bioelectrochemical systems are also evaluated for their potential to enhance acidogenesis. The review concludes with perspectives on microbial engineering, hybrid systems, and the economic feasibility of cold-adapted fermentation technologies, emphasizing their respective roles in advancing the carboxylate platform and sustainable bio-based production in cold regions.

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1. Introduction

Prominent challenges worldwide stem from the adverse environmental impacts of tremendous waste generation and its subsequent pollution, along with increasing demand for energy. A waste-to-energy approach could offer an effective and sustainable route for managing the annual two billion tonnes of waste problem.^{1,2} Acidogenic fermentation of organic waste offers a sustainable route for waste management and resource recovery through the production of volatile fatty acids (VFA). These intermediate compounds are monocarboxylic acids comprising of two to six carbon atoms (C₂ to C₆), *i.e.* acetic, propionic, butyric, valeric, and caproic acid in the order of increasing carbon chain. VFA have a vital role as building blocks in a broad range of applications in food, pharmaceutical, and plastic production industries and wastewater treatment.^{3,4} The

acidogenic fermentation process thus provides an environment-friendly alternative to the conventional petroleum-derived VFA. Furthermore, the use of waste streams as feedstock for mixed-culture fermentation can be employed by the conversion of existing anaerobic digestion systems, saving both energy and the associated costs of producing biogas. While anaerobic digestion is a well-established technology, there are several challenges to the process, such as extended residence times (exceeding 4 weeks), large reactor volume, inefficient carbon conversion (40–60%) and slow digestion rates.^{5–7} This subsequently increases the overall capital costs of the system. In contrast, VFA-oriented acidogenic fermentation achieves shorter residence times, higher carbon conversion efficiencies (~70%), and greater economic returns,⁸ with revenue from food waste estimated at \$23.62 per tonne for VFAs compared to \$12.07 per tonne for biogas.⁹

Predominantly research on VFA production has been conducted under mesophilic (25–37 °C) and thermophilic (45–55 °C) conditions, with mesophilic temperatures generally found to be optimal for microbial activity. However, in cold climate countries such as Canada, the average ambient temperature is below 20 °C. Thus, the energy expenditure for maintaining higher operational temperatures is a significant operational cost.^{10,11} Psychrophilic

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fermentation is emerging as a promising approach to address these challenges. This approach takes advantage of naturally occurring microorganisms that thrive in cold environments. Hence, it reduces the time required for acclimatising the microbial diversity to lower temperatures by optimizing other operational parameters and enhancing hydrolysis. Moreover, with psychrophilic fermentation, it is possible to reduce the high energy demands associated with mesophilic or thermophilic processes while maintaining efficient VFA production in cold countries. Furthermore, psychrophilic fermentation can contribute to sustainable waste management practices. Despite the advantages, research in this area remains limited, with most reviews emphasizing psychrophilic biogas production.^{12–14} To the knowledge of the authors, no comprehensive review has specifically examined VFA production under psychrophilic conditions, representing a critical gap this work aims to address.

2. Overview of acidogenic fermentation and insights from psychrophilic conditions

The organic matter, comprising carbohydrates, proteins, and lipids, first undergoes hydrolysis where complex compounds

are broken down into soluble forms, increasing the soluble chemical oxygen demand (sCOD). During this step, extracellular enzymes are secreted by hydrolytic bacteria from the Proteobacteria, Firmicutes, and Bacteroidetes phyla.¹⁵ The soluble compounds are then quickly fermented into pyruvate *via* glycolysis and subsequently into VFAs, with hydrogen, carbon dioxide, and small amounts of alcohol as by-products during the acidogenesis phase.¹⁶ This stage is facilitated by acidogenic bacteria, primarily belonging to the families *Bacillus*, Enterobacteriaceae, Clostridia and Bacteroides.¹⁵ Within the digester, methanogenic activity is suppressed to prevent VFAs from being further utilized to produce biogas. This can be achieved by (i) modifying operational parameters such as retention time, pH, and inoculum-to-substrate ratio;^{17,18} (ii) applying chemical inhibitors such as carbon monoxide, 2-bromoethanesulfonate, or 2-mercaptoethanesulfonate;^{19,20} or (iii) using substrate pretreatments to selectively restrict methanogen activity.²¹ The primary VFAs generated include acetic acid, propionic acid, butyric acid, valeric acid, and caproic acid. These acids serve as renewable carbon sources for numerous biological applications, such as biopolymer production, bioenergy generation, and biological nutrient removal. Additionally, they find use in industries such as food additives, pharmaceuticals, cosmetics, and chemical manufacturing.²¹ The distribution of VFAs

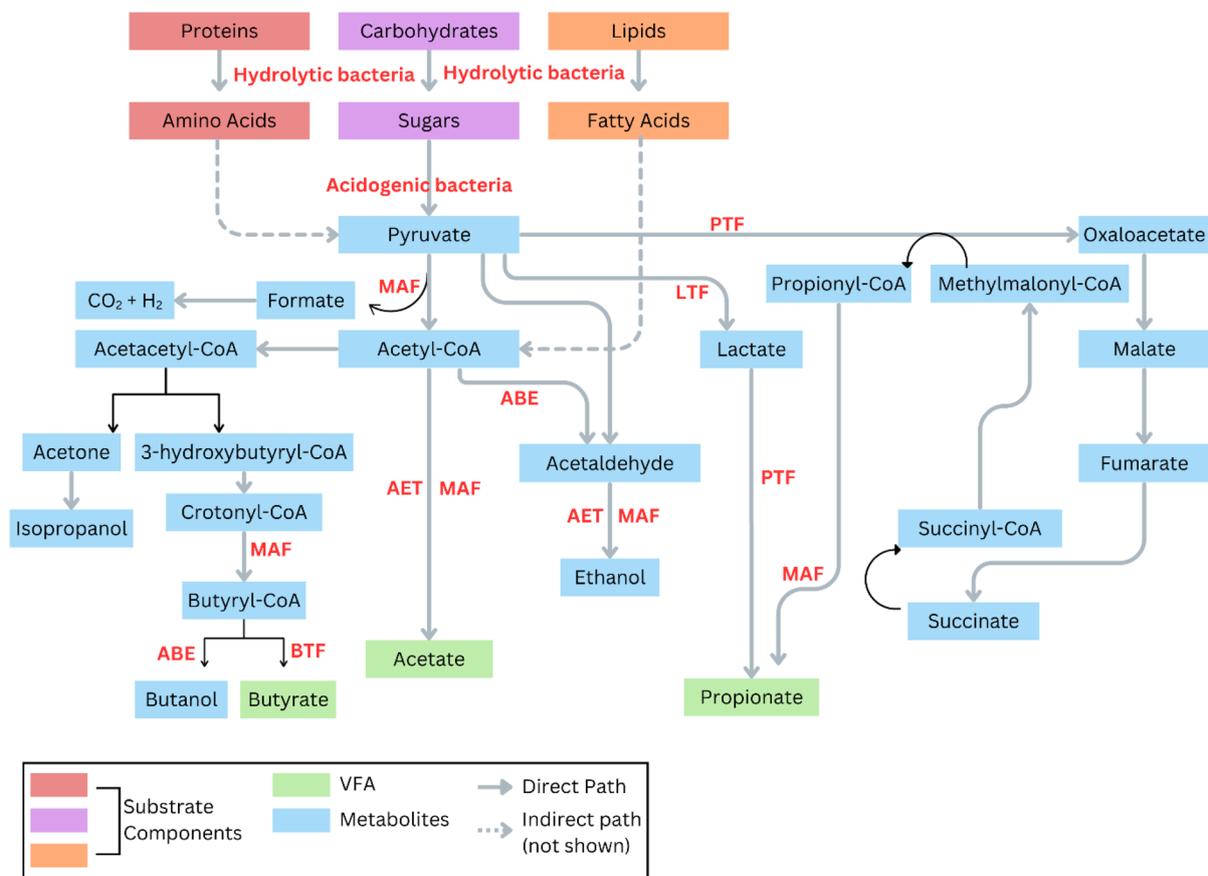


Fig. 1 Key metabolic pathways of acidogenic fermentation – acetate ethanol-type fermentation (AET), acetone-butanol-type fermentation (ABE), butyrate-type fermentation (BTF), lactate-type fermentation (LTF), propionate-type fermentation (PTF), mixed-acid fermentation (MAF) (adapted from Dahiya *et al.*, 2018 (ref. 23) and Zhou *et al.*, 2018 (ref. 24)).



depends on the dominant metabolic pathways, which are influenced by environmental factors like pH and temperature. Variations in these conditions drive the development of distinct microbial communities, leading to different fermentation profiles.²² Depending on the main fermentation product, acidogenic fermentation can be categorized into types such as acetate-ethanol, propionate, butyrate, mixed-acid, or lactate fermentation (Fig. 1).

Acetate is synthesized either *via* the acetyl-CoA pathway or through the syntrophic oxidation of ethanol and long-chain fatty acids. Ethanol formation involves the decarboxylation of pyruvate into acetaldehyde, which is then reduced to ethanol. Certain *Clostridium* species, such as *Clostridium acetobutylicum*, can also produce acetone and butanol during this process *via* acetone-butanol-ethanol (ABE) fermentation.^{25,26} Propionate can be produced through two distinct metabolic routes. In one pathway, pyruvate is first converted into lactate by lactate dehydrogenase, and lactate is subsequently reduced to propionate by the enzyme propionate dehydrogenase. Alternatively, the *trans*-carboxylation pathway can also lead to propionate formation. Butyrate production follows the Embden-Meyerhof-Parnas pathway, starting with glucose conversion to pyruvate. Pyruvate is then metabolized into butyryl-CoA through a series of intermediates including acetoacetyl-CoA, 3-hydroxybutyryl-CoA, and crotonyl-CoA. This sequence involves the enzymes thiolase, 3-hydroxybutyryl-CoA dehydrogenase, and butyryl-CoA dehydrogenase. Finally, butyryl-CoA is transformed into butyrate by the action of phosphotransbutyrylase and butyrate kinase.²⁵

In lactic acid fermentation, pyruvate produced from glycolysis is reduced to lactate by lactate dehydrogenase. This process occurs in two forms: homolactic fermentation, which yields two moles of lactate per mole of glucose, and heterolactic fermentation, where lactate is produced together with by-products such as acetate or ethanol *via* pathways like the phosphoketolase and Bifidus pathways.^{27,28} Mixed-acid fermentation is another metabolic route where a variety of fermentation products are generated through two or more pyruvate catabolic pathways. In this case, there is no single abundant VFA in the fermentation product mixture, though acetic acid, propionic acid, and butyric acid are generally present in relatively high concentrations, with other products like lactate and ethanol appearing in lower amounts.²⁵

Among operational parameters, temperature is one of key importance due to its effect on physiochemical and biochemical processes such as mass transfer, enzymatic activity, microbial growth, *etc.* In addition, temperature directly influences microbial activity, particularly the hydrolytic and acidogenic bacteria kinetics.²⁴ However, the thermal adaptation by microorganisms drives the change in microbial community structure and its impact on the fermentation process.^{29,30} While psychrophilic temperature retards the activity of hydrolytic bacteria, a community shift by controlled temperature can favour the abundance and function of acidogenic bacteria.³¹ As previously reviewed,³² using -omics technologies such as metagenomics and metatranscriptomics can help understand the interplay of microbial communities at a functional level. Thus,

by applying strategic methodologies to enhance hydrolysis and optimize conditions for acidogenic bacteria, VFA production can be increased under psychrophilic temperatures, as observed in anaerobic digestors producing methane.

Temperature can also influence the biochemical activity of acid-producing microorganisms by modulating the dynamic balance between glycolysis and the pentose-phosphate pathway.³³ *Enterobacter* and *Bacillus* species have been observed to show increased pentose-phosphate pathway activity at lower temperatures and increased glycolysis activity at higher temperatures.³⁴ The slower metabolic rate extends the time required for the complete breakdown of organic matter in the food waste. Additionally, specific VFAs can be preferentially produced at certain temperatures, as different microbial species and waste materials respond uniquely to temperature variations. Identifying the optimal temperature for specific microbial species or consortium is crucial since many acidogenic bacteria are unable to withstand extreme temperatures.^{35,36} Acidogenic bacteria can maintain their population during the microbial diversity shift under low temperatures. In psychrophilic anaerobic digestors, the most commonly found bacteria genera are *Bacteroides*, *Clostridium*, *Syntrophus*, *Syntrophomonas*, *Geobacter*, and *Treponema*.³⁷ Since low temperatures do not support the methanogenic activity, this further supports the acidogenic bacteria to produce the VFAs. These dominant acidogenic microbial genera observed in psychrophilic digestors show their abundance at temperatures generally above 10 °C. Below 10 °C, their activity is hindered due to reduced membrane fluidity, protein misfolding in enzymes,³⁸ and impairment of intracellular transfer. Thus, in such conditions, true psychrophiles are able to persist.

While temperature can impact the type of VFA produced, current findings are inconsistent,³⁹ likely due to a limited understanding of the interactions between various parameters. For instance, temperature may influence ammonia release,⁴⁰ complicating the isolation of its independent effects. It is also observed that fermentation of food waste at 17 °C favoured the accumulation of butyric acid than under mesophilic temperatures.⁴¹ The abundant bacteria of genera *Sporosarcina* and *Solibacillus* supported substrate hydrolysis under low temperature and *Clostridia* spp., in producing butyric acid *via* protein degradation pathway. However, studies such as those by Garcia-Aguirre *et al.* observed that temperature did not significantly affect product distribution in the treatment of slaughterhouse wastewater and paper mill wastewater.⁴² Similarly, Yu and Fang reported no notable impact of temperature on product distribution in protein-rich wastewater.⁴³

Although mesophilic temperature conditions are found to be optimal for VFA production, it is still an energy-intensive process. Moreover, in cold countries such as Canada, the energy expenditure for maintaining higher operational temperatures can be significant. At an industrial scale, VFAs have been produced through sludge fermentation at temperatures between 20–25 °C for downstream applications like nitrogen removal from wastewater. However, research on acidogenic fermentation at temperatures below 30 °C, or in psychrophilic ranges, is limited. Psychrophilic conditions can



result in incomplete degradation along with the accumulation of other metabolites such as alcohols and lactic acid which can be inhibitory for eventual VFA generation. This happens because the syntrophy between hydrolytic and acidogenic bacteria gets affected when interspecies electron transfer gets affected.⁴⁴ Moreover, the active microbial diversity is limited which may require additional process optimization. Certain strategies such as pretreatment of substrate could be used to increase the rate of hydrolysis since it is known to promote substrate hydrolysis by increasing the surface area of food waste constituents for effective biodegradation and lowering the degree of polymerization.^{45,46}

3. Strategies for enhancing VFA production in psychrophilic conditions

3.1 Optimization of fermentation conditions

3.1.1 pH. During acidogenic fermentation, pH plays a critical role in hydrolysis and acidogenesis, making it essential to maintain an optimal range that supports both processes. pH significantly impacts microbial enzymatic activity during the hydrolytic and acidogenic stages of fermentation.²⁴ Specifically, pH variations alter microbial community composition, leading to changes in metabolite profiles.⁴⁷ Acidogenic bacteria thrive at a pH of 5.5–6.5, while methanogens are most active around pH 7.0. Maintaining the pH in the acidic range can effectively inhibit methanogens, promoting VFA accumulation.⁴⁸ Additionally, pH regulates the transport of undissociated acids across cell membranes, with acidic conditions requiring more energy for permeation and alkaline conditions facilitating energy gain.⁴⁹ A comprehensive study examining the effects of pH, ranging from acidic (pH 4–6) to alkaline (pH 8–11), found that the highest VFA production of 53.87 gCOD L⁻¹ occurred at pH 6 using food waste (FW).⁵⁰ Conversely, during the AD of spent mushroom compost, the maximum VFA concentration of 3.8 g L⁻¹ was recorded at pH 10 when pH conditions ranged from 4 to 12.⁵¹ Across these studies, acetic acid consistently accounted for more than 50% of the total VFAs.

