Resources, Conservation & Recycling 182 (2022) 106327

Contents lists available at ScienceDirect

Resources, Conservation & Recycling

journal homepage: www.elsevier.com/locate/resconrec

Review

Separation and purification techniques for the recovery of added-value biocompounds from waste activated sludge. A review

Daniel Núñez, Paula Oulego, Sergio Collado, Francisco A. Riera, Mario Díaz *

Department of Chemical and Environmental Engineering, University of Oviedo, c/ Julián Clavería 8, Oviedo 33006, Spain

ARTICLE INFO

Keywords:
Adsorption
Biorefinery
Circular economy
Membrane filtration
Precipitation
Solvent extraction

ABSTRACT

The need of developing a new growth model based on circular economy has led to an increasing interest in the revalorization of urban and industrial wastewaters in order to use the resources efficiently. The most stablished way of valorising these residues implies the energy production in the form of biomethane. However, urban and industrial wastewaters can also be considered promising raw sources for the recovery of valuable chemical compounds. Especially, waste activated sludge from water treatment plants is a fantastic source of biomolecules such as lipids (triglycerides or fatty acids), proteins and enzymes, carbohydrates, and humic and fulvic acids. However, prior to the recovery of these biocompounds, sludge solubilization processes (thermal hydrolysis, sonication and acidification, among others) must be conducted, in order to break the cell walls and release the protoplasmic content into the liquid media, thus obtaining a matrix of high complexity, which condition the possible strategies to be applied.

This review gathered and discussed in-depth the studies that deal with the recovery of valuable biocompounds from secondary waste activated sludge. Furthermore, other types of sludge comparable to the activated one, such as cell cultures and food-related sources, have been also discussed here, in order to be used as a starting point for further research on the valorisation of waste activated sludge.

1. Introduction

Efficient wastewater management has become a topic of prime concern, due to increasing pressure derived from rapid population growth and increasing public and governmental environmental awareness. One of the main wastewater management challenges is related to waste activated sludge (WAS), which is generated during the secondary treatment stage in the water treatment process . Indeed, around 10 million tons (dry weight basis) of sewage sludge were generated in Europe in 2019 (Eurostat, 2022), and WAS disposal volume is intended to be reduced by 50% by 2050 (as compared with the production rates recorded in 2000) (Fytili and Zabaniotou, 2008). Hence, the development and application of optimized procedures for WAS management will turn even more critical in the upcoming years. Standard methods for WAS management include agricultural use, forestry and land reclamation, incineration, carbonization, co-composting or landfill disposal (Mateo-Sagasta et al., 2015; Shi et al., 2018). All of these treatments pose important health and environmental hazards, such as heavy metals (Hsiau and Lo, 1998), organic pollutants (Dai et al., 2007) or pathogens (Lewis and Gattie, 2002) contamination of soil; increase of antibiotic resistance genes in soil (Chen et al., 2016); greenhouse gas emissions (Awasthi et al., 2016) and air pollution (Werther and Ogada, 1999); or terrestrial acidification and eutrophication (Zhao et al., 2015).

In the latter years, tendencies in environmental management have evolved from eliminating wastes to recycling and valorising them, within the context of circular economy (Geissdoerfer et al., 2017). Many processes for WAS valorisation have focused on total oxidation of sludge, mainly producing biofuels (Bharathiraja et al., 2014; Manara and Zabaniotou, 2012; Rulkens, 2008; Zhao et al., 2014). Nevertheless, this approach leads to the loss of added-value products present in the sludge, since it is mainly composed of bacteria and other microorganisms (Urbain et al., 1993). Therefore, WAS is considered a fantastic

Abbreviation: ADH, alcohol dehydrogenase; APTES, (3-aminopropyl)triethoxysilane; CAGR, compound annual growth rate; CFV, crossflow velocity; MF, microfiltration; MWCO, molecular weight cut-off; NF, nanofiltration; PES, polyethersulphone; PS, polysulphone; RC, regenerated cellulose; RO, reverse osmosis; SCFAs, short-chain fatty acids; TCOD, total COD; TH, thermal hydrolysis; TMP, tansmembranar pressure; UF, ultrafiltration; VCR, volume concentration ratio; VSS, volatile suspended solids; WAS, waste activated sludge; WO, wet oxidation; PVP, polyvinylpyrrolidone.

