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A review on energy and cost effective phase separated pretreatment of biosolids

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A B S T R A C T

Extracellular Polymeric Substances (EPS) existent in anaerobic sludge proves to be a barrier for sludge liquefaction and biomass lysis efficiency. Hence EPS deaggregation heightens the surface area for the subsequent pretreatment thereby uplifting the sludge disintegration and biomethanation rate. This review documents the role of EPS and its components which inhibits sludge hydrolysis and also the various phase separated pretreatment methods available with its disintegration mechanism to enhance the biomass lysis and methane production rate. It also illustrates the effects of phase separated pretreatment on the sludge disintegration rate which embodies two phases-floc disruption and cell lysis accompanied by their computation through biomethane potential assay and fermentation analysis comprehensively. Additionally, energy balance study and cost analysis requisite for successful implementation of a proposed phase separated pretreatment on a pilot scale level and their challenges are also reviewed. Overall this paper documents the potency of phase separated pretreatment for full scale approach.

1. Introduction

Enormous amount of waste activated sludge (WAS) emanating from wastewater treatment plant (WWTPs) are stabilized extensively through aerobic and anaerobic digestion (AD). Here, AD is weighed down as a dynamic method considering its green-energy recovery in the form of biogas in a profitable manner. However, hydrolytic and acidogenic phases plays a transitional role in the bioconversion of WAS to biogas wherein hydrolysis is premeditated as a hindering aspect in AD. This warrants an admissible sludge pretreatment method prior to AD which stimulates the sludge disintegration rate (Kavitha et al., 2016a). In recent times, numerous pretreatment methods have been recorded by legion of researchers such as physical (thermal and microwave) (Ebenezer et al., 2015a; Kavitha et al., 2018, 2016b; Yan et al., 2013) mechanical (ultrasonication and disperser), (Banu et al., 2018a; Gayathri et al., 2015; Kavitha et al., 2016c,2016d, 2014a) chemical (acid, alkali and ozone), (Banu et al., 2012; Kannah et al., 2017a; Kavitha et al., 2015a) advanced oxidation process (AOP) (Sharmila et al., 2017) biological (enzyme and microbes) (Kavitha et al., 2017b; Yang et al., 2010; Kannah et al., 2020) and combinative disintegrations (Banu et al., 2018b; Kannah et al., 2017b; Kavitha et al., 2015b, 2017a) have been employed to augment sludge biodegradation. These methods cause a fissure in cell walls of sludge biomass which emancipates the strongly bound organic materials from the cell in-house into the aqueous phase. Soluble extracellular polymeric substance (EPS) existent in sludge matrix plays a vital role in curbing the accessibility of substrates for sludge disintegration whereas release of EPS actuates the surface area exposure for the microbial cell disintegration process in a prominent manner (Merrylin et al., 2014). EPS are highly composed of extracellular biopolymers such as protein, carbohydrates, lipid, humic and nucleic acids. The concentration of biopolymer may vary with respect to certain environmental factors during formation of biosolids EPS. It act as a protective layer of the intracellular compounds present in the biosolids.

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It forms a complex net structure with plenty of water that reduces biosolids dewatering capacity. EPS which binds over the surface of biosolids limits the hydrolysis, which directly affects the anaerobic biodegradability. Therefore removal of EPS from biosolids prior to AD will be more effective in enhancing the anaerobic biodegradability and biogas yield. The anaerobic biodegradability assay is found to be an advantageous method for critical evaluation of biogas production among diverse substrates which gets amplified by an assortment of pretreatment methods available lately (Packyam et al., 2015). On the other hand, the EPS removal from biosolids significantly reduces pretreatment input energy and accelerates the biosolids liquefaction, which indirectly supports the profitable output yield. Besides, the energy demand of a process should be at least counterbalanced by the volume of methane produced which in turn necessitates an energy balance and economic assessment to ascertain the practicability of implementing the pretreatment process in a massive scale. Therefore extraction of EPS prior to sludge pretreatment is found to be imperative in heightening the process efficiency, the speed and extent of liquefaction, curtailment of cost and also contracts the time consumed during the process (Kavitha et al., 2020a). Accordingly, the two step disintegration/phase separation process which consists of floc disintegration (FD) and cell lysis (CL) phase conceptualized by Tiehm et al. (2001) and Show et al. (2007) was utilized to study the solubilization rate. The disruption of floc matrix solubilizes the intercellular organic components and is utilized as substrates by microbes present in sludge biomass and strengthens the sludge pretreatment process. The passive hydrolysis of particulate organics in AD caps the disintegration rate of biosolids which necessitates pretreatment process prior to AD. An integral function of pretreatment is to agitate the EPS matrix thereby ousting the available intercellular components into the aqueous phase thus quickening the organic solids conversion rate and increase the methane productivity volume. So far, very limited literatures are available reporting phase separated sludge pretreatment process and this review gives an encyclopedic insight of the process. This review mainly focus on understanding the mechanism of EPS formation and the biopolymer that cause hindrance towards biosolids hydrolysis. Considering the above facts, the review concerning the role of EPS in sludge matrix, importance of EPS removal, different techniques employed for EPS disruption, techno economic aspect of EPS removal prior to pretreatment of biosolids were discussed comprehensively.

2. Extracellular polymeric substances (EPS) in sludge biomass

2.1. Role of EPS in sludge matrix

EPS which is a fundamental component in biofilms contributes to about 50% to 80% (w/w) of total biofilms weight which is ascertained through various electron microscopy techniques (Meng et al., 2016). At present, a proportionate amount of reviews is available demonstrating the constructive and adverse aspects of EPS in sludge biomass which influence the performance of biological wastewater treatment systems (Salama et al., 2016; Sheng et al., 2010). EPS have a paramount impact on the physicochemical properties of sludge biomass such as structure, surface charge, flocculation, settling properties, stability, adhesion, dewatering properties, hydrophobicity / hydrophilicity and adsorption ability. Presence of multivalent cations in EPS plays an immense role on sludge biomass structure thereby influencing their flocculation rate (Li et al., 2012). EPS are amphoteric to certain extent as it embodies profuse amount of charged functional groups and nonpolar groups (e.g., aromatics, aliphatic in proteins and hydrophobic region in polysaccharides). Besides, the hydrophobic regions in EPS yields to organic pollutants adsorption as an augmented hydrophobicity in general brings about better flocculation (Shi et al., 2017). The dewatering potentiality of activated sludge was found to improve with an upsurge in EPS content owing to its lower shear sensitivity and reduced degree of dispersion. On the contrary, a magnified EPS level was not favorable for substrate mass transfer by virtue of the decrease in permeability of anaerobic sludge granules (Sheng et al., 2010). Plus, EPS are scrutinized as an imperative intermediary in the adhesion of bacteria to the sludge surface in the course of biofilm formation, upholding the structural integrity of biofilms and consequently leads to the overall stability of biofilm. Withal, several studies have reported that sludge settling, bioflocculation and dewatering properties offers a huge correlation with EPS components and its spatial distribution. Additionally, DNA and protein contents in EPS have an affirmatory relationship with sludge volume index (SVI) in comparison to polysaccharides (Peng et al., 2014; Zhang et al., 2015a).

According to spatial distribution, EPS can be classified depending on the extraction methods as soluble EPS and bound EPS. Soluble EPS comprises of soluble macromolecules, colloids, and slimes that can voluntarily move amidst the surrounding liquor and sludge flocs. While bound EPS comprising capsule polymers, gelatin coacervates, loose polymers and attached organic compounds firmly adheres to cells and form a discrete cover layer (Yu etal., 2009). The various constituents of Soluble and bound EPS instigated variation in the metal biosorption activity and organic pollutant binding capacity. Furthermore, bound EPSs are subdivided into loosely bound EPSs (LB-EPSs) and tightly bound EPSs (TB-EPSs), which forms a dynamic double-layer structure (Yu et al., 2008). Tsai et al. (2008) found that large molecules (MW > 20000Da) in TB-EPS were protein-like substances of high aromaticity which amounted to 22% of the total dissolved organic carbon (DOC) as to LB-EPS. Likewise, Yu et al. (2009) reported that the TB-EPS extracted from sludge displayed huge amount of macromolecules content (330-1200 kDa) and trivalent cation in comparison to other fractions, which delivers larger flocculating rate to kaolin suspensions. Thus variant EPS components demonstrate a specific role which chiefly serves as an influential function of EPS and microbial aggregates. In a study by Zhao et al. (2015) LB-EPS had an outstanding effect on sludge flocculation, sludge settling and dewaterability characteristic as to the TB-EPS and thus improving potentiality of cell-cell interactions. Conjointly, nutritional parameters, such as C/N ratio, serve as an influential parameter in the activated sludge systems and have a significant impact on the nature and EPS matter present in sludge biomass (Ye et al., 2011). Along these lines, EPS plays a crucial role in sludge granulation, and couples cells through ion bridging interaction, hydrophobic interaction and polymer entanglement, which intensifies the formation of sludge granules (Sheng et al., 2010). Concisely, EPS has a definitive influence on sludge properties and are profoundly interconnected which creates a great impact on the performance of activated sludge systems.

2.2. Components of extracellular polymers and its hindrance towards hydrolysis

Sludge biomass is built up of diverse microbes that are susceptible to environmental conditions like temperature, organic load etc there by altering the physical, chemical, and biological properties of activated sludge (AS). For this reason the flocculation mechanisms of AS are considered to be complex as to colloidal particles. The intermolecular interaction theories such as polymer bridging, Derjaguin–Landau–Verwey–Overbeek (DLVO) theory, hydrophobic interactions, multivalent bridging theory, and steric interactions elucidates the binding of sludge biomass elaborately (Li and Yu, 2014). Whilst the Classical DLVO theory and extended DLVO theory which is based on colloid and surface chemistry, illustrates the cell-to-cell interactions and bio-flocculation process.

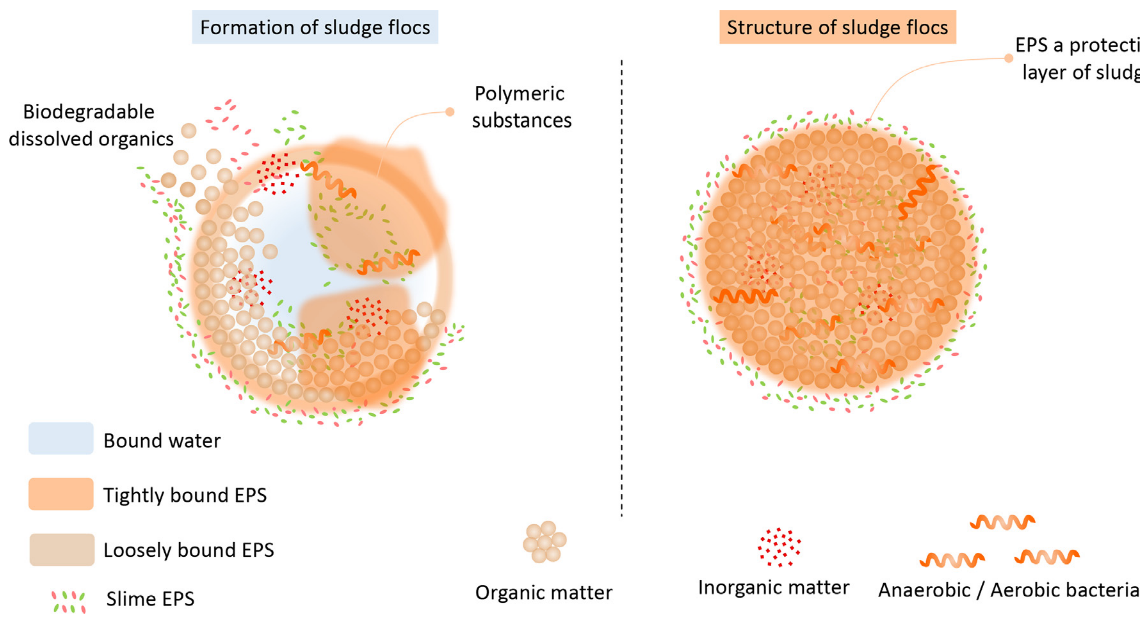

Fig. 1. Structure and formation of sludge floc.

It suggests energy assessment owing to the overlying of electric double layers (generally repulsion) and the Vander Waals energy (typically attraction) in terms of inter-particle distance. Additionally Lewis acid–base interactions were taken into consideration in extended DLVO theory. According to this theory, sludge cells support one another as they advance closer and cause a decline in the double layer thickness. Furthermore, the repulsion between particles also contracts and thus concedes short range attractive forces to bolster sludge aggregation (Liu et al., 2010, 2008). The aforesaid DLVO theory and multivalent bridging theory are strongly associated to cations since multivalent cations can bridge EPS and thereby reduces the effect of negative charges on the AS surface (Wilén et al., 2008). Usually, divalent cations such as Ca^{2+} and Mg^{2+} play a crucial role in bioflocculation process and keeps the cations adhesive to EPS in a three dimensional matrix. Other significant inter-particle forces consist of hydrogen bonds, thermodynamic forces, covalent bonds etc. The extended DLVO theory could quantify the non-bioparticles; still, it has a definite application in bioaggregates, particularly when EPS expulsion influences the cell surface characteristics. Moreover, gelation, divalent cation bridging, and lectin binding have been extensively probed relative to the mechanism of cell aggregation, which are physico-chemical interactions involving the production and function of EPS (Ding et al., 2015). Likewise the hydrophobicity of the cell surface was partially due to the hydrophobic portion of EPS macromolecules. Besides, the above mentioned theories accentuate the significance of EPS since they stockpile on the cell surface initially and gets engaged in sludge flocs interactions and subsequently used to analyze the physical and chemical characteristics of AS.