Substrate type largely determines the VFA composition, but pH also significantly influences it during acidogenic fermentation.⁵² Wang *et al.* showed that butyric acid dominated (80%) at

pH < 5.0 during food waste digestion, whereas at exactly pH 5.0, acetic acid became predominant, followed by butyric, propionic, and valeric acids.^{40,53} Similar shifts have been reported, with propionic acid favored at pH 6.5 and butyric acid at pH 8.0.^{54,55} Begum *et al.* found acetic acid dominance at pH 5.5, but a shift toward butyric acid at pH 11, accompanied by lower fractions of acetic, formic, and propionic acids.¹⁷ Cheah *et al.* reported VFA concentrations >10 g L⁻¹ at pH 9 from OFMSW, with acetic acid comprising over 48%. They also observed the highest yield (0.36 g VFA/COD_{in}) in sludge–artichoke co-fermentation at the same pH, where pathways shifted from butyrate at pH 5.5 to acetate at pH 9.⁵⁶ Li *et al.* tested pH 6–8 and found maximum VFA production of 19.92 g L⁻¹ at pH 8, with acetic and butyric acids accounting for 86.4%.⁵⁷ Protein fermentation studies likewise showed acetate dominance at neutral to alkaline pH, making up 55–60% of VFAs from casein and 65–75% from gelatin.⁵⁸ Khatami *et al.* further highlighted that acidic pH 5 reduced acetate but increased propionic and valeric acids, whereas alkaline pH 10 enhanced acetate and completely suppressed caproic acid production.⁵⁹ Dominant acid types and bacterial phylum/genus under different pH ranges are summarized in Table 1. Collectively, these findings show that acidic conditions generally favor butyrate, propionate, or valerate pathways, whereas alkaline pH shifts metabolism toward acetate and higher overall VFA yields.

While acidic pH was more frequently documented to show higher VFA generation, alkaline pH in the range 8–10 also showed enhanced VFA production and neutralising the resultant acidity. Metagenomic insights into anaerobic fermentation, largely based on mesophilic systems, indicate that pH significantly shapes microbial functional gene expression, providing scope for baseline interpretation under psychrophilic conditions (Fig. 2). At acidic pH values (4.5–6.5), there is a clear enrichment of genes involved in acidogenesis and hydrolysis, including *ldh* (lactate dehydrogenase), *ackA/pta* (acetate kinase/phosphotransacetylase), and *hydA* (hydrogenase), aligning with the dominance of fermentative bacteria such as *Clostridium* and *Bacteroides*.^{63,64} Neutral pH (6.8–7.5) facilitates a more balanced microbial ecosystem where genes linked to syntrophic metabolism and acetoclastic methanogenesis such as *mcrA* (methanogenesis marker), *fhs* (formate metabolism), and *por*

Table 1 Dominant VFA types under different ranges of pH and substrate conditions

pH range	Dominant VFA	Dominant phyla/genera	Substrate conditions	References
<5.0	Acetic acid, butyric acid	<i>Clostridium</i> , <i>Lactobacillus</i> , <i>Bacteroides</i>	Food waste, protein-rich waste	60
5.0–6.5	Acetic acid, propionic/butyric acid	<i>Syntrophomonas</i> , <i>Treponema</i> , <i>Geobacter</i>	Food waste, organic fraction of municipal solid waste	60
6.5–7.5	Propionic acid	<i>Syntrophus</i> , <i>Desulfovibrio</i> , <i>Methanosaeta</i> , <i>Methanoculleus</i>	Organic fraction of municipal solid waste, sewage sludge	60
8–9	Mixed acetic and butyric acid	<i>Syntrophomonas</i> , <i>Desulfovibrio</i> , <i>Anaerostipes</i>	Sewage sludge, artichokes	61
9–10	Acetic and butyric acid	<i>Anaerobrance</i> , <i>Alkalibacterium</i> , <i>Oscillospira</i>	Alkaline co-fermentation	62
>10	Butyric and acetic acid	<i>Alkaliphilus</i> , <i>Thermovibrio</i>	Protein fermentation	62



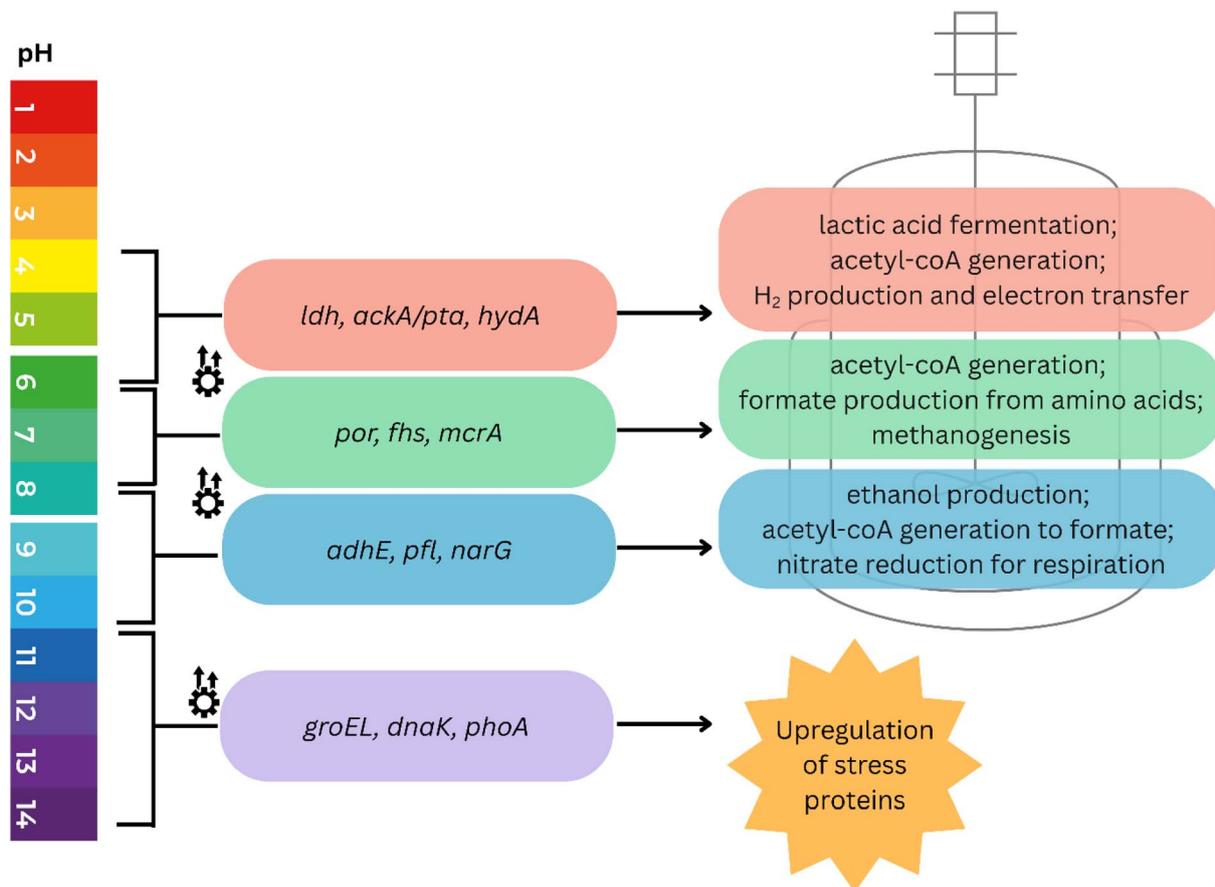


Fig. 2 Effect of different pH values on regulating specific genes and their respective roles in fermentation mechanisms. The pH scale on the left shows different ranges of acidic, neutral, alkaline, and highly alkaline pH values. These four ranges correspond to their effect on gene groups. The gene group – *ldh*, *ackA/pta*, and *hydA* – are upregulated under acidic pH and their subsequent effect is enhanced in lactic acid fermentation, acetyl-coA generation, and hydrogen production and electron transfer respectively. Under a neutral pH range, the gene group – *por*, *fhs*, *mcrA* – are upregulated and promote acetyl-coA generation, amino acid-derived formate production, and methanogenesis respectively. The alkaline pH upregulates the gene group – *adhE*, *pfl*, *narG* – resulting in ethanol production, acetyl-CoA to formate conversion, and nitrate reduction respectively. Highly alkaline conditions induce a stress response by upregulating genes, *groEL*, *dnaK*, and *phoA*.

(pyruvate-ferredoxin oxidoreductase) become active, enabling conversion of VFAs to methane.^{61,62} In alkaline ranges (8–10), the system favors expression of redox-balancing and stress-adapted genes like *adhE* (alcohol dehydrogenase), *pfl* (pyruvate-formate lyase), and *narG* (nitrate reductase),⁶⁵ while extremely alkaline pH (>10) induces survival pathways through upregulation of stress proteins (*groEL*, *dnaK*) and alkaline enzymes like *phoA*.⁶⁶ Thus, strategic pH control in psychrophilic fermentation can serve as a lever to direct microbial gene activity toward targeted volatile fatty acid production.

3.1.2 Substrate concentration. The organic loading rate (OLR) refers to the quantity of organic material, measured in volatile solids (VS), introduced per unit volume of the reactor per day. It determines the availability of substrates for fermentation, with higher OLRs providing more substrates, potentially benefiting VFA production. However, excessively high OLRs can cause the reactor medium to become highly viscous, leading to process instability.⁶⁷ For anaerobic digestion (AD), recommended OLR values typically range from 2 to 7 g VS L⁻¹ d⁻¹.^{68,69} Exceeding these values can inhibit methane

production and favour acidogenesis. When the OLR surpasses the AD threshold of 7 g VS L⁻¹ d⁻¹, higher VFA concentrations are observed, though yields tend to decrease.⁷⁰ Studies have shown that VFA production increases with OLR up to a certain threshold, beyond which it gradually declines. This indicates the existence of an optimal OLR for maximum VFA production.⁷¹ Using dry substrates with a total solids content of approximately 20% can slow methanogen activity and enhance VFA production, although this approach may result in lower VS destruction.⁷² In moderate OLRs (2–5 g VS L⁻¹ d⁻¹), microbial diversity tends to be higher with fermentative bacteria expressing genes like *ldh* and *ackA*.⁷³ Functional metagenomics reveals that at high OLRs, bacteria express stress-related and acid-tolerance genes, including *dnaK*, *groEL*, and *phoA*, alongside upregulation of formate and lactate fermentation pathways (*pfl*, *adhE*).^{74,75} Additionally, OLR influences the composition of VFAs, with lower OLRs favouring the production of propionic and butyric acids, while higher OLRs lead to increased production of acetic and valeric acids.⁴⁰



Despite the known relevance of OLR in regulating acidogenesis, the current understanding of its role under psychrophilic conditions remains limited. Most insights are derived from mesophilic systems and extrapolated to cold environments. In particular, very few studies explicitly design experiments to explore VFA production across a gradient of OLRs at temperatures below 20 °C, leaving a significant gap in research. Where low-temperature studies do exist, they often focus broadly on COD removal or methane inhibition, with VFA profiles reported as secondary metrics. This makes it difficult to discern how substrate concentration affects not just total VFA yield, but the composition and selectivity toward specific VFAs under cold-adapted fermentation. Moreover, microbial and functional responses to OLR in psychrophilic environments remain largely unresolved. While high OLRs are assumed to induce stress pathways and acid tolerance genes as shown in mesophilic systems, this has rarely been confirmed using metagenomic or transcriptomic tools under psychrophilic conditions. There is a need to ascertain whether cold-active acidogens follow the same saturation and inhibition kinetics as observed in mesophilic systems.

3.1.3 Retention time. Hydraulic retention time (HRT) is a critical factor in acidogenic fermentation. As with substrate concentration, studies report the effect of HRT in anaerobic digestion systems, thus providing insight which are extrapolated to comprehend its influence on VFA production. This presents a rather skewed metric since the VFAs are not accumulated but are actively converted into methane. However, these studies still offer a valuable foundational understanding of HRT dynamics and hydrolysis limitations, particularly identifying the minimum HRT required to sustain fermentative activity before methanogenesis begins. Short HRTs, typically less than 10 days, are favoured as they help eliminate slower-growing methanogens.^{68,69} However, for solid waste substrates, shorter retention times can lead to reduced yields since hydrolysis is often the rate-limiting step.⁷⁶ Furthermore, under psychrophilic temperatures, due to the already reduced microbial activity, short HRT would not be efficient. According to Pant *et al.*, a minimum HRT of 3 days is necessary to achieve optimal conversion during the fermentation process.⁷⁷ In batch operations, peak VFA concentrations are generally reached within 4 to 9 days, suggesting that relatively short retention times are sufficient for effective fermentation.

One study found increased VFA production when the HRT was extended from 1 day to 2 days during Co-digestion of mixed waste. However, further increasing the HRT to 3 or 4 days did not significantly improve VFA concentration.⁷⁸ Specific acid production can be influenced by HRT, as longer retention times allow slower-growing organisms to dominate while faster-growing microbes are washed out at shorter HRTs. For example, in whey fermentation, propionic acid production increased as the HRT was extended from 20 h to 95 h, whereas butyric acid production was suppressed.⁷⁹ In contrast, co-fermentation of waste active sludge and fruit/vegetable waste showed little change in the proportion of VFAs within an HRT range of 1 to 4 days.⁸⁰ Together, these findings suggest that while longer HRT can enable the enrichment of slower-growing,

propionate-producing organisms, the benefits plateau beyond a substrate-specific threshold. Therefore, optimal HRT should be determined based on feedstock characteristics rather than assuming a linear relationship with VFA yield.

3.2 Substrate pretreatment methods to enhance hydrolysis

Achieving effective substrate hydrolysis under psychrophilic temperatures poses challenges due to reduced microbial activity and slower biochemical reactions. Pretreatment of the substrate could be used to increase the rate of hydrolysis since it is known to promote substrate hydrolysis and solubilization by increasing the surface area of substrate constituents for effective biodegradation and lowering the degree of polymerization.⁴⁶ This, in turn, can increase VFA production by increasing the availability of simpler or smaller-sized molecules in the aqueous phase in less process time. However, studies focusing on VFAs using pretreatment are scarce. The pretreatment studies for anaerobic digestion could be used to infer the effect on VFA, though these are not optimized for VFAs. Pre-treatment technologies are employed for various purposes, including the production of methane (CH₄), hydrogen (H₂), glucose (C₆H₁₂O₆), fermentable soluble sugars, and bioethanol. Pretreatment strategies can be classified as mechanical, physical, chemical, physicochemical, and biological.

