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Review

A review on waste activated sludge pretreatment for improved volatile fatty acids production and their upcycling into polyhydroxyalkanoates

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ABSTRACT

Waste activated sludge (WAS), a byproduct of wastewater treatment (WWTPs) facilities is challenging to manage because of its high organic content. Most of WAS is managed via anaerobic digestion (AD) to produce biogas, which is not deemed economically viable. The AD of WAS into volatile fatty acids (VFA) and their subsequent upcycling into polyhydroxyalkanoates (PHA) is gaining popularity due to their high value and uses. However, the fundamental issue with WAS is its low solubility, and pretreatment is required to increase it. Pretreatment disintegrates sludge floc and enhances its solubility, supports acetogens, and inhibits methanogens, leading to increased VFA synthesis in the AD process. The key factors influencing VFA yield include the size of the sludge granules, the mixing rate, and the presence of resistant organic components. Fermented broth containing VFA from AD can be utilized directly as a feedstock for microbial fermentation to produce PHA using both pure as well as mixed cultures. Utilisation of mixed cultures is useful since they are robust, able to consume a wide range of substrates, and do not require sterility. In addition, the VFA, which is made up of various organic acids, impacts the structure, productivity, characteristics, and type of PHA produced by microbial communities. Considering the importance of WAS management through VFA production and its integration with PHA production process this review article discusses the WAS pretreatment strategies, various factors that influence the AD process, trends in VFA to PHA production technologies with challenges, and possible solutions for integrated process development.

1. Introduction

Waste activated sludge (WAS) is produced during the wastewater treatment in a wastewater treatment plant (WWTPs). Around 1.9 billion tons of WAS is produced globally every year and requires appropriate methods for its treatment to avoid its hazardous effect on the environment and human health [1]. It contains high organic content (proteins and carbohydrates), and its management is a challenge. Anaerobic digestion (AD) of WAS is a carbon-neutral and effective way of waste management. Conversion of WAS into products such as methane and hydrogen is a complex process with low revenue while volatile fatty acid (VFA) production seems a more promising approach [2]. The AD process is comprised of different steps like hydrolysis, acidogenesis, acetogenesis, and methanogenesis. The first stage is hydrolysis, and the main challenges are inadequate sludge solubilization and the presence of resistant organic components [3]. Various pretreatment methods including physical (thermal and ultrasonic), chemical (alkaline and oxidising agent), and biological (enzymatic) have been reported to increase WAS solubilization [4–6]. In addition to enhancing solubilization, pretreatment influences the composition of microbial communities, promotes the growth of acetogenesis and hydrolysing species, and inhibits the growth of methanogens [4]. Volatile fatty acids have many commercial applications and are used as building blocks for polymer synthesis, food beverages, and pharmaceutical production [7]. Recovery and downstreaming of VFA from anaerobic digest is challenging so its direct use as a feedstock for microbial fermentation to produce other valuable products is the preferred process.

Polyhydroxyalkanoates (PHA) are biodegradable polymers produced by various microbes and are considered a suitable alternative to petro-based plastics [8,9]. It has applications in the packaging industry,

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preparation of adhesives, scaffold and tissue engineering, drug delivery, and paper coating [10,11]. The global PHA market is expected to touch 6.73 billion dollars by 2025 however production cost of PHA ($4000–15,000/Mt) is high compared to synthetic plastic ($1000–1500/Mt) [12]. Feedstock costs around 40 % of the total production cost [1]. There have been reports of using a variety of waste products as a feedstock for microbial fermentation in order to make the process more cost-effective, including lignocellulosic biomass, used cooking oil, glycerol from the biodiesel sector, dairy waste, etc. [13–15]. Waste activated sludge is produced massively and can be used as a raw material for AD to produce VFA. Bioplastic can be made more cost-effective by further integrating the VFA and PHA production processes. Volatile fatty acids are composed of various organic acids like acetic acid, propionic acid, butyric acid, and valeric acid, and their content varies with the type of substrate, method of fermentation, and microbial community involved [16,17]. A variety of microbes such as *Cupriavidus necator*, *Halomonas* sp. YLGW01, *Bacillus megaterium*, *Salinivibrio* sp. TGB19 have been reported with the capability of VFA utilisation and accumulating PHA [18–20]. The use of mixed culture also had been demonstrated to make VFA upcycling into PHA less expensive

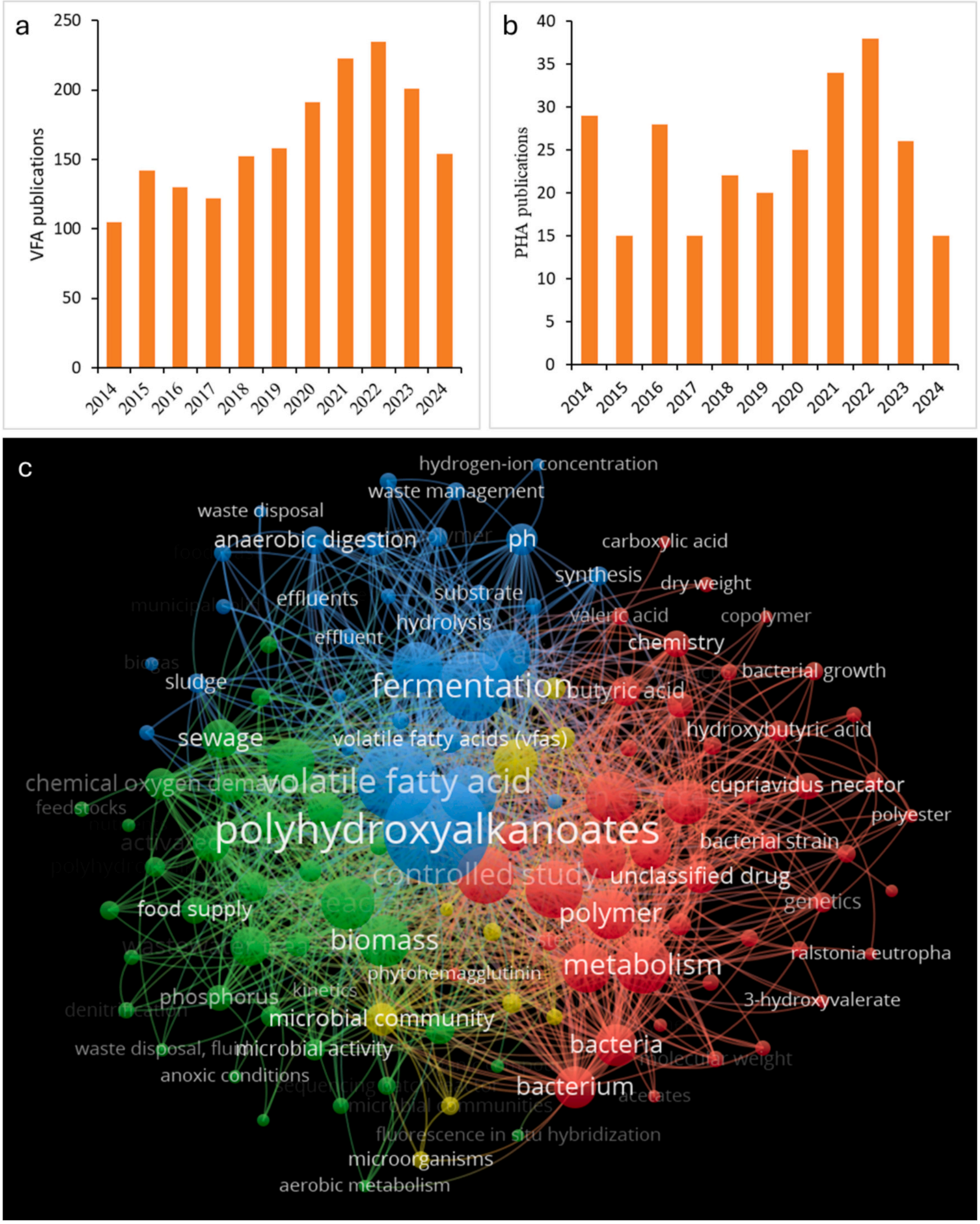

Fig. 1. Waste activated sludge (WAS) fermentation into volatile fatty acid (VFA) and its upcycling into polyhydroxyalkanoates (PHA) publication data from Scopus (January 2014 to May 2024). a) WAS to VFA production data, b) VFA to PHA related publication, c) keyword map of articles published (minimum number of occurrences 10).

[21]. Feast and famine is the key strategy used to develop a stable microbial mixed culture for PHA production [22,23]. Production of different types of PHA like short chain length PHA (*scl*-PHA) and medium chain length PHA (*mcl*-PHA) have been reported. Polyhydroxybutyrate (PHB) is the most commonly produced PHA and has high crystallinity and brittleness that limits its application [24]. Medium chain length PHA is less crystalline and more ductile and can be used for different purposes. PHA productivity and properties are affected by the VFA composition as even chain VFA supports PHA with even chain length monomers while odd chain incorporates odd chain monomers in PHA [25]. Other factors like C/N ratio, aeration rate, and pH also affect the PHA accumulation capability of microbes and all the process parameters should be optimised carefully to achieve efficient conversion and yield [26,27].