Typically, production of EPS by microorganisms seems to occur by diverse mechanisms which is inclusive of secretion, degradation, cell death and adsorption of environmental substances in wastewater treatment systems (Sun et al., 2016). EPS are thus construed as high-molecular-weight polymers generated by microorganisms that are placed either on microbial cell surfaces or in the enclosing environment. The properties of EPS components extracted had preeminent impact on certain functions of sludge biomass. They have been proclaimed as heterogeneous structure characterized by distinct aspects such as extraction and characterization methods utilized, sludge type, process parameter, culture and growth conditions (Basuvaraj et al., 2015) and Fig. 1 illustrates the structure and formation of sludge flocs. A few researchers have documented that the effect of substrate greatly influences the biosynthesis and composition of EPS existent in the surface of biosolids. The two major substrates such as carbon source (in terms of COD concentration) and nitrogen plays an essential role in biosynthesis of EPS (Nouha et al., 2018). EPS typically consist of proteins, polysaccharides, humic-like substances, uronic acids, nucleic acids and lipids. Geyik et al. (2016) have reported the difference in concentration of protein and carbohydrates which influences EPS hydrophobicity and is correlated with the substrate provided to the microbial communities. The greatest part of EPS comprises of carbohydrates/exo-polysaccharides, which are chiefly linear or branched long molecules, embodies either homo-polysaccharides or hetero-polysaccharides. Howbeit, most exo-polysaccharides found in EPS are hetero-polysaccharides, like glucose, mannose and N-acetylgalactosamine, pertaining to amalgamation of neutral and charged sugar residues. Further organic or inorganic matter existence alters the physical and biological properties of EPS (Flemming and Wingender, 2010). Distinctively, polysaccharide owing to the accretion of few functional groups, such as carboxyl (eCOO−), plays a major role in cell aggregation which gets enhanced due to the multivalent ions present in them (Yin et al., 2015). Pan et al. (2010) have reported that the EPS and protein plays a different functional role during formation of biosolids and extraction of EPS. The EPS fills the gap between the biosolids to form stable floc or aggregates. The EPS matrixes act as a multiuse functional part of microbial communities, which includes binding agents, protective layer which indicates stability of biosolids and its physiology. Next to polysaccharides, another important extracellular polymers such as protein, plays a vital role in the formation of biosolids EPS (Yin et al., 2015). According to Zhang et al. (2015b) the concentration of protein in waste activated sludge and anaerobic granular sludge depends on hydrophobic interactions and elevated the aggregation of microbial activity in biosolids. The proteins originated from internal and external surface of biosolids has different molecular functions based on their catalytic activity and binding activity. The protein found in the outer surface of the biosolids tends to increase the floc binding capacity. Apart from the authentic form of polysaccharides and proteins, those molecules existent in hybrid form in the course of covalent bonds, and their configuration and structure have proven

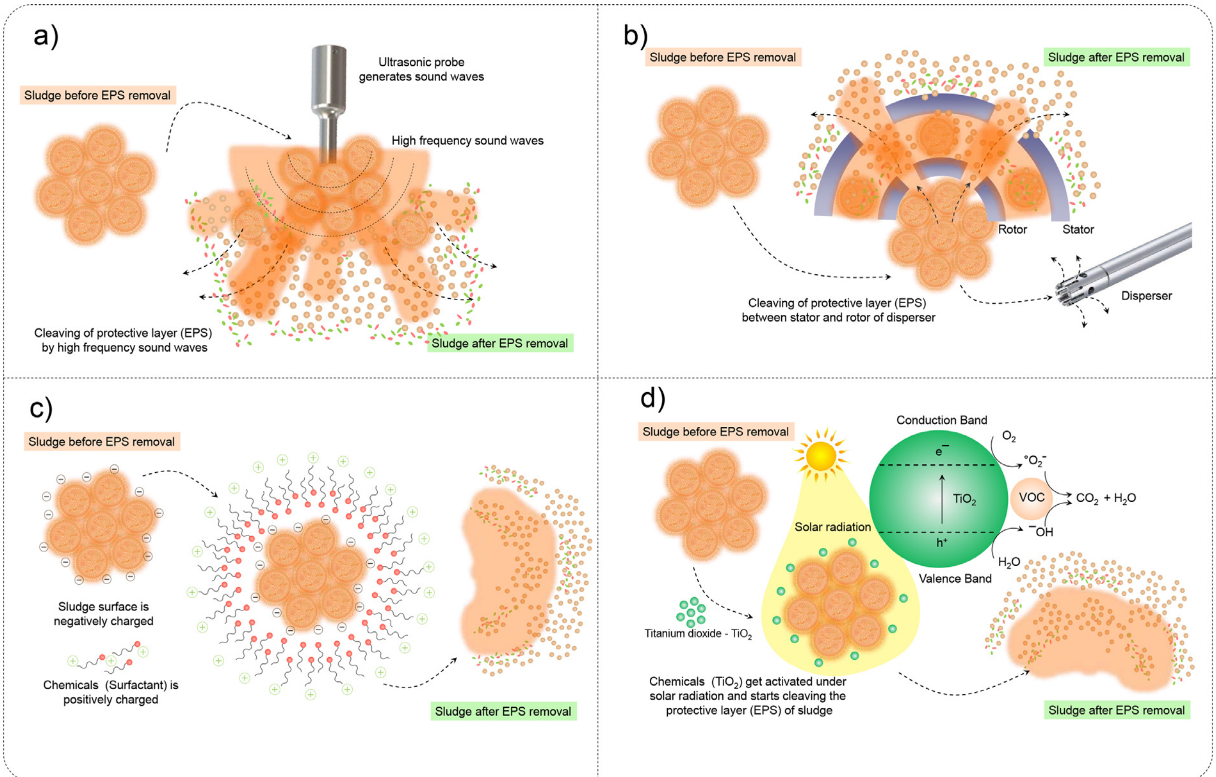

Fig 2. Mechanism of different EPS removal (deflocculation) methods a) ultrasonic b) disperser c) chemical d) advance oxidation process.

to have disseminated effects on the formation and stability of microbial aggregates (Bourven et al., 2015). Extracellular DNA (eDNA) serve as a decisive factor to establish the three-dimensional structure of biofilms thereby providing structural integrity to the EPS matrix. Also, it clouted the zeta potential and hydrodynamic diameter of bacterial cell, and the cell surface hydrophobicity. Moreover the thermodynamic analyses stated that eDNA brought about affirmative acid-base interaction accountable for bacterial aggregation and adhesion to surface, in conjunction with biofilm formation (Das et al., 2011). Extracellular lipids with surface-active properties have substantiated to safeguard bacteria from strong surface tension of adjacent water in the surface environment, thus accelerating the bacterial growth on solid surfaces (Flemming and Wingender, 2010).

3. Phase separated pretreatments of sludge and its disintegration mechanism

As aforestated phase separated pretreatment incorporates deaggregation of EPS matrix, as it hinders the accessibility of intracellular organic matter for the consequent pretreatment thereby augmenting the biosolids disintegration rate. Variant phase separated pretreatment signifies diverse disintegration mechanism employed to liquefy the particulate organics existent in biosolids and provokes biogas generation and highlighted in Table 1. Most commonly thermal and free nitrous acids (FNA) method of EPS extraction is followed by many researcher (Wu et al., 2018; Wang et al. 2017). Antonelli et al. (2011) have reported that the thermal EPS extraction enhance the sludge hydrolysis, release EPS, and reduced sludge viscosity which improves the biosolids dewatering and digestibility. Zhang et al. (2015c) have documented that the FNA method of EPS extraction is renewable and low cost chemical, which also accelerates the biosolids biodegradability. It also stimulate the production of volatile fatty acid from AD of biosolids. Fig 2 illustrates the mechanism of EPS removal using different pretreatment methods while Fig 3 depicts the mechanism of various pretreatment methods which are discussed comprehensively in the forthcoming sections.

3.1. Chemical induced biological pretreatments

Chemical induced biological pretreatment is carried out by usage of chemical such as acid, alkalis or oxidants as deflocculating agent in advance to bacterial disintegration with the desideratum of amplifying the sludge solubilization rate and biogas volume (Kavitha et al., 2015c). The monovalent (sodium) and divalent cation (particularly magnesium and calcium) are coupled with EPS matrix are detached by ion exchange thereby mitigating the floc strength and stimulates floc disruption. Kavitha et al. (2015a) found out an optimal dosage of 0.03 g/g SS of NaCl liberated the intracellular components into liquid medium and effectuated maximum sludge liquefaction rate of 23% when succeeded by bacterial disaggregation. Likewise pH plays a vital role in bacterial growth and inclusion of chemical escalates sludge ionic strength. No significant impact on pH was seen on usage of $CaCl_2$ as deflocculating agent because the bacteria adopted for pretreatment maintained an optimum pH of 6.5 throughout the study (Kavitha et al., 2015d). Conversely, sodium dodecyl sulfate (SDS) ameliorates sludge pH to alkaline condition and necessitates adjusting pH to optimal conditions for attaining efficient liquefaction rate in the sub sequential bacterial pretreatment. This was found to be in congruent with Ushani et al. (2018) where sodium thiosulphate (STS) when employed as a deflocculating agent aided with protease secreting bacterial pretreatment affirmed a greater sCOD release (22%) and lofty methane production. Also chemical deflocculation strengthened the sludge enzymatic activity and functions as enzyme effectors in sludge particle bringing about higher disintegration rate. It has been proclaimed that deflocculating agents used

Table 1
Various EPS extraction techniques, its advantages and disadvantage.

S No	Mode of extraction	Extraction technique	Operation conditions	Reduced bound EPS yield (mg/L)	Soluble EPS yield (mg/L)	Extracellular enzyme activity (U/mL)	Advantages	Disadvantages	References
1.	Physical	Thermal	Temperature - 80 °C Time - 30 mins	27.5	786.5	-	Reduces the sludge viscosity	Energy intensive	Wang et al. (2017)
2.	Physical	Thermal	Temperature - 80 °C Time - 10 mins	6.7	70.6	-	Improve the sludge dewatering capacity	High energy demand	Antonelli et al. (2011)
3.	Chemical	Zeolite	0.04g/g SS dosage	13.8	123.6	0.088	Enhances the enzyme activity of sludge matrix and improves biodegradability	May chelate sludge biomass	Banu et al. (2019)
4.	Chemical	Sodium citrate	0.05 g/g SS dosage	28	80	0.33	Easily biodegradable	Possibility of drop in sludge pH	Sharmila et al. (2019)
5.	Chemical	Sodium thiosulphate	0.08 g/g SS dosage	19	178	0.07	Effectively chelates metal ions and act as enzyme cofactor	Alters the pH condition of sludge medium	Ushani et al. (2018)
6.	Chemical	$MgSO_4$	0.1 g/g SS dosage	110	185	0.72	Act as enzyme activator at low concentration	Act as enzyme inhibitor at higher dosage	Ushani et al. (2017)
7.	Chemical	Free nitrous acid	Dosage - 0.06 mg/L * Time – 24 h	2.0	7.0	-	Improving sludge biodegradability	Negative effects on the performance of ammonium oxidizing bacteria	Wu et al. (2018)
8.	Chemical	Sodium citrate	0.1 g/g SS dosage	31	42	0.18	Easily biodegradable	Drop in sludge pH	Ebenezer et al. (2015b)
9.	Chemical	Free nitrous acid	Dosage - 2.0 mg/L Time - 24 h	19.1	38.3	-	Renewable and low cost chemical	Higher dosage and contact time leads to inhibition of ammonium oxidization	Zhang et al. (2015c)
10.	Mechanical	sonication	Specific energy-1.12 of kJ/kg TS Treatment time-30s Sonic power-30W	13	490	-	Devoid of chemical contaminants	Increment of medium temperature	Kavitha et al. (2019)
11.	Mechanical	Disperser	9.5 kJ/kg TS	5.4	128	-	No possibility of chemical contaminants	Reflocculation	Banu et al. (2018a)
12.	Mechanical	Sonicator	3.5 kJ/kg TS	3	90	-	Devoid of refractory compound formation	Reflocculation	Kavitha et al. (2018)
13.	Mechanical	Disperser	30 s and 3000 rpm	15	265	-	Devoid of chemical refractory compounds	Collection of sample is difficult	Kannah et al. (2017a)
14.	Physiochemical	Low temperature thermo-chemical	pH- 8 temperature- 60 °C treatment time-3mins specific energy- 0.06 kJ/L	14	71.1	-	Less energy consumption	Alteration in sludge pH	Kavitha et al. (2017a)