3.2.1 Mechanical methods. Reducing particle size through processes like milling, grinding, and chipping enhances surface area, thereby improving substrate solubility and biodegradability. In anaerobic digestion, finer particle sizes can hasten VFA production, as shown by studies indicating that smaller particles (*e.g.*, 0.4 mm) promote the acetic acid formation, whereas larger particles (*e.g.*, 0.9 mm) favour butyric acid production.^{81–83} Mechanical pretreatment offers the benefits of low energy requirements for dry feedstocks, ease of implementation, and improved dewaterability. However, it is less effective for breaking down lignin, does not aid in pathogen removal,⁸¹ and involves significant equipment maintenance costs.⁸²

One of the mechanical methods, the crashing method, reduces food waste (FW) particle size, facilitating microbial degradation of organic solids for enhanced gas production. Agyeman and Tao observed that reducing FW to a particle size of 2.5 mm significantly improved digestate dewaterability and increased methane production rates.⁸⁴ Similarly, crashing FW to ≤30 mm particle size enhanced methane production by 30% and improved process stability.⁸⁵ This method is also widely applied in the anaerobic digestion of other substrates, including agricultural and animal wastes, due to its effectiveness in reducing particle size.⁸⁶

3.2.2 Chemical methods. Acid treatment primarily hydrolyzes hemicellulose, improving substrate digestibility and enhancing yields of hydrogen and VFAs, particularly for protein-rich wastes. For example, using hydrochloric acid increased VFA yields from waste-activated sludge by 153%, while free nitrous acid achieved a 370% increase.^{87,88} This method is particularly effective compared to other pretreatments for protein-rich substrates.^{81,82} Acid pre-treatment of food waste (FW) has been



conducted using HCl and H₂SO₄, with H₂SO₄ concentrations ranging from 0.1 to 1 M and HCl at 3 M. Optimal pH values for this process are typically between 1 and 4, as lower pH levels can lead to the formation of toxic compounds and inhibitors that disrupt FW management processes like anaerobic digestion (AD). Zhang *et al.*, pre-treated FW with HCl to achieve a pH of 1.0 for 24 h, followed by batch anaerobic fermentation at 108 rpm and 37 °C, significantly enhancing butanol production. Similarly, treating FW with 98% (w/w) H₂SO₄ at pH 1 before fermentation increased hydrogen production by 62.8%.^{89–91}

Alkali treatment targets lignocellulosic materials by dissolving lignin, increasing substrate solubility and buffering capacity, which helps maintain stable pH during acidogenesis. While studies focusing on VFA production are limited, alkaline pretreatment has shown promising results. For instance, a 19% solubilization rate of lignocellulosic feedstocks led to over a 40% increase in hydrogen production.⁹² Optimizing the alkali concentration is crucial in pre-treatment, as excessive Na⁺ and K⁺ cations can inhibit microbial growth and cause toxicity in subsequent processes, while insufficient concentrations may fail to achieve the desired pre-treatment outcomes. Studies have utilized NaOH concentrations ranging from 0.1 to 3 M at pH levels of 8 to 12, with varying time duration of exposure and temperatures depending on the characteristics of the FW and the process objectives.⁹³ These conditions have resulted in differing levels of improvement in methane or hydrogen production. NaOH pretreatment increased acetic and butyric acid production sixfold, although the highest VFA concentrations remained under 2 g L⁻¹.⁹⁴ In primary sludge, using sodium carbonate (Na₂CO₃) quadrupled VFA yields, with the success attributed to an initial pH of 10 and the disintegration of sludge flocs.⁹⁵

Ozonation employs ozone (O₃) to oxidize and degrade feedstocks, effectively delignifying substrates and sterilizing materials by damaging microbial cell walls. It is environmentally friendly, as ozone decomposes into oxygen, but it is energy-intensive, requiring approximately 12 kWh per kilogram of O₃ produced.^{96,97} While ozonation increased hydrogen production by 158% for lignocellulosic wastes,⁹⁸ it negatively impacted the dark fermentation of food waste by degrading proteins and carbohydrates.⁹⁹

3.2.3 Thermal methods. Thermal treatment enhances the hydrolysis phase of acidogenic fermentation by changing the structure of insoluble fractions, lowering viscosity, and increasing soluble chemical oxygen demand. This adjustment favours acidogenesis while suppressing methanogens, making it ideal for VFA production.¹⁰⁰ Thermal pre-treatment of FW was initially introduced as a substrate preparation process to enhance digestion.¹⁰¹ This method relies on temperature as a key operating factor to accelerate the solubilization of FW compounds.¹⁰² A wide temperature range, typically between 50 and 220 °C, has been shown to improve the bioavailability of soluble organic substances, with exposure times ranging from 5 min to 48 h.¹⁰³

Ali *et al.* observed that thermal pre-treatment increased propionic acid production from FW by 38%. However, they noted that this method is not commercially viable for large-

scale waste streams.¹⁰⁴ For waste-activated sludge, thermal treatment at 100 °C for 60 min resulted in a 680% increase in VFA yield at a fermentation pH of 9, whereas neutral pH yielded a smaller increase of approximately 300%.⁵³ However, the lack of control pH data makes it difficult to attribute these increases solely to the pretreatment method. Food waste exhibited a more modest improvement (~55%), but combining thermal treatment with enzymatic or pre-fermentation methods enhanced yields by 380% and 200%, respectively.^{54,105}

Microwave irradiation integrates thermal and non-thermal effects by disrupting crystalline structures while heating the aqueous environment, effectively enhancing solubilization. However, this approach is energy-intensive and costly. For sludge, microwave treatment increased hydrogen production by 66%.¹⁰⁶ When combined with alkaline treatment, microwave irradiation resulted in a 30% increment in solubilization and a 400% boost in VFA and hydrogen production from lignocellulosic waste.¹⁰⁷ Ortigueira *et al.* reported that microwave treatment of the FW has accelerated the H₂ production rate by 62.8%.¹⁰⁸

Ultrasound is frequently identified as one of the most effective physical pretreatment techniques.^{102,109} Ultrasonication pre-treatment of FW involves using sound energy to agitate and break down particles, enhancing the solubilization of the matrix. It combines physical and chemical degradation, utilizing cavitation bubble collapse and free radical generation to break down substrates. Ultrasound can enhance enzyme activity or promote enzyme production, depending on the application. However, the high energy demand and maintenance costs are significant limitations.⁸² For waste-activated sludge, ultrasound pretreatment increased the sCOD by 28-fold, significantly boosting acidogenesis. When applied to food waste, ultrasound achieved a disintegration degree of 57% with the highest VFA yield of 0.98 g COD/g VS.¹¹⁰ This improves the digestion stability of FW and optimizes the overall process. Li *et al.* reported that ultrasonication significantly increased interactions with organic matter and enzymes by over 10%, highlighting its crucial role in maximizing waste utilization.¹⁰²

3.2.4 Physiochemical methods. Thermochemical treatment combines heat with chemical agents to improve substrate solubilization. For vegetable waste, using 1% sulfuric acid and autoclaving at 121 °C for 15 min increased solubilization 4.7-fold, yielding 0.62 g VFA per g of reducing sugars at pH 6.¹¹¹ However, a comparison to untreated substrates was not provided. Pretreatment with diluted nitric acid on lignocellulosic waste like corn stover showed partial success, acidifying less than 10% of soluble sugars.¹¹²

Ionic liquids (ILs) dissolve cellulose or extract lignin, enhancing substrate biodegradability. This physiochemical method typically operates at 80–180 °C and has been extensively studied for bioethanol production from lignocellulosic materials.¹¹³ For anaerobic digestion, IL pretreatment has improved biogas yields by 64–140% from lignocellulosic substrates.¹¹⁴ However, in some cases, inhibitory compounds like melanoidins and *n*-derivative amides have negated benefits.¹¹⁵ While there is potential for ILs to improve VFA production, direct studies are needed to confirm their efficacy.



Table 2 Substrate pretreatment strategies and their implications for psychrophilic fermentation

Pretreatment	Mechanism	Impact on substrate	Implication for VFA	Key considerations
Mechanical	Surface area increases by reduced particle size stimulates hydrolytic and acidogenic bacteria (e.g., <i>Firmicutes</i> , <i>Clostridium</i> spp.)	Improves accessibility	Enhances early acidogenesis, and may shift VFA composition	Limited lignin breakdown
Acid	Hydrolyzes cellulose, releases sugars and breaks down proteins	Significant solubilization	Favours acetic acid and butyric acid production	Needs pH control, possible inhibitor generation (e.g., furans and phenolics)
Alkaline	Dissolves lignin, disintegrates sludge flocs	Increases solubility and buffering capacity	Stimulates VFA production, buffers pH	Na ⁺ /K ⁺ toxicity is possible at low microbial growth rates
Thermal	Disrupts polymer structure, increases sCOD	Boosts soluble substrate fraction	Stimulates VFA production	Energy-intensive
Ultrasound/microwave	Enhances solubilization	Rapid disintegration and COD solubilization	Enables rapid fermentation onset	High energy demand, need for optimization of exposure to avoid over-degradation
Biological	Enzymatic degradation of complex carbohydrates, proteins and fats	Gentler and selective hydrolysis	Environmentally friendly option for slow but sustained acidogenesis	Slow rate, risk of enzyme inactivation

3.2.5 Biological methods. Biological pre-treatment is an eco-friendly method that avoids harmful environmental impacts, significant capital expenditure, and energy usage.¹⁰² It uses biological organisms to degrade FW, facilitating enzymatic hydrolysis, an advantage not offered by physical or chemical pre-treatment methods. Unlike other methods, biological pre-treatment does not require high temperatures, pressure, acids, alkalis, or reactive chemicals. However, it has a longer processing time. This approach can use enzymes such as protease, amylase, Viscozyme™ (carbohydrases blend), Flavourzyme™ (endo- and exo-peptidases blend), *S. cerevisiae* KA4, and Palatase™ (lipase). Alternatively, fungi such as *Aspergillus awamori*, *Aspergillus oryzae*, and *Monascus* can also be employed. Enzymes primarily break down proteins and carbohydrates into amino acids and mono sugars, while fungal pre-treatment targets the decomposition of complex FW compounds.¹¹⁶ Enzyme performance under psychrophilic conditions is strongly temperature-dependent. For example, cellulases retain only ~20–30% of their maximum activity at 5 °C, but activity improves to ~50–60% at 15 °C.¹¹⁷ Similarly, cold-active proteases have been shown to display lower V_{\max} values but higher catalytic efficiency (k_{cat}/K_m) at 10–15 °C compared to mesophilic counterparts, reflecting their adaptation to cold environments. These kinetic constraints highlight why enzymatic pretreatment under psychrophilic conditions remains less efficient than in mesophilic systems.

Fungi are adept at breaking down substrates not easily degraded by fermentation or anaerobic digestion species. Although fungal pretreatment for fermentation has not been explored, enzymes tailored to specific substrates are an alternative. For example, proteases like trypsin are effective for hydrolyzing protein-rich materials but can harm acidogenic bacteria by degrading their proteins.¹¹⁸ As a result, enzymatic

pretreatment is generally discouraged for variable feedstocks like organic fraction municipal solid waste. Enzymatic pretreatment can either precede or occur during anaerobic digestion/fermentation. However, when done concurrently, enzyme activity may decline due to endogenous proteases from anaerobic digestion microbes.¹¹⁹ Despite this limitation, biological treatments are advantageous because they are eco-friendly and do not generate additional waste streams, as enzymes and biological agents naturally degrade during fermentation.^{81,82}

While substrate pretreatment addresses enhancing the slower hydrolysis under psychrophilic fermentation, there is limited understanding of whether pretreatment-enhanced substrates result in faster VFA accumulation, altered VFA composition, or shifts in microbial selection in psychrophilic environments. Furthermore, potential inhibitors, such as phenolics from alkaline pretreatment or free radicals from ozone treatment, may have different toxicity profiles at lower temperatures. The key implications derived from the mentioned pretreatment strategies are summarized in Table 2.

3.3 Design of reactors for psychrophilic fermentation

Designing bioreactors to operate efficiently under psychrophilic conditions (<20 °C) requires strategic adaptations to overcome the reduced enzymatic activity and slower microbial metabolism. Various reactor configurations have been developed or modified to maintain high biomass retention, enhance hydrolysis, and optimize the conversion of organics into VFAs or methane despite low temperatures. Broadly, reactors for VFA production fall into two categories: attached growth systems and suspended growth systems. Packed bed reactors, an example of an attached growth system, involve microbes



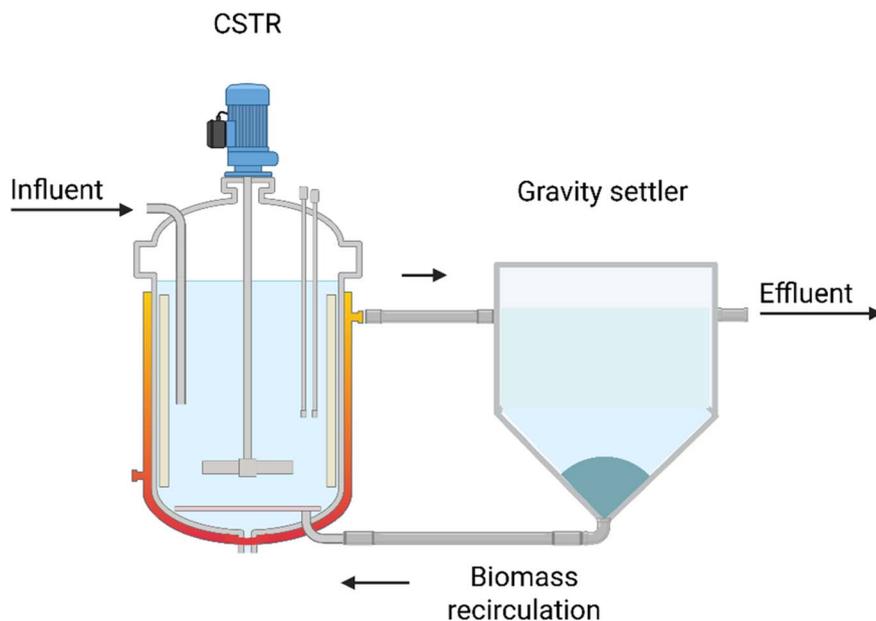


Fig. 3 CSTR coupled with gravity settler for biomass recirculation.

growing on media such as plastic or stones. These are good for retaining biomass and ensuring process stability, especially under cold conditions. However, they can suffer from clogging, especially when treating solid-rich waste streams.¹²⁰ Fluidized bed reactors solve this problem by using small suspended media like sand iron and nickel nanoparticles that provide surface area for microbial attachment while staying mobile in the reactor flow. This helps maintain high mass transfer and avoids clogging issues.¹²¹ In suspended growth systems, the continuous stirred tank reactor (CSTR) is a commonly used option. It offers complete mixing of microbes and substrates and is ideal for treating wastewater with solids. These systems can be coupled with a gravity settler to recycle biomass (Fig. 3), which is helpful at low temperatures where microbial growth is slow.¹²²

Some bioreactors have been designed specifically to maximize VFA production rather than methane, and many of them can perform well at low temperatures. For instance, the anaerobic sequencing batch reactor (ASBR) allows operation in cycles so the process can be stopped before methanogens become active. Under psychrophilic conditions, ASBRs have shown around 60% COD removal using dairy waste as substrate.¹²³ The simplicity of the design is attractive, though the batch-mode operation may not suit large-scale or variable flow systems.

Anaerobic membrane bioreactors (AnMBRs) have emerged as leading options for cold VFA production. By using membranes to retain all biomass, they can allow slow-growing acidogenic microbes to persist. These systems operate at high retention times and decouple HRT from SRT, which would be advantageous in cold conditions for effective substrate degradation. Studies have shown COD removal efficiencies over 90% and stable VFA production at 12–18 °C.¹²⁴ However, membrane fouling becomes a concern due to higher viscosity and EPS

release.^{125,126} Biofilm-based systems like anaerobic immobilized bio-plate reactors (AnIBPRs) promote biofilm growth on carrier media, which helps buffer against thermal fluctuations and retain functional fermenters over long periods. These systems have demonstrated high COD removal and stable VFA profiles at 15–18 °C, even when methane formation is negligible.¹²⁷ Several other reactor types have also shown promise for VFA production, including packed-bed biofilm columns¹²⁸ and anaerobic leach bed reactors.¹²⁹ In these systems, hydrolysis and acidogenesis are separated from methanogenesis, giving greater control over process outputs, particularly when targeting specific VFA profiles or producing VFAs as a byproduct.

Thus, for VFA enhancement under psychrophilic temperatures, the bioreactor design needs to be adapted especially by focusing on biomass retention and minimizing process disruptions like clogging and membrane fouling. The use of biofilm systems and membrane-based reactors, which facilitate high retention times and support slow-growing microbial communities, appears particularly promising for maintaining effective VFA production despite the sluggish microbial activity at low temperatures. These approaches cleverly leverage physical separation and retention mechanisms, allowing microbes to persist and efficiently process substrates without being washed out, which is crucial in cold environments. However, translating these promising lab-scale results into practical, large-scale applications may encounter significant hurdles. For instance, membrane fouling remains a major concern, especially as increased viscosity and the accumulation of EPS at lower temperatures can reduce membrane lifespan and increase maintenance costs. Similarly, biofilm reactors, while advantageous for biomass stability, require careful control of biofilm growth and detachment to prevent clogging or uneven distribution, which can be more difficult under colder



conditions. Batch systems like ASBRs, though effective in limiting methanogen activity and focusing on acidogenesis, might face challenges related to process scalability and operational continuity. They are inherently less suited for continuous large-volume processing. Although these reactor designs and operational strategies appear promising for enhancing VFA yields at low temperatures, further research into their long-term stability, economic viability, and ease of operation is needed.