Research area related to WAS anaerobic digestion into VFA is getting attention around the globe and 1813 articles have been published in the last decade (Scopus data: January 2014–May 2024). Volatile fatty acids utilisation as a feedstock for PHA production has also become an important area of research as 267 articles have been published since 2014 (Fig. 1a, b). Keyword analysis from the published articles was performed using Vosviewer and polyhydroxyalkanoates, volatile fatty acids, fermentation are the most common keywords (Fig. 1c). This article is completely focused on the WAS pretreatment for VFA production and its integration with the PHA production process. Considering the importance of the research area this article was planned to provide an overview of recent advances in WAS pretreatment, various factors that influence the AD process, and trends in VFA to PHA production technologies with challenges and possible solutions.

2. Waste activated sludge: Composition and characteristics

Municipal wastewater (MWW) is the spent water that originates from residences, commercial facilities, and industrial activities. This wastewater is treated in various WWTPs and residual byproducts are termed as WAS. It can be categorized based on the stage of treatment i.e. primary (physical and chemical), secondary (biological), and tertiary (final processing). Characteristics and composition of WAS depend on the source, geographical location, and process applied for the treatment. Dewatered WAS typically has a higher percentage of organic matter (50–70 %), mineral components (30–50 %), phosphorus (0.5–2.5 %), nitrogen (3.4–4.0 %), and a significant amount of micronutrients [28]. According to an estimate 360–380 billion m^3 of MWW was produced in 2020 across the world and is expected to increase by 27 % till 2030 [29]. The Central Pollution Control Board (CPCB), New Delhi estimated that around 38,354 MLD (million liters per day) of wastewater is produced in India, and only half of it is treated and the rest is discharged directly [30]. European Union alone produces 10 million tons of WAS dry weight annually out of which 47.5 % is reused in agriculture, 27.2 % is incinerated, 8.3 % is disposed of in landfills and the rest is managed by other ways [31]. Annual wet WAS production was about 30 million tons in China in 2016 [32]. In the US >8 million tons of dry WAS is produced and its disposal by landfill is banned in 37 out of 50 states [33]. Australia and Canada use >60 % of WAS in agriculture [34]. Waste activated sludge contains pathogens, parasites, toxins, and pollutants and poses a threat to humans and the environment making its management a challenging task. A study reported >27 types of human viruses (adenovirus, coronavirus, HIV, etc.) from WAS of five US cities [35]. For decades the majority of WAS has been managed by thickening, hygienization, landfilling, incineration, or used as fertilizer in agriculture. Waste activated sludge contributes ~1 % and ~ 5 % of the annual N and P fertilizers used in the US [33]. Initially, in the European Union, the use of WAS in agriculture was regulated only by metal ion concentration (cadmium, copper, mercury, nickel, led, and zinc) but now many countries have introduced more regulations related to synthetic compounds and microbial contamination [36]. In Amsterdam it is illegal to use WAS in agriculture so they started to export WAS and around 27,500 tons of WAS was transported to the UK for use as fertilizer [37]. South Korea moved to ocean dumping of WAS in 1993 due to the prohibition of landfilling to protect water resources. A rapid increase in ocean dumping affected the marine environment and the Ministry of Ocean and Fisheries (MOF) of ROK raised concern and prohibited this practice in 2006. This implementation resulted in an increase in WAS recycling from 5 % to 43 % in the period of 2006 to 2012 [38]. Direct use of WAS as fertilizer causes odor problems and pathogens (*Escherichia coli, Salmonella*) may enter food which affects human health. To deal with these challenges researchers are exploring gasification and pyrolysis methods to produce oil, gas, and solid residue (biochar). This process can reduce waste volume by up to 50 % and produced biochar can be used as a fertilizer for soil amendment [39]. Anaerobic digestion is a popular method for managing WAS, and approximately 1300 WAS-based anaerobic digestion facilities are operating worldwide. [40]. Volatile fatty acids is one of the products produced during the AD process and can be used as a feedstock to produce a variety of products.

3. Polyhydroxyalkanoates and its production strategies

Polyhydroxyalkanoates are biodegradable biopolymers accumulated by various microbes as a reserve food material under excess carbon source and nutrient stress conditions [41]. These polymers have properties similar to polystyrene and are considered as an alternative to petrol-based plastic. There are >150 types of monomeric units involved in PHA production have been reported. The characteristics of PHA are determined by their monomeric composition and are divided into two categories based on the length of the carbon chain of the monomeric unit: short chain length PHA (3–5 carbon, *scl*-PHA), and medium chain length PHA (6–14 carbon, *mcl*-PHA) [42]. Polyhydroxybutyrate (PHB) is the most commonly produced *scl*-PHA with the ability to easily crystallise at higher temperatures and shows higher water and oxygen barrier properties. Due to these properties, its use in the packaging industry is getting attention, and around 35,870 t of PHB has been introduced in the packaging industry and is estimated to increase by 5 to 10 times in the next five years [24]. Polyhydroxybutyrate has limited applications due to its high crystallinity, brittleness, and lower ductility. Researchers are working on the production of different copolymers of PHB by introducing various monomer units as precursors during fermentation. Propionic acids act as a precursor for hydroxyvalerate in Poly(3-hydroxybutyrate-*co*-3-hydroxyvalerate) P(3HB-*co*-3HV), 4-hydroxybutyric, caprolactone or γ-butyrolactone act as a precursor for hydroxybutyrate subunit in Poly(3-hydroxybutyrate-*co*-4-hydroxybutyrate) P(3HB-*co*-4HB), while copolymer Poly(3-hydroxybutyrate-*co*-3-hydroxyhexanoate) P(3HB-*co*-3HHx) can be produced using oil based carbon source where various fatty acids are metabolized through β-oxidation pathway and provide the precursor for polymer production [43–45]. The properties of copolymers depend on the monomeric fraction of different subunits. The introduction of HV fraction reduces the crystallinity, improves flexibility, and makes it more amorphous, vulnerable to biodegradation, and suitable for biomedical applications such as drug delivery and implant preparation [46].

Various carbon sources like sugars, lipids, and proteins are metabolized through different pathways and provide monomer units. Acetyl-CoA is produced by the glycolytic pathway and beta-oxidation of fatty acids. Propionyl-CoA is produced through amino acid metabolism or odd chain fatty acids β-oxidation. Various enzymes are involved in PHA synthesis such β-ketothiolase (PhaA), acetoacetyl-CoA reductase (PhaB), and PHA synthase (PhaC) (Fig. 2). β-ketothiolase condenses acetyl-CoA with another molecule of acetyl-CoA or with propionyl-CoA to produce acetoacetyl-CoA or 3-ketovaleryl-CoA, respectively which are further converted to 3HB and 3HV by reductase [47]. Lastly, the PHA synthase enzyme polymerizes these monomeric units to create the different polymers P(3HB), P(3HB-*co*-3 HV). PHA synthase is the key enzyme involved in the polymerization of different monomeric units into the polymer and the monomers incorporated depend on the substrate