* Calculated

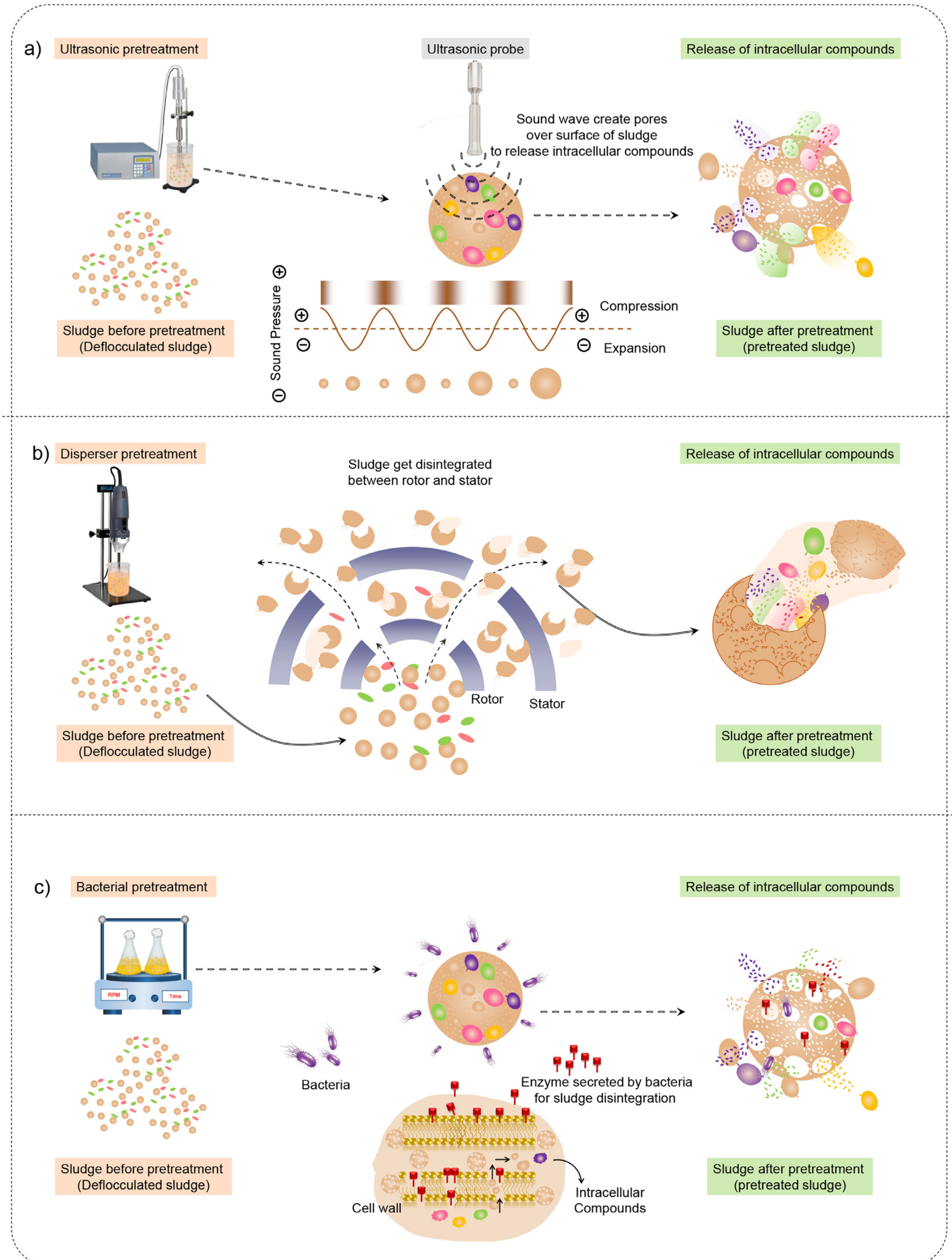

Fig 3. Mechanism of different pretreatment methods a) ultrasonic b) disperser c) bacterial.

namely citric acid, $MgCl_2$, SDS and $MgSO_4$at low dosage acted as enzyme activator. Whilst at higher dosage they enacted to be enzyme inhibitor thereby contributing to increased solubilization rate (Gayathri et al., 2015; Kavitha et al., 2016c, 2015c, 2014b; Ushani et al., 2017).

3.2. Physiochemical induced biological pretreatments

Physical pretreatment method such as thermal and microwave which when combined with chemical disintegration deflocculates the EPS thereby increasing the surface area requisite for the succeeding biological pretreatment. Thus they lead to an enhancement in the sludge solubilization rate. Biological disintegration garners low energy for pretreatment analogous to other pretreatment methods (physical, mechanical) for cell lysis and can be carried out using commercially available enzymes, biosurfactant and antibiotics. Due to their pricey nature, cultured bacterial strains are put to use which attests to be cost effective. Studies have revealed that thermo-chemical pre-treatment at temperatures nearly lower than 80°C accrued an efficacious sludge solubilization (Kavitha et al., 2014a, 2015b). Low temperature thermo-chemical (LTTC) treatment was adopted by Kavitha et al. (2017b) for EPS disaggregation tailed by bacterial pretreatment. It was proclaimed that LTTC pretreatment had a significant impact on EPS fractionation which induced solubilization of the LB-EPS and TB-EPS into liquid medium at a minimal thermo-chemical energy of 0.06 kJ/L. Antibiotic secreting bacteria (ASB) was exploited for cell lysis as it rifts apart the peptidoglycan layer and proteins confined in the sludge cell membrane and liberates the accessible intercellular components into the liquid phase. Withal, floc deaggregation by LTTC plummet the pH value from 8 to 6.8 fomenting a favorable condition for cell lysis by ASB and attained an effectual sludge disintegration rate.

3.3. Solar photo catalysis induced biological pretreatment

In the midst of advanced oxidation processes (AOP's) available solar photo catalysis process chiefly adopted for waste water treatment is lately used in sludge pretreatment studies (Banu et al., 2008; Liu et al., 2014). The catalyst employed can be used under suspension or immobilized during pretreatment process. Solar photo catalysis is mediated with bacterial disintegration to enhance the biodegradability of sludge biomass and proves to be an economical one. TiO_2 used as photo catalyst for EPS disruption admitted into the sludge clings onto the EPS surface and gets agitated and forms hydroxyl ions due to solar radiation (Liu et al., 2013). On the grounds of the above photo catalytic reaction, EPS deflocculation at TiO_2 dosage of 0.03 g/g SS discharges the intracellular components with negligible cell lysis up to 15 min irradiation time into the aqueous phase elevating liquefaction level of the succeeding biological pretreatment (Sharmila et al., 2015). Moreover the impact of sludge enzymatic activity by solar photo catalysis interceded by $TiO_2$was imperative as the enzymes entrapped within the sludge biosolids curtails the amplitude of sludge disintegration. Oxidization of the detached sludge flocs into CO_2elevates the discharge rate of enmeshed enzymes until 15min of irradiation time. Furthermore a fall in enzyme level was seen beyond the optimal time in view of mineralization of liberated enzymes by the oxidative radical (Liu et al., 2013; Sharmila et al., 2015). Moreover phase separation by TiO_2 induced biosurfactant secreting bacterial pretreatment attained a liquefaction rate of 22.9%. A similar solubilization rate was accomplished when a thin filmed TiO_2 layer was employed prior to bacterial pretreatment (Sharmila et al., 2017).

3.4. Chemical induced mechanical pretreatments

Considering the extensive amount of energy dissipated by mechanical pretreatments (namely ultrasonication and disperser) parity between energy and sludge disintegration efficiency demands a suitable pretreatment in the form of phase separation. Chemicals like acid, alkali, surfactants and oxidation reagents applied to pretreatment attacks the EPS cell wall releasing the soluble organic matter and hydrolytic enzymes confined in sludge matrix thereby magnifying the sludge solubilization rate of the consecutive pretreatment. Cell lysis brought out by mechanical pretreatment such as sonication and disperser enforces cavitational forces which propagates hydrodynamic shear forces to solubilize the particulate organic matter. The particles thus liberated during EPS deaggregation causes an increase in the sludge disintegration and methane production rate. A synergistic effect was observed when chemo mediated ultrasonication pretreatment was employed to attain an accelerated sludge solubilization rate at an optimal specific energy input (SEI)of 171.9 kJ/ kg TS (Gayathri et al., 2015). Here cation binding agent like citric acid disrupts the EPS which bridges the divalent cations Ca^{2+} and Mg^{2+}liberating the intracellular components into the liquid medium. Hence the subsequent sonication pretreatment elevates the accessibility of bacterial cell for sludge biodegradation due to hydro-mechanical shear forces generated by ultrasonic irradiation at an optimal sonic pretreatment time of 1 min. Contrarily, EPS solubilization induced a slump in pH value due to acid synthesis during chemo mediated sonic pretreatment which contradicted Kavitha et al. (2015d). Diversely, SDS used for floc disruption ebbs the surface tension of the sludge medium which is counteracted by the tantamount of cavitational forces emanated by disperser pretreatment. This further intensifies the sludge liquefaction rate (26%) and biomethanation at an energy input of 5013 kJ/kg TS (Devi et al., 2014).

3.5. Mechanical induced biological pretreatment

Mechanical pretreatment namely disperser and ultrasonication occurs under various mechanical action such as shear stress, turbulence, thrust and cavitational forces. The said mechanical actions were held responsible for breaking down the large sized particulate matter into soluble matter thus enhancing the surface area of contact for the sub sequential biological pretreatment. EPS existent on surface of sludge biosolids are disaggregated using disperser prior to bacterial pretreatment operates on stator-rotor principle. Owing to the expeditious force and infinitesimal space amidst rotor and stator, cavitational forces are developed which segregates the soluble EPS from sludge biomass and transfers it into the liquid medium (Banu et al., 2018a). Moreover mechanical pretreatment decreases the sludge pH value as the disassociated liquefying organics undergo hydrolysis or evolution of acidic compounds (Kavitha et al., 2017b). Withal, no such occurrences were detected during the study, as the pretreatment was carried out at moderate conditions at a low SEI of 9.5 kJ/kg TS. The pH value declined from 6.8 to 6.45 and was found favorable for the succeeding bacterial pretreatment. In addition the enzymatic action of the inoculated bacteria causes a fissure in the cell wall releasing the soluble organics into the liquid phase and achieved a higher biomass lysate liquefaction rate of 22.4%.

3.6. Chemical induced physical pretreatments

Elimination of the EPS present in the peripheral layer of sludge biomass liberated the intracellular organics and enzymes into the liquid medium which brings about a protraction in surface area for the consequential physical pretreatment. EPS deaggregation further admits direct infiltration of microwaves into the sludge cells which shorten the treatment time. This led to proliferation in the potency of microwave liquefaction rate. Amidst the available chemical methods, cation binding agents testifies to be effective for EPS disassociation as it ousts out the coalesced divalent cations which scales down the energy expenditure when subjected to the posterior physical pretreatment. Zeolite, a chelating agent utilized by Banu et al. (2019) ensued maximal EPS disassociation of 93% at 0.04 g/g SS dosage, by virtue of its water adsorbing capacity. This evoked congenital heat severing the sludge cell walls thereby releasing the intracellular components which ameliorates the sludge biodegradability rate. Thus zeolite mediated microwave pretreatment (Ze-MWP) negotiated a maximum COD solubilization rate of 42.8%at a SEI of 16,200 kJ/kg TS resulting in an upsurge in biomethanation production value. In an equivalent study by Eswari et al. (2017) lower dosage of oxidant like H_2O_2 (1.0 g/g SS) was sufficient for OH radical engenderment which was found efficacious for EPS deaggregation of sludge flocs under acidic pH conditions facilitating methanogenesis. Additionally, it curbs the energy expenditure during the ensuing microwave pretreatment (MP). Furthermore a larger solubilization rate (46.6%) was obtained in H_2O_2 mediated microwave pretreatment at a lower SEI of 10,810 kJ/kg TS akin to the combined pretreatment by Eswari et al. (2016) where SEI of 18,600 kJ/kg TS was requisite for attaining maximal liquefaction.

3.7. Mechanical induced physical pretreatments

As stated earlier, ejection of EPS residing on the surface of sludge biosolids heightens the diffusion efficiency of microwaves (physical pretreatment) endorsing high cell biomass lysate rate. Thus EPS removal curtails the energy disbursed for enhancing the sludge disintegration and methane potential rate. Disaggregation of EPS by mechanical methods like sonication applies acoustic cavitational phenomenon emanating hydroxyl radicals which infiltrates the cell wall emancipating the soluble intracellular molecules into the liquid phase prior to microwave pretreatment (Kavitha et al., 2018; Tyagi et al., 2014). Cell disintegration by microwave pretreatment is interceded by the thermal effect generated by the microwaves permeates the sludge cell wall. This rifts apart the hydrogen bond and as a consequence contributes to a rise in the sludge liquefaction rate. Likewise SEI plays an integral role in physical/mechanical pretreatment which has an aftereffect on the sludge solubilization rate. A study by Kavitha et al. (2018) revealed that ultrasonic mediated microwave pretreatment (UMMP) accomplished a higher sludge liquefaction rate (33.2%) at a lower SEI of 16,700 kJ/kg TS with enhanced biomethanation level.

3.8. Mechanical induced chemical pretreatments

As known earlier, phase separation pretreatment embodies EPS disaggregation pursued by cell biomass lysate for achieving a competent sludge disintegration of sludge biomass. EPS prevalent on the peripheral of sludge flocs amasses bound EPSs in the form of LB-EPSs and TB-EPSs as a double layered structure. This bound EPS curbs the diffusion of intracellular hydrolytic enzymes for the consequent pretreatment contracting the performance of sludge solubilization and biomethanation potency (Cho et al., 2012). Mechanical pretreatment such as sonication, disperser are used to disrupt the EPS stratum which improves the solubility rate of particulate organics into the aqueous phase. Ozonation, another mode of chemical pretreatment degenerates into hydroxyl radical and infiltrates through the biosolids sheath revealing the liquefiable organics susceptible for sludge biodegradation (Cesaro and Belgiorno, 2013). This method is generally used with mechanical pretreatment to curtail the energy demand. A study by Kannah et al. (2017a) explored the impact of disperser mediated ozone pretreatment (DOP)for phase separation. Disperser pretreatment operates on the principle of rotor-stator effect which induces tenacious shear stress and thrust on the sludge biosolids. Furthermore this effectuates dissolution of soluble EPS at a lower rpm (3000rpm) with 30s treatment time into the supernatant phase with trivial biomass lysate. Further EPS stratification was also assayed at the optimal rpm which divulged that maximal emulsifiable bound EPS occurred at 30s dispersion pretreatment time. This when superseded by ozonation pretreatment brought about proficient biomass disintegration at lower ozone dosage of 0.014 gO_3/g SS. Akin to the aforestated study, Packyam et al. (2015) employed ultrasonication supervened by ozonation pretreatment was found to be cogent for phase separation with reduced SEI (76.4 (log1.88) kJ/kg TS). Also the above phase separation pretreatment process cuts down the ozone dosage which improved the sludge biodegradability rate and biomethane potential.