3.4 Bioaugmentation

Bioaugmentation, the process of introducing specific microbial strains into a system, is an effective strategy to enhance volatile fatty acid (VFA) production. It can be particularly valuable under psychrophilic conditions (≤ 20 °C), where lower temperatures typically reduce microbial activity, slowing down hydrolysis and acidogenesis, the key steps in VFA production. By introducing psychrotolerant or acidogenic microorganisms, bioaugmentation can overcome these limitations by enhancing enzymatic activity and shifting the microbial community towards acidogenesis, thereby improving substrate utilization and VFA yields.

For example, species like *Clostridium butyricum* and *Clostridium tyrobutyricum* are notable for their ability to sustain metabolic activity under a broad range of temperatures from 8–40 °C. Diez *et al.* reported a 20% increase in butyric acid production when *C. butyricum* was bioaugmented into a fermentation system using food waste as a substrate mainly for hydrogen production.¹³⁰ Similarly, De Maayer *et al.*, highlighted that the introduction of cold-adapted acidogenic microbes improved substrate hydrolysis by producing cold-active enzymes, which addressed the bottleneck of reduced hydrolysis rates under psychrophilic conditions.¹³¹ A study by Atasoy *et al.* assessed bioaugmentation as a strategy for tailored production of VFA.¹³² *Propionibacterium acidipropionici* was used to bioaugment mixed microbial cultures in anaerobic sequencing batch reactors treating cheese wastewater under alkaline pH. Bioaugmentation increased propionic acid production nearly fourfold (3779 ± 201 mgCODEq L⁻¹ in the reactor bioaugmented with *P. acidipropionici* vs. 942 ± 172 mgCODEq L⁻¹ in the control) without significantly altering VFA composition. The gene copy number of *P. acidipropionici* was observed to increase 20-fold and showed a positive correlation with total VFA and isovaleric acid concentrations. Additionally, the abundance of Flavobacteriaceae increased, likely due to syntrophic interactions with *P. acidipropionici*. Another study investigated bioaugmentation with homoacetogenic bacteria to enhance volatile fatty acid (VFA) production during lignocellulose fermentation.¹³³ Methanogenesis in wet-exploded corn stover fermentation was inhibited using 10 mM 2-bromoethanesulfonate (BES), which reduced acetic acid yield by 24% but increased headspace hydrogen from 1% to 60%. Bioaugmentation with *Acetitomaculum ruminis* and *Acetobacterium woodii* resulted in hydrogen consumption and increased acetic acid production by 45% and 70%, respectively.

One of the critical benefits of bioaugmentation is its potential to alter the microbial community structure. By selectively

increasing the population of acidogenic bacteria, bioaugmentation can suppress competing methanogenic pathways, leading to higher VFA accumulation. Goud *et al.*, showed that bioaugmentation could effectively balance microbial consortia by fostering acidogenic bacteria while minimizing methanogen activity, ensuring VFAs are the predominant metabolites.¹³⁴ Furthermore, bioaugmentation can optimize specific VFA profiles by selecting strains with metabolic pathways tailored to produce specific VFAs, such as acetic or butyric acids.

Operational parameters such as inoculum dosage, timing of augmentation, and the substrate's compatibility with the bioaugmented strains significantly influence the success of this strategy. For instance, Chi *et al.* demonstrated that adding *C. tyrobutyricum* at the onset of fermentation led to enhanced butyric acid production due to its competitive growth advantage over native microbes during the early acidogenesis phase.¹³⁵ However, timing must be carefully optimized; adding strains too early or late may result in poor integration or reduced efficacy due to unfavourable community dynamics.

Despite its promise, bioaugmentation faces challenges in practical applications. Ensuring the survival, activity, and dominance of the introduced strains in complex microbial communities remains a critical hurdle. Additionally, the cost of culturing and maintaining specific microbial strains at scale can be significant. Addressing these challenges involves optimizing inoculum size, improving the resilience of bioaugmented strains, and exploring synthetic consortia that mimic naturally occurring microbial interactions. Another limitation is that most bioaugmentation studies have been conducted in short-term batch systems, and the long-term stability of the introduced strains has not yet been verified. Continuous or semi-continuous experiments are needed to assess microbial retention, competition with native populations, and the sustained contribution of bioaugmented strains under psychrophilic conditions.

Future advancements in synthetic biology and microbial engineering could further refine bioaugmentation for psychrophilic VFA production. Engineered microbes could be tailored for specific substrates, environmental conditions, or desired VFA profiles. Coupled with metagenomic and metabolomic analyses, these approaches can provide insights into microbial interactions, enabling the design of more effective bioaugmentation strategies. As global interest in sustainable waste-to-resource technologies grows, bioaugmentation offers a promising avenue to maximize the efficiency of psychrophilic fermentation systems for VFA production.

3.5 Co-Digestion of substrates

Co-Digestion of substrates has emerged as an effective strategy to enhance the efficiency and productivity of anaerobic digestion processes, including psychrophilic fermentation. By combining multiple feedstocks, Co-digestion leverages the complementary characteristics of different substrates to improve microbial activity and biochemical yields. For instance, mixing high-carbon feedstocks like food waste with nitrogen-



Table 3 Recent MET configuration operated under psychrophilic temperature

MET configuration	Temperature (°C)	Scale (L)	Cathode/anode	Substrate	References
Double chambered MFC	0–10	0.064	Graphite/graphite	Cow manure	145
Photothermal MFC	7 ± 2	0.028	Air cathode/Janus anode	Synthetic wastewater, brewery wastewater	144
Wetland MFC	5–25	420	Graphite plates/carbon fibre brushes	Synthetic wastewater	160
Soil-based MFC	5–40	0.4	Stainless steel wool/stainless steel wool	Soil organics	161
MES	10	1.3	Graphite granules/graphite granules	H ₂ and CO ₂	162
MES	20	1.5	Graphite/stainless steel	Cow manure	163
Dual and single-chamber MES	0.4	15	Carbon brush/carbon brush	Dog food	164

rich materials such as sewage sludge helps balance the carbon-to-nitrogen (C/N) ratio, which is crucial for optimal microbial metabolism.¹³⁶ This synergistic effect can mitigate the inhibitory effects of excess ammonia or volatile fatty acids often observed with single-substrate digestion. Additionally, Co-digestion increases the diversity of organic compounds available to microbial communities, promoting the enrichment of functional microbial consortia capable of efficient hydrolysis and acidogenesis at low temperatures.¹³⁷ Studies have also shown that Co-digestion improves the buffering capacity of the system, maintaining stable pH levels and reducing the risk of process failure.¹³⁸ Moreover, Co-digestion enhances substrate solubilization and biogas yields due to the interactions between microbial populations optimized for diverse feedstocks.¹³⁹ Advances in pretreatment technologies, such as thermal-alkaline or enzymatic methods, further enhance the compatibility of substrates for Co-digestion, enabling better hydrolysis and fermentation efficiency under psychrophilic conditions.¹⁴⁰ However, achieving optimal performance requires careful selection and proportioning of substrates, as imbalances can lead to issues such as foaming, scum formation, or accumulation of inhibitory compounds. Co-digestion not only enhances the sustainability of fermentation processes by enabling the simultaneous treatment of multiple waste streams but also contributes to resource recovery and circular economy goals by improving the overall yield of valuable products like volatile fatty acids and biogas. Future research should focus on exploring novel substrate combinations, microbial interactions, and system optimization to fully realize the potential of Co-digestion as a robust strategy for enhancing psychrophilic fermentation processes.

3.6 Bioelectrochemical systems

Microbial electrochemical technologies (MET) have emerged as a promising option to transform the organic matter present in waste to produce valuable products with the use of electrodes either a single set-up, *i.e.* microbial fuel cell (MFC) or coupled with other anaerobic digestion (AD) systems in hybrid configurations, *i.e.* microbial electrolysis cells (MEC) and microbial electrosynthesis (MES) cells.^{141,142} Operation of anaerobic reactors under psychrophilic temperature has always been challenging since the metabolic activity may be adversely affected.

However, in recent times some promising hybrid AD configurations assisted with electrodes have successfully navigated the adverse impacts of the cold temperature.¹⁴³ Although not directly linked to VFA production, these hybrid reactor configurations can be redirected to enhance VFA production under cold temperatures by suppressing methanogens, stimulating hydrolytic and acidogenic activity, and redirecting electron flow toward acidogenic pathways (Table 3).

For instance, microbial fuel cells (MFCs) equipped with modified electrodes have demonstrated that psychrophilic operation is feasible when electrode design supports efficient electron transfer. A notable example is the use of a photothermal Janus anode, which combined a waterproof nonporous layer with an electroconductive porous layer to prevent algal growth and improve electron transfer. This configuration enhanced pollutant removal and power density by 1.6- and 24-fold, respectively, compared to traditional porous anodes.¹⁴⁴ Similarly, a MFC utilizing cow manure as substrate could achieve a high-power density of 57.387 mW m⁻² and open circuit voltage of 204.9 ± 0.1 mV despite being operated in cold temperatures.¹⁴⁵ A unique MFC which exploits the interaction between plant and microbe in the rhizosphere for *in situ* bioelectricity and biomass production could achieve stable operation in the winter. The various applications of MFC even under psychrophilic temperature are owing to the interaction of diverse groups of microorganisms which is able to counteract the overpotential losses at low temperatures. A different configuration of METs, the psychrophilic MEC have also been successfully operated to achieve hydrogen production from simple substrates such as glucose¹⁴⁶ and molasses wastewater¹⁴⁷ as well as complex substrates such as domestic wastewater and landfill leachate.¹⁴⁸ The syntropic interaction between two processes occurring simultaneously in MECs, *i.e.* electrogenic oxidation and fermentation, and glucose metabolism was responsible for hydrogen production apart from the primary pathway of direct glucose oxidation.¹⁴⁶ In the case of landfill leachate, Pseudomonadaceae, Geobacteraceae and Comamonadaceae were found enriched in the anode biofilm. It was expected that Rhodospirillaceae and Rhodocyclaceae might have contributed to hydrogen production. MEC has also been integrated with constructed wetlands to improve ammonia removal by 11.7% in comparison to a conventional wetland system. Even long-term operation of large-scale MECs with domestic



wastewater such as 120 L MEC operated at 5–20 °C for 3 months and 100 L MEC operated for 12 months at 1–22 °C could achieve daily production of 0.015 L and 0.6 L hydrogen per day, respectively.^{149,150} While MECs chiefly advance H₂ production, microbial electrosynthesis cells (MES) have achieved methane generation from wastewater under cold conditions and, when coupled with AD, further enhance performance. Applying a cathodic potential in MES promotes extracellular polymer formation, boosting the activity/retention of methanogens (e.g., *Methanobacterium*, *Methanoregula*, *Methanospirillum*).¹⁵¹ In hybrid MES-AD systems at 10 °C, methane increased 5.3–6.6 times *versus* standalone AD, and at ~20 °C, cathodic enrichment of hydrogenotrophic methanogens yielded 2–3 times higher methane than controls.¹⁵²

The adoption of scaled-up METs over conventional approaches for waste treatment and energy recovery is governed by two key factors *i.e.* the comparative cost of the system and net energy balance. The capital cost component is dominated by cost of electrode material and the proton exchange membrane. A lab-scale study estimated the contribution from the electrodes, specifically the platinized cathode to be highest at 47% of the total capital cost in addition to the 37% contribution from the membrane.¹⁵³ In a large scale 200 L modularized MFC, around 60% of the material cost was attributed to cation exchange membrana alone.¹⁵⁴ Again, wastewater contains embedded energy in the range of 0.7 kWh m⁻³ and 1.79 kWh m⁻³ which can be recovered from the nutrient and organic matter fraction.¹⁵⁵ In terms of energy balance, municipal wastewater with an average load of 300–400 mg COD L⁻¹ could potentially generate around 1.2 kWh m⁻³, yet MFCs operated in liter scale have typically achieved energy recoveries three orders lower to the estimated value.¹⁵⁶ Hence, the development of low-cost sustainable reusable non platinized electrodes, electrocatalysts and membranes, and inoculum pretreatment strategies to tackle bottlenecks of cost and upscaling can further assist in anaerobic digestion.^{142,143,157–159} In addition, it is expected that optimizing reactor configuration, and mitigating the ohmic, concentration and activation losses could further improve the energy balance of the system and hence result in a worthwhile net energy gain.

4. Perspectives and applications

Psychrophilic fermentation presents a promising avenue for energy-efficient and sustainable waste management, particularly in regions with cold climates. By leveraging microbial activity at low temperatures, these systems can significantly reduce heating costs, aligning with ambient conditions and minimizing energy inputs. This makes psychrophilic fermentation particularly suitable for remote or high-altitude areas, where maintaining mesophilic or thermophilic conditions would be resource-intensive. However, VFA production under psychrophilic conditions faces several inherent challenges, such as slower hydrolysis rates, reduced microbial activity, and limited metabolic versatility of cold-adapted consortia. Addressing these bottlenecks requires integrated strategies that simultaneously optimize substrate accessibility, microbial community structure, and reactor performance. For instance,

substrate pretreatment can mitigate the rate-limiting step of hydrolysis by enhancing the solubilization of complex organics. Similarly, tailoring microbial communities through enrichment or bioaugmentation with psychrotolerant hydrolytic and acidogenic strains can improve process stability and VFA yields. Advances in microbial ecology aided by -omics technologies such as metagenomics and metatranscriptomics, allow for the identification and functional profiling of key psychrophilic taxa involved in VFA production, supporting the design of more resilient consortia. Furthermore, integrated multi-omics approaches allow for correlating microbial activity with operational parameters in real-time, paving the way for predictive modelling and process control in psychrophilic fermentation systems.¹⁶⁵ Moreover, metabolic modelling can be leveraged to predict flux distributions and optimize operational parameters (e.g., HRT, pH, substrate loading) to steer fermentation pathways toward desired VFA profiles. These models can help resolve trade-offs between production rate and selectivity, especially under the kinetic constraints of low-temperature fermentation.¹⁶⁶ Real-time monitoring and control systems, integrating sensors and machine learning algorithms, could enable dynamic adjustments in response to process fluctuations.¹⁶⁷ This can be particularly valuable in psychrophilic systems, where microbial processes are more sensitive to environmental perturbations. In addition, regulating strategies have gained attention for mitigating inhibitory effects that suppress VFAs production. Accumulation of substances such as free ammonia, sulfides, phenolics, and long-chain fatty acids can damage microbial cells, inhibit key enzymes, and disrupt electron transfer, leading to reduced acidogenesis.¹⁶⁸ Several interventions, including Co-digestion, trace metal supplementation, and the use of adsorbents, have been shown to relieve these inhibitory effects and restore VFAs yields. More recently, quorum sensing-based regulation has emerged as a novel approach to steer microbial communities by supplementing signaling molecules such as acyl-homoserine lactones and AI-2.¹⁶⁹ These molecules enhance microbial cooperation, strengthen acidogenic pathways, and suppress competing methanogens, resulting in higher VFAs accumulation and process stability. While these strategies have been validated primarily under mesophilic conditions, they hold considerable promise for psychrophilic systems, where slow kinetics often exacerbate inhibitory stress. Future work should explore how regulatory interventions can be combined with process optimization to maximize VFAs production under low-temperature fermentation. Furthermore, combining bioreactor design innovations (e.g., hybrid anaerobic systems or membrane bioreactors) with microbial and process engineering holds promise for scaling up psychrophilic VFA production. When these strategies are cohesively applied, psychrophilic fermentation could evolve to transform low-value waste streams into valuable VFAs with reduced energy input.