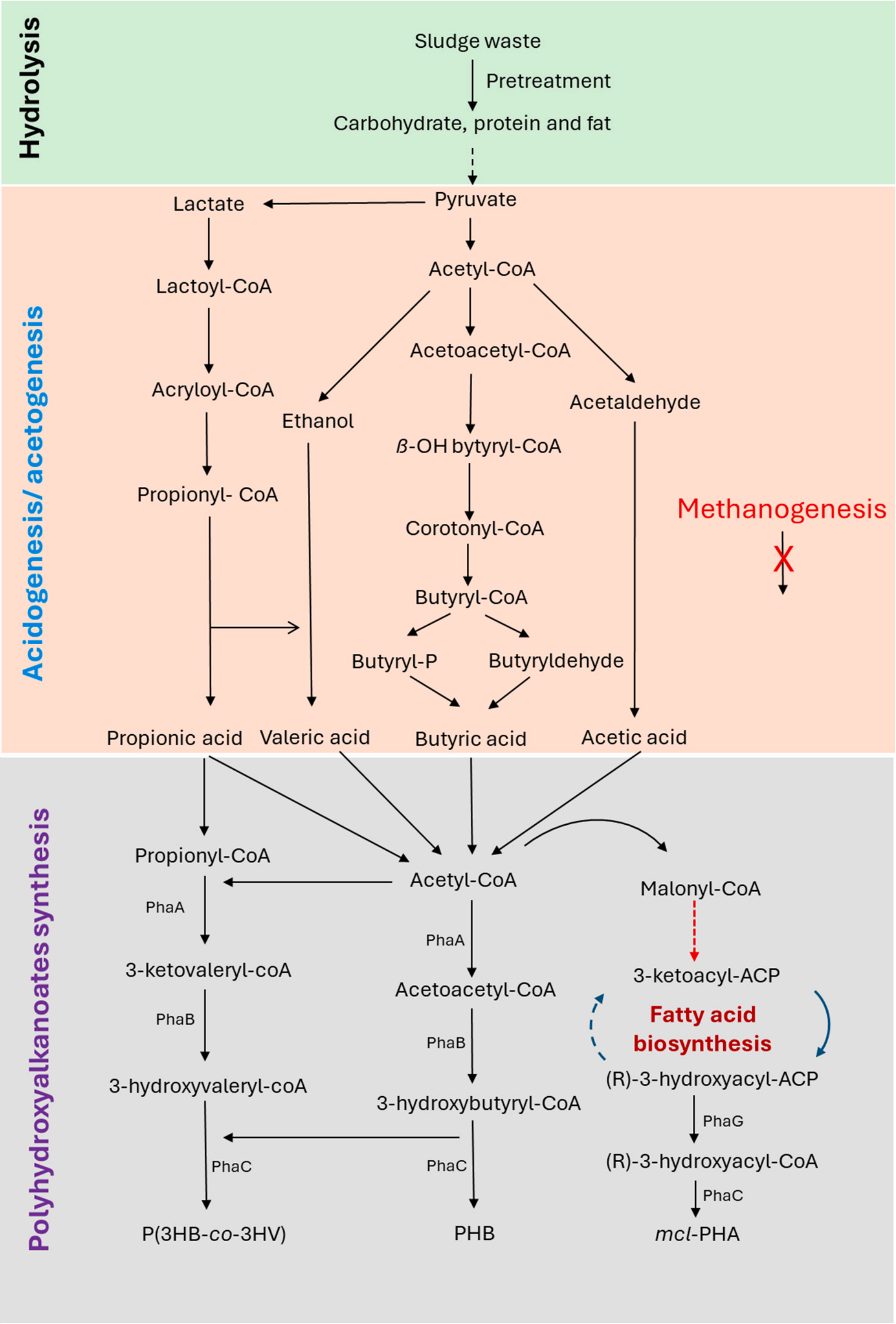

Fig. 2. Pathways involved in WAS fermentation into VFA and its valorisation into PHA.

specificity of the synthase. There are four classes of PhaC have been reported where only class II can polymerise *mcl*-PHA while I, III, and IV have more specificity for *scl*-PHA. PHA synthases can polymerise only *scl* monomers found in *C. necator* H16, others are involved in the synthesis of *mcl* polymers found in *Pseudomonas* sp. while some can polymerise both *scl* and *mcl* monomers found in *Chrmobacterium* sp. [48–50].

Almost 40 % of PHA production cost is due to the raw material and there is a need to explore various wastes as a feedstock for microbial fermentation. The use of lignocellulosic waste, waste cooking oils, animal fats, etc. have been explored but these feedstocks have their own drawbacks [51,52]. Lignocellulosic biomass requires pretreatment to produce fermentable sugars and also generate various side products that affect microbial growth and their PHA accumulating activity [13]. Utilisation of waste cooking oil and animal fat is also challenging as these have low solubility in a liquid medium and require various treatments and the addition of surfactant to improve the oil solubility which makes the production process costly [53]. Waste activated sludge is produced in huge quantities during wastewater treatment and its management is an issue. Volatile fatty acids are considered a suitable feedstock for PHA production and can be produced from the waste activated sludge AD process.

4. Waste activated sludge conversion into VFA

Waste activated sludge is a complex and recalcitrant structure composed of microbial biomass and exopolysaccharides (EPS) and pretreatment is needed to release the intracellular and extracellular organic material and make it accessible for microbes during AD. Pretreatment of WAS enhances the hydrolysis of complex compounds into simpler ones and allows fast conversion into VFA. Direct use of WAS as a feedstock for microbial fermentation results in lower PHA production due to the growth of non-PHA accumulating microbes. The use of a two-step process where WAS is first fermented into VFA and then into PHA seems a promising approach with high yield (Fig. 3).

4.1. Waste activated sludge pretreatment

Anaerobic digestion of WAS into VFA is advantageous as these can be used as feedstock to produce other valuable products on the other hand the process has low sludge retention time, high resistance to environmental factors, and is easy to maintain [54]. Microbes can utilize VFA and produce different kinds of PHA (Fig. 2). The AD process comprises of various steps including hydrolysis, acidogenesis, acetogenesis, and methanogenesis carried by diverse microbial communities. The WAS exists in the form of complex flocs and its solubilization is the main limiting factor in the AD process (Fig. 3a). In a normal AD process, only 30–50 % of the organic content present in WAS is utilized while the rest remains unutilized due to its poor solubilization [55]. Solubilization of WAS can be increased using various treatment methods i.e. physical, chemical, and biological treatment (Table. 1).

4.1.1. Physical treatment

4.1.1.1. Thermal treatment:. The temperature has a main role in thermal treatment, Xiang et al., explored a range of temperatures (100–160 °C)

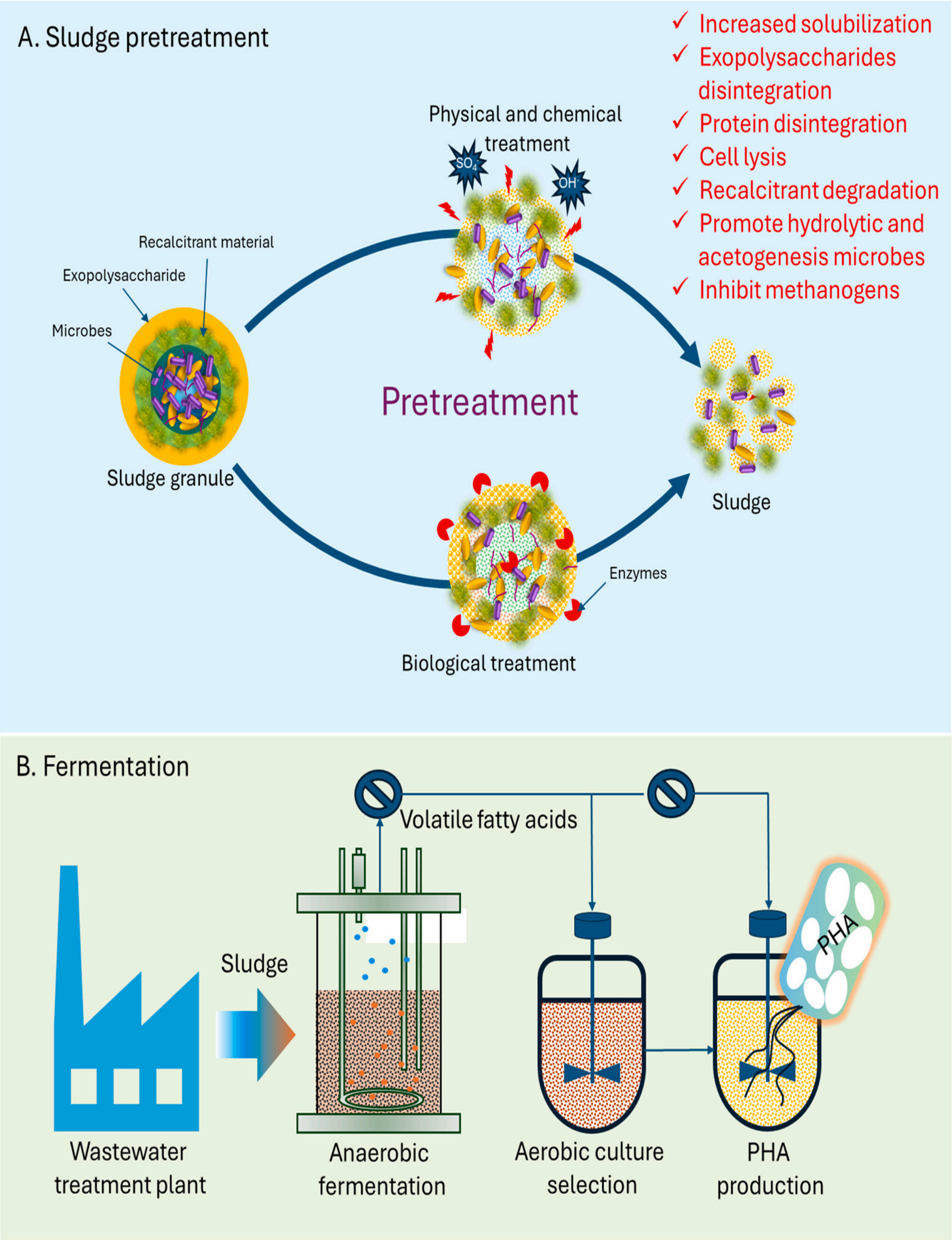

Fig. 3. Waste activated sludge (WAS) pretreatment and its fermentation, a) various pretreatment methods, b) fermentation of WAS into VFA and its valorisation into PHA.

Table. 1
Various methods reported for the pretreatment of waste activated sludge (WAS) and its anaerobic digestion (AD) for volatile fatty acid (VFA) production.