* Corresponding author.
E-mail address: mariodiaz@uniovi.es (M. Díaz).

https://doi.org/10.1016/j.resconrec.2022.106327
Received 23 November 2021; Received in revised form 21 February 2022; Accepted 28 March 2022
Available online 5 April 2022

Table 1
Biocompounds content in WAS.

Parameter	Units	Value
pH		6.5–8.0
TSS	g/L	8.3–33
VSS	%TSS	59–88
COD	gO_2/L	5.88–43.82
Carbohydrates	mg/L	506.3–3234
Proteins	mg/L	2656–13530
Lipids	mg/L	166–3960
Humic acids	mg/L	196.71–5849
SCFAs	mg/L as acetic acid	16–1700

source of biorefinery products (Raheem et al., 2018). Its main constituents are summarized in Table 1 (Chen et al., 2007; Contreras et al., 2002; García et al., 2017; Gascó and Lobo, 2007; Li et al., 2013; Suárez-Iglesias et al., 2017). To that end, it is required a controlled breaking of the biological structures to generate these bio-based products. Less aggressive solubilization processes enable the release of enzymes (Karn and Kumar, 2019; Nabarlatz et al., 2011, 2010; Ni et al., 2017; Yu et al., 2009), bioplastics (Chen, 2017; Liu et al., 2019; Morgan-Sagastume et al., 2010; Pittmann and Steinmetz, 2014), proteins (Hwang et al., 2008; Jimenez et al., 2013; Pervaiz and Sain, 2011; Wei et al., 2016), humic acids (Li et al., 2014a, 2009; Motojima et al., 2012; Wei et al., 2016) and lipids (Dong et al., 2019; Olkiewicz et al., 2014; Revellame et al., 2012; Siddiquee and Rohani, 2011) to the liquid media. The market size of all these biocompounds is forecasted to increase in the following years due to their numerous industrial applications, as it is indicated below. Therefore, WAS valorisation through the recovery of biocompounds such as proteins, enzymes, humic acids, lipids or short-chain fatty acids (SCFAs) appears to be an interesting economic opportunity.

Proteins are biological heteropolymers, composed by subunits of peptides linked by peptide bonds. They have a massive number of sequential and structural arrangements, greatly varying in size (the majority of the proteins are comprised between 20 and 100 kDa (Sokatch, 1969)) and structure (Nussinovitch, 2013; Pollock, 2007). Bulk proteins have several market applications, including food and beverages, personal care and cosmetics, pharmaceuticals, and animal feed, this last application being the most common one for the proteins from microbial sources (Ritala et al., 2017). Protein market size was valued in USD 33.9 billion in 2020, and is expected to grow up to USD 48.1 billion by 2026 (Expert Market Research, 2020a).

Regarding enzymes (a subtype of proteins characterised for catalysing chemical reactions), (Litalien and Beaulieu, 2011), hydrolytic enzymes (proteases, lipases, amylases and cellulases) are the most commercially used ones in the field of cleaning product, food and beverage, biofuel production, and animal feed, among others. Besides, other enzymes, such as dehydrogenases, galactosidases, other glucosidases, and phosphatases, can also be produced and recovered from WAS (Liu and Smith, 2019; Nabarlatz et al., 2010). In this sense, enzymes are considered a valuable product, with a market price near USD 10 billion in 2020 and expected to grow at a compound annual growth rate (CAGR) of 7.5% for the next five years (Expert Market Research, 2020b).

Humic acids are a loosely defined group of molecules, consisting of the fraction of precipitated compounds after the acidification of strong-base extracted natural organic matter (the fraction of organic matter that remains dissolved corresponds to the fulvic acids) (Bleam, 2017). Chemically, they have an undefined composition, varying both in chemical composition and size (comprised between 2.0 and 1300 kDa), but essentially they are amphiphilic weak acidic electrolytes with carboxylic, phenolic or quinone groups, which give them their useful properties, such as being anti-inflammatory, antioxidant, fungicide and bactericide (De Melo et al., 2016). From an economic point of view, they are applied in agriculture, animal feed, ecological remediation, and in more innovative fields promoted by the growing awareness of the health benefits of humic acids, like dietary supplements or pharmaceuticals. However, its commercial use is somehow hindered by the inconsistent efficacy of humic acid products (Market Research Future, 2019). Humic acids market is more restrained, this being valued at USD 503million in 2020 and expected to grow at CAGR of 11.2% during the forecast period 2020–2026 (Mordor Intelligence, 2021).