5. Conclusion

Psychrophilic fermentation presents a promising and sustainable strategy for waste valorization, particularly in cold regions



where energy-intensive heating can be avoided. To enhance performance, strategies can be prioritized according to their technical maturity. Reactor design improvements and operational optimization (e.g., pH, HRT, inoculum-to-substrate ratio) are the most advanced, with proven ability to stabilize hydrolysis and fermentation under low temperatures, making them suitable for near-term industrial application. Pretreatment methods and Co-digestion are moderately mature; while extensively validated in mesophilic and thermophilic systems, they require further adaptation to cold conditions but offer strong potential in the medium term. Bioaugmentation with psychrotolerant strains and bioelectrochemical systems remain at earlier stages. These approaches show high promise for long-term deployment by enabling targeted pathway control and enhanced electron transfer but still require more research on microbial stability, cost, and scale-up.

An industrial application roadmap can therefore be envisioned as follows: short-term efforts should focus on optimizing reactor configurations and process parameters within existing anaerobic digestion facilities; medium-term strategies may integrate pretreatments and Co-digestion to maximize yields; and long-term innovation will rely on bioaugmentation and bioelectrochemical systems to fine-tune product selectivity and efficiency. This staged approach provides a practical path toward industrial adoption of psychrophilic fermentation, advancing energy-efficient VFA recovery within the circular economy.

Conflicts of interest

There are no conflicts to declare.

Data availability

No primary research results, software or code have been included and no new data were generated or analysed as part of this review.

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References

- 1 *Waste-to-Energy Incineration*, International Environmental Technology Centre, available: <https://www.unep.org/ietc/resources/toolkits-manuals-and-guides/waste-energy-incineration>, accessed: Jan. 07, 2025, [online].
- 2 *Global Waste Management Outlook 2024*, UNEP – UN Environment Programme, available: <https://www.unep.org/resources/publications/global-waste-management-outlook-2024>, accessed: Jan. 07, 2025, [online].
- 3 W. Qin, *et al.*, Bridging waste stream complexity and VFAs applications: a review of recovery technologies, *Sep. Purif. Technol.*, 2025, 374, 133690, DOI: [10.1016/j.seppur.2025.133690](https://doi.org/10.1016/j.seppur.2025.133690).
- 4 S. Agnihotri, *et al.*, A Glimpse of the World of Volatile Fatty Acids Production and Application: A review, *Bioengineered*, 2022, 13(1), 1249–1275, DOI: [10.1080/21655979.2021.1996044](https://doi.org/10.1080/21655979.2021.1996044).
- 5 S. Nagarajan, R. J. Jones, L. Oram, J. Massanet-Nicolau and A. Guwy, Intensification of Acidogenic Fermentation for the Production of Biohydrogen and Volatile Fatty Acids—A Perspective, *Fermentation*, 2022, 8, 325, DOI: [10.3390/fermentation8070325](https://doi.org/10.3390/fermentation8070325).
- 6 C. Shi, K. Wang, M. Zheng, Y. Liu, J. Ma and K. Li, The efficiencies and capacities of carbon conversion in fruit and vegetable waste two-phase anaerobic digestion: ethanol-path vs. butyrate-path, *Waste Manage.*, 2021, 126, 737–746, DOI: [10.1016/j.wasman.2021.04.010](https://doi.org/10.1016/j.wasman.2021.04.010).
- 7 J. Gao, J. Li, A. C. Wachemo, H. Yuan, X. Zuo and X. Li, Mass conversion pathway during anaerobic digestion of wheat straw, *RSC Adv.*, 2020, 10(46), 27720–27727, DOI: [10.1039/d0ra02441d](https://doi.org/10.1039/d0ra02441d).
- 8 W. Zhao, *et al.*, Towards high carbon conversion efficiency by using a tailored electrodialysis process for *in situ* carboxylic acids recovery, *J. Cleaner Prod.*, 2021, 297, 126431, DOI: [10.1016/j.jclepro.2021.126431](https://doi.org/10.1016/j.jclepro.2021.126431).
- 9 A. Castro-Fernandez, L. Rodríguez-Hernández, C. M. Castro-Barros, J. M. Lema and A. Taboada-Santos, Scale-up and economic assessment of volatile fatty acids production from food waste, *Biomass Bioenergy*, 2024, 182, 107112, DOI: [10.1016/j.biombioe.2024.107112](https://doi.org/10.1016/j.biombioe.2024.107112).
- 10 L. M. Rodríguez-Jiménez, A. Pérez-Vidal and P. Torres-Lozada, Research trends and strategies for the improvement of anaerobic digestion of food waste in psychrophilic temperatures conditions, *Heliyon*, 2022, 8(10), e11174, DOI: [10.1016/j.heliyon.2022.e11174](https://doi.org/10.1016/j.heliyon.2022.e11174).
- 11 S. Dev, *et al.*, Perspective on anaerobic digestion for biomethanation in cold environments, *Renewable Sustainable Energy Rev.*, 2019, 103, 85–95, DOI: [10.1016/j.rser.2018.12.034](https://doi.org/10.1016/j.rser.2018.12.034).
- 12 B. R. Tiwari, T. Rouissi, S. K. Brar and R. Y. Surampalli, Critical insights into psychrophilic anaerobic digestion: novel strategies for improving biogas production, *Waste Manage.*, 2021, 131, 513–526, DOI: [10.1016/j.wasman.2021.07.002](https://doi.org/10.1016/j.wasman.2021.07.002).
- 13 L. M. Rodríguez-Jiménez, A. Pérez-Vidal and P. Torres-Lozada, Research trends and strategies for the improvement of anaerobic digestion of food waste in psychrophilic temperatures conditions, *Heliyon*, 2022, 8(10), DOI: [10.1016/j.heliyon.2022.e11174](https://doi.org/10.1016/j.heliyon.2022.e11174).
- 14 M. A. Akindolire, H. Rama and A. Roopnarain, Psychrophilic anaerobic digestion: a critical evaluation of microorganisms and enzymes to drive the process, *Renewable Sustainable Energy Rev.*, 2022, 161, 112394, DOI: [10.1016/j.rser.2022.112394](https://doi.org/10.1016/j.rser.2022.112394).



- 15 E. S. Salama, S. Saha, M. B. Kurade, S. Dev, S. W. Chang and B. H. Jeon, Recent trends in anaerobic Co-digestion: fat, oil, and grease (FOG) for enhanced biomethanation, *Prog. Energy Combust. Sci.*, 2019, **70**, 22–42, DOI: [10.1016/j.peccs.2018.08.002](https://doi.org/10.1016/j.peccs.2018.08.002).
- 16 Y. Lu, Q. Zhang, X. Wang, X. Zhou and J. Zhu, Effect of pH on volatile fatty acid production from anaerobic digestion of potato peel waste, *Bioresour. Technol.*, 2020, **316**, 123851, DOI: [10.1016/j.biortech.2020.123851](https://doi.org/10.1016/j.biortech.2020.123851).
- 17 K. Kuruti, S. Nakkasunchi, S. Begum, S. Juntupally, V. Arelli and G. R. Anupoju, Rapid generation of volatile fatty acids (VFA) through anaerobic acidification of livestock organic waste at low hydraulic residence time (HRT), *Bioresour. Technol.*, 2017, **238**, 188–193, DOI: [10.1016/j.biortech.2017.04.005](https://doi.org/10.1016/j.biortech.2017.04.005).
- 18 M. Atasoy, I. Owusu-Agyeman, E. Plaza and Z. Cetecioglu, Bio-based volatile fatty acid production and recovery from waste streams: current status and future challenges, *Bioresour. Technol.*, 2018, **268**, 773–786, DOI: [10.1016/j.biortech.2018.07.042](https://doi.org/10.1016/j.biortech.2018.07.042).
- 19 S. Esquivel-Elizondo, J. Miceli, C. I. Torres and R. Krajmalnik-Brown, Impact of carbon monoxide partial pressures on methanogenesis and medium chain fatty acids production during ethanol fermentation, *Biotechnol. Bioeng.*, 2018, **115**(2), 341–350, DOI: [10.1002/bit.26471](https://doi.org/10.1002/bit.26471).
- 20 B. Eryildiz, L. Lukitawesa and M. J. Taherzadeh, Effect of pH, substrate loading, oxygen, and methanogens inhibitors on volatile fatty acid (VFA) production from citrus waste by anaerobic digestion, *Bioresour. Technol.*, 2020, **302**, 122800, DOI: [10.1016/j.biortech.2020.122800](https://doi.org/10.1016/j.biortech.2020.122800).
- 21 A. H. Bhatt, Z. (Jason) Ren and L. Tao, Value Proposition of Untapped Wet Wastes: Carboxylic Acid Production through Anaerobic Digestion, *iScience*, 2020, **23**(6), DOI: [10.1016/j.isci.2020.101221](https://doi.org/10.1016/j.isci.2020.101221).
- 22 S. Wainaina, M. Parchami, A. Mahboubi, I. S. Horváth and M. J. Taherzadeh, Food waste-derived volatile fatty acids platform using an immersed membrane bioreactor, *Bioresour. Technol.*, 2019, **274**, 329–334, DOI: [10.1016/j.biortech.2018.11.104](https://doi.org/10.1016/j.biortech.2018.11.104).
- 23 S. Dahiya, A. N. Kumar, J. Shanthi Sravan, S. Chatterjee, O. Sarkar and S. V. Mohan, Food waste biorefinery: sustainable strategy for circular bioeconomy, *Bioresour. Technol.*, 2018, **248**, 2–12, DOI: [10.1016/j.biortech.2017.07.176](https://doi.org/10.1016/j.biortech.2017.07.176).
- 24 M. Zhou, B. Yan, J. W. C. Wong and Y. Zhang, Enhanced volatile fatty acids production from anaerobic fermentation of food waste: a mini-review focusing on acidogenic metabolic pathways, *Bioresour. Technol.*, 2018, **248**, 68–78, DOI: [10.1016/j.biortech.2017.06.121](https://doi.org/10.1016/j.biortech.2017.06.121).
- 25 M. Zhou, B. Yan, J. W. C. Wong and Y. Zhang, Enhanced volatile fatty acids production from anaerobic fermentation of food waste: a mini-review focusing on acidogenic metabolic pathways, *Bioresour. Technol.*, 2018, **248**, 68–78, DOI: [10.1016/j.biortech.2017.06.121](https://doi.org/10.1016/j.biortech.2017.06.121).
- 26 N. K. N. Al-Shorgani, M. S. Kalil, W. M. W. Yusoff and A. A. Hamid, Impact of pH and butyric acid on butanol production during batch fermentation using a new local isolate of *Clostridium acetobutylicum* YM1, *Saudi J. Biol. Sci.*, 2018, **25**(2), 339–348, DOI: [10.1016/j.sjbs.2017.03.020](https://doi.org/10.1016/j.sjbs.2017.03.020).
- 27 K. Feng, H. Li and C. Zheng, Shifting product spectrum by pH adjustment during long-term continuous anaerobic fermentation of food waste, *Bioresour. Technol.*, 2018, **270**, 180–188, DOI: [10.1016/j.biortech.2018.09.035](https://doi.org/10.1016/j.biortech.2018.09.035).
- 28 Y. Wu, H. Ma, M. Zheng and K. Wang, Lactic acid production from acidogenic fermentation of fruit and vegetable wastes, *Bioresour. Technol.*, 2015, **191**, 53–58, DOI: [10.1016/j.biortech.2015.04.100](https://doi.org/10.1016/j.biortech.2015.04.100).
- 29 *Microbes and Climate Change – Science, People & Impacts*, 2022, DOI: [10.1128/aamcol.nov.2021](https://doi.org/10.1128/aamcol.nov.2021).
- 30 A. Mateos-Rivera, J. C. Yde, B. Wilson, K. W. Finster, L. J. Reigstad and L. Øvreås, The effect of temperature change on the microbial diversity and community structure along the chronosequence of the sub-arctic glacier forefield of Styggealdsbreen (Norway), *FEMS Microbiol. Ecol.*, 2016, **92**(4), DOI: [10.1093/femsec/fiw038](https://doi.org/10.1093/femsec/fiw038).
- 31 S. Wei and Y. Guo, Comparative study of reactor performance and microbial community in psychrophilic and mesophilic biogas digesters under solid state condition, *J. Biosci. Bioeng.*, 2018, **125**(5), 543–551, DOI: [10.1016/j.jbiosc.2017.12.001](https://doi.org/10.1016/j.jbiosc.2017.12.001).
- 32 R. Kumar, R. Kumar, S. K. Brar and G. Kaur, Next-generation omics approaches to drive carboxylate production by acidogenic fermentation of food waste: a review, *Bioengineered*, 2022, **13**(7–12), 14987–15002, DOI: [10.1080/21655979.2023.2180583](https://doi.org/10.1080/21655979.2023.2180583).
- 33 P. Dijkstra, S. C. Thomas, P. L. Heinrich, G. W. Koch, E. Schwartz and B. A. Hungate, Effect of temperature on metabolic activity of intact microbial communities: evidence for altered metabolic pathway activity but not for increased maintenance respiration and reduced carbon use efficiency, *Soil Biol. Biochem.*, 2011, **43**(10), 2023–2031, DOI: [10.1016/j.soilbio.2011.05.018](https://doi.org/10.1016/j.soilbio.2011.05.018).
- 34 Y. E. Luo, *et al.*, Analysis of metabolic flux in *Escherichia coli* expressing human-like collagen in fed-batch culture, *Biotechnol. Lett.*, 2008, **30**(4), 637–643, DOI: [10.1007/s10529-007-9593-1/tables/3](https://doi.org/10.1007/s10529-007-9593-1/tables/3).
- 35 G. Strazzer, F. Battista, B. Tonanzi, S. Rossetti and D. Bolzonella, Optimization of short chain volatile fatty acids production from household food waste for biorefinery applications, *Environ. Technol. Innovation*, 2021, **23**, 101562, DOI: [10.1016/j.eti.2021.101562](https://doi.org/10.1016/j.eti.2021.101562).
- 36 S. H. Kim and H. S. Shin, Effects of base-pretreatment on continuous enriched culture for hydrogen production from food waste, *Int. J. Hydrogen Energy*, 2008, **33**(19), 5266–5274, DOI: [10.1016/j.ijhydene.2008.05.010](https://doi.org/10.1016/j.ijhydene.2008.05.010).
- 37 S. Wei and Y. Guo, Comparative study of reactor performance and microbial community in psychrophilic and mesophilic biogas digesters under solid state condition, *J. Biosci. Bioeng.*, 2018, **125**(5), 543–551, DOI: [10.1016/j.jbiosc.2017.12.001](https://doi.org/10.1016/j.jbiosc.2017.12.001).
- 38 G. Feller, Protein folding at extreme temperatures: current issues, *Semin. Cell Dev. Biol.*, 2018, **84**, 129–137, DOI: [10.1016/j.semcdb.2017.09.003](https://doi.org/10.1016/j.semcdb.2017.09.003).