Method/reagent	Function	WAS solubility	Fermentation	VFA yield	Reference
Peroxymonosulfate with Fe-loaded sodium alginate hydrogel beads (Fe-SA)	Fe-SA induces $SO_4^{\bullet-}$, and $^{\bullet}OH$ reactive radicals which contribute to sludge disintegration. Sludge particle size decreased by 14 %.	SCOD increased from 868 to 3368 mg/L (3.8 fold increase)	200 mL, 30 °C, 5 days	2013 mg COD/L	[73]
Peroxydisulphate	Enhance organic matter disintegration, and promote VFA production and phosphorus removal.	SCOD increased from 138 to 3115 mg/L (22.6 fold increase)	200 mL, 37 °C, 7 days	690.41 mg COD/L	[74]
Benzethonium chloride	Disintegrate protein structure and release amino acids. Stimulate EPS production and reduce surface tension. Facilitate enrichment of hydrolytic and acidogens microbes.	SCOD increased from 400 to 3367 mg/L (8.41 fold increase)	300 mL scale at 37 °C, 8 days	2441.01 mg COD/L	[75]
CaO_2 and nitrous acid	Both the reagents act synergistically for the degradation of EPS and the release of soluble organic material.	SCOD increased from 106 to 3319 mg/L (31.31 fold increase)	200 mL, 10 days	150 mg/g VSS	[76]
Plasma/CaO_2	Plasma enhances free radical formation from CaO_2 which leads to increased solubilization.	SCOD increased from 2074 to 3888 mg/L (1.87 fold increase)	1 L, 35 °C, 16 days	3888.6 mg/L	[77]
Potassium permanganate	Provide an alkaline environment and its oxidant nature increases the disruption of organic sludge.	–	Batch fermentation for 14 days	144–196 mg COD/g VSS	[78]
Potassium permanganate	Promote growth of VFA producing microbes related to Proteobacteria, Firmicutes, and Bacteroidetes phyla and inhibit VFA consumers.	SCOD increased from 120 to 1263 mg/L (10.52 fold increase)	1.1 L, 18 days	664.2 mg COD/L	[5]
Potassium persulphate	Improve bioavailability of organics and upregulated expression of various genes involved in carbohydrate, amino acids, and fatty acids metabolism.	SCOD increased from 154 to 362 mg/L (2.35 fold increase)	600 mL, 35 °C, 8 days	1808.4 mg COD/L	[79]
Polysorbate-80	Act as a surfactant and reduce the surface tension of the solvent. Promote solubilization and stimulate microbial metabolism.	SCOD increased from 88 to 4200 mg/L (47.72 fold increase)	450 mL, 35 °C, 12 days	2958.35 mg COD/L	[55]
Ferrate	Ferrate degrades EPS and cytoderm by electron transfer, reactive oxygen species, and elevating alkalinity.	SCOD increased from 370 to 6060 mg/L (16.37 fold increase)	300 mL, 35 °C, 16 days	8106.3 mg COD/L	[80]
Ferrate and percarbonate	Percarbonate provides bicarbonate and carbonate for the oxidation of ferrate and reduces the amount required. Increase sludge solubilization.	SCOD increased from 740 to 6120 mg/L (8.27 fold increase)	300 mL, 35 °C, 8 days	3670.2 mg COD/L	[81]
Ferric chloride and nitrite	Ferric chloride induces the conversion of nitrite to nitrous acid which promotes the conversion of recalcitrant organic material to biodegradable one.	SCOD increased from 852 to 3456 mg/L (4.05 fold increase)	1 L, 35 °C, 8 days	211.3 mg COD/gVSS	[82]
Fe(II)-sulphite	Fe induces $SO_4^{\bullet-}$, and •OH which promotes the biodegradability of organic matter. Activities of hydrolytic and acidogenic enzymes also elevated.	SCOD increased from 200 to 1523 mg/L (7.61 fold increase)	300 mL, 35 °C, 10 days	1459.2 mg COD/L	[83]
Nitrate	Nitrate is photosensitive which produces various reactive species that increase soluble protein and enrich functional consortia.	SCOD increased from 735 to 1364 mg/L (1.85 fold increase)	400 mL, 10 days	280.7 mg/g VSS	[84]
Sodium percarbonate and free ammonia	Synergistically improve sludge disintegration, hydrolysis, acidification and inhibit methanogens.	–	500 mL, 12 days	347.1 mg COD/g	[85]
Peracetic acid and free ammonia	Improve sludge solubilization, and promote hydrolysis and acidification.	SCOD increased from 346 to 2983 mg/L (8.62 fold increase)	450 mL, 3 days	491.35 mg COD/g VSS	[86]
Zinc pyrithione	Accelerate solubilization, hydrolysis, acidification, and inhibit methanogenesis.	SCOD increased from 27 to 206 mg/L (7.62 fold increase)	300 mL, 35 °C, 12 days	3318 mg COD/L	[87]
Protease and amylase	Enzymatic pretreatment increases exposure of hydrophilic and hydrophobic groups and sludge surfaces and promotes repulsion forces between sludge particles.	SCOD increased from 283 to 7728 mg/L (27.30 fold increase)	150 mL, 20 days	5700 mg COD/L	[4]
Protease and lysozyme	The synergistic effect of both enzymes improved solubilization and hydrolysis.	SCOD increased from 98 to 325 mg/L (3.31 fold increase)	150 mL, 35 °C, 4 days	451–455 mg COD/L	[88]

effect on WAS solubilization and optimal VFA production (3297.6 mg COD/L) was recorded at 140 °C with a 26.7 % increase in acetic acid content [6]. Protein and polysaccharide concentrations rise with temperature, indicating the solubilization of complex organic materials from WAS. Further, temperature impacts the distribution of bacterial communities and with the increase of temperature dominance of Firmicutes increases (23.6 % to 53.3 %). Firmicutes have a thick cell wall and able to withstand higher temperatures and are responsible for high VFA production. High temperature increases hydrolysis, improves membrane transport and metabolism of carbohydrates and lipids, impacts the abundance of functional microbes, and improves the expression of functional genes involved in VFA metabolic pathways [6]. Wen et al., also explored the thermal treatment and 1 h treatment able to release >30.5 % of organics in the liquid phase and process able to produce 22.8 % more VFA compared to the control [56]. Before subjecting hydrolysate to AD, solid residues are separated to avoid any interference of these. Creating hydrolysate of high soluble organic content is a challenge as it requires dewatering and higher temperature treatment which may lead to the production of unwanted compounds. Freezing with thawing at lower temperatures is also helpful in improving the release of soluble polysaccharides and proteins from WAS as investigated by He et al., where this method leads to 212 mg COD/g VSS volatile fatty acids production in 5 days [57]. Zhou et al., applied the thermal treatment method with alkaline treatment and were able to achieve maximum release of SCOD i.e. 8788.74 mg/L which increased VFA production of 4596 mg/L [58]. All these studies prove that thermal treatment has the potential to increase soluble organic content which further supports microbial fermentation and improves VFA production.

4.1.1.2. Ultrasonic and electrochemical treatment:. Organic components are embedded in cells and EPS and restrict solubilization, which affects the AD process. Ultrasonic pretreatment can disintegrate sludge flocs and release polysaccharides and proteins. Peng et al., explored ultrasonic treatment and were able to increase soluble protein (4173 mg/L) and carbohydrate (853 mg/L), respectively [59]. Three-dimensional excitation-emission matrix results showed ultrasonic treatment effectively decomposes the tightly bound EPS matrix and lyse microbial cell wall. The anaerobic digestion process of ultrasonic pretreated WAS resulted in a high content of VFA i.e. 10,816 mg COD/L [59]. The electrochemical method is also getting attention due to its chemical-free nature, high efficiency, and generation of minimum side products. Electrochemical treatment disintegrates WAS flocs structure and causes cell lysis by direct electric force and indirect oxidation mediated by free radicals. Ki et al. used pulsed-field electric treatment for WAS and were able to produce VFA containing 2.6 fold higher content of acetate [60]. Lin et al., applied this method for WAS and food waste mixture and reported the production of reactive chlorine species from chloride present in food waste which further helped in cell lysis and increased soluble organic matter. In the AD process acetogen became dominant compared to methanogen due to their robust nature and the process resulted in 40.8 % higher VFA compared to the control [61].

4.1.2. Chemical treatment

4.1.2.1. Alkaline treatment:. Alkaline treatment of WAS was studied by Yuan et al., who reported that maintaining pH 10 at the initial stage increases VFA production with a maximum yield of 256.2 mg COD/g VSS with 40–55 % acetic acid content [62]. Under alkaline conditions soluble COD increases and utilisation of VFA by methanogens is decreased. VFA production (323 mg COD/g VSS) was further improved by He et al., using stepwise alkaline treatment coupled with ammonia stripping. pH was maintained at 10 on the first and third days and then ammonia stripping was done [54]. Ammonia is formed during the fermentation which can inhibit acetogens and methanogens and using ammonia stripping this can be avoided and high VFA production can be achieved. The presence of metals and EPS in WAS stabilise its structure and suppresses the AD process. Supplementation of EDTA during alkaline treatment improved solubilization of WAS by 62.8 % releasing 21.8 % more soluble COD and the AD process resulted in 477.4 mg COD/g VSS that was 6.13 times higher compared to the control [63].