Lipids are a heterogeneous group of biomolecules with the common property of being soluble in non-polar solvents (IUPAC, 2014a). The most relevant lipids are glycerides (esters of glycerol and fatty acids [aliphatic carboxylic acids] (IUPAC, 2008)), phospholipids (lipids with phosphoric acids as mono- or di-esters (IUPAC, 2014b)) or non-saponifiable lipids like steroids (IUPAC, 2014c). Lipids have a wide range of industrial applications, such as personal care, cosmetics, agrochemicals, animal feed, and biodiesel. Among them, SCFAs (carboxylic acids with an aliphatic chain length of up to six carbon atoms (Silva et al., 2020)) have been widely applied in food, chemical, biochemical, textile, cosmetic or pharmaceutical industries (Panda et al., 2019). The global market for lipids was estimated at USD 6.4 billion in 2020, and it is forecasted to grow at a CAGR of 5.2% to reach USD 9.4 billion by 2027 (MarketWatch, 2022). As for fatty acids, their global market value was USD 134.2 billion in 2020, and it is expected to grow at a CAGR of 4% to reach USD 148.2 billion by 2023 (Research and Markets, 2021).

Saccharides are polymers of highly variable length and with linear or ramified structures, composed of monosaccharides and that respond to the general formula of $(CH_2O)_n$, (Castro-Puyana et al., 2013). Bacteria are the main source for saccharides in the industry, where are used in the areas of beverages, savoury and snacks or animal feed, among others. Saccharides had a USD 13.5 billion market value in 2020, and are expected to grow at a CAGR of over 5% in the period between 2020 and 2030 (Fact.MR, 2020).

To sum up, the bioproducts that can be obtained from WAS have a current global market size of almost USD 200 billion, proving that its revalorization is economically interesting, and essential to make more sustainable WAS management.

After solubilization, a complex stream is obtained, consisting of a solid fraction, which can also be valorised (Bridle and Pritchard, 2004); and a liquid fraction containing a mixture of the above-mentioned biorefinery products, in addition to inorganic N, P, K, Ca, S and Mg, heavy metals, dioxins, furans and other organic and inorganic matter (Raheem et al., 2018). P and N can be simultaneously recovered through the struvite precipitation method (Tong and Chen, 2009), while larger biocompounds can be purified by a variety of techniques. This last purification step, however, has been scarcely studied for WAS, and only a few papers focused on WAS valorisation discuss this topic. Thus, it is difficult to accurately assess the best recovery strategy based on the existing literature, when WAS is revalorized through the purification of biocompounds. Nonetheless, the separation of biorefinery products is more common in other industries, including water management (Chishti et al., 1992; Olkiewicz et al., 2015; Talebi et al., 2020; Zhou et al., 2018); and farming (Wilken et al., 2016; Wu et al., 2007; Zhang et al., 2015), biotechnology (Boychyn et al., 2000; Costa et al., 2018; Hansson et al., 1994; Johansson et al., 1996), food (Abejón et al., 2016; Białas et al., 2015; Tahergorabi et al., 2011; Xu et al., 2001), alcohol (Hegazi et al., 1973; Li et al., 2018) or textile (Capar et al., 2008; Feairheller et al., 1972; Li et al., 2015) industries; those techniques being applicable in WAS treatment. Classic separation methods, such as extraction (Li et al., 2018; Tabtabaei and Diosady, 2013), adsorption (Wang et al., 2009), precipitation (Li et al., 2019; Motojima et al., 2012; Tahergorabi et al., 2011), membrane separation (Li et al., 2009; Nguyen et al., 2016; Wu et al., 2007) or chromatography (Dong et al., 2019) have been used for this purpose, along with more specific techniques. These technologies are readily available for WAS revalorization, so the reported results in these analogous streams can serve as a starting point for further research into the recovery of biocompounds from WAS.