- 39 P. Zhang, Y. Chen and Q. Zhou, Waste activated sludge hydrolysis and short-chain fatty acids accumulation under mesophilic and thermophilic conditions: effect of pH, *Water Res.*, 2009, **43**(15), 3735–3742, DOI: [10.1016/j.watres.2009.05.036](https://doi.org/10.1016/j.watres.2009.05.036).
- 40 J. Jiang, Y. Zhang, K. Li, Q. Wang, C. Gong and M. Li, Volatile fatty acids production from food waste: effects of pH, temperature, and organic loading rate, *Bioresour. Technol.*, 2013, **143**, 525–530, DOI: [10.1016/j.biortech.2013.06.025](https://doi.org/10.1016/j.biortech.2013.06.025).
- 41 R. Kumar, G. Kaur and S. K. Brar, Tailored production of butyric acid from mixed culture fermentation of food waste, *Food and Bioproducts Processing*, 2025, **150**, 1–11, DOI: [10.1016/J.fbp.2024.12.013](https://doi.org/10.1016/J.fbp.2024.12.013).
- 42 J. Garcia-Aguirre, E. Aymerich, J. González-Mtnez de Goñi and M. Esteban-Gutiérrez, Selective VFA production potential from organic waste streams: assessing temperature and pH influence, *Bioresour. Technol.*, 2017, **244**, 1081–1088, DOI: [10.1016/j.biortech.2017.07.187](https://doi.org/10.1016/j.biortech.2017.07.187).
- 43 H. Q. Yu and H. H. P. Fang, Acidogenesis of gelatin-rich wastewater in an upflow anaerobic reactor: influence of pH and temperature, *Water Res.*, 2003, **37**(1), 55–66, DOI: [10.1016/S0043-1354\(02\)00256-7](https://doi.org/10.1016/S0043-1354(02)00256-7).
- 44 C. Liu, L. Ren, B. Yan, L. Luo, J. Zhang and M. K. Awasthi, Electron transfer and mechanism of energy production among syntrophic bacteria during acidogenic fermentation: a review, *Bioresour. Technol.*, 2021, **323**, 124637, DOI: [10.1016/j.biortech.2020.124637](https://doi.org/10.1016/j.biortech.2020.124637).
- 45 M. Carlsson, A. Lagerkvist and F. Morgan-Sagastume, The effects of substrate pre-treatment on anaerobic digestion systems: a review, *Waste Manage.*, 2012, **32**(9), 1634–1650, DOI: [10.1016/j.wasman.2012.04.016](https://doi.org/10.1016/j.wasman.2012.04.016).
- 46 R. Y. Kannah, C. Velu, J. Rajesh Banu, K. Heimann and O. P. Karthikeyan, Food Waste Valorization by Microalgae, *Energy Environ. Sustainability*, 2018, 319–342, DOI: [10.1007/978-981-10-7431-8_14/cover](https://doi.org/10.1007/978-981-10-7431-8_14/cover).
- 47 S. K. Bhatia and Y. H. Yang, Microbial production of volatile fatty acids: current status and future perspectives, *Rev. Environ. Sci. Bio/Technol.*, 2017, **16**(2), 327–345, DOI: [10.1007/S11157-017-9431-4](https://doi.org/10.1007/S11157-017-9431-4).
- 48 C. Liu, G. Luo, W. Wang, Y. He, R. Zhang and G. Liu, The effects of pH and temperature on the acetate production and microbial community compositions by syngas fermentation, *Fuel*, 2018, **224**, 537–544, DOI: [10.1016/j.fuel.2018.03.125](https://doi.org/10.1016/j.fuel.2018.03.125).
- 49 R. I. Rodríguez-Pimentel, S. Rodríguez-Pérez, O. Monroy-Hermosillo and F. Ramírez-Vives, Effect of organic loading rate on the performance of two-stage anaerobic digestion of the organic fraction of municipal solid waste (OFMSW), *Water Sci. Technol.*, 2015, **72**(3), 384–390, DOI: [10.2166/wst.2015.223](https://doi.org/10.2166/wst.2015.223).
- 50 J. Ma, *et al.*, pH shaped kinetic characteristics and microbial community of food waste hydrolysis and acidification, *Biochem. Eng. J.*, 2019, **146**, 52–59, DOI: [10.1016/j.bej.2019.03.004](https://doi.org/10.1016/j.bej.2019.03.004).
- 51 W. Fang, J. Ye, P. Zhang, X. Zhu and S. Zhou, Solid-state anaerobic fermentation of spent mushroom compost for volatile fatty acids production by pH regulation, *Int. J. Hydrogen Energy*, 2017, **42**(29), 18295–18300, DOI: [10.1016/j.ijhydene.2017.04.148](https://doi.org/10.1016/j.ijhydene.2017.04.148).
- 52 D. H. Kim, S. H. Kim, K. W. Jung, M. S. Kim and H. S. Shin, Effect of initial pH independent of operational pH on hydrogen fermentation of food waste, *Bioresour. Technol.*, 2011, **102**(18), 8646–8652, DOI: [10.1016/j.biortech.2011.03.030](https://doi.org/10.1016/j.biortech.2011.03.030).
- 53 J. Yin, K. Wang, Y. Yang, D. Shen, M. Wang and H. Mo, Improving production of volatile fatty acids from food waste fermentation by hydrothermal pretreatment, *Bioresour. Technol.*, 2014, **171**, 323–329, DOI: [10.1016/j.biortech.2014.08.062](https://doi.org/10.1016/j.biortech.2014.08.062).
- 54 H. J. Kim, Y. G. Choi, D. Y. Kim, D. H. Kim and T. H. Chung, Effect of pretreatment on acid fermentation of organic solid waste, *Water Sci. Technol.*, 2005, **52**(1–2), 153–160, DOI: [10.2166/wst.2005.0511](https://doi.org/10.2166/wst.2005.0511).
- 55 W. Han, P. He, L. Shao and F. Lü, Road to full bioconversion of biowaste to biochemicals centering on chain elongation: a mini review, *J. Environ. Sci.*, 2019, **86**, 50–64, DOI: [10.1016/j.jes.2019.05.018](https://doi.org/10.1016/j.jes.2019.05.018).
- 56 Y. K. Cheah, C. Vidal-Antich, J. Dosta and J. Mata-Álvarez, Volatile fatty acid production from mesophilic acidogenic fermentation of organic fraction of municipal solid waste and food waste under acidic and alkaline pH, *Environ. Sci. Pollut. Res.*, 2019, **26**(35), 35509–35522, DOI: [10.1007/S11356-019-05394-6/FIGURES/7](https://doi.org/10.1007/S11356-019-05394-6/FIGURES/7).
- 57 Y. Li, *et al.*, Acidogenic properties of carbohydrate-rich wasted potato and microbial community analysis: effect of pH, *J. Biosci. Bioeng.*, 2019, **128**(1), 50–55, DOI: [10.1016/j.jbiosc.2018.12.009](https://doi.org/10.1016/j.jbiosc.2018.12.009).
- 58 R. Bevilacqua, A. Regueira, M. Mauricio-Iglesias, J. M. Lema and M. Carballa, Steering the conversion of protein residues to volatile fatty acids by adjusting pH, *Bioresour. Technol.*, 2021, **320**, 124315, DOI: [10.1016/j.biortech.2020.124315](https://doi.org/10.1016/j.biortech.2020.124315).
- 59 K. Khatami, M. Atasoy, M. Ludtke, C. Baresel, Ö. Eyice and Z. Cetecioglu, Bioconversion of food waste to volatile fatty acids: impact of microbial community, pH and retention time, *Chemosphere*, 2021, **275**, 129981, DOI: [10.1016/j.chemosphere.2021.129981](https://doi.org/10.1016/j.chemosphere.2021.129981).
- 60 Y. C. Liu, J. Ramiro-Garcia, L. M. Paulo, C. M. Braguglia, M. C. Gagliano and V. O'Flaherty, Psychrophilic and mesophilic anaerobic treatment of synthetic dairy wastewater with long chain fatty acids: process performances and microbial community dynamics, *Bioresour. Technol.*, 2023, **380**, 129124, DOI: [10.1016/j.biortech.2023.129124](https://doi.org/10.1016/j.biortech.2023.129124).
- 61 A. Giongo, *et al.*, Microbial communities in anaerobic digesters change over time and sampling depth, *Braz. J. Microbiol.*, 2020, **51**(3), 1177–1190, DOI: [10.1007/S42770-020-00272-7/figures/7](https://doi.org/10.1007/S42770-020-00272-7/figures/7).
- 62 G. Collins, A. Woods, S. McHugh, M. W. Carton and V. O'Flaherty, Microbial community structure and methanogenic activity during start-up of psychrophilic anaerobic digesters treating synthetic industrial



- wastewaters, *FEMS Microbiol. Ecol.*, 2003, **46**(2), 159–170, DOI: [10.1016/S0168-6496\(03\)00217-4](https://doi.org/10.1016/S0168-6496(03)00217-4).
- 63 X. Zhang, *et al.*, Optimizing anaerobic digestion: benefits of mild temperature transition from thermophilic to mesophilic conditions, *Environ. Sci. Ecotechnology*, 2024, **21**, 100440, DOI: [10.1016/j.ese.2024.100440](https://doi.org/10.1016/j.ese.2024.100440).
- 64 A. Li, *et al.*, A pyrosequencing-based metagenomic study of methane-producing microbial community in solid-state biogas reactor, *Biotechnol. Biofuels*, 2013, **6**(1), 1–17, DOI: [10.1186/1754-6834-6-3/figures/6](https://doi.org/10.1186/1754-6834-6-3/figures/6).
- 65 J. Chen, Y. Yan, L. Cui, B. Zhang, Q. Fei and Y. Ma, Impact of pH-mediated *in situ* enzymatic pretreatment on food waste decomposition and biomethane production: performance, mechanism, and key metabolic pathways, *Chem. Eng. J.*, 2024, **490**, 151931, DOI: [10.1016/j.cej.2024.151931](https://doi.org/10.1016/j.cej.2024.151931).
- 66 J. K. Vester, M. A. Glaring and P. Stougaard, Discovery of novel enzymes with industrial potential from a cold and alkaline environment by a combination of functional metagenomics and culturing, *Microb. Cell Fact.*, 2014, **13**(1), 1–14, DOI: [10.1186/1475-2859-13-72/tables/7](https://doi.org/10.1186/1475-2859-13-72/tables/7).
- 67 P. C. Hallenbeck and J. R. Benemann, Biological hydrogen production; fundamentals and limiting processes, *Int. J. Hydrogen Energy*, 2002, **27**(11–12), 1185–1193, DOI: [10.1016/S0360-3199\(02\)00131-3](https://doi.org/10.1016/S0360-3199(02)00131-3).
- 68 M. H. Gerardi, Introduction to Operational Conditions, *The Microbiology of Anaerobic Digesters*, 2003, pp. 77–80, DOI: [10.1002/0471468967](https://doi.org/10.1002/0471468967).
- 69 Anaerobic Digestion; Modern Theory and Practice, ed. P. N. Hobson and A. D. Wheatley. 269pp. 1993. Elsevier Applied Science, London, *Int. J. Biochem.*, 1994, vol. **26**, no. 6, p. 856, doi: DOI: [10.1016/0020-711X\(94\)90122-8](https://doi.org/10.1016/0020-711X(94)90122-8).
- 70 H. Liu, P. Han, H. Liu, G. Zhou, B. Fu and Z. Zheng, Full-scale production of VFAs from sewage sludge by anaerobic alkaline fermentation to improve biological nutrients removal in domestic wastewater, *Bioresour. Technol.*, 2018, **260**, 105–114, DOI: [10.1016/j.biortech.2018.03.105](https://doi.org/10.1016/j.biortech.2018.03.105).
- 71 C. H. Hung, Y. T. Chang and Y. J. Chang, Roles of microorganisms other than Clostridium and Enterobacter in anaerobic fermentative biohydrogen production systems - A review, *Bioresour. Technol.*, 2011, **102**(18), 8437–8444, DOI: [10.1016/j.biortech.2011.02.084](https://doi.org/10.1016/j.biortech.2011.02.084).
- 72 F. Liotta, *et al.*, Effect of total solids content on methane and volatile fatty acid production in anaerobic digestion of food waste, *Waste Manage. Res.*, 2014, **32**(10), 947–953, DOI: [10.1177/0734242x14550740/asset/images/large/10.1177_0734242x14550740-fig6.jpeg](https://doi.org/10.1177/0734242x14550740/asset/images/large/10.1177_0734242x14550740-fig6.jpeg).
- 73 E. Gunnigle, *et al.*, Functional responses and adaptation of mesophilic microbial communities to psychrophilic anaerobic digestion, *FEMS Microbiol. Ecol.*, 2015, **91**(12), 1–15, DOI: [10.1093/femsec/fiv132](https://doi.org/10.1093/femsec/fiv132).
- 74 M. Yan, C. Wang, Y. Li, H. Tian and Y. Sun, Effect of bioaugmentation on psychrotrophic anaerobic digestion: bioreactor performance, microbial community, and cellular metabolic response, *Chem. Eng. J.*, 2023, **455**, 140173, DOI: [10.1016/j.cej.2022.140173](https://doi.org/10.1016/j.cej.2022.140173).
- 75 M. Casallas-Ojeda, *et al.*, Understanding the acidification risk of cheese whey anaerobic digestion under psychrophilic and mesophilic conditions, *Heliyon*, 2024, **10**(5), 26476, DOI: [10.1016/j.heliyon.2024.e26476](https://doi.org/10.1016/j.heliyon.2024.e26476).
- 76 L. A. Fdez-Güelfo, C. Álvarez-Gallego, D. Sales and L. I. Romero, The use of thermochemical and biological pretreatments to enhance organic matter hydrolysis and solubilization from organic fraction of municipal solid waste (OFMSW), *Chem. Eng. J.*, 2011, **168**(1), 249–254, DOI: [10.1016/j.cej.2010.12.074](https://doi.org/10.1016/j.cej.2010.12.074).
- 77 D. Pant, *et al.*, Integrated conversion of food waste diluted with sewage into volatile fatty acids through fermentation and electricity through a fuel cell, *Environ. Technol.*, 2013, **34**(13–14), 1935–1945, DOI: [10.1080/09593330.2013.828763](https://doi.org/10.1080/09593330.2013.828763).
- 78 Y. Li, *et al.*, Anaerobic acidogenic fermentation of food waste for mixed-acid production, *Energy Sources, Part A*, 2017, **39**(7), 631–635, DOI: [10.1080/15567036.2015.1120824](https://doi.org/10.1080/15567036.2015.1120824).
- 79 S. G. Langer, C. Gabris, D. Einfalt, B. Wemheuer, M. Kazda and F. R. Bengelsdorf, Different response of bacteria, archaea and fungi to process parameters in nine full-scale anaerobic digesters, *Microb. Biotechnol.*, 2019, **12**(6), 1210, DOI: [10.1111/1751-7915.13409](https://doi.org/10.1111/1751-7915.13409).
- 80 I. O. Bolaji and D. Dionisi, Acidogenic fermentation of vegetable and salad waste for chemicals production: effect of pH buffer and retention time, *J. Environ. Chem. Eng.*, 2017, **5**(6), 5933–5943, DOI: [10.1016/j.jece.2017.11.001](https://doi.org/10.1016/j.jece.2017.11.001).
- 81 L. Romero-Cedillo, *et al.*, A review of the potential of pretreated solids to improve gas biofuels production in the context of an OFMSW biorefinery, *J. Chem. Technol. Biotechnol.*, 2017, **92**(5), 937–958, DOI: [10.1002/jctb.5116](https://doi.org/10.1002/jctb.5116).
- 82 A. Cesaro and V. Belgiorno, Pretreatment methods to improve anaerobic biodegradability of organic municipal solid waste fractions, *Chem. Eng. J.*, 2014, **240**, 24–37, DOI: [10.1016/j.cej.2013.11.055](https://doi.org/10.1016/j.cej.2013.11.055).
- 83 K. Izumi, Y. ki Okishio, N. Nagao, C. Niwa, S. Yamamoto and T. Toda, Effects of particle size on anaerobic digestion of food waste, *Int. Biodeterior. Biodegrad.*, 2010, **64**(7), 601–608, DOI: [10.1016/j.ibiod.2010.06.013](https://doi.org/10.1016/j.ibiod.2010.06.013).
- 84 I. Owusu-Agyeman, E. Plaza and Z. Cetecioglu, Production of volatile fatty acids through Co-digestion of sewage sludge and external organic waste: effect of substrate proportions and long-term operation, *Waste Manage.*, 2020, **112**, 30–39, DOI: [10.1016/j.wasman.2020.05.027](https://doi.org/10.1016/j.wasman.2020.05.027).
- 85 E. Castelló, *et al.*, Feasibility of biohydrogen production from cheese whey using a UASB reactor: links between microbial community and reactor performance, *Int. J. Hydrogen Energy*, 2009, **34**(14), 5674–5682, DOI: [10.1016/j.ijhydene.2009.05.060](https://doi.org/10.1016/j.ijhydene.2009.05.060).
- 86 T. Akhtar, A. S. Hashmi, M. Tayyab, A. A. Anjum, S. Saeed and S. Ali, Bioconversion of Agricultural Waste to Butyric Acid Through Solid State Fermentation by Clostridium tyrobutyricum, *Waste Biomass Valorization*, 2020, **11**(5), 2067–2073, DOI: [10.1007/S12649-018-0475-7/figures/4](https://doi.org/10.1007/S12649-018-0475-7/figures/4).
- 87 X. Li, *et al.*, An efficient and green pretreatment to stimulate short-chain fatty acids production from waste activated sludge anaerobic fermentation using free nitrous acid,