4.1.2.2. Oxidising agent:. Utilisation of various oxidising agents like peroxides, ozone, potassium permanganate, persulfate, and potassium ferrate also getting attention for WAS pretreatment due to their high efficiency and low cost. Peroxymonosulphate ($2KHSO_5$-K_2SO_4-$KHSO_4$) (PMS) is a strong oxidising agent and has the ability to solubilise the WAS flocs. Yang et al., studied PMS pretreatment and reported VFA production from 29.69 mg COD/g VSS to 311.67 mg COD/g VSS with acetic acid as the major product (43.4 %) and low content of methane [64]. Larger flocs break down into smaller ones with PMS treatment, which also raises the amount of soluble protein, carbohydrates, and TOC and turns resistant organic material into biodegradable material. Further, its mechanism was investigated, and Yang et al., found that PMS decomposed into K^+ and HSO_5^- when comes in contact with hydrous media and further degraded into $SO_4^{\bullet -}$, $^{\bullet}OH$, and $^1O_2$. These free radicals cause damage to cellular components like protein, lipids, carbohydrates, and other macromolecules and increase VFA production [64]. In another study Zeng et al., combined PMS treatment with alkaline fermentation and were able to achieve 401.1 mg COD/g VSS which was 7.3 and 2.1 fold higher compared to PMS and alkaline treatment alone, respectively. Soluble organic content present in the hydrolysate is directly related to the VFA yield. He et al., used sludge derived biochar (SBC) to activate PMS, and found that biochar active sites possess metal ions and C=O groups which decompose PMS into various radicals and further increase WAS solubilization and production of VFA. The use of PMS/SBC (0.14 g/g VSS/0.24 g/g VSS) in pretreatment improved VFA production by 801 % by increasing the hydrolysis rate and expression of various genes [3]. Calcium peroxide (CaO_2) also acts as an oxidant and produces free radicals like hydroperoxyl radical ($HO_2^{\bullet}$), hydroxy radical ($^{\bullet}OH$), and superoxide radical ($^{\bullet}O_2^-$) which helps in the destruction of the extracellular polymeric matrix and provide more substrate for acidification. Li et al., investigated the effect of CaO_2 on WAS pretreatment and AD process and reported VFA production up to 284 mg COD/g VSS which was 3.9 times higher as compared to control. CaO_2 pretreatment improved hydrolysis, inhibited methane production, and produced VFA comprised of 60.2 % of acetic acid [65]. Zhao et al., combined CaO_2 pretreatment with freezing cycles and was able to achieve 438.5 mg COD/g VSS. Mechanism investigation showed that co-pretreatment triggered sludge solubilization, provided a more digestible substrate for fermentation, promoted acidogens, and inhibited methanogens [66]. In another study, Wang et al., used CaO_2 + free ammonia pretreatment method for WAS treatment and were able to produce 338.6 mg COD/gVSS that was 2.5 fold and 1.5 fold higher compared to CaO_2 and fee ammonia treatment, respectively [67].

4.1.2.3. Ionic liquid and deep eutectic solvents:. Ionic liquids are salts of organic cations and inorganic anions which are liquid at room temperature. Choline chloride (ChCl) is well reported for its capability to degrade biomass due to the potential of hydrogen bond acceptance and interaction with diverse functional groups like amino, carboxyl, and carbonyl. Cao et al., used ChCl for WAS treatment and reported 3884 mg COD/L VFA production which was 723.3 % higher compared to the control. Fourier transforms infrared spectrometer and molecular docking results proved that ChCl attacks and disrupts extracellular polymeric matrix by forming hydrogen bonds with organic moieties [68]. Practical applications of IL is limited due to their toxicity, cost, and low degradability. Natural deep eutectic solvents (NADES) are composed of Lewis or Bronsted acids and bases that contain a variety of anionic and cationic species and have low toxicity. Liu et al., investigated NADES (choline chloride: oxalic acid anhydride) for WAS treatment at different temperatures and found 180 °C is the optimum temperature with a maximum yield of VFA i.e. 2728.71 mg COD/L. The NADES treatment in combination with thermal increases soluble polysaccharides and protein contents and upregulates fatty acids biosynthesis genes [69].

4.1.3. Biological method

In WAS extracellular polymeric substances with uronic components of alginate and polygalacturonic acid form a gel-like structure and resist biodegradation. Biological pretreatment offers high selectivity and lower operating costs. Hu et al., enriched WAS with alginate degrading consortium and observed 38 % increase in hydrolysis efficiency and 72 % increase in acidification with main metabolite acetate (1.6 g/L) and propionate (0.7 g/L) [70]. Metals ions like Mg^{2+}, Ca^{2+}, etc. present in WAS contribute to the structural skeleton and bioflocculation of sludge by linking negatively charged organic components with EPS. The use of metal-removing resin can improve hydrolysis. Lysozyme can hydrolyse mucopolysaccharides and lyse microbial cells. Pang et al., used lysozyme and cation exchange resin for WAS treatment which triggered EPS rupture and resulted in increased VFA (3651.14 mg COD/L) after 48 h AD process [71]. Zhao et al., explored the enzymatic pretreatment method to improve WAS hydrolysis. Initially, glucose was added to activate the acidifying microbes, and different enzymes lysozyme, protease, and amylase were added individually. Enzymes modulated the hydrolysis process and affected VFA production which resulted in varied amounts of VFA production lysozyme (4076 mg.COD/L), protease (3883.9 mg.COD/L), amylase (1577.2 mg.COD/L) compared to control (1949.7 mg.COD/L). Types of enzymes used also affected produced VFA composition as propionic acid was the major product with lysozyme (58.75 %) and protease (58.58 %), and acetic acid was the main product with amylase (64.4 %) and control (89.8 %), respectively [1]. To increase enzyme activity and stability protease, pectinase, and laccase were immobilized on $Fe_3O_4@SiO_2\text{-}NH_2$ and further utilized for WAS pretreatment. The use of immobilized enzymes resulted in 85 % increase in soluble COD due to the increased release of EPS and proteins from sludge and the AD process led to the production of 10.6 g/L VFA production [72]. The ζ-potential value of raw sludge is 16.9 ± 1.1 mV after enzymatic treatment i.e. amylase and protease increased to −22.5 and − 25.6 mV as treatment helps in the exposure of negatively charged (-COOH) groups of flocs. Enzymatic treatment reduces the contact angle from 80.0 ± 2.7° to 47.6 ± 2.9° and makes the surface more hydrophilic and enhances acidogenic fermentation [4].

4.2. VFA production and affecting factors

This section goes into detail about the several parameters that influence the conversion of waste activated sludge into VFA, including its composition, pretreatment techniques, AD process, and microbial communities.

4.2.1. The fermentation process and microbial community

Pretreated WAS is first subjected to AD and produced VFA is further used as a feedstock for PHA production (Fig. 3). Different strategies like batch, continuous, and semi-continuous can be utilized for VFA production from WAS. Mieno et al., used batch fermentation for VFA production where WAS was pretreated with 0.1 g $KMNO_4$/g of total suspended solids, and the process resulted in 452 mg COD/g VSS [89]. Zhang et al., used an up-flow anaerobic sludge blanket (UASB) for VHA production where WAS first pretreated and separated sludge liquid further subjected to fermentation with online nitrogen and phosphorus removal efficiency. AD process was performed in three phases (I, II, III) with OLR of 4.0 kg COD/m^3/d, 6.5 kg COD/m^3/d, and 10.0 kg COD/m^3/d, respectively. Batch and semicontinuous fermentation were also performed for comparison. A high VFA productivity (3.19 kg/m^3/d) was achieved with AD process of higher OLR (10.0 kg COD/m^3/d) [90]. Alkaline fermentation (pH ~ 10) is a preferred process for VFA production as it amplifies the electrostatic repulsion forces between exopolysaccharides and microbes which cause the disintegration of sludge. Under alkaline conditions, VFA production can be increased by 3–5 folds, but maintaining alkaline conditions requires continuous alkaline addition which makes it costly [56]. The microbial community has an important role in VFA production. Raw sludge possesses various microbes related to phyla such as Proteobacteria (50.1 %), Bacteroidetes (30.0 %), Chloroflexi (5.5 %), and Planctomycetes (4.2 %). Pretreatment shifts the microbial community as reported by Li et al., with CaO_2 treatment i.e. Firmicutes (43.2 %), Proteobacteria (26.8 %), Bacteroidetes (14.7 %) and Planctomycetes (5.7 %) [65]. Firmicutes produce various enzymes like protease, lipase, cellulase, etc., and are involved in organic material degradation and acid production. Other dominant microbes like Proteobacteria and Bacteroidetes play important role in hydrolysis and acetogenesis. Enzymatic pretreatment also affects the microbial community as reported by Song et al., where under amylase treatment Firmicutes are dominant (37 %) at the initial stage and Proteobacteria dominant (73 %) at a later stage [4]. In the initial stage, Firmicutes consume organics and perform hydrolysis and Proteobacteria at a later stage are involved in the oxidation of propionate and butyrate to produce acetate as the main product. Under protease treatment, the Bacteroidetes population increases in the mid (60.8 %) and late phase (113.3 %) while the Chlorflexi population decreases [4]. Bacteroidetes play an important role in carbohydrate and protein hydrolysis and participate in the chain elongation of acetate and propionate to produce butyrate and valerate [91]. The pretreatment process also regulates enzyme production and activity during the AD process. In a study by Zhang et al., where persulphate was used for pretreatment of WAS it was observed that genes related to substrate (carbohydrate and amino acids), pyruvate metabolism, and fatty acids synthesis are upregulated due to variation in the population of various fermentative bacteria (*Clostridium* and *Fonticella*). Further mutual cooperation among microbes is crucial for robustness and efficacy of AD process. Bacterial communication through quorum sensing is dependent on cell density, gene expression, and community level. Microbes transmit information using various signalling molecules and when their concentration reaches a certain level, expression of certain genes is triggered to regulate microbial behaviour for extracellular substances production and biofilm formation [92]. Genes encoding oxidative defense enzymes (SOD_2 and KatE) are also activated to protect the anaerobes and maintain metabolic function [79]. Waste activated sludge is also explored to improve the fermentation of other waste. Huang et al., used WAS as a cosubstrate for kitchen waste fermentation in a membrane separation system and reported a 2 times increase in VFA production. The WAS helps in alleviating salt stress and acid inhibition through ammonia buffering [93].