3.9. Advanced oxidation process (AOP) induced mechanical pretreatments

AOP namely Fenton process leverages short reaction time as to other processes such as ultraviolet and solar photo catalysis method. Addition of Fenton reagent to sludge biomass brings forth a vicious cyclic reaction amid Fe^{2+} and H_2O_2which actuated chemical oxidation stimulating hydroxyl radicals. Plus, the EPS present in sludge surface gets severed facilitating the liquefiable organics into liquid medium (Kavitha et al., 2016e). Besides lower dosage of Fenton reagent was found to be propitious as higher quantity accumulates ferrous ion concentration in biosolids. Kavitha et al. (2016a) affirmed in their study that 0.2 g/ g SS Fe^{2+} with 0.8 g/g SS of H_2O_2 of Fenton reagent successfully deflocculated EPS with treatment time of 5min.Further when pursued by ultrasonication for effective cell lysis pretreatment dissevers the cell membrane of biosolids as a result of the hydro mechanical shear forces brought about by bubble implosion and attained a high sludge liquefaction rate of 34.4% bettering the performance of methane generation.

4. Effect of phase separated pretreatment on sludge characteristics and its evaluation

As aforesaid in the previous section, phase separated pretreatment encompasses deflocculation and cell lysis stage and its effects on a range of parameters are summated in the subsequent section and also tabulated in Table 2.

4.1. COD liquefaction

sCOD release was used as indicant parameter to gauge the sludge solubilization rate relatively by numerous researchers. Hence it was used to assess the sludge disintegration efficiency in phase separated pretreatment studies. In chemo mediated bacterial pretreatment, sCOD rate was found to elevate (21.4%) up to a treatment time of 42h, owing to the riddance of EPS by $MgCl_2$. This instigates the enzymes secreting bacteria to discharge the intracellular compounds entrapped in the cell wall as to the flocculated one (10.5%) (Kavitha et al., 2015c). The sCOD rate ceased aftermath of 42h in flocculated and deflocculated sludge to 2.9% and 4.3% as the inoculated bacterial strain exploits the soluble organic matter released. Also, lower ozone dosage (0.014 gO_3/g SS) was utilized to procure high sCOD rate (32.8%) during cell disintegration in disperser induced ozone pretreatment. This tested out to be beneficial compared to ozone pretreatment (0.042 gO_3/g SS) carried out solely (Kannah et al., 2017a). This was further attributed to the disintegration of larger size sludge particles which enlarge the surface area and offers easier admittance for ozone to strike (Tian et al., 2015). A prominent decline in sCOD level was seen when the dosage exceeds the above limit in view of ozone oxidation. Plus, specific energy input plays a vital role in energy intensive treatment process such as microwave and thermal. In a comparable study, sludge deflocculation effectuated by sodium tri-polyphosphate (STPP) prior to microwave pretreatment emanated a surge in COD solubilization rate (28%) when subjected to diverse microwave irradiation (Ebenezer et al., 2015a). Besides, maximum COD liquefaction rate was noted at a minimal SEI of 14,000 kJ/kg which proved quite profitable when compared to MW pretreatment done single-handedly where higher specific energy were used to attain the corresponding COD liquefaction rate (Ariunbaatar et al., 2014). At SEI farther to 45,000 kJ/kg TS, a decline in sCOD value was espied owing to the sectional vaporization

Table 2
Various types of phase separated pretreatment and its methane yield.

S. No	Type of Phase separated pretreatment	EPS removal condition	Pretreatment Condition	Mode of action	Pretreatment outcome	Methane yield	References
1.	TiO_2 embedded chitosan thin film followed by bacterial pretreatment	Dosage – 0.05 g TiO_2/g SS Time – 25 mins	pH – 6.5 Time – 42 h Temp – 40 °C	Enzyme mediated disintegration. Bacteria secret protease and amylase enzyme to improve the organic release	SR – 808 mg/L COD Sol.– 16% SSC– 3500 mg/L SSR – 12.5 % SP – 452 mg/L SC – 290 mg/L	140.40 mL/g COD	Banu et al. (2020)
2.	Zeolite mediated microwave pretreatment	Dosage – 0.04 g Zeolite /g SS	SE – 16,200 kJ/kg TS IF – 2450 MHz Time – 12 mins	Maillard reactions occur during microwave pretreatment, it reduced the concentration between nitrogenous substance such as amino acids, proteins and liquefied sugars	SR – 10272 mg/L COD Sol.– 42.8% SSC– 12200 mg/L SSR – 33.1 % SP – 4390 mg/L SC – 1175 mg/L	200 mL/gCOD	Banu et al. (2019)
3.	Ultrasonic mediated bacterial pretreatment	SE – 1.12 kJ/kg TS Power – 60 W Time – 30 sec	pH – 6.5 Time – 42 h Temp – 40 °C	Biosurfactant action mediated by the inoculated bacteria. Decrease the surface tension of hydrophobic components and H_2O in biosolids	SR – 1980 mg/L COD Sol.– 20.3% SSC– 6215 mg/L SSR – 17.1 % SP – 540 mg/L SC – 1388 mg/L	0.247 g COD/g COD	Kavitha et al. (2019)
4.	Disperser mediated bacterial pretreatment	Dispersion rpm – 3000 rpm Time – 50 secs	pH – 6.5 Time – 42 h Temp – 40 °C	The enzyme protease solubilizes the amino acids, its derivatives, proteins and lipoproteins of the cell walls of sludge biomass.	SR – 1750 mg/L COD Sol. – 23.9% SSC – 8090 mg/L SSR – 19.1 % SP – 860 mg/L SC – 1720 mg/L	220 mL/g COD	Rufus et al. (2019)
5.	Sodium citrate mediated immobilized TiO_2 pretreatment	Dosage – 0.05 g sodium citrate /g SS Time – 60 mins	UV intensity of 5.89–6.09 kWh/m^2 Time – 50 mins	TiO_2 generates enormous amount of hydroxyl radicals ($^{-}$OH) during pretreatment to disintegrate biosolids. It has high oxidation potential	SR – 1347 mg/L COD Sol.– 19.25% SSC–3000 mg/L SSR –25.00%	0.24 gCOD/gCOD	Sharmila et al. (2019)
6.	Disperser-induced bacterial pretreatment	SE – 9.5 kJ/kg TS	pH – 6.5 Time – 42 h Temp – 40 °C	The protease and amylase enzymes adhere to the glycan tetra peptide of peptidoglycan	SR – 1971 mg/L COD Sol.– 22.4 % SSC– 5550 mg/L SSR – 20.7% SP – 1379.8 mg/L SC – 394.2 mg/L	0.279 gCOD/gCOD	Banu et al. (2018a)
7.	EGTA mediated microwave pretreatment	Dosage – 0.012 g EGTA /g SS	SE – 13,500 kJ/kg TS IF – 2450 MHz	The hydrogen bonds and other chemical bonds in the cell walls of sludge biomass were subjected to breakage through athermal effect	SR – 9528 mg/L COD Sol.– 39.7% SSC – 15290 mg/L SSR – 30.5% SP – 4330 mg/L SC – 2430 mg/L	235.3 mL/g VS	Banu et al. (2018c)
8.	Ultrasonic assisted microwave pretreatment	SE – 3.5 kJ/kg TS	SE – 16,700 kJ/kg TS IF – 2450 MHz	Thermal effect of microwave cleaves the N-acetlyglucosamine polymer of cell walls of sludge biomass and leads to cleavage of biomass. Rapid heat beyond the boiling point of water by molecular rotation of macromolecules in an altering electromagnetic field.	SR – 3818 mg/L COD Sol.– 33.2% SSC – 7750 mg/L SSR – 22.5% SP – 2400 mg/L SC – 1418 mg/L	0.3 L/gCOD	Kavitha et al. (2018)
9.	Nano-layered TiO_2 for effective bacterial pretreatment	Layer thickness – 10 nm Particle size – 3.576 nm	pH – 6.5 Time – 42 h Temp – 40 °C	Bacteria secret enzyme to breaks the complex cell wall of sludge and improves the organic release.	SR – 1603 mg/L COD Sol.– 22.9% SSC – 3180mg/L SSR – 20.5 %	0.28 gCOD/gCOD	Sharmila et al. (2018)

(*continued on next page*)

Table 2 (*continued*)

S. No	Type of Phase separated pretreatment	EPS removal condition	Pretreatment Condition	Mode of action	Pretreatment outcome	Methane yield	References
10.	Sodium thiosulphate induced immobilized bacterial pretreatment	Dosage – 0.08 g Sodium thiosul-phate /g SS Time – 60 mins	pH – 6.5 Time – 36 h Temp – 35 °C	The amylase enzyme secreted by the inoculated bacteria solubilizes the glycan layer and polysaccharides.	SR – 2200 mg/L COD Sol.– 22% SSC– 5660 mg/L SSR –19.1 % SP – 1070 mg/L SC – 146 mg/L	0.32 gCOD/gCOD	Ushani et al. (2018)
11.	Potassium hydroxide induced immobilized bacterial pretreatment	Dosage – 0.025 g KOH /g SS	pH – 6.5 Time – 36 h Temp – 35 °C	The increase in dissolved organic matter was due to the action of enzymes which leads to the release of intracellular materials into sludge.	SR – 2300 mg/L COD Sol.– 23 % SSC – 5600mg/L SSR – 20 %	232 mL/g COD	Banu et al. (2017)
12.	Sodium citrate mediated microwave pretreatment	Dosage – 0.1 g sodium citrate /g SS Time – 60 mins	SE – 14,000 kJ/kg TS IF – 2450 MHz	Propagation of electromagnetic waves and the heat dissipation governed by heat and mass transfer.	SR – 4000 mg/L COD Sol.– 31 % SSC – 6300 mg/L SSR – 37 % SP – 552 mg/L SC – 68 mg/L	0.4841 L/g VS	Ebenezer et al. (2015b)

SR – SCOD Release; COD Sol. – COD solubilization; SSC – Suspended solids concentration; SSR – Suspended solids reduction; SP – Soluble protein; SC – Soluble carbohydrate; SE - Specific Energy; IF – Irradiation Frequency; EGTA – Ethylene glycol tetra acetic acid;

of sludge organics. Further, studies by Kavitha et al. (2016a) confirmed that sole ultrasonication pretreatment (18936.9 kJ/kg TS) requisites specific energy input (SEI) about 29 times more than that required by Fenton mediated ultrasonication pretreatment process (641 kJ/kg TS) to acquire 30% COD solubilization rate. As a result of the total energy being depleted on cell lysis, Fenton mediation sonic pretreatment displayed a rise in sCOD level whereas in sole ultrasonic pretreatment certain amount of SE was dissipated towards EPS detachment causing a decline in sCOD. These findings discernibly illustrate a significant distinction between deflocculated and flocculated sludge in terms of COD liquefaction rate.

4.2. Solids reduction

Solids reduction is often correlated with COD liquefaction to be used as another fundamental index to measure the efficiency of sludge disintegration. Solids reduction is requisite in a liquefaction process as it deduces the sludge disintegration of non-biodegradable organic matter into liquefiable organic matter (Tian et al., 2015). Solids reduction was found maximal (17.14%) in sludge deflocculated by $CaCl_2$ prior to bacterial pretreatment up to treatment time of 42h than sole bacterially pretreated sludge (13.57%) (Kavitha et al., 2015d). The results were the consequence of the combinative effect of floc disintegration and biosurfactant activity of inoculated bacterial strain. Beyond 42h, solids reduction was found to be equable as the cultural action of inoculated strain obtained saturation stage. Likewise a collateral study by Kavitha et al. (2015a) detected an enhancement in solids reduction (26%) at the end of 42h when deflocculated by NaCl prior to bacterial pretreatment. From the above research outcome, it can be inferred that solids reduction during bacterial pretreatment ensue a dual phase progression viz Phase 1 -rapid disintegration stage (0 to 42 h) and Phase 2-passivedisintegration stage (48 to 84 h).Furthermore, SEI plays a prominent role which proliferate the efficiency of phase separated pretreatment(Banu et al., 2018a). Rate of solids reduction percentage was examined in both sonicated (SD) and Fenton induced sonicated sludge (FSD) with gradual rise in SEI at both phases (Kavitha et al., 2016a). A paramount solids reduction was seen in FSD (25%) as to SD (16%) at SEI of about 641 kJ/kg TS as anticipated in Phase 1. EPS disaggregation by Fenton in sludge biomass acts as a precursor to ultrasonic pretreatment and heightened the potency of solids reduction. Additionally to accomplish the aforesaid maximum solids reduction, SD sludge requisites SEI that surpasses optimum SEI value. Also, Phase 2 delineates a stable solids reduction percentage as diminutive quantity of solids remnant existed in the sludge biomass. Correspondingly, sludge deflocculated by disperser prior to MW pretreatment (17.33%) draws out a higher solids liquefaction rate in Phase 1. The liquefaction rate obtained when correlated with lone MW pretreatment was 9.3% up to SEI of 18,000 kJ/ kg TS while Phase 2 observed only a slender rise in solids reduction level in spite of higher SEI. It was then disclosed that tremendous increase in MW SEI had only a trivial impact on solids liquefaction rate (Kavitha et al., 2016b). Conjointly, the solids reduction rate attained in phase separated pretreatment were found superior to than that obtained by Tian et al. (2015) in the course of sole sonication (38.3% solids reduction) and ultrasonic combined ozone post-treatment (42.1% solids reduction).