- Chemosphere*, 2016, **144**, 160–167, DOI: [10.1016/j.chemosphere.2015.08.076](https://doi.org/10.1016/j.chemosphere.2015.08.076).
- 88 L. Wu, *et al.*, Phosphorus and short-chain fatty acids recovery from waste activated sludge by anaerobic fermentation: effect of acid or alkali pretreatment, *Bioresour. Technol.*, 2017, **240**, 192–196, DOI: [10.1016/j.biortech.2017.03.016](https://doi.org/10.1016/j.biortech.2017.03.016).
- 89 Q. Zhang, R. Li, B. Guo, L. Zhang and Y. Liu, Thermophilic Co-digestion of blackwater and organic kitchen waste: impacts of granular activated carbon and different mixing ratios, *Waste Manage.*, 2021, **131**, 453–461, DOI: [10.1016/j.wasman.2021.06.024](https://doi.org/10.1016/j.wasman.2021.06.024).
- 90 L. Zhang, K. C. Loh, J. W. Lim and J. Zhang, Bioinformatics analysis of metagenomics data of biogas-producing microbial communities in anaerobic digesters: a review, *Renewable Sustainable Energy Rev.*, 2019, **100**, 110–126, DOI: [10.1016/j.rser.2018.10.021](https://doi.org/10.1016/j.rser.2018.10.021).
- 91 Q. Wang, *et al.*, Volatile fatty acid production in anaerobic fermentation of food waste saccharified residue: effect of substrate concentration, *Waste Manage.*, 2023, **164**, 29–36, DOI: [10.1016/j.wasman.2023.03.045](https://doi.org/10.1016/j.wasman.2023.03.045).
- 92 L. Ozkan, T. H. Erguder and G. N. Demirer, Effects of pretreatment methods on solubilization of beet-pulp and bio-hydrogen production yield, *Int. J. Hydrogen Energy*, 2011, **36**(1), 382–389, DOI: [10.1016/j.ijhydene.2010.10.006](https://doi.org/10.1016/j.ijhydene.2010.10.006).
- 93 D. Chuanchuan, L. Yuling, L. Shuaishuai and J. Yang, Study on the effect of alkali on the production of volatile fatty acids from rhamnolipid-alkali-thermal pretreated waste activated sludge, *Environ. Prog. Sustainable Energy*, 2024, **43**(4), e14377, DOI: [10.1002/ep.14377](https://doi.org/10.1002/ep.14377).
- 94 P. Guo, *et al.*, Effects of different pretreatment strategies on corn stalk acidogenic fermentation using a microbial consortium, *Bioresour. Technol.*, 2011, **102**(16), 7526–7531, DOI: [10.1016/j.biortech.2011.04.083](https://doi.org/10.1016/j.biortech.2011.04.083).
- 95 L. Lin, R. hong Li and X. yan Li, Recovery of organic resources from sewage sludge of Al-enhanced primary sedimentation by alkali pretreatment and acidogenic fermentation, *J. Cleaner Prod.*, 2018, **172**, 3334–3341, DOI: [10.1016/j.jclepro.2017.11.199](https://doi.org/10.1016/j.jclepro.2017.11.199).
- 96 Y. Rosen, H. Mamane and Y. Gerchman, Short Ozonation of Lignocellulosic Waste as Energetically Favorable Pretreatment, *Bioenergy Res.*, 2019, **12**(2), 292–301, DOI: [10.1007/s12155-019-9962-3/tables/3](https://doi.org/10.1007/s12155-019-9962-3/tables/3).
- 97 J. Gomes, D. Frasson, R. M. Quinta-Ferreira, A. Matos and R. C. Martins, Removal of Enteric Pathogens from Real Wastewater Using Single and Catalytic Ozonation, *Water*, 2019, **11**, 127, DOI: [10.3390/w11010127](https://doi.org/10.3390/w11010127).
- 98 C. Yirong, S. Heaven and C. J. Banks, Effect of a Trace Element Addition Strategy on Volatile Fatty Acid Accumulation in Thermophilic Anaerobic Digestion of Food Waste, *Waste Biomass Valorization*, 2015, **6**(1), 1–12, DOI: [10.1007/s12649-014-9327-2/figures/6](https://doi.org/10.1007/s12649-014-9327-2/figures/6).
- 99 L. Yue, J. Cheng, J. Hua, H. Dong, J. Zhou and Y. Y. Li, Improving fermentative methane production of glycerol trioleate and food waste pretreated with ozone through two-stage dark hydrogen fermentation and anaerobic digestion, *Energy Convers. Manage.*, 2020, **203**, 112225, DOI: [10.1016/j.enconman.2019.112225](https://doi.org/10.1016/j.enconman.2019.112225).
- 100 J. Ariunbaatar, A. Panico, L. Frunzo, G. Esposito, P. N. L. Lens and F. Pirozzi, Enhanced anaerobic digestion of food waste by thermal and ozonation pretreatment methods, *J. Environ. Manage.*, 2014, **146**, 142–149, DOI: [10.1016/j.jenvman.2014.07.042](https://doi.org/10.1016/j.jenvman.2014.07.042).
- 101 R. Jariyaboon, *et al.*, Thermophilic Dark Fermentation for Simultaneous Mixed Volatile Fatty Acids and Biohydrogen Production from Food Waste, *Fermentation*, 2023, **9**, 636, DOI: [10.3390/fermentation9070636](https://doi.org/10.3390/fermentation9070636).
- 102 A. M. Yeneneh, S. Chong, T. K. Sen, H. M. Ang and A. Kayaalp, Effect of ultrasonic, microwave and combined microwave-ultrasonic pretreatment of municipal sludge on anaerobic digester performance, *Water, Air, Soil Pollut.*, 2013, **224**(5), 1–9, DOI: [10.1007/S11270-013-1559-4/figures/8](https://doi.org/10.1007/S11270-013-1559-4/figures/8).
- 103 X. Gong, M. Wu, Y. Jiang and H. Wang, Effects of different temperatures and pH values on volatile fatty acids production during Co-digestion of food waste and thermal-hydrolysed sewage sludge and subsequent volatile fatty acids for polyhydroxyalkanoates production, *Bioresour. Technol.*, 2021, **333**, 125149, DOI: [10.1016/j.biortech.2021.125149](https://doi.org/10.1016/j.biortech.2021.125149).
- 104 X. Huang and Z. Xiang, Enhanced volatile fatty acids production in thermal hydrolysis pretreatment with constant pH regulation: effects evaluation and mechanism elucidation, *J. Environ. Chem. Eng.*, 2024, **12**(6), 114830, DOI: [10.1016/j.jece.2024.114830](https://doi.org/10.1016/j.jece.2024.114830).
- 105 X. Yu, J. Yin, K. Wang, D. Shen, Y. Long and T. Chen, Enhancing Food Waste Hydrolysis and the Production Rate of Volatile Fatty Acids by Prefermentation and Hydrothermal Pretreatments, *Energy Fuels*, 2016, **30**(5), 4002–4008, DOI: [10.1021/acs.energyfuels.6b00077/asset/images/large/ef-2016-000774_0002.jpeg](https://doi.org/10.1021/acs.energyfuels.6b00077/asset/images/large/ef-2016-000774_0002.jpeg).
- 106 G. Yanga and J. Wang, Fermentative hydrogen production from sewage sludge, *Crit. Rev. Environ. Sci. Technol.*, 2017, **47**(14), 1219–1281, DOI: [10.1080/10643389.2017.1348107](https://doi.org/10.1080/10643389.2017.1348107).
- 107 L. Ozkan, T. H. Erguder and G. N. Demirer, Effects of pretreatment methods on solubilization of beet-pulp and bio-hydrogen production yield, *Int. J. Hydrogen Energy*, 2011, **36**(1), 382–389, DOI: [10.1016/j.ijhydene.2010.10.006](https://doi.org/10.1016/j.ijhydene.2010.10.006).
- 108 M. Ortiz-Sanchez, P. J. Inocencio-García, A. F. Alzate-Ramírez and C. A. C. Alzate, Potential and Restrictions of Food-Waste Valorization through Fermentation Processes, *Fermentation*, 2023, **9**, 274, DOI: [10.3390/fermentation9030274](https://doi.org/10.3390/fermentation9030274).
- 109 B. Deepanraj, V. Sivasubramanian and S. Jayaraj, Effect of substrate pretreatment on biogas production through anaerobic digestion of food waste, *Int. J. Hydrogen Energy*, 2017, **42**(42), 26522–26528, DOI: [10.1016/j.ijhydene.2017.06.178](https://doi.org/10.1016/j.ijhydene.2017.06.178).
- 110 Q. Wu, W. Guo, S. Yang, H. Luo, S. Peng and N. Ren, Effects of ultrasonic and acid pretreatment on food waste disintegration and volatile fatty acid production, *J. Harbin Inst. Technol., New Ser.*, 2015, **22**(3), 1–6, DOI: [10.11916/j.issn.1005-9113.2015.03.001](https://doi.org/10.11916/j.issn.1005-9113.2015.03.001).



- 111 A. N. Kumar and S. V. Mohan, Acidogenic valorization of vegetable waste for short chain carboxylic acids and biohydrogen production: influence of pretreatment and pH, *J. Cleaner Prod.*, 2015, **203**, 1055–1066, DOI: [10.1016/j.jclepro.2018.08.198](https://doi.org/10.1016/j.jclepro.2018.08.198).
- 112 L. Zhang, K. C. Loh, Y. Dai and Y. W. Tong, Acidogenic fermentation of food waste for production of volatile fatty acids: bacterial community analysis and semi-continuous operation, *Waste Manage.*, 2020, **109**, 75–84, DOI: [10.1016/j.wasman.2020.04.052](https://doi.org/10.1016/j.wasman.2020.04.052).
- 113 A. A. Elgharrawy, M. Z. Alam, M. Moniruzzaman and M. Goto, Ionic liquid pretreatment as emerging approaches for enhanced enzymatic hydrolysis of lignocellulosic biomass, *Biochem. Eng. J.*, 2016, **109**, 252–267, DOI: [10.1016/j.bej.2016.01.021](https://doi.org/10.1016/j.bej.2016.01.021).
- 114 J. Gao, L. Chen, K. Yuan, H. Huang and Z. Yan, Ionic liquid pretreatment to enhance the anaerobic digestion of lignocellulosic biomass, *Bioresour. Technol.*, 2013, **150**, 352–358, DOI: [10.1016/j.biortech.2013.10.026](https://doi.org/10.1016/j.biortech.2013.10.026).
- 115 B. J. Allison, J. C. Cádiz, N. Karuna, T. Jeoh and C. W. Simmons, The Effect of Ionic Liquid Pretreatment on the Bioconversion of Tomato Processing Waste to Fermentable Sugars and Biogas, *Appl. Biochem. Biotechnol.*, 2016, **179**(7), 1227–1247, DOI: [10.1007/s12010-016-2061-4/tables/6](https://doi.org/10.1007/s12010-016-2061-4/tables/6).
- 116 P. Li, *et al.*, Biological pretreatment of corn straw for enhancing degradation efficiency and biogas production, *Bioengineered*, 2020, **11**(1), 251–260, DOI: [10.1080/21655979.2020.1733733](https://doi.org/10.1080/21655979.2020.1733733).
- 117 S. D'Amico, T. Collins, J. C. Marx, G. Feller and C. Gerday, Psychrophilic microorganisms: challenges for life, *EMBO Rep.*, 2006, **7**(4), 385–389, DOI: [10.1038/sj.embor.7400662](https://doi.org/10.1038/sj.embor.7400662).
- 118 J. Plácido and Y. Zhang, Production of volatile fatty acids from slaughterhouse blood by mixed-culture fermentation, *Biomass Convers. Biorefin.*, 2018, **8**(3), 621–634, DOI: [10.1007/S13399-018-0313-Y/figures/6](https://doi.org/10.1007/S13399-018-0313-Y/figures/6).
- 119 A. Odnell, M. Recktenwald, K. Stensén, B. H. Jonsson and M. Karlsson, Activity, life time and effect of hydrolytic enzymes for enhanced biogas production from sludge anaerobic digestion, *Water Res.*, 2016, **103**, 462–471, DOI: [10.1016/j.watres.2016.07.064](https://doi.org/10.1016/j.watres.2016.07.064).
- 120 C. P. L. Grady Jr, G. T. Daigger, N. G. Love and C. D. M. Filipe, *Biological Wastewater Treatment*, available: https://books.google.ca/books?hl=en&lr=&id=stjLBQAAQBAJ&oi=fnd&pg=PP1&ots=r-bVJB8qG7&sig=8pblKdi-HetZP2dzvAFku-pj9YA&redir_esc=y#v=onepage&q&f=false, accessed: Apr. 22, 2025, [online].
- 121 A. G. L. Moura, T. P. Delforno, C. A. B. S. Rabelo, G. Kumar, E. L. Silva and M. B. A. Varesche, Iron and nickel nanoparticles role in volatile fatty acids production enhancement: functional genes and bacterial taxonomy in an anaerobic fluidized bed reactor, *Biochem. Eng. J.*, 2022, **187**, 108656, DOI: [10.1016/j.bej.2022.108656](https://doi.org/10.1016/j.bej.2022.108656).
- 122 J. Yun and K. S. Cho, Effects of organic loading rate on hydrogen and volatile fatty acid production and microbial community during acidogenic hydrogenesis in a continuous stirred tank reactor using molasses wastewater, *J. Appl. Microbiol.*, 2016, **121**(6), 1627–1636, DOI: [10.1111/jam.13316](https://doi.org/10.1111/jam.13316).
- 123 R. R. Dague, G. C. Banik and T. G. Ellis, Anaerobic sequencing batch reactor treatment of dilute wastewater at psychrophilic temperatures, *Water Environ. Res.*, 1998, **70**(2), 155–160, DOI: [10.2175/106143098x126991](https://doi.org/10.2175/106143098x126991).
- 124 E. Maleki, Psychrophilic anaerobic membrane bioreactor (AnMBR) for treating malting plant wastewater and energy recovery, *J. Water Proc. Eng.*, 2020, **34**, 101174, DOI: [10.1016/j.jwpe.2020.101174](https://doi.org/10.1016/j.jwpe.2020.101174).
- 125 P. van den Brink, O. A. Satpradit, A. van Bentem, A. Zwijnenburg, H. Temmink and M. van Loosdrecht, Effect of temperature shocks on membrane fouling in membrane bioreactors, *Water Res.*, 2011, **45**(15), 4491–4500, DOI: [10.1016/j.watres.2011.05.046](https://doi.org/10.1016/j.watres.2011.05.046).
- 126 R. Watanabe, Y. Nie, S. Wakahara, D. Komori and Y. Y. Li, Investigation on the response of anaerobic membrane bioreactor to temperature decrease from 25 °C to 10 °C in sewage treatment, *Bioresour. Technol.*, 2017, **243**, 747–754, DOI: [10.1016/j.biortech.2017.07.001](https://doi.org/10.1016/j.biortech.2017.07.001).
- 127 P. H. Wu, K. K. Ng, P. K. A. Hong, P. Y. Yang and C. F. Lin, Treatment of low-strength wastewater at mesophilic and psychrophilic conditions using immobilized anaerobic biomass, *Chem. Eng. J.*, 2017, **311**, 46–54, DOI: [10.1016/j.cej.2016.11.077](https://doi.org/10.1016/j.cej.2016.11.077).
- 128 S. J. Lim, Y. H. Ahn, E. Y. Kim and H. N. Chang, Nitrate removal in a packed bed reactor using volatile fatty acids from anaerobic acidogenesis of food wastes, *Biotechnol. Bioprocess Eng.*, 2006, **11**(6), 538–543, DOI: [10.1007/bf02932080](https://doi.org/10.1007/bf02932080).
- 129 D. Cysneiros, C. J. Banks, S. Heaven and K. A. G. Karatzas, The effect of pH control and “hydraulic flush” on hydrolysis and Volatile Fatty Acids (VFA) production and profile in anaerobic leach bed reactors digesting a high solids content substrate, *Bioresour. Technol.*, 2012, **123**, 263–271, DOI: [10.1016/j.biortech.2012.06.060](https://doi.org/10.1016/j.biortech.2012.06.060).
- 130 M. P. Díez, *et al.*, Enhanced hydrogen production from food waste via bioaugmentation with *Clostridium* and *Lactobacillus*, *Biomass Convers. Biorefin.*, 2025, 1–13, DOI: [10.1007/s13399-025-06628-z/figures/6](https://doi.org/10.1007/s13399-025-06628-z/figures/6).
- 131 P. De Maayer, D. Anderson, C. Cary and D. A. Cowan, Some like it cold: understanding the survival strategies of psychrophiles, *EMBO Rep.*, 2014, **15**(5), 508–517, DOI: [10.1002/embr.201338170](https://doi.org/10.1002/embr.201338170).
- 132 M. Atasoy and Z. Cetecioglu, Bioaugmented Mixed Culture by *Clostridium acetivum* to Manipulate Volatile Fatty Acids Composition From the Fermentation of Cheese Production Wastewater, *Front. Microbiol.*, 2021, **12**, 658494, DOI: [10.3389/fmicb.2021.658494/bibtex](https://doi.org/10.3389/fmicb.2021.658494/bibtex).
- 133 N. Murali, K. Srinivas and B. K. Ahring, Increasing the Production of Volatile Fatty Acids from Corn Stover Using Bioaugmentation of a Mixed Rumen Culture with Homoacetogenic Bacteria, *Microorganisms*, 2021, **9**, 337, DOI: [10.3390/microorganisms9020337](https://doi.org/10.3390/microorganisms9020337).
- 134 R. K. Goud, O. Sarkar, P. Chiranjeevi and S. Venkata Mohan, Bioaugmentation of potent acidogenic isolates:



- a strategy for enhancing biohydrogen production at elevated organic load, *Bioresour. Technol.*, 2014, **165**(C), 223–232, DOI: [10.1016/j.biortech.2014.03.049](https://doi.org/10.1016/j.biortech.2014.03.049).
- 135 X. Chi, J. Li, X. Wang, Y. Zhang, S. Y. Leu and Y. Wang, Bioaugmentation with *Clostridium tyrobutyricum* to improve butyric acid production through direct rice straw bioconversion, *Bioresour. Technol.*, 2018, **263**, 562–568, DOI: [10.1016/j.biortech.2018.04.120](https://doi.org/10.1016/j.biortech.2018.04.120).
- 136 M. K. Ibro, V. R. Ancha and D. B. Lemma, Impacts of Anaerobic Co-Digestion on Different Influencing Parameters: A Critical Review, *Sustainability*, 2022, **14**, 9387, DOI: [10.3390/su14159387](https://doi.org/10.3390/su14159387).
- 137 S. Shrestha, R. Pandey, N. Aryal and S. P. Lohani, Recent advances in Co-digestion conjugates for anaerobic digestion of food waste, *J. Environ. Manage.*, 2023, **345**, 118785, DOI: [10.1016/j.jenvman.2023.118785](https://doi.org/10.1016/j.jenvman.2023.118785).
- 138 B. S. Xing, S. Cao, Y. Han, J. Wen, K. Zhang and X. C. Wang, Stable and high-rate anaerobic Co-digestion of food waste and cow manure: optimisation of start-up conditions, *Bioresour. Technol.*, 2020, **307**, 123195, DOI: [10.1016/j.biortech.2020.123195](https://doi.org/10.1016/j.biortech.2020.123195).
- 139 F. Ameen, J. Ranjitha, N. Ahsan and V. Shankar, Co-digestion of microbial biomass with animal manure in three-stage anaerobic digestion, *Fuel*, 2021, **306**, 121746, DOI: [10.1016/j.fuel.2021.121746](https://doi.org/10.1016/j.fuel.2021.121746).
- 140 P. K. Karthikeyan, H. C. H. Bandulasena and T. Radu, A comparative analysis of pre-treatment technologies for enhanced biogas production from anaerobic digestion of lignocellulosic waste, *Ind. Crops Prod.*, 2024, **215**, 118591, DOI: [10.1016/j.indcrop.2024.118591](https://doi.org/10.1016/j.indcrop.2024.118591).
- 141 B. R. Tiwari, M. T. Noori and M. M. Ghangrekar, Carbon supported nickel-phthalocyanine/MnOx as novel cathode catalyst for microbial fuel cell application, *Int. J. Hydrogen Energy*, 2017, **42**(36), 23085–23094, DOI: [10.1016/j.ijhydene.2017.07.201](https://doi.org/10.1016/j.ijhydene.2017.07.201).
- 142 Md. T. Noori, A. Ganta and B. R. Tiwari, Recent Advances in the Design and Architecture of Bioelectrochemical Systems to Treat Wastewater and to Produce Choice-Based Byproducts, *J. Hazard. Toxic Radioact. Waste*, 2020, **24**(3), 04020023, DOI: [10.1061/\(asce\)hzh.2153-5515.0000510/asset/87d4b783-30dd-4905-95d1-c2317b3b31b5/assets/images/large/hzeng-801f05.jpg](https://doi.org/10.1061/(asce)hzh.2153-5515.0000510/asset/87d4b783-30dd-4905-95d1-c2317b3b31b5/assets/images/large/hzeng-801f05.jpg).
- 143 B. R. Tiwari, T. Rouissi, S. K. Brar and R. Y. Surampalli, Critical insights into psychrophilic anaerobic digestion: novel strategies for improving biogas production, *Waste Manage.*, 2021, **131**, 513–526, DOI: [10.1016/j.wasman.2021.07.002](https://doi.org/10.1016/j.wasman.2021.07.002).
- 144 J. Zhang, *et al.*, Photothermal Janus Anodes: Photothermal Janus Anode with Photosynthesis-Shielding Effect for Activating Low-Temperature Biological Wastewater Treatment, *Adv. Funct. Mater.*, 2020, **30**(7), 2070045, DOI: [10.1002/adfm.202070045](https://doi.org/10.1002/adfm.202070045).
- 145 N. Çek, A. Erensoy, N. Ak and H. Uslu, High power microbial fuel cell operating at low temperature using cow dung waste, *Int. J. Chem. React. Eng.*, 2022, **20**(6), 661–666, DOI: [10.1515/ijcre-2021-0150/asset/graphic/j_ijcre-2021-0150_fig_004.jpg](https://doi.org/10.1515/ijcre-2021-0150/asset/graphic/j_ijcre-2021-0150_fig_004.jpg).
- 146 L. Lu, D. Xing, N. Ren and B. E. Logan, Syntrophic interactions drive the hydrogen production from glucose at low temperature in microbial electrolysis cells, *Bioresour. Technol.*, 2012, **124**, 68–76, DOI: [10.1016/j.biortech.2012.08.040](https://doi.org/10.1016/j.biortech.2012.08.040).
- 147 Y. Wang, W. Q. Guo, D. F. Xing, J. S. Chang and N. Q. Ren, Hydrogen production using biocathode single-chamber microbial electrolysis cells fed by molasses wastewater at low temperature, *Int. J. Hydrogen Energy*, 2014, **39**(33), 19369–19375, DOI: [10.1016/j.ijhydene.2014.07.071](https://doi.org/10.1016/j.ijhydene.2014.07.071).
- 148 M. Hassan, A. S. Fernandez, I. San Martin, B. Xie and A. Moran, Hydrogen evolution in microbial electrolysis cells treating landfill leachate: dynamics of anodic biofilm, *Int. J. Hydrogen Energy*, 2018, **43**(29), 13051–13063, DOI: [10.1016/j.ijhydene.2018.05.055](https://doi.org/10.1016/j.ijhydene.2018.05.055).
- 149 E. S. Heidrich, J. Dolfing, K. Scott, S. R. Edwards, C. Jones and T. P. Curtis, Production of hydrogen from domestic wastewater in a pilot-scale microbial electrolysis cell, *Appl. Microbiol. Biotechnol.*, 2013, **97**(15), 6979–6989, DOI: [10.1007/S00253-012-4456-7/tables/1](https://doi.org/10.1007/S00253-012-4456-7/tables/1).
- 150 E. S. Heidrich, S. R. Edwards, J. Dolfing, S. E. Cotterill and T. P. Curtis, Performance of a pilot scale microbial electrolysis cell fed on domestic wastewater at ambient temperatures for a 12 month period, *Bioresour. Technol.*, 2014, **173**, 87–95, DOI: [10.1016/j.biortech.2014.09.083](https://doi.org/10.1016/j.biortech.2014.09.083).
- 151 T. Tian, S. Qiao, C. Yu and J. Zhou, Bio-electrochemically assisting low-temperature anaerobic digestion of low-organic strength wastewater, *Chem. Eng. J.*, 2018, **335**, 657–664, DOI: [10.1016/j.cej.2017.11.016](https://doi.org/10.1016/j.cej.2017.11.016).
- 152 D. Liu, L. Zhang, S. Chen, C. Buisman and A. Ter Heijne, Bioelectrochemical enhancement of methane production in low temperature anaerobic digestion at 10 °C, *Water Res.*, 2016, **99**, 281–287, DOI: [10.1016/j.watres.2016.04.020](https://doi.org/10.1016/j.watres.2016.04.020).
- 153 R. A. Rozendal, T. H. J. A. Sleutels, H. V. M. Hamelers and C. J. N. Buisman, Effect of the type of ion exchange membrane on performance, ion transport, and pH in biocatalyzed electrolysis of wastewater, *Water Sci. Technol.*, 2008, **57**(11), 1757–1762, DOI: [10.2166/wst.2008.043](https://doi.org/10.2166/wst.2008.043).
- 154 Z. Ge and Z. He, Long-term performance of a 200 liter modularized microbial fuel cell system treating municipal wastewater: treatment, energy, and cost, *Environ. Sci.*, 2016, **2**(2), 274–281, DOI: [10.1039/c6ew00020g](https://doi.org/10.1039/c6ew00020g).
- 155 P. L. McCarty, J. Bae and J. Kim, Domestic wastewater treatment as a net energy producer-can this be achieved?, *Environ. Sci. Technol.*, 2011, **45**(17), 7100–7106, DOI: [10.1021/es2014264/asset/images/large/es-2011-014264_0001.jpeg](https://doi.org/10.1021/es2014264/asset/images/large/es-2011-014264_0001.jpeg).
- 156 L. Xiao, Z. Ge, P. Kelly, F. Zhang and Z. He, Evaluation of normalized energy recovery (NER) in microbial fuel cells affected by reactor dimensions and substrates, *Bioresour. Technol.*, 2014, **157**, 77–83, DOI: [10.1016/j.biortech.2014.01.086](https://doi.org/10.1016/j.biortech.2014.01.086).
- 157 M. T. Noori, G. D. Bhowmick, B. R. Tiwari, M. M. Ghangrekar and C. K. Mukhrejee, Application of Low-Cost Cu-Sn Bimetal Alloy as Oxygen Reduction Reaction Catalyst for Improving Performance of the



- Microbial Fuel Cell, *MRS Adv.*, 2018, 3(13), 663–668, DOI: [10.1557/adv.2018.163/metrics](https://doi.org/10.1557/adv.2018.163/metrics).
- 158 B. R. Tiwari and M. M. Ghangrekar, Enhancing electrogenesis by pretreatment of mixed anaerobic sludge to be used as inoculum in microbial fuel cells, *Energy Fuels*, 2015, 29(5), 3518–3524, DOI: [10.1021/ef5028197/asset/images/large/ef-2014-028197_0004.jpeg](https://doi.org/10.1021/ef5028197/asset/images/large/ef-2014-028197_0004.jpeg).
- 159 B. R. Tiwari and M. M. Ghangrekar, Electricity Production during Distillery Wastewater Treatment in a Microbial Fuel Cell Equipped with Low Cost PVA-Nafion-Borosilicate Membrane, *J. Clean Energy Technol.*, 2018, 6(2), 155–158, DOI: [10.18178/jocet.2018.6.2.452](https://doi.org/10.18178/jocet.2018.6.2.452).
- 160 X. Wang and Y. Tian, Seasonal variations of pollutants removal and microbial activity in integrated constructed wetland–microbial fuel cell systems, *Water Reuse*, 2021, 11(2), 312–328, DOI: [10.2166/wrd.2021.094](https://doi.org/10.2166/wrd.2021.094).
- 161 L. Gong, M. Abbaszadeh Amirdehi, A. Miled and J. Greener, Practical increases in power output from soil-based microbial fuel cells under dynamic temperature variations, *Sustainable Energy Fuels*, 2021, 5(3), 671–677, DOI: [10.1039/d0se01406k](https://doi.org/10.1039/d0se01406k).
- 162 H. Y. Yang, *et al.*, Mixed-culture biocathodes for acetate production from CO₂ reduction in the microbial electrosynthesis: impact of temperature, *Sci. Total Environ.*, 2021, 790, 148128, DOI: [10.1016/j.scitotenv.2021.148128](https://doi.org/10.1016/j.scitotenv.2021.148128).
- 163 Y. Jiao, Y. Yuan, C. He, L. Liu, X. Pan and P. Li, Enrichment culture combined with microbial electrochemical enhanced low-temperature anaerobic digestion of cow dung, *Bioresour. Technol.*, 2022, 360, 127636, DOI: [10.1016/j.biortech.2022.127636](https://doi.org/10.1016/j.biortech.2022.127636).
- 164 H. Wang, *et al.*, Dual-chamber differs from single-chamber microbial electrosynthesis in biogas production performance under low temperature (15 °C), *Bioresour. Technol.*, 2021, 337, DOI: [10.1016/j.biortech.2021.125377](https://doi.org/10.1016/j.biortech.2021.125377).
- 165 S. Wan, X. Liu, W. Sun, B. Lv and C. Li, Current advances for omics-guided process optimization of microbial manufacturing, *Bioresour. Bioprocess.*, 2023, 10(1), 1–18, DOI: [10.1186/S40643-023-00647-2](https://doi.org/10.1186/S40643-023-00647-2).
- 166 M. Atasoy, W. T. Scott, A. Regueira, M. Mauricio-Iglesias, P. J. Schaap and H. Smidt, Biobased short chain fatty acid production - Exploring microbial community dynamics and metabolic networks through kinetic and microbial modeling approaches, *Biotechnol. Adv.*, 2024, 73, 108363, DOI: [10.1016/j.biotechadv.2024.108363](https://doi.org/10.1016/j.biotechadv.2024.108363).
- 167 J. Zou, F. Lü, L. Chen, H. Zhang and P. He, Machine learning for enhancing prediction of biogas production and building a VFA/ALK soft sensor in full-scale dry anaerobic digestion of kitchen food waste, *J. Environ. Manage.*, 2024, 371, 123190, DOI: [10.1016/j.jenvman.2024.123190](https://doi.org/10.1016/j.jenvman.2024.123190).
- 168 X. Liu, *et al.*, Unveiling the mechanisms of how cationic polyacrylamide affects short-chain fatty acids accumulation during long-term anaerobic fermentation of waste activated sludge, *Water Res.*, 2019, 155, 142–151, DOI: [10.1016/j.watres.2019.02.036](https://doi.org/10.1016/j.watres.2019.02.036).
- 169 X. Liu, J. Luo, Q. Xu, Q. Lu, B. J. Ni and D. Wang, Roles and opportunities of quorum sensing in natural and engineered anaerobic digestion systems, *Water Res.*, 2025, 275, 123190, DOI: [10.1016/j.watres.2025.123190](https://doi.org/10.1016/j.watres.2025.123190).