It is also difficult to collect VFA from the WAS acidogenic ferment because the mixture contains a lot of biopolymers, stable heterogeneous colloids, and EPS, which makes it difficult to separate. Different methods like distillation, stripping, and electrodialysis have been reported for VFA separation [94,95]. Membrane based systems can be used inline recovery of VFA but also face challenges like several organic compounds and microbial biomass causing membrane fouling and reducing the membrane permeability and selectivity [96]. For PHA production there is no need to recover the VFA and anaerobic digestate can be directly used for microbial fermentation after some treatment if needed. Mengmeng et al., used WAS from WWTP and subjected it to AD for VFA production in the presence of sodium dodecylbenzene sulfonate (SDBS) for substrate solubilization and to decrease the methanogenic activity. After the AD process, the VFA containing digestate was centrifuged and processed for ammonia removal, autoclaved, and directly inoculated with activated sludge to produce PHA (56.5 % *w*/w) [97].

4.2.2. WAS composition and AD process parameters affect on VFA production

Waste activated sludge may contain some inorganic material along with organic one. Superfine sand is a particle of size <200 μm present in WAS and can affect AD process. Jiang et al., studied the effect of superfine dust on WAS anaerobic digestion and reported an increase in VFA production from 2513 mg COD/L to 3002 mg COD/L [98]. It was reported that superfine dust facilitates solubilization, acidification, and abundance of anaerobic functional microbes. Humic acid is present in up to 6–20 % of VSS, which is produced during the decay of organic substances and may affect the AD process. Li et al., investigated humic acid's effect on WAS to VFA conversion and reported an inhibitory effect on hydrolysis efficiency (38.2 %), methanogenesis efficiency (52.2 %), and a positive effect on acetogenesis efficiency (101.5 %) [99]. Lignin is also present in WAS and recalcitrant in nature. He et al. examined its impact on VFA formation and found that 15 % lignin increases the amount of short chain fatty acids from 129.1 to 223.14 mg COD/g VSS [100]. It was concluded that lignin enhances acidogenesis by perfecting the electron transfer chain and the quinone structure in lignin competes for electrons with methanogens and inhibits VFA consumption [100]. Lignin also causes microbial community shift and promotes *Proteiniclasticum* sp., *Acetoanaerobium* sp., growth specialized in VFA production. Waste activated sludge granule size and structure also affect VFA production. Zou et al., investigated the effect of granule size (200 to >1600 μm) on hydrolysis and acidification. The soluble COD and VFA production were higher with medium-sized granules (500–1600 μm) compared to small-sized (200–500 μm) and larger granules (>1600) due to higher hydrolysis and acidification [101]. The granule structure is loose and porous when the size is >1430 μm. The average-sized granules have a higher anaerobic zone and an abundance of hydrolytic and acidogenic microbes. The presence of toxic recalcitrant compounds i.e. pharmaceuticals, personal care products, hormones, steroids, flame retardants, and other organic pollutants in the WAS also affects the AD process. These compounds are not removed during the wastewater treatment in conventional wastewater treatment plants as these are not designed to do so and all these compounds accumulate in WAS which ultimately affects AD process. Zheng et al., analysed WAS for toxic compounds and about 24 types of compounds with concentrations of 3.488–5601 ng/g VSS were found. Among these pharmaceuticals are the most dominant e.g. hydrochlorothiazide (167.8 ng/g VSS), gemfibrozil (30.46 ng/g VSS), sulfamethoxazole (79.94 ng/g VSS), diphenhydramine (38.10 ng/g VSS), trimethoprim (23.21 ng/g VSS), etc. UV/CaO_2 pretreatment was applied and able to remove 19 detected compounds with 50 % efficiency and was considered as a potential method to remove recalcitrant and improve VFA production (421 %) [102].

Anaerobic digestion process parameters also affect VFA production. Gong et al., studied the effect of temperature and pH on WAS anaerobic digestion and reported that at 35 °C and pH 7 mixed acid types were produced while at high pH (10) and high temperature (55 °C) butyric acid is the main product [16]. Similarly, in one other study Perez-Esteban et al., found that at higher temperatures (55 °C) acetate (40 %) and butyrate (40 %) are dominant products while at lower temperatures (35 °C) acetate (37 %), butyrate (31 %) and propionate (17 %) produced while at 45 °C additional caproic acid is also produced due to diversity in microbial community at a different temperature [103]. Atasoy et al., studied pH effects and reported that at acidic pH (5) there is a high yield of VFA (0.92 g COD/gVSS) with acetic acid (23 %), butyric acid (22 %), propionic acid (21 %) and valeric acid (15 %) as dominant product while propionate (64–72 %) was the dominant product at neutral (7) and basic pH (10) [17]. This change in VFA profile is due to the dominance of different microbial communities at different pH i.e. at acidic pH Firmicutes (47.64 %), neutral pH Proteobacteria (40.74 %), and at basic pH, Bacteroidetes (50 %) are the main microbes. Mixing is also an important parameter as evaluated by Ma et al., where continuous and intermittent mixing at 150 rpm enhances the hydrolysis of sludge and favors the growth of Firmicutes and *Tissierella* which increases VFA production [104]. Mixing intensity influences the shearing force faced by the sludge flocs which cause erosion of extracellular polymeric substances and increase dissolved organic matter. Hydraulic retention time (HRT) for VFA fermentation is shorter compared to the conventional AD process as the methanogenesis process step is eliminated. HRT can be reduced by optimizing the hydrolysis process and acidogenesis.

5. Integration of VFA to PHA production

One of the most promising applications of these bio-based VFA is as substrates for the polyhydroxyalkanoates (PHAs) production. PHAs are microbial polyesters that accumulate intracellularly as carbon and energy storage materials under nutrient-limited conditions. They are biodegradable and biocompatible, making them attractive for sustainable plastic production.

5.1. Fermentation strategies

Volatile fatty acids produced in the AD process can be further upcycled into PHA by fermentation processes using pure culture as well as mixed culture (Fig. 3, Table. 2). The pure culture ensures high efficiency in converting VFA into PHA, uniform composition and properties of PHA, and can accumulate high PHA content (>90 %). Further pure culture can be easily engineered to improve substrate utilisation or alter monomeric composition for production of customized bioplastic with specific properties. Gracia et al., used WAS derived VFA for PHA production using *Burkholderia cepacia* 2G-57 and mixed culture and were able to produce 91 % *w*/w PHA with pure culture and 86 % w/w with mixed culture at the same VFA concentration [105]. The use of pure culture requires the maintenance of sterilized conditions and impacts the production cost. Development of mixed culture for PHA production requires enrichment under feast/famine conditions. Sequential reactors under the feast/famine approach together with the pressure of nutrient and oxygen limitation lead to the accumulation of microbes able to produce PHA. Microbes which not able to accumulate food (PHA) cannot bear a longer starvation period and become extinct. For a better selection of PHA accumulating culture feast/famine ratio should be equal or <0.33 is considered a better approach [22]. Mixed cultures are appropriate for the low-cost, industrial-scale synthesis of PHA because they are durable and can use a variety of substrates without the need for sterile conditions. Kumar et al. used a feast/famine approach (48 h for each step) where the feast phase was performed with acetate/butyrate, nitrate, and phosphate with an undersupply of air while the famine phase was performed without nitrate, phosphate, and oxygen to provide stress conditions [106]. A high content of PHA accumulation was recorded in the 8th cycle of the famine phase (0.36 g/L PHB/g VFA) while in the 8th cycle of the feast phase, only 0.034 g/L PHB/g VFA production was recorded. In the first famine cycle, there was only 0.14 PHB/g VFA was observed, and repeated cycles of nutrient and oxygen

Table 2
Upcycling of volatile fatty acids into PHA.