4.3. Biopolymers liquefaction

Proficiency of sludge solubilization efficiency is also valuated based on intracellular biopolymer liquefaction rate such as protein and carbohydrate encapsulated within the cell wall which significantly contributes towards biomethane enhancement. Akin to the COD liquefaction and solids reduction trend, biopolymer liquefaction also pursues a comparable profile which is condensed to rapid solubilization and passive degradation phase during bacterial disintegration. A study by Kavitha et al. (2015d) compared the soluble protein and carbohydrate release rate amidst sludge deflocculated by $CaCl_2$ preceding to bacterial pretreatment, lone bacterially pretreated sludge and control. Though there was a gradual increment in both protein and carbohydrate concentration up to a disintegration time of 42h there was a drastic fall in the release rate beyond it. As foreseen in the above liquefaction parameters, deflocculated sludge exhibited maximum biopolymer release in comparison with flocculated sludge because of floc disruption by $CaCl_2$. While the successive bacterial pretreatment leads to escalation of the biopolymer release level thereby boosting the cell lysis rate. The results of the biopolymer concentration obtained were persistent with the findings by Ushani et al. (2017) attained during bacterial disintegration. The decline in biopolymer concentration after 42h was the result of disaggregation of the soluble protein and carbohydrate into peptides, amino acids and simple sugars (Yang et al., 2010). In an analogous phase separated study, disperser induced ozone pretreatment (DOP) demonstrated exemplary protein (1896.8 mg/L) and carbohydrate release (675.2 mg/L)at minimal ozone dosage of 0.014 gO_3/g SS as to the ozone pretreatment (OP) where maximal protein (1251.2 mg/L) and carbohydrate (386.8 mg/L) level was obtained only at a higher dosage level of 0.042 gO_3/g SS (Kannah et al., 2017a). This was accredited to the fact that EPS deflocculation by disperser engendered the ozone to permeate into sludge biomass unleashing the intracellular biopolymer component into the aqueous medium. In conjunction with the above findings, it can be seen that protein release rate surpasses the release level of carbohydrate. This was conformed to the fact that readily soluble carbohydrates were liberated along with proteins as glycol-proteins through sludge solubilization (Kavitha et al., 2020a). In addition to this, Li et al. (2013) have reported that the concentration of biopolymer obtained by phase separated pretreatment (protein 4.4 mg/L and polysaccharides 136 mg/L) is relatively higher than the individual pretreatment (protein 2.2 mg/L and polysaccharides 72 mg/L) of sludge. The increasing profile of biopolymer exposes the noteworthy of phase separated pretreatment.

4.4. Sludge Enzyme Activity

Hydrolytic enzymes (such as protease and amylase) that are entrapped in sludge flocs play a pivotal role in hydrolyzing the complex macromolecules existent in sludge biosolids (Lin and Li, 2018). Thus, it is imperative to emancipate these intracellular enzymes into the aqueous medium through EPS deaggregation brought about by phase separated pretreatment. This in turn intensifies the sludge disintegration rate. Customarily, protease is liable for rupturing the peptide bonds of protein whereas amylase is held accountable for the dissolution of polysaccharides like carbohydrates (Kavitha et al., 2013). An amplified protease and amylase activity (0.17 U/mL and 0.14 U/mL) was detected in a disperser mediated bacterially pretreated sludge up to 42 h. This was due to the compiled activity of EPS expulsion which unleashes the hydrolytic enzymes enmeshed in sludge flocs and the enzymes secreted by the inoculated bacteria (Banu et al., 2018a). This was further affirmed in a analogous study by Kavitha et al., (2015c) where EPS disruption by $MgCl_2$ followed by bacterial pretreatment observed a rise in the protease and amylase activity. In short, EPS disaggregation stimulated by phase separated pretreatment has a significant impact on the exoneration of intracellular enzymes present on sludge cell walls and thereby proves to be beneficial for sludge disintegration. Xiao et al., (2017) have documented that the microbes exploited during extracellular electron transfer (EET) results in biomass productivity and communication exchange with peripheral environments or with different cells. Each and every microbial cell is enclosed and adheres together by transparent adhesion fluid, which is referred as EPS. Liang et al. (2021) have studied the effect of electron transfer and interaction between extracellular electron transfer and enzyme activity. EET is directed by groups of electron transfer microbes, in which humic substances is considered as an electron acceptor to oxidize biodegradable organic fraction. It also utilized for the microbial growth, energy generation and supports development of new humic substances. The concentration of humus substances is the most important factor that governs the electron exchange capacity and biosolids enzyme activity. Currently the study on electron transfer of humic substance is a remarkable field of research. The humus is the considered as an electron acceptor and redox mediator during digestion processes. It also accelerates the activity of enzyme involved in the redox reaction, particularly the hydrolytic bacteria, which promotes the digestibility of organic biosolids and simultaneously degradation of recalcitrant compounds occurs. It was documented that the addition of additives and pretreatment to the biosolids energetically enhances the hydrolysis and increase the accumulation of volatile fatty acids (VFA) concentration during AD.

4.5. Evaluation of phase separated pretreatments

4.5.1. Biochemical methane potential assay (BMP assay)

The vitality of employing phase separated pretreatment to uplift the anaerobic biodegradability of sludge biosolids was accomplished by conducting biochemical methane potential assay (BMP).Generally BMP is delineated as the maximal methane volume generated per g of VS substrate symbolizing the biodegradability potency of sludge. The outcome of BMP assays relies upon diverse parameters namely temperature, pressure, inoculum to substrate ratio (ISR), substrate preferred, stirring, and composition of flush gas (Wang, 2016). A diploid curve is pursued for methane generation which is found pertinent with the bacterial growth curve and assorted as lag phase, exponential phase and flattening phase (Kavitha et al., 2015d). The methane yield of sludge biosolids can be indirectly gauged by the COD liquefaction rate (Jingura and Kamusoko, 2017). The initial biomethane yield was found to be low at the time of acclimatization in the sludge. An augment rise in methane yield was evident at the exponential stage whilst disaggregated sludge (Ultrasonically mediated Microwave pretreated sludge) proffered a heightened methane production rate of 0.2 L/g COD as to aggregated one (Microwave pretreated sludge) which showed 0.078 L/g COD. This was attributed to the surge in the solubilization rate of particulate organic matter into the liquid medium and resorted to as substrate by the methanogens thereby boosting its biomethanation value. Furthermore methane volume increased during the treatment and was characterized in terms of COD. Thus 0.8114g of COD extracted in deagglomerated pretreated sludge was approximal to the methane transmuted all along the pretreatment process (Kavitha et al., 2018). Concurrently, the liquefied COD retrieved in the course of anaerobic digestion pares down the acidogenesis which in turn curtails the methanogenesis. In conjunction with the preceding statement, extrication of EPS unveils the biopolymers existent in the sludge biomass to the acidogenic biomass which eventually hydrolyzes the solubilized intracellular organic matter ousted into the aqueous phase. This generates a substantial amount of VFA thus accelerating the rate of biomethanation (Kavitha et al., 2015c). This result was congruent to the one obtained by Kavitha et al. (2016b) while using disperser mediated microwave pretreatment. Likewise a harsh pretreatment condition renders indocile compounds to hinder the permutation of soluble organic matter into methane (Kim et al., 2013). This was affirmed by Eswari et al. (2017) where the biomethanation value of H_2O_2 mediated microwave pretreatment elevated up to 85.6% whilst microwave pretreatment showed up 76% under optimal treatment conditions. Conclusively, a surpassing biomethanation yield was noteworthy in phase separated pretreatment when correlated with microwave disintegration coupled with H_2O_2 acidic sludge pretreatment (Eswari et al., 2016).

4.5.2. Assessment of hydrolysis and acidogenesis

Acidification also known as fermentation is a transitional process which transpires during anaerobic fermentation and metamorphoses the hydrolyzed organics into amassment of VFA. The engendered acidified products namely short chain fatty acids (like acetic acid, propionic acid, etc), CO_2, H_2 are made available to the anaerobic methanogens present in the sludge biomass which subsequently enhances the methane yield. Moreover the end product of VFA which originates by hydrolysis and acidogenesis during acidogenic fermentation brings out minuscule quantity of VFA. Sludge pretreatment therefore quickens the hydrolysis rate and decreases the macromolecules existent in the sludge matrix to micro molecules concomitantly by the extracellular hydrolytic enzyme of acidogens. EPS overlying sludge biosolids matrix embeds the intracellular organic matter available to the microbes for cell disintegration. Along these lines, Kavitha et al. (2015c) interpreted that EPS which holds up the biopolymer components are detached from the sludge cell wall by the aid of $MgCl_2$ and ejects them into the liquid medium. This resulted in VFA accretion which when employed in succession with bacterial pretreatment. As a result of EPS deflocculation, the concentration of VFA was found to be higher in chemo mediated bacterially pretreated sludge (640 mg/L) when compared to bacterially pretreated (345 mg/L) and control (55 mg/L). Similarly another researcher Devi et al. (2014) have reported after 72 h of anaerobic fermentation a highest VFA yield of 700 mg/L, 350 mg/L and 60 mg/L was obtained from deaggregated sludge followed by disperser pretreatment, disperser pretreatment alone and control respectively.

4.6. Economics and scale up of phase separated pretreatments

Despite the demand to aggrandize the proficiency of biomethanation through phase separated pretreatment by an array of lab-scale approaches, a techno-economic valuation is indispensable for extensive usage of the technology. Economic analysis thus aids as the guidance to comprehend the factual reasons and the source of cost in an energy process. Moreover, the energy outcome proliferated as methane and heat have got to be superior to the input energy requisite in a phase separated pretreatment which upholds the expediency and viability of the proffered method.

4.6.1. Energy balances

The applicability and like lines of a sludge pretreatment process to be implemented at pilot scale level generally complies with the heightened volume of biogas yield and solids reduction. Along these lines, a thorough energy analysis on the energy expended and procured during the liquefaction process is imperative to decipher a befitting phase separated pretreatment. Pretreatment methods such as sonication, dispersion, high pulse electric field, ozonation, microwave etc. employs electricity as a synergist to effectuate the sludge liquefaction process. Broadly sludge pumping, mechanical stirring exploited for deaggregation, pretreatment and anaerobic digestion are laid out as energy expended howbeit biomethane produced are intended as energy procured in the course of phase separated pretreatment. The energy spent is calculated as per the work of Kavitha et al. (2020b) and Kannah et al. (2021) and the equation given is below:

$$ES = (P \times T)/\ (V \times TS) \tag{1}$$

Where ES is the energy spent (kWh/kg); P is the power consumed (kW); T is the pretreatment time (h); V is the volume of substrate sample (m^3); and TS is the total solid concentration of sample (kg/m^3).

Moreover COD liquefaction rate was used as the criterion for deducing the energetic and economic feasibility of a sludge disintegration process. In a study by Kannah et al. (2017a) DOP requisites lessened amount of energy (−247.35 kWh/ton) to retrieve 20% COD solubilization rate as to the OP (−412.42 kWh/ton). This insinuated the fact that a substantial quantity of energy was resorted to EPS disaggregation together with an equivalent amount of energy procured as biomethane. Correspondingly, the energy procured as methane (616 kWh) to attain 30% biomass lysis was proclaimed to be equitable in the course of microwave (MW) and Zeolite mediated microwave (Ze-MW) pretreatment study. Whilst MW pretreatment demanded a twofold energy expenditure to obtain the aforesaid lysis level (Banu et al., 2019). In addition to the above, energy ratio beyond 1 and an affirmatory net energy production is vital for bringing forth an energy efficient phase separated pretreatment. The energy ratio is the ratio of total output energy (energy gain during AD in the form of biogas) to total input energy (energy spent to produce biogas which includes deflocculation, pretreatment, pumping and AD energy). The equation of energy ratio is given in Fig 4. Kavitha et al. (2019) accomplished a positive net energy and energy ratio (20.5 kWh/ton and 1.04) during fragmented anaerobic granules bacterial pretreatment (FAG-BP) as to the bacterial pretreatment (−307.2 kWh/ton and 0.4). Nevertheless, a section of energy was famished at the time of EPS deaggregation and it could be counterpoised by the volume of methane procured thereby depicting the energetic competency of FAG-BP. These results were found consistent by Kavitha et al. (2018) where UMMP divulged the energy ratio to be 1.08 and accomplished an affirmative net energy. Contrarily, a negative net energy was accomplished when EPS was disaggregated by sodium citrate and preceded by immobilized TiO_2 thin film pretreatment process while it resulted in a 85% net energy savings than the flocculated one (Sharmila et al., 2019).

Plus, the desideratum of energy by thermal pretreatments takes into account the temperature endorsed all along the pretreatment and the energy expended is calculated as per the work of Kannah et al. (2019) and the equation is given below:

$$TE = M \times SH \times (T_{fi}\text{- } T_{in}) \tag{2}$$

Where TE is the thermal energy of biomass (kJ), SH is the specific heat of biomass (for example, sludge −4.2 kJ/kg°C), M is the mass of biomass (kg), T_{in} is the initial temperature of biomass (°C), and $T_{fi}$is the final temperature of biomass (°C). A detailed energy analysis of disperser deflocculation and ultrasonic pretreatment of biosolids elucidated in this section is further depicted in Fig 4.

Furthermore the heat recovered by cooling down the pretreated sludge at the same time is calculated as per the work of Banu et al. (2018d) and the equation is given below:

$$HR = M \times SH \times (\mathrm{Tp\ Tin})\ M \times SH\ (T_p\text{- } T_d) \tag{3}$$

Where HR is the heat energy recovered from biomass (kJ), SH is the specific heat of biomass (4.2 kJ/kg°C), M is the mass of biomass (kg), T_p is the pretreatment temperature (°C), T_d is the digestion temperature (°C), and ϕ is the efficiency of heat exchanger.

An energetic probe on LTTC phase separated pretreatment revealed a positive net energy profit of 48.26kWh/ton owing to the EPS disruption at an optimal temperature of 60°C.