In order to gather all the knowledge applicable to WAS revalorization through the recovery of biocompounds, and to facilitate further

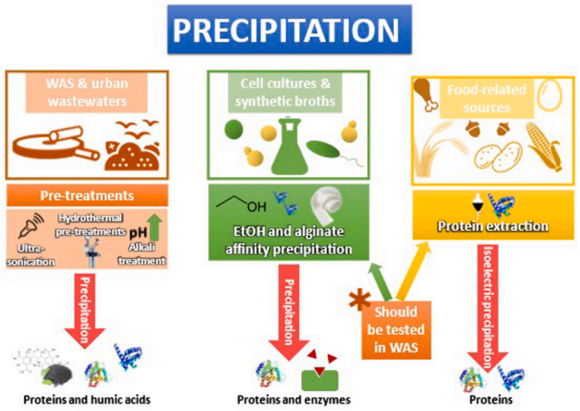

Fig. 1. Uses of precipitation technology for the recovery of added-value biomolecules.

investigation into this matter, the current efforts in separation and purification of these valuable products by the most established techniques, either from WAS or from other comparable sources, such as primary sludge (Melero et al., 2015; Olkiewicz et al., 2015, 2014), landfill leachate (Iskander et al., 2019; Talebi et al., 2020; Zhou et al., 2018), cell cultures (Djamai et al., 2019; Lorenzo-Hernando et al., 2019; Richardson et al., 1990; Ward et al., 2016), or complex aqueous streams from animal or vegetal sources (Li et al., 2013; Saidi et al., 2013; Strætkvern and Schwarz, 2012; Taskila et al., 2017), have been thoroughly reviewed in this work.

2. Precipitation

Precipitation is a well-known separation method in the municipal wastewater treatment (Wang et al., 2005), pharmaceutical (Linn, 2009; Martinez et al., 2019) and food (Hoare and Dunnill, 1984) industries. This technique relies on the separation of a component of a solution either by changing the solubility of the molecule (converting the compound to an insoluble form) or the nature of the solvent. It is mainly used for the precipitation of metallic ions (Wang et al., 2005), but also for biological molecules, such as phenols, oily emulsions (Wang et al., 2005), proteins (Burgess, 2009), or humic substances (Chen et al., 2021). There are different strategies to achieve this, such as changing pH; salting-out by the addition of certain salts that interact with the compound of interest and increase its hydrophobicity, which eventually makes it insoluble; heating; or phase partitioning with organic polymers or solvents (Burgess, 2009; Ojovan et al., 2019; Waglay et al., 2014). The advantages of precipitation over other methods lie in the fact that it is relatively simple, effective, selective and easily implemented. However, it usually presents higher costs, and may require further purification steps (Linn, 2009). Precipitation studies have been conducted for the recovery of biorefinery products (mainly bulk proteins, but also for enzymes and humic acids) in a broad range of sources, namely from WAS and other urban residues; from cell cultures, culture broths and synthetic solutions; and from food and other animal-related industries (Fig. 1).

2.1. Precipitation in urban wastewater

Regarding the use of precipitation techniques for the revalorization of WAS, the use of precipitation is mainly focused on the recovery of phosphate and ammonium as struvite (Akmehmet Balcioglu et al., 2017; Mazlum and İkizoğlu, 2018), and only a few papers dealing with the recovery of biological biorefinery products can be found. All four papers consulted (García et al., 2017; Hwang et al., 2008; Pervaiz and Sain, 2011; Wei et al., 2016) recovered soluble proteins, while (Wei et al., 2016) also recovered humic acids from the supernatant through membrane filtration.