Microbes	Key points	PHA type	Biomass (g/L)	PHA g/L, %	Reference
C. necator	Valerate is the preferred source for HV fraction.	P(3HB-*co*-3 HV (40.80 %))	1.51	1.20, 79.47	[18]
C. necator	A continuous stirred tank reactor was used.	P(3HB-*co*-3HV)	1.5	0.84, 56	[119]
Salinivibrio sp. TGB19	Fed batch fermentation was performed at 5 L scale.	P(3HB)	60.03	53.23, 88.67	[120]
Halomonas sp. YLGW01	pH-stat fed batch fermentation method was used.	P(3HB)	7.0	5.1, 73	[19]
Rhodopseudomonas palustris	Even chain acids utilized first then odd chain.	P(3HB-*co*-3HV)	0.76	0.18, 24	[121]
Thauera mechernichensis TL1	Suitable for VFA contains acetate and propionate.	P(3HB-*co*-3HV)	2.16	0.52, 24	[122]
Bacillus megaterium	A microfiltration membrane system was used for cell recycle. System is used when VFA is less concentrated. 4 L.	P(3HB)	19.05	13.37, 70.21	[20]
Mixed culture	Mixed culture was isolated from waste treatment plant sludge	P(3HB)	0.85	0.77, 90	[21]
Mixed culture	Fed-batch fermentation was performed. 2 L.	P(3HB-*co*-3HV (41 %))	–	-, 54.8	[16]
Mixed culture	A sequencing batch reactor was used for the enrichment of PHA producer.	P(3HB)	7.05	1.2, 17	[123]
Mixed culture	Salt tolerant mixed culture was developed using an enrichment procedure.	PHA	–	0.55–0.62 g/g	[124]
Mixed culture	VFA was used as a cosubstrate with wood hydrolysate.	P(3HB-*co*-3HV) (51 %)	–	3.0	[125]
Mixed culture	VFA affects microbial community as well as substrate utilisation rate.	P(3HB-*co*-3HV (5.2 %))	7.58	3.3, 43.5	[126]

limitation enriched the PHB accumulating microbes and led to increased PHB production. Under nutrients and oxygen presence in the feast, the excess carbon source is utilized for biomass production rather than for PHB. Conca et al., performed a pilot scale study at a wastewater treatment plant where cellulosic primary sludge was recovered from the plant and fermented to VFA. The SBR reactor was used to establish aerobic feast and anoxic famine conditions for isolation of PHA accumulation culture and the process resulted in 1.2 kg/PHA/PE y [107]. Lorini et al., applied thermal pretreatment and thermal fermentation to WAS to produce VFA and a feast/famine approach to select PHA accumulating microbes and the overall process led to 56 g PHA/kg VSS [108].

Different fermentation methods can be employed for VFA transformation into PHA. In a batch system, all the required nutrients are added to the culture at the start, and the product is recovered at the end of the process. Sun et al., studied the synergistic effect of peracetic acid and free ammonia pretreatment on anaerobic digestion of sludge. Cotretreatment resulted in a high content of VFA 491.35 mg.COD/g VSS which was higher compared to a single treatment. Batch fermentation was performed using mixed culture and the process led to a PHA yield of 55 wt% in five days [86]. Batch fermentation is a laborious and time-consuming process as after each batch it requires washing, sterilization, and preparation of new inoculum which adds in cost. The continuous process is advantageous as it is easy to operate and ensures continuous harvesting and PHA production. Parroquin-Gonzalez et al., used a continuous culture system for *Haloferax mediterranei* cultivation by maintaining the flow of inlet and outlet. A continuous system was able to achieve PHBV with 15.8 mg/h with a controlled monomeric HV (30 mol%) fraction [109]. PHA production is hindered by the VFA high concentration as it is composed of different acids that act synergistically and impact the microbe's physiology. Semi-continuous mode can be helpful where VFA is added at different time intervals to maintain an appropriate level. Washout of cells may occur during the removal of samples and affect the conversion rate. An immersed membrane reactor can be used to overcome substrate inhibition, wash out of cells, and improve PHA production under high VFA concentration. Vu et al., studied semicontinuous and semi-continuous immersed membrane reactors and reported higher biomass (6.6 g/L) and PHA (2.8 g/L) production in later which was 1.5 and 1.4 times higher compared to semicontinuous [110]. Zhao et al., also used a membrane bioreactor for *Paracoccus* sp. TOH cultivation and PHA production where enzyme pretreated anaerobically digested WAS hydrolysate containing 4076 mg. COD/L was used as a feedstock and reported 119.1 mg/L P(3HB-*co*-3 HV) production with 20.3 HV mol% [1]. Burniol-Figols et al., studied two different membrane bioreactor systems i.e. pressure driven and diffusion-based MBR, and observed lower PHA accumulation in later due to low VFA diffusion compared to utilisation rate [111]. Pressure-driven MBR resulted in high PHA accumulation (0.87–1.44 g/L). In another study, a comparison between pulse fed culture and continuous feeding for VFA conversion into PHA using *Haloferax mediterranei* reported a two fold increase in PHA productivity (5 g/L) for continuous fermentation. Fermentation time was reduced to half compared to the pulsed fed batch and produced polymer P(3HB-*co*-3 HV) containing 40 mol% HV fraction [112].

After fermentation, microbial biomass needs to be separated from the supernatant for PHA recovery. PHA recovery and purification contribute 30–50 % of total manufacturing costs, making them critical to industrial feasibility. The method includes biomass harvesting, pretreatment, PHA recovery, and formulation. Cell lysis methods include physical (e.g., bead milling), chemical (e.g., solvent extraction), and combined procedures, such as microwave-EDTA treatments, which improve recovery rates and polymer purity [113]. Solvent-based extraction is chosen for industrial scalability since it can handle enormous volumes and allows for solvent recycling. Non-chlorinated solvents such as cyclohexanone, γ-butyrolactone, dimethyl carbonate (DMC), and acetone have higher yields than chloroform, making them safer and more environmentally friendly [114,115]. Solvent-antisolvent systems, such as DMC/ethanol, improve purity while increasing process complexity.

5.2. Factors affect PHA accumulation

Volatile fatty acid is composed of different types of acids that directly affect microbial biomass production and the composition of produced PHA. Even carbon chain number VFA supports the production of even chain PHA while odd carbon chain VFA responsible for odd chain PHA. Bhatia et al., investigated the effect of individual VFA and a mixture of VFA on PHA accumulation and composition using engineered *R. eutropha* Re2133/pCB81 [116]. The use of acetate and lactate as a carbon source led to the production of P(3HB) (10 % *w*/w and 20 % w/w, respectively), with butyrate P(3HB-*co*-3HHx) (59 % *w*/w), while propionate resulting in P(3HB-*co*-3HV) (63.4 % w/w). *R. eutropha* shows a different preference and utilisation rate for different acids in VFA mixture i.e. lactate> butyrate >propionate> acetate. Use of VFA-enriched ferment as feedstock resulted in 0.57 g/L PHA (52 % w/w) [116]. Zhang et al., reported that mixed VFA leads to 1.6 times higher PHA production compared to single substrate in mixed culture [25]. Butyrate is the dominant substrate followed by valerate and supports PHA production up to 72.08 % and 61.57 % respectively [25]. VFA utilisation also varies with its composition as reported by Li et al., studying the even: odd chain fatty acid ratio i.e. VFAs (88:12) > VFAs (63:37) > VFAs(54:46) > VFAs (48:52) [27]. Bacteroidetes, Proteobacteria, Actinobacteria, Chloroflexi, Acidobacteria, Dependentiae, and Firmicutes are present in all samples of diverse VFA ratio. Bacteroidetes and Proteobacteria account for 97.4 %, 91.0 %, 90.2 %, and 89.1 % of the total microbial community at four different VFA ratios and are responsible for PHA production [27]. This study demonstrated that VFA composition has no obvious impact on microbial community type but has an influence on relative abundance. The selective pressure exerted by the feast/famine approach alters microbial community structure and favors the growth of PHA producers. Despite of many research on the feast/famine approach still, information related to population succession dynamics during different time points is lacking. pH also affects PHA accumulations as reported by Li et al., and the following trend related to PHA accumulation noticed pH 7 > pH 9 > pH 5. At acidic pH (5) acetate remains in an undissociated state to maintain equilibrium and easily diffuses into cells and dissociates into protons lowering the intracellular pH and resulting in lower PHA production. VFA consumption is higher (70.8 %) at pH 7 and reduced to 68.8 % at pH 9 [27]. The C/N ratio also affects PHA production as Li et al., found that C/N of 33 led to higher PHA (6.9 g/L) compared to lower C/N (14) and it was 2.3 times higher. At lower C/N (14) volatile fatty acids are diverted to the TCA cycle and result in more biomass production while at higher C/N (33) there is lower production of enzymes required for growth, and VFA is transformed into PHA [27]. Feeding strategy also affects PHA accumulation as reported by Chen et al., studying one-time feeding and pulsed feeding which resulted in 51.5 % and 64.5 % PHA accumulation, respectively. In one-time feeding, VFA concentration increased rapidly and maximum PHA was observed in 330 min. and decreased thereafter due to exhaustion of VFA while in pulsed feeding the PHA synthesis activity of microbes triggered during each feeding and resulted in increased PHA accumulation [117]. In the feast/famine strategy, microbial diversity is high at the initial stage and decreases later. Morya et al., studied feast/famine for 8 cycles using waste-activated sludge as an inoculant [118]. Bacterial communities in the zero-hour sample were diverse, containing numerous phyla. Proteobacteria were the most prevalent phylum, with an average relative abundance of 55.2 %. Firmicutes (21 %), Nitrospirae (6.8 %), Actinobacteria (4.1 %), and Bacteroidetes (4.1 %) were other prominent phyla. Chlorobi (0.7 %) and Chloroflexi (1.6 %) were also present in smaller concentrations [118]. During the feast/famine cycles only three bacterial phyla Firmicutes, Actinobacteria, and Proteobacteria able to survive, and in 4th famine cycle which showed the highest PHA accumulation Firmicutes, and