Additionally mass balancing was carried out to gauge the sludge reduction potential of the preferential phase separated pretreatment at pilot scale level. Banu et al. (2020) attained a 47.8% of solids reduction in the course of anaerobic digestion of TiO_2embedded chitosan thin film (TCTF) mediated bacterially pretreated sludge while the net solids disposal amounted to about 693.75kg/ton which was superior to the flocculated sludge. As a consequence EPS fractionation cuts down the quantity of sludge to be cast off thereby narrowing down the landfill disposal cost. Also, the energy analysis of different phase separated pretreatment methods is tabulated in Table 3. The energy analysis is carried out based on experimental conditions of Banu et al. (2018a) and Kavitha et al. (2020a) for disperser deflocculation and ultrasonic pretreatment. The flow rate of pretreated biosolids to anaerobic digestion is fixed as 100 m^3/d (4.2 m^3/h). The energy required per day for deflocculation, ultrasonic pretreatment, solids pump-

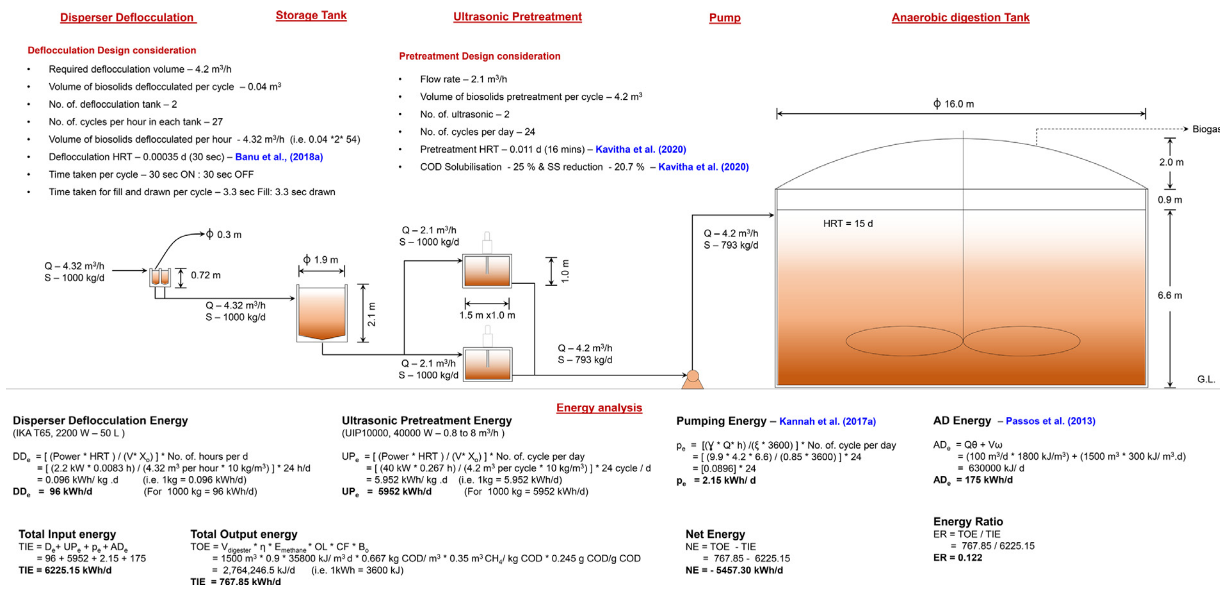

Fig 4. A detailed energy analysis of disperser deflocculation and ultrasonic pretreatment of biosolids.

Table 3
Energy analysis of different phase separated pretreatment methods.

S. No	Type of Phase separated pretreatment	Plant capacity	Input energy	Output energy	Net energy production	Energy ratio	References
1.	TiO_2 embedded chitosan thin film followed by bacterial pretreatment	Pilot scale 1 ton of Solids	– 442 kWh	+ 468.44 kWh	+ 26.44 kWh	1.05	Banu et al. (2020)
2.	Zeolite mediated microwave pretreatment	Pilot scale 1 ton of Solids	– 361.5 kWh	+ 616 kWh	+ 254.2 kWh	1.70	Banu et al. (2019)
3.	Ultrasonic mediated bacterial pretreatment	Pilot scale 1 ton of Solids	–507.5 kWh	+ 528 kWh	+ 20.5 kWh	1.04	Kavitha et al. (2019)
4.	Sodium citrate mediated immobilized TiO_2 pretreatment	Pilot scale 1 ton of Solids	– 463.1 kWh	+ 398.99 kWh	- 64.11 kWh	0.86	Sharmila et al. (2019)
5.	EGTA mediated microwave pretreatment	Pilot scale 1 ton of Solids	– 373.3 kWh	+ 616 kWh	+ 242.7 kWh	1.65	Banu et al. (2018c)
6.	Ultrasonic assisted microwave pretreatment	Pilot scale 1 ton of Solids	– 425.86 kWh	+ 461.81 kWh	+ 35.95 kWh	1.08	Kavitha et al. (2018)
7.	Nano-layered TiO_2 for effective bacterial pretreatment	Pilot scale 1 ton of Solids	– 459.21 kWh	+ 502.32 kWh	+ 43.11 kWh	1.09	Sharmila et al. (2018)
8.	Sodium thiosulphate induced immobilized bacterial pretreatment	Pilot scale 1 ton of Solids	– 431.62 kWh	+ 295 kWh	– 136.62 kWh	0.68	Ushani et al. (2018)
9.	Potassium hydroxide induced immobilized bacterial pretreatment	Pilot scale 1 ton of Solids	– 418.1 kWh	+ 240.5 kWh	– 177.6 kWh	0.57	Banu et al. (2017)
10.	Sodium citrate mediated microwave pretreatment	Pilot scale 1 ton of Solids	– 2777 kWh	+ 3754 kWh	+ 977 kWh	1.35	Ebenezer et al. (2015b)

Loss (-ve) and Profit (+ve)

ing and energy demand in AD is calculated as 96, 5952, 2.15 and 175 kWh/d respectively. Therefore the total input energy (TIE) is calculated to be 6225.15 kWh/d and the total output energy (TOE) is calculated as 767.85 kWh/d, then the net energy is obtained as – 5457.30 kWh (-ve indicates energy loss). However the TOE is less than TIE in this case (phase separated pretreatment). For direct ultrasonic pretreatment to achieve 25 % COD solubilization, it demands high input energy when compared with previous case (Kavitha et al., 2016a).

4.6.2. Cost evaluation

Along with the energy and mass balance investigation, a comprehensive study on cost benefits of the contemplated phase separated pretreatment is crucial before enforcing it at a pilot scale level. Discrete costs like sludge biosolids disposal cost, operational cost (inclusive of chemical and energy cost) and the disposal cost of sludge biosolids reduced after treatment were probed to assess the practicability of the adopted pretreatment method. In view of this, energy cost (biomethane) and cost of biosolids abatement were envisaged as profitable cost (gain) whereas chemical cost and leftover biosolids cost after treatment were conceded to be non-profit (loss).In accordance with a study by Kavitha et al. (2015c), the energy cost for one kWh was recorded to be $0.23 and sludge biosolids disposal cost was noted to be 0.28 $/kg solids. Banu et al. (2019) found that the net profit cost of Zeolite microwave pretreated (Ze-MWP) sludge (26 €/Ton of SS) surpassed the microwave (MW) pretreated sludge (-27€/Ton of SS) and proved to be beneficial. Equivalently a profitable net cost of $107.74 was taped in a TCTF induced bacterially pretreated sludge with an energy ratio of 1.04 (Banu et al., 2020) which outperformed the net profit cost ($2.6/ton of sludge) incurred by an analogous phase separated BP study (Ushani et al., 2018) and also the net profit ($83.39/ton of sludge) obtained through combinative pretreatment of alkaline and microwave by Chi et al. (2011).

4.7. Future Perspectives

The current encyclopedic review gives an overall insight about how deflocculation of EPS influences the pretreatment efficiency thereby augmenting the biomethanation production and sludge disintegration rate. Nonetheless, a plausible experimental study should be carried out to figure out the presence of objectionable compounds during a pretreatment process. Recently, many researchers have focused their research in discovering a cost effective solution to reduce the heavy metal contamination associated with wastewater treatment and biosolids management. Gupta and Diwan (2017) have reviewed about the possibility and technique associated with heavy metal ions for EPS disruption. Typically, there are several expensive techniques followed widely for EPS disruption and heavy metal removal such as ultrasonication, filtration (nano & ultra), electrodialysis, chemical (coagulation & precipitation), and ion exchange. Even though these techniques are effective, they cause certain adverse effect on environment by generating toxic and harmful by product. Inorder to safeguard the environment from these adverse effects, an interesting biological approach was studied by Cui et al. (2020). In their study, the authors have checked the possibility of biosorption mechanism of metal ions on EPS disruption. As a result, highest adsorption efficiency of 95%, 94%, and 78% were achieved for lead, cadmium, and nickel EPS disruption respectively. Another researcher Hu et al. (2021) have documented that the simultaneous heavy metal removal and EPS disruption enhances the sludge dewatering capacity. They studied the effect of Fe (II) coupled with electro oxidation technology to achieve excellent dewatering performance and higher sludge stability. In addition to this, the authors have stated that an increase in the heavy metal removal percentage indicates the stability of the dewatered sludge and its effectiveness in EPS disruption and fragmentation of sludge floc. Additionally, a valuable quantification strategy should be figured out while adopting a method calibrating its adverse facets. Likewise, WAS engaged for phase separated pretreatments is subjective to digestion in batch scale process. Thereupon effect of phase separated sludge pretreatment on semi-continuous/continuous digesters should be thoroughly probed and if possible in a pilot scale level in the near future.

5. Conclusion

In a nutshell this review explores the role of EPS in curbing the hydrolysis rate necessitating its removal for subsequent pretreatment in a protracted manner. Also it catalogues the disintegration mechanism of discrete phase separated pretreatment technology employed and its impact on sludge characteristics. Plus, the assessment of the technique through BMP assays, WAS fermentation and techno-economic analysis affirmed that EPS deflocculation prior to the subsequent pretreatment elevated the sludge disintegration efficiency and biomethane production rate. However, an appropriate explanation is vital to waive out the aforesaid challenges for full scale implementation of a specific phase separated pretreatment.

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 review article.

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References

Antonelli, M., Bialek, K., Teli, A., Citterio, S., Malpei, F., 2011. Influence of thermal extraction of extracellular polymeric substances on cell integrity in activated sludge and membrane bioreactor samples. Water Environ. Res. 83 (2), 100–106. doi:10.2175/106143010X1260, 9736966568.

Ariunbaatar, J., Panico, A., Esposito, G., Pirozzi, F., Lens, P.N.L., 2014. Pretreatment methods to enhance anaerobic digestion of organic solid waste. Appl. Energy 123, 143–156. doi:10.1016/j.apenergy.2014.02.035.

Banu, J.R., Anandan, S., Kaliappan, S., Yeom, I.-T., 2008. Treatment of dairy wastewater using anaerobic and solar photocatalytic methods. Sol. Energy 82, 812–819. doi:10.1016/j.solener.2008.02.015.

Banu, J.R., Do Khac, U., Kumar, S.A., Ick-Tae, Y., Kaliappan, S., 2012. A novel method of sludge pretreatment using the combination of alkalis. J. Environ. Biol. 33, 249.

Banu, J.R., Ushani, U., Rajkumar, M., Naresh Kumar, R., Parthiba Karthikeyan, O., 2017. Impact of mild alkali dosage on immobilized Exiguobacterium spp. mediated cost and energy efficient sludge disintegration. Bioresour. Technol. 245, 434–441. doi:10.1016/j.biortech.2017.08.216.

Banu, J.R., Kannah, R.Y., Kavitha, S., Gunasekaran, M., Yeom, I.T., Kumar, G., 2018a. Disperser-induced bacterial disintegration of partially digested anaerobic sludge for efficient biomethane recovery. Chem. Eng. J. 347, 165–172. doi:10.1016/j.cej.2018.04.096.

Banu, J.R., Sugitha, S., Kannah, R.Y., Kavitha, S., Yeom, I.T., 2018b. Marsilea spp.—A novel source of lignocellulosic biomass: Effect of solubilized lignin on anaerobic biodegradability and cost of energy products. Bioresour. Technol. 255, 220–228. doi:10.1016/j.biortech.2018.01.103.

Banu, J.R., Eswari, A.P., Saratale, G.D., Rani, R.U., Kaliappan, S., Yeom, I.T., 2018c. Enhancing biomethanation from dairy waste activated biomass using a novel EGTA mediated microwave disintegration. J. Environ. Manage. 223, 644–651. doi:10.1016/j.jenvman.2018.06.079.

Banu, J.R., Kannah, R.Y., Kavitha, S., Gunasekaran, M., Kumar, G., Novel insights into scalability of biosurfactant combined microwave disintegration of sludge at alkali pH for achieving profitable bioenergy recovery and net profit. Bioresour. Technol. 255, 220–228. 10.1016/j.biortech.2018.07.046.

Banu, J.R., Eswari, A.P., Kavitha, S., Kannah, R.Y., Kumar, G., Jamal, M.T., Saratale, G.D., Nguyen, D.D., Lee, D.G., Chang, S.W., 2019. Energetically efficient microwave disintegration of waste activated sludge for biofuel production by zeolite: Quantification of energy and biodegradability modelling. Int. J. Hydrogen Energy 44, 2274–2288. doi:10.1016/j.ijhydene.2018.06.040.

Banu, J.R., Sharmila, V.G., Kavitha, S., Rajajothi, R., Gunasekaran, M., Angappane, S., Kumar, G., 2020. TiO_2 - chitosan thin film induced solar photocatalytic deflocculation of sludge for profitable bacterial pretreatment and biofuel production. Fuel 273, 117741. doi:10.1016/j.fuel.2020.117741.