Both solubilization and precipitation methods were found to affect the final recovery yield. García et al. (2017) studied two hydrothermal pre-treatments (wet oxidation [WO] and thermal hydrolysis [TH]) and four precipitation methods (ammonium sulphate precipitation, acetone precipitation, trichloroacetic acid precipitation and pH adjustment to values of 2.0, 3.0, 4.5, 5.5 and 9.0), and concluded that TH followed by ammonium sulphate precipitation attained the best purification result, yielding 87% protein recovery. Furthermore, pH adjustment was found to be the worst precipitation strategy, with yields lower than 35% in all cases. Recovery yields obtained with this strategy greatly differ between those reported by García et al. (2017). and the rest of the studies: Wei et al. (2016) obtained 78.3% separation yield at pH 2.0, Pervaiz and Sain (2011) reported that 92% of soluble protein precipitated at pH 3, and Hwang et al. (2008) measured a maximum precipitation of 80.5% of soluble protein at pH 3.3. In all of these studies, 2 M H_2SO_4 was used for the acid pH adjustment and short times of precipitation (15 min) were selected. Therefore, the extraction yields were not affected by these parameters. Nevertheless, García et al. (2017) considered the interference of humic acid when measuring proteins. The methods used by the other authors overestimate the presence of proteins: Foulin-Ciocalteu reagents employed in the Lowry method can react with the phenolic groups of humic acids (Vakondios et al., 2014), resulting in overestimations of protein concentration by approximately 40% (Frølund et al., 1995). Kjeldahl method measures total N content, and protein content is then calculated by a conversion factor of 6.25 (average N presence in protein is 16% w/w, so 1 g of N would be equivalent to 6.25 g of protein (Mæhre et al., 2018)). N content in humic acids ranges between 1.7 and 5.6% (Kumada, 1955; Piper and Posner, 1972), so its presence would lead to an overestimation of protein content. García et al. (2017) measured humic acids and proteins with a modification of the Lowry method proposed by Frølund et al. (1995), which helped correct this overestimation. These significant differences between the extraction yields can also be explained taking into account the pre-treatment methods employed for the WAS solubilisation, which conditioned the efficiency obtained. In this sense, Hwang et al. (2008) compared the effects of alkali treatment, ultra-sonication and alkali treatment followed by ultra-sonication as pre-treatments on protein extraction performance. The highest solubilisation rate was achieved after alkali treatment and ultra-sonication (2626 mg/L), followed by ultra-sonication (1818 mg/L) and alkali treatment (932 mg/L). Precipitation yields greatly changed as a function of the solubilization method: only 41.7% of soluble protein obtained after alkali treatment precipitated at pH 3.3, while the values was 80.5% when alkali treatment and ultra-sonication were used at the same pH. García et al. (2017) also found differences between the yields achieved after WO and TH. For a given treatment time, the presence of an oxidising atmosphere fastened protein solubilisation, reaching a concentration of 7700 mg/L of soluble protein after WO versus 7200 mg/L after TH. Depending on the precipitation technique, protein precipitation was favoured by one treatment or the other: ammonium sulphate precipitation was slightly more effective after TH (87% TH, 80% WO), and acetone precipitation after WO (77% WO, 70%TH). Trichloroacetic acid precipitation was favoured by TH, and the difference between the recovery yields was higher than in other cases (53% TH, 40% WO). Precipitation through pH adjustment was higher after TH at pH values of 2.0, 3.0 and 4.5; similar at pH 5.5; and higher after WO at pH 9.0. These variations can be explained by: (i) variations in protein soluble concentration due to distinct degrees of solubilisation achieved by different treatments, and (ii) possible changes in protein structure or chemical characteristics (e.g., oxidation degree, geometry and conformation, size, surface functional groups…) that can alter the protein proneness to precipitation.

It should be noted that most of these methods: pH adjustment, acetone, trichloroacetic acid and ammonium sulphate precipitations, led to the precipitation humic acids together with the proteins. Therefore, it is also interesting to determine the selectivity factor for proteins against

Table 2
Consulted studies about precipitation in urban wastewater.