Actinobacteria were the dominant phyla. At the genus level *Pseudomonas*, *Corynebacterium*, *Paenibacillus*, and *Bacillus* are dominant and their relative abundance varies with feast/famine cycle. The relative abundance of *Corynebacterium* was high (40.5 %), followed by *Bacillus* (23 %) in famine phase 4 which represents their role in PHA production [118].

6. Life cycle assessment and techno-economic analysis

Anaerobic digestion is a well-known and widely used process for renewable energy production utilising various wastes. Advances in the AD process can reduce methane production costs up to 0.1$ per m^3 but its role as a greenhouse gas and reduced prices of other renewable energy sources like solar energy raising questions on its sustainability for a long time. Economically wastewater treatment and biogas plants are struggling for existence and shifting towards more valuable products like VFA and PHA. Bastidas-Oyanedel et al., performed a technoeconomic analysis of the AD process at 50 tons/day waste conversion into methane and organic acids and reported methane production selling can generate 19 $/t.VS of profit while production of acetic and butyric acid can give 296 $/t.VS [127]. Life cycle assessment (LCA) is important for decision-making and validation of the sustainability of any production process. It involves analysis of the environmental effects of various steps like raw material, production process, distribution, use, and disposal of product.

WAS to VFA conversion poses environmental challenges due to high energy use, emissions, and chemical residues. Thermal and mechanical methods are energy-intensive, increasing greenhouse gas emissions. Chemical pretreatments generate toxic byproducts and can contaminate water and soil. Release of residual heavy metals and hazardous gases further impacts air and effluent quality. The full scale study to demonstrate the life cycle assessment and ecotoxic effect of the pretreatment process is not yet reported, therefore a lifecycle assessment is essential to ensure the benefits of VFA and PHA production outweigh these impacts. Gracia et al., performed LCA assessment of VFA production from WAS generated from a municipal wastewater treatment plant. Anaerobic digestion was performed in a semi-continuous reactor at an organic loading of 14 gVS/L, under 25 °C, pH 10 for 7 and 16 days. The AD process resulted in a high content of VFA in 16 days (6048 mg COD/L) compared to 7 days (5472 mg COD/L) [128]. The LCA analysis was performed using ReCiPe Midpoint method and Simapro software and concluded that VFA production in 7 days is more sustainable with a smaller ecological impact compared to 16 days. Fernando-foncillas et al., investigated different scenarios for sewage sludge valorisation with joint production of acids, biogas, and fertilizers with two downstream processes including anion exchange chromatography and biological separation with PHA production [129]. From analysis they found that none of the processes is profitable without subsidies as almost 50 % production cost is due to facility and labour and 40 % cost is due to utilities. The downstream process involves a higher capital cost due to costly equipment. Integration of VFA production with the PHA production process seems a more promising approach as operating costs are close to total revenues. Amabile et al., studied technoeconomic assessment for P(3HB-*co*-3HV) production from VFA and analysed different scenario effects like the use of mixed consortia and reagent recycling used during downstream. The study was performed at 100 to 100,000 t/y and the selling price was assessed. This study concluded that the selling price of P(3HB-*co*-3HV) was 18.4 €/kg for a 100,000 capacity plant and by increasing the suspended solid concentration from 5 to 30 g/L this cost can be reduced up to 8.6 €/kg.

7. Challenges and future perspectives

Despite advancements in technology, there are still numerous problems with the conversion of WAS into VFA and their fermentation into PHA. The efficiency of AD is low due to WAS's complex composition and low biodegradability, caused by the inclusion of microbial cell walls, and extracellular polymeric substances (EPS). The WAS composition may vary significantly with the wastewater source and method of treatment applied. Due to the varying compositions of the WAS, different pretreatment techniques are needed, and additional research input is always required to standardize the procedure. Further, the presence of heavy metals or hazardous compounds complicates the process by limiting microbial activity and lowering VFA yields [63,102]. Although many physical, chemical, and biological pretreatment techniques have been established, a more cost-effective and environmentally friendly technique is still required to guarantee the maximum solubilization of WAS. The combination of various treatment methods has the potential to improve sludge solubilization so efforts should be made to develop methods that act synergistically. The anaerobic digestion process also requires the optimization of many parameters like sludge floccule size, pH, temperature, and HRT as all these factors have a direct effect on microbial community succession and selection which affects hydrolysis, acetogenesis, and methanogenesis steps that further affect VFA production and composition. All these parameters should be properly optimised and controlled to achieve uniform results and VFA of appropriate composition each time. During acidogenesis, competing pathways (e.g., methanogenesis) can consume VFAs, reducing their yield unless these pathways are inhibited. There is a need to develop strategies like the use of methanogen inhibitors or pretreatment methods that can promote acetogens and restrict the growth of methanogens. For PHA production anaerobic digestate containing VFA can be directly used as a feedstock but for other applications VFA purification and recovery are important. VFA recovery is also challenging and there is a need to develop a process that can be applied at a larger scale more readily and can ensure high recovery with minimum energy input. The use of an inefficient process may result in the loss of VFA and reduce the overall yield of a process.

Valorisation of VFA into PHA is also challenging as this process is affected by the VFA composition, microbes used, fermentation method, operation parameters, and downstream process used. Volatile fatty acid is composed of different fatty acids i.e., acetic acids, propionic acid, butyric acid, and valeric acid, and the content of these acids depends on WAS composition, pretreatment method used, AD process, and microbial community involved. Microbes have different VFA utilisation capabilities and preferences with varying PHA accumulation capacities. There is a need to find microbes able to tolerate a higher content of VFA and transform these into PHA more efficiently. The use of mixed culture is more advantageous but needs the development of a strategy that can lead to stable consortia. Feast/famine approach can develop a stable mixed microbial consortium but requires a more complex experimental approach and time. VFA produced in AD processes have low concentration so there is a need to develop a process that can support high cell density culture. The use of membrane bioreactors has been reported but membrane fouling is a challenge and its applicability at a larger scale is not feasible, so there is a need to develop another suitable strategy to overcome this. The VFA fermentation process also requires operation control and proper nutrient management. Microbes require a suitable C/N ratio to support PHA production. The composition and properties of produced PHA depend on microbes, VFA used and downstream process applied. To achieve the PHA with uniform composition and properties it is very important to control the VFA composition used as feedstock. The downstream process is also challenging as it involves the use of a variety of solvents for cell lysis, solubilization, and recovery of PHA. The use of solvents for large-scale production is not an economical and eco-friendly approach. There is a need to find a more efficient approach to ensure maximum recovery of PHA with the preservation of native properties. The utilisation of WAS into VFA and upcycling into PHA not only solves the waste management problem but also generates revenue and solves the plastic-related issue.

8. Conclusion

Waste activated sludge anaerobic digestion into VFA and its valorisation into PHA seems a realistic approach for large-scale production. From the literature survey, it's evident that no single pretreatment method is efficient and the use of combined methods increases sludge solubilization and VFA yield. Most of the studies, till reported, are limited to WAS to VFA conversion or the use of VFA for PHA production there are very few studies where efforts have been made to develop an integrated process for WAS to PHA conversion. Applying PHA production directly at waste treatment plants may help to reduce production costs. More research input is required to increase sludge solubilization, control the microbial community, and inhibit VFA consumers. The development of a robust strain able to tolerate high VFA and have the capacity to produce various PHA copolymers is also needed.

CRediT authorship contribution statement

Shashi Kant Bhatia: Writing – review & editing, Writing – original draft, Visualization, Validation, Resources, Methodology, Investigation, Formal analysis, Conceptualization. **Ranjit Gurav:** Writing – review & editing, Writing – original draft, Visualization, Software. **Yung-Hun Yang:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Methodology, Investigation, Formal analysis, Conceptualization.

Declaration of competing interest

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

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Data availability

Data will be made available on request.

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