Basuvaraj, M., Fein, J., Liss, S.N., 2015. Protein and polysaccharide content of tightly and loosely bound extracellular polymeric substances and the development of a granular activated sludge floc. Water Res 82, 104–117. doi:10.1016/j.watres.2015.05.014.

Bourven, I., Bachellerie, G., Costa, G., Guibaud, G., 2015. Evidence of glycoproteins and sulphated proteoglycan-like presence in extracellular polymeric substance from anaerobic granular sludge. Environ. Technol. 36, 2428–2435. doi:10.1080/09593330.2015.1034186.

Cesaro, A., Belgiorno, V., 2013. Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste. Ultrason. Sonochem. 20, 931–936. doi:10.1016/j.ultsonch.2012.10.017.

Chi, Y., Li, Y., Fei, X., Wang, S., Yuan, H., 2011. Enhancement of thermophilic anaerobic digestion of thickened waste activated sludge by combined microwave and alkaline pretreatment. J. Environ. Sci. 23, 1257–1265. doi:10.1016/S1001-0742(10)60561-X.

Cho, S.-K., Shin, H.-S., Kim, D.-H., 2012. Waste activated sludge hydrolysis during ultrasonication: Two-step disintegration. Bioresour. Technol. 121, 480–483. doi:10.1016/j.biortech.2012.07.024.

Cui, D., Tan, C., Deng, H., Gu, X., Pi, S., Chen, T., Zhou, L., Li, A., 2020. Biosorption mechanism of aqueous Pb2+, Cd2+, and Ni2+ ions on extracellular polymeric substances (EPS). Archaea 2020. doi:10.1155/2020/8891543.

Das, T., Sharma, P.K., Krom, B.P., van der Mei, H.C., Busscher, H.J., 2011. Role of eDNA on the Adhesion Forces between Streptococcus mutans and Substratum Surfaces: Influence of Ionic Strength and Substratum Hydrophobicity. Langmuir 27, 10113–10118. doi:10.1021/la202013m.

Devi, T.P., Vimala Ebenezer, A., Adish Kumar, S., Kaliappan, S., Rajesh Banu, J., 2014. Effect of deflocculation on the efficiency of disperser induced dairy waste activated sludge disintegration and treatment cost. Bioresour. Technol. 167, 151–158. doi:10.1016/j.biortech.2014.06.004.

Ding, Z., Bourven, I., Guibaud, G., van Hullebusch, E.D., Panico, A., Pirozzi, F., Esposito, G., 2015. Role of extracellular polymeric substances (EPS) production in bioaggregation: application to wastewater treatment. Appl. Microbiol. Biotechnol. 99, 9883–9905. doi:10.1007/s00253-015-6964-8.

Ebenezer, A.V., Arulazhagan, P., Adish Kumar, S., Yeom, I.-T., Rajesh Banu, J., 2015a. Effect of deflocculation on the efficiency of low-energy microwave pretreatment and anaerobic biodegradation of waste activated sludge. Appl. Energy 145, 104–110. doi:10.1016/j.apenergy.2015.01.133.

Ebenezer, A.V., Kaliappan, S., Adish Kumar, S., Yeom, I.-T., Banu, J.R., 2015b. Influence of deflocculation on microwave disintegration and anaerobic biodegradability of waste activated sludge. Bioresour. Technol. 185, 194–201. doi:10.1016/j.biortech.2015.02.102.

Eswari, A.P., Kavitha, S., Kaliappan, S., Yeom, I.T., Banu, J.R., 2016. Enhancement of sludge anaerobic biodegradability by combined microwave-H_2O_2 pretreatment in acidic conditions. Environ. Sci. Pollut. Res. 23, 13467–13479. doi:10.1007/s11356-016-6543-2.

Eswari, A.P., Kavitha, S., Banu, J.R., Karthikeyan, O.P., Yeom, I.-T., 2017. H_2O_2 induced cost effective microwave disintegration of dairy waste activated sludge in acidic environment for efficient biomethane generation. Bioresour. Technol. 244, 688–697. doi:10.1016/j.biortech.2017.07.078.

Flemming, H.-C., Wingender, J., 2010. The Biofilm Matrix. Nat. Rev. Microbiol. 8, 623–633. doi:10.1038/nrmicro2415.

Gayathri, T., Kavitha, S., Kumar, S.A., Kaliappan, S., Yeom, I.T., Banu, J.R., 2015. Effect of citric acid induced deflocculation on the ultrasonic pretreatment efficiency of dairy waste activated sludge. Ultrason. Sonochem. 22, 333–340. doi:10.1016/j.ultsonch.2014.07.017.

Geyik, A.G., Kılıç, B., Çeçen, F., 2016. Extracellular polymeric substances (EPS) and surface properties of activated sludges: effect of organic carbon sources. Environ. Sci. Pollut. Res. 23, 1653–1663. doi:10.1007/s11356-015-5347-0.

Gupta, P., Diwan, B., 2017. Bacterial Exopolysaccharide mediated heavy metal removal: A Review on biosynthesis, mechanism and remediation strategies. Biotechnol. Reports 13, 58–71. doi:10.1016/j.btre.2016.12.006.

Hu, S., Hu, J., Sun, Y., Zhu, Q., Wu, L., Liu, B., Xiao, K., Liang, S., Yang, J., Hou, H., 2021. Simultaneous heavy metal removal and sludge deep dewatering with Fe(II) assisted electrooxidation technology. J. Hazard. Mater. 405, 124072. doi:10.1016/j.jhazmat.2020.124072.

Jingura, R., Kamusoko, R., 2017. Methods for determination of biomethane potential of feedstocks: A review. Biofuel Res. J. 4, 573–586. doi:10.18331/BRJ2017.4.2.3.

Kannah, R.Y., Kavitha, S., Rajesh Banu, J., Karthikeyan, O.P., Sivashanmugham, P., 2017a. Dispersion induced ozone pretreatment of waste activated biosolids: Arriving biomethanation modelling parameters, energetic and cost assessment. Bioresour. Technol. 244, 679–687. doi:10.1016/j.biortech.2017.08.001.

Kannah, R.Y., Kavitha, S., Rajesh Banu, J., Yeom, I.T., Johnson, M., 2017b. Synergetic effect of combined pretreatment for energy efficient biogas generation. Bioresour. Technol. 232, 235–246. doi:10.1016/j.biortech.2017.02.042.

Kannah, R.Y., Kavitha, S., Sivashanmugham, P., Kumar, G., Nguyen, D.D., Chang, S.W., Banu, J.R., 2019. Biohydrogen production from rice straw: effect of combinative pretreatment, modelling assessment and energy balance consideration. Int. J. Hydrogen Energy 44 (4), 2203–2215. doi:10.1016/j.ijhydene.2018.07.201.

Kannah, R.Y., Merrylin, J., Devi, T.P., Kavitha, S., Sivashanmugham, P., Kumar, G., Banu, J.R., 2020. Food waste valorization: Biofuels and value added product recovery. Bioresour. Technol. Rep., 100524 doi:10.1016/j.biteb.2020.100524.

Kannah, R.Y., Kavitha, S., Sivashanmugam, P., Kumar, G., Banu, J.R., 2021. Ultrasonic induced mechanoacoustic effect on delignification of rice straw for cost effective biopretreatment and biomethane recovery. Sustain. Energy Fuels. 5, 1832–1844. doi:10.1039/D0SE01814G.

Kavitha, S., Kumar, S.A., Yogalakshmi, K.N., Kaliappan, S., Banu, J.R., 2013. Effect of enzyme secreting bacterial pretreatment on enhancement of aerobic digestion potential of waste activated sludge interceded through EDTA. Bioresour. Technol. 150, 210–219. doi:10.1016/j.biortech.2013.10.021.

Kavitha, S., Jayashree, C., Kumar, S.A., Kaliappan, S., Banu, J.R., 2014a. Enhancing the functional and economical efficiency of a novel combined thermo chemical disperser disintegration of waste activated sludge for biogas production. Bioresour. Technol. 173, 32–41. doi:10.1016/j.biortech.2014.09.078.

Kavitha, S., Jayashree, C., Kumar, S.A., Yeom, I.T., Banu, J.R., 2014b. The enhancement of anaerobic biodegradability of waste activated sludge by surfactant mediated biological pretreatment. Bioresour. Technol. 168, 159–166. doi:10.1016/j.biortech.2014.01.118.

Kavitha, S., Kaliappan, S., Adish Kumar, S., Yeom, I.T., Rajesh Banu, J., 2015a. Effect of NaCl induced floc disruption on biological disintegration of sludge for enhanced biogas production. Bioresour. Technol. 192, 807–811. doi:10.1016/j.biortech.2015.05.071.

Kavitha, S., Kannah, R.Y., Yeom, I.T., Do, K.U., Banu, J.R., 2015b. Combined thermo-chemo-sonic disintegration of waste activated sludge for biogas production. Bioresour. Technol. 197, 383–392. doi:10.1016/j.biortech.2015.08.131.

Kavitha, S., Kumar, S.A., Kaliappan, S., Yeom, I.T., Banu, J.R., 2015c. Achieving profitable biological sludge disintegration through phase separation and predicting its anaerobic biodegradability by non linear regression model. Chem. Eng. J. 279, 478–487. doi:10.1016/j.cej.2015.05.051.

Kavitha, S., Saranya, T., Kaliappan, S., Adish Kumar, S., Yeom, I.T., Banu, J.R., 2015d. Accelerating the sludge disintegration potential of a novel bacterial strain Planococcusjake 01 by $CaCl_2$ induced deflocculation. Bioresour. Technol. 175, 396–405. doi:10.1016/j.biortech.2014.10.122.

Kavitha, S., Banu, J.R., IvinShaju, C.D., Kaliappan, S., Yeom, I.T., 2016a. Fenton mediated ultrasonic disintegration of sludge biomass: Biodegradability studies, energetic assessment, and its economic viability. Bioresour. Technol. 221, 1–8. doi:10.1016/j.biortech.2016.09.012.

Kavitha, S., Banu, J.R., Kumar, J.V, Rajkumar, M., 2016b. Improving the biogas production performance of municipal waste activated sludge via disperser induced microwave disintegration. Bioresour. Technol. 217, 21–27. doi:10.1016/j.biortech.2016.02.034.

Kavitha, S., Stella, P.B.C., Kaliappan, S., Yeom, I.T., Banu, J.R., 2016c. Enhancement of anaerobic degradation of sludge biomass through surfactant-assisted bacterial hydrolysis. Process Saf. Environ. Prot. 99, 207–215. doi:10.1016/j.psep.2015.11.009.

Kavitha, S., Saji Pray, S., Yogalakshmi, K.N., Kumar, S.S., Yeom, I.T., Banu, J.R., 2016d. Effect of chemo-mechanical disintegration on sludge anaerobic digestion for enhanced biogas production. Environ. Sci. Pollut. Res. 23, 2402–2414. doi:10.1007/s11356-015-5461-z.

Kavitha, S., Karthika, P., Banu, J.R., Yeom, I.T., Kumar, S.A., 2016e. Enhancement of waste activated sludge reduction potential by amalgamated solar photo-Fenton treatment. Desalin. Water Treat. 57, 13144–13156. doi:10.1080/19443994.2015.1055810.

Kavitha, S., Banu, J.R., Priya, A.A., Uan, D.K., Yeom, I.T., 2017a. Liquefaction of food waste and its impacts on anaerobic biodegradability, energy ratio and economic feasibility. Appl. Energy 208, 228–238. doi:10.1016/j.apenergy.2017.10.049.

Kavitha, S., Preethi, J., Banu, J.R., Yeom, I.T., 2017b. Low temperature thermochemical mediated energy and economically efficient biological disintegration of sludge: Simulation and prediction studies for anaerobic biodegradation. Chem. Eng. J. 317, 481–492. doi:10.1016/j.cej.2017.02.092.

Kavitha, S., Banu, J.R., Kumar, G., Kaliappan, S., Yeom, I.T., 2018. Profitable ultrasonic assisted microwave disintegration of sludge biomass: Modelling of biomethanation and energy parameter analysis. Bioresour. Technol. 254, 203–213. doi:10.1016/j.biortech.2018.01.072.

Kavitha, S., Kannah, R.Y., Gunasekaran, M., Nguyen, D.D., Al-Muhtaseb, A.H., Park, J.H., Banu, J.R., 2019. Effect of low intensity sonic mediated fragmentation of anaerobic granules on biosurfactant secreting bacterial pretreatment: Energy and mass balance analysis. Bioresour. Technol. 279, 156–165. doi:10.1016/j.biortech.2019.01.118.

Kavitha, S., Kannah, R., Gunasekaran, M., Banu, J.R., Kumar, G., 2020a. Rhamnolipid induced deagglomeration of anaerobic granular biosolids for energetically feasible ultrasonic homogenization and profitable biohydrogen. Int. J. Hydrogen Energy. 45, 5890–5899. doi:10.1016/j.ijhydene.2019.04.063.

Kavitha, S., Kannah, R.Y., Kasthuri, S., Gunasekaran, M., Pugazhendi, A., Rene, E.R., Pant, D., Kumar, G., Banu, J.R., 2020b. Profitable biomethane production from delignified rice straw biomass: the effect of lignin, energy and economic analysis. Green Chem 22, 8024–8035. doi:10.1039/D0GC02738C.

Kim, D.-H., Cho, S.-K., Lee, M.-K., Kim, M.-S., 2013. Increased solubilization of excess sludge does not always result in enhanced anaerobic digestion efficiency. Bioresour. Technol. 143, 660–664. doi:10.1016/j.biortech.2013.06.058.

Li, H., Wen, Y., Cao, A., Huang, J., Zhou, Q., Somasundaran, P., 2012. The influence of additives (Ca2+, Al3+, and Fe3+) on the interaction energy and loosely bound extracellular polymeric substances (EPS) of activated sludge and their floccula-

tion mechanisms. Bioresour. Technol. 114, 188–194. doi:10.1016/j.biortech.2012.03.043.
Li, H., Zou, S., Li, C., Jin, Y., 2013. Alkaline post-treatment for improved sludge anaerobic digestion. Bioresour. Technol. 140, 187–191. doi:10.1016/j.biortech.2013.04.093.
Li, W.-W., Yu, H.-Q., 2014. Insight into the roles of microbial extracellular polymer substances in metal biosorption. Bioresour. Technol. 160, 15–23. doi:10.1016/j.biortech.2013.11.074.
Liang, T., Elmaadawy, K., Liu, B., Hu, J., Hou, H., Yang, J., 2021. Anaerobic fermentation of waste activated sludge for volatile fatty acid production: Recent updates of pretreatment methods and the potential effect of humic and nutrients substances. Process Saf. Environ. Prot. 145, 321–339. doi:10.1016/j.psep.2020.08.010.
Lin, L., Li, X., 2018. Effects of pH adjustment on the hydrolysis of Al-enhanced primary sedimentation sludge for volatile fatty acid production. Chem. Eng. J. 346, 50–56. doi:10.1016/j.cej.2018.04.005.
Liu, X.-M., Sheng, G.-P., Wang, J., Yu, H.-Q., 2008. Quantifying the surface characteristics and flocculability of Ralstoniaeutropha. Appl. Microbiol. Biotechnol. 79, 187–194. doi:10.1007/s00253-008-1426-1.
Liu, X.-M., Sheng, G.-P., Luo, H.-W., Zhang, F., Yuan, S.-J., Xu, J., Zeng, R.J., Wu, J.-G., Yu, H.-Q., 2010. Contribution of Extracellular Polymeric Substances (EPS) to the Sludge Aggregation. Environ. Sci. Technol. 44, 4355–4360. doi:10.1021/es9016766.
Liu, C., Lei, Z., Yang, Y., Wang, H., Zhang, Z., 2013. Improvement in settleability and dewaterability of waste activated sludge by solar photocatalytic treatment in Ag/TiO_2-coated glass tubular reactor. Bioresour. Technol. 137, 57–62. doi:10.1016/j.biortech.2013.03.071.
Liu, C., Shi, W., Li, H., Lei, Z., He, L., Zhang, Z., 2014. Improvement of methane production from waste activated sludge by on-site photocatalytic pretreatment in a photocatalytic anaerobic fermenter. Bioresour. Technol. 155, 198–203. doi:10.1016/j.biortech.2013.12.041.
Meng, L., Xi, J., Yeung, M., 2016. Degradation of extracellular polymeric substances (EPS) extracted from activated sludge by low-concentration ozonation. Chemosphere 147, 248–255. doi:10.1016/j.chemosphere.2015.12.060.
Merrylin, J., Adish Kumar, S., Kaliappan, S., Yeom, I.T., Rajesh Banu, J., 2014. Effect of EPS removal on the sludge reduction potential of B. licheniformis on its optimized pH conditions. Water Env. J. 28, 95–103. doi:10.1111/wej.12014.
Nouha, K., Kumar, R.S., Balasubramanian, S., Tyagi, R.D., 2018. Critical review of EPS production, synthesis and composition for sludge flocculation. J. Environ. Sci. 66, 225–245. doi:10.1016/j.jes.2017.05.020.
Packyam, G.S., Kavitha, S., Kumar, S.A., Kaliappan, S., Yeom, I.T., Banu, J.R., 2015. Effect of sonically induced deflocculation on the efficiency of ozone mediated partial sludge disintegration for improved production of biogas. Ultrason. Sonochem. 26, 241–248. doi:10.1016/j.ultsonch.2015.01.015.
Pan, X., Liu, J., Zhang, D., Chen, X., Li, L., Song, W., Yang, J., 2010. A comparison of five extraction methods for extracellular polymeric substances (EPS) from biofilm by using three-dimensional excitation-emission matrix (3DEEM) fluorescence spectroscopy. Water SA 36, 111–116. doi:10.4314/wsa.v36i1.50914.
Peng, Z., Fang, F., Chen, Y.-P., Shen, Y., Zhang, W., Yang, J.-X., Li, C., Guo, J.-S., Liu, S.-Y., Huang, Y., Li, S., Gao, X., Yan, P., 2014. Composition of EPS fractions from suspended sludge and biofilm and their roles in microbial cell aggregation. Chemosphere 117, 59–65. doi:10.1016/j.chemosphere.2014.05.070.
Rufus, D.P., Banu, J.R., Kannah, R.Y., Nguyen, D.D., Kumar, G., Chang, S.W., Raja, S.A., 2019. Effect of Dispersion Treatment on Dairy Waste Activated Sludge to Hasten the Production of. Biogas. Front. Energy Res. 7, 136. doi:10.3389/fenrg.2019.00136.
Salama, Y., Chennaoui, M., Sylla, A., Mountadar, M., Rihani, M., Assobhei, O., 2016. Characterization, structure, and function of extracellular polymeric substances (EPS) of microbial biofilm in biological wastewater treatment systems: a review. Desalin. Water Treat. 57, 16220–16237. doi:10.1080/19443994.2015.1077739.
Sharmila, V.G., Kavitha, S., Rajashankar, K., Yeom, I.T., Banu, J.R., 2015. Effects of titanium dioxide mediated dairy waste activated sludge deflocculation on the efficiency of bacterial disintegration and cost of sludge management. Bioresour. Technol. 197, 64–71. doi:10.1016/j.biortech.2015.08.038.
Sharmila, V.G., Dhanalakshmi, P., Banu, J.R., Kavitha, S., Gunasekaran, M., 2017. Effect of deflocculation on photo induced thin layer titanium dioxide disintegration of dairy waste activated sludge for cost and energy efficient methane production. Bioresour. Technol. 244, 776–784. doi:10.1016/j.biortech.2017.08.030.
Sharmila, V.G., Banu, J.R., Gunasekaren, M., Subramanian, A., Yeom, I.T., 2018. Nano layered TiO_2 for effective bacterial disintegration of waste activated sludge and biogas production: Immobilized TiO_2 mediated bacterial pretreatment of WAS for anaerobic digestion. J. Chem. Technol. Biotechnol. 93, 2701–2709. doi:10.1002/jctb.5626.
Sharmila, V.G., Gunasekaran, M., Angappane, S., Zhen, G., Tae Yeom, I., Rajesh Banu, J., 2019. Evaluation of photocatalytic thin film pretreatment on anaerobic degradability of exopolymer extracted biosolids for biofuel generation. Bioresour. Technol. 279, 132–139. doi:10.1016/j.biortech.2019.01.124.
Sheng, G.-P., Yu, H.-Q., Li, X.-Y., 2010. Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: A review. Biotechnol. Adv. 28, 882–894. doi:10.1016/j.biotechadv.2010.08.001.
Shi, Y., Huang, J., Zeng, G., Gu, Y., Chen, Y., Hu, Y., Tang, B., Zhou, J., Yang, Y., Shi, L., 2017. Exploiting extracellular polymeric substances (EPS) controlling strategies for performance enhancement of biological wastewater treatments: An overview. Chemosphere 180, 396–411. doi:10.1016/j.chemosphere.2017.04.042.
Show, K.-Y., Mao, T., Lee, D.-J., 2007. Optimisation of sludge disruption by sonication. Water Res 41, 4741–4747. doi:10.1016/j.watres.2007.07.017.
Sun, J., Guo, L., Li, Q., Zhao, Y., Gao, M., She, Z., Wang, G., 2016. Structural and functional properties of organic matters in extracellular polymeric substances (EPS) and dissolved organic matters (DOM) after heat pretreatment with waste sludge. Bioresour. Technol. 219, 614–623. doi:10.1016/j.biortech.2016.08.042.
Tian, X., Trzcinski, A.P., Lin, L.L., Ng, W.J., 2015. Impact of ozone assisted ultrasonication pre-treatment on anaerobic digestibility of sewage sludge. J. Environ. Sci. 33, 29–38. doi:10.1016/j.jes.2015.01.003.
Tiehm, A., Nickel, K., Zellhorn, M., Neis, U., 2001. Ultrasonic waste activated sludge disintegration for improving anaerobic stabilization. Water Res 35, 2003–2009. doi:10.1016/S0043-1354(00)00468-1.
Tsai, B., Chang, C., Lee, D., 2008. Fractionation of soluble microbial products (SMP) and soluble extracellular polymeric substances (EPS) from wastewater sludge. Environ. Technol. 29, 1127–1138. doi:10.1080/09593330802217740.
Tyagi, V.K., Lo, S.-L., Appels, L., Dewil, R., 2014. Ultrasonic Treatment of Waste Sludge: A Review on Mechanisms and Applications. Crit. Rev. Environ. Sci. Technol. 44, 1220–1288. doi:10.1080/10643389.2013.763587.
Ushani, U., Banu, J.R., Kavitha, S., Kaliappan, S., Yeom, I.T., 2017. Immobilized and $MgSO_4$ induced cost effective bacterial disintegration of waste activated sludge for effective anaerobic digestion. Chemosphere 175, 66–75. doi:10.1016/j.chemosphere.2017.02.046.
Ushani, U., Kavitha, S., Kannah, R.Y, Gunasekaran, M., Kumar, G., Nguyen, D.D., Chang, S.W., Rajesh Banu, J., 2018. Sodium thiosulphate induced immobilized bacterial disintegration of sludge: An energy efficient and cost effective platform for sludge management and biomethanation. Bioresour. Technol. 260, 273–282. doi:10.1016/j.biortech.2018.03.118.
Wang, B., 2016. Factors that influence the biochemical methane potential (BMP) test. Lund Univ. ISBN978-91-7422-436-8.
Wang, L.F., Qian, C., Jiang, J.K., Ye, X.D., Yu, H.Q., 2017. Response of extracellular polymeric substances to thermal treatment in sludge dewatering process. Environ. Pollut. 231, 1388–1392. doi:10.1016/j.envpol.2017.08.119.
Wilén, B.-M., Lumley, D., Mattsson, A., Mino, T., 2008. Relationship between floc composition and flocculation and settling properties studied at a full scale activated sludge plant. Water Res 42, 4404–4418. doi:10.1016/j.watres.2008.07.033.
Wu, J., Yang, Q., Luo, W., Sun, J., Xu, Q., Chen, F., Zhao, J., Yi, K., Wang, X., Wang, D., Li, X., 2018. Role of free nitrous acid in the pretreatment of waste activated sludge: extracellular polymeric substances disruption or cells lysis? Chem. Eng. J. 336, 28–37. doi:10.1016/j.cej.2017.11.038.
Xiao, Y., Zhang, E., Zhang, J., Dai, Y., Yang, Z., Christensen, H.E.M., Ulstrup, J., Zhao, F., 2017. Extracellular polymeric substances are transient media for microbial extracellular electron transfer. Sci. Adv. 3. doi:10.1126/sciadv.1700623.
Yan, Y., Chen, H., Xu, W., He, Q., Zhou, Q., 2013. Enhancement of biochemical methane potential from excess sludge with low organic content by mild thermal pretreatment. Biochem. Eng. J. 70, 127–134. doi:10.1016/j.bej.2012.10.011.
Yang, Q., Luo, K., Li, X., Wang, D., Zheng, W., Zeng, G., Liu, J., 2010. Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes. Bioresour. Technol. 101, 2924–2930. doi:10.1016/j.biortech.2009.11.012.
Ye, F., Ye, Y., Li, Y., 2011. Effect of C/N ratio on extracellular polymeric substances (EPS) and physicochemical properties of activated sludge flocs. J. Hazard. Mater. 188, 37–43. doi:10.1016/j.jhazmat.2011.01.043.
Yin, C., Meng, F., Chen, G.-H., 2015. Spectroscopic characterization of extracellular polymeric substances from a mixed culture dominated by ammonia-oxidizing bacteria. Water Res 68, 740–749. doi:10.1016/j.watres.2014.10.046.
Yu, G.-H., He, P.-J., Shao, L.-M., 2009. Characteristics of extracellular polymeric substances (EPS) fractions from excess sludges and their effects on bioflocculability. Bioresour. Technol. 100, 3193–3198. doi:10.1016/j.biortech.2009.02.009.
Yu, G.-H., He, P.-J., Shao, L.-M., He, P.-P., 2008. Stratification Structure of Sludge Flocs with Implications to Dewaterability. Environ. Sci. Technol. 42, 7944–7949. doi:10.1021/es8016717.
Zhang, W., Peng, S., Xiao, P., He, J., Yang, P., Xu, S., Wang, D., 2015. Understanding the evolution of stratified extracellular polymeric substances in full-scale activated sludges in relation to dewaterability. RSC Adv 5, 1282–1294. doi:10.1039/C4RA13379J.
Zhang, P., Shen, Y., Guo, J.-S., Li, C., Wang, H., Chen, Y.-P., Yan, P., Yang, J.-X., Fang, F., 2015. Extracellular protein analysis of activated sludge and their functions in wastewater treatment plant by shotgun proteomics. Sci. Rep. 5, 1–11. doi:10.1038/srep12041.
Zhang, T., Wang, Q., Khan, J., Yuan, Z., 2015. Free nitrous acid breaks down extracellular polymeric substances in waste activated sludge. RSC Adv 5 (54), 43312–43318. doi:10.1039/C5RA06080J.
Zhao, W., Yang, S., Huang, Q., Cai, P., 2015. Bacterial cell surface properties: Role of loosely bound extracellular polymeric substances (LB-EPS). Colloids Surf. B. 128, 600–607. doi:10.1016/j.colsurfb.2015.03.017.