Source	Compounds	Content	Pre-treatment	Precipitation method	Main results	Ref
WAS	Proteins	181 mg/L	TH or WO	(a) pH precipitation (2.0, 3.0, 4.5, 5.5 and 9.0,) (b) acetone precipitation, (c) trichloroacetic acid (TCA) precipitation, (d) ammonium sulphate precipitation	Recoveries: Ammonium sulphate 87% (TH), 86% (WO) Acetone 75% (TH), 77% (WO) TCA 53% (TH), 43% (WO) pH adjustment <35% (WO and TH)	García et al., 2017)
WAS	Proteins, humic acids	1345.4-1633.7 mg/L (proteins) 1199.1-1456.05 mg/L (humic acids)	Sonication, ultrafiltration (UF) (for humic acid recovery)	pH precipitation (1.0, 2.0, 3.0, 4.0 and 5.0)	Recoveries of 78.3% for protein and 88.6% for humic acid	Wei et al., 2016)
WAS	Proteins	800 mg/L	Alkali treatment	pH precipitation (1.5, 3.0, 4.5 and 5.5)	Recovery of 92% at pH 3.0	Pervaiz and Sain (2011)
WAS	Proteins	932.3 mg/L	Alkali treatment, ultra-sonication and alkali treatment followed by ultra-sonication	pH precipitation (1.0, 3.3 and 5.0)	80.5% recovery at pH 3.3 after alkali treatment and ultra-sonication	Hwang et al. (2008)
Landfill leachate and synthetic humic acid solutions	Humic acids	50-1000 mg/L	-	Coagulation by (3-aminopropyl) triethoxysilane (APTES)	Maximum humic acid separation efficiency at pH 3.0-5.0 Maximum recovery yield of 95.5% at 35°C, 4.0 mL/L of APTES, contact time of 60 min and HA concentration of 250 mg/L.	Zhou et al. (2018)
Landfill leachate	Humic acids	763 mg/L	Concentration by forward osmosis	Acidic precipitation (pH 1.5 or 2).	No recovery differences due to pH. 2.45 g/L raw leachate recovered.	Iskander et al. (2019)
Primary sewage sludge	Proteins	3.2-21.3 g/L	Alkaline solubilisation (NaOH, pH 12.5)	Hydrochloric acid, sodium lignosulphonate, sulphuric acid, acetic acid and ammonium sulphate precipitations	Maximum protein recovery of 91%, obtained with ammonium sulphate (40%).	Chishti et al. (1992)

Table 3
Consulted studies about precipitation in cell cultures and synthetic broths.

Source	Compounds	Content	Pre-treatment	Precipitation method	Main results	ref
S. cerevisiae	Enzymes (ADH)	444 U/mL	High-pressure homogenization	Ammonium sulphate precipitation	Maximum ADH precipitation yield of 100% (Ammonium sulphate 60%sat).	Richardson et al. (1990)
S. cerevisiae	Proteins, enzymes (ADH)	17300 U/mL (ADH), 1270 g/L (proteins)	High-pressure homogenization	Ammonium sulphate precipitation	Protein recovery yield of 27.53%. ADH recovery yield of 7.7%	Boychyn et al. (2000)
Microalgae	Proteins	46.7% (dry-weight ash-free basis)	Alkaline hydrolysis	Acidic precipitation (HCl, pH 2.5)	Maximum protein extraction yield of 16.9%.	Lorenzo-Hernando et al. (2019)
Aspergillus niger submerged fermentation broths	Proteins, enzymes (xylanases)	7.68 U/mL (xylanases), 120 mg/L (proteins)	Filtration and centrifugation	Ethanol precipitation	Maximum recoveries 85.3% of protein and of 68.7% of xylanase activity	Costa et al. (2018)
Aspergillus allahabadii X26 culture supernatant	Proteins, enzymes (dextranase)	110.0 U/mL (dextranase), 641 mg/L (proteins)	Filtration and centrifugation	Ammonium sulphate precipitation	Protein recovery yield of 57.6%, total activity recovery yield of 82.0%.	Netsopa et al. (2019)

humic acids for each of the pre-treatments and precipitation methods evaluated. This was only reported by García et al. (2017), who found that the use of ammonium sulphate, TCA and pH 9 had better performance for TH, whereas the precipitation at pH 3, 4 and 5.5 worked greater on WO. Besides, only short precipitation times were analysed for acid precipitation once the desired pH value was reached. Moreover, it could be convenient to study longer times in order to analyse its influence on protein extraction.

Based on the existing literature, it is difficult to determine which pre-treatment, sonication or alkali treatment, is considered the best one, as Hwang et al. (2008) observed that sonication better solubilised sludge than alkali treatment, but did not study their effect on protein precipitation yield (only compared alkali treatment vs sonication and alkali treatment combined). On the other hand, Pervaiz and Sain (2011) achieved a higher protein recovery yield using alkali treatment as a pre-treatment than Wei et al. (2016) with sonication.

As for the feasibility of the application of the different pre-treatments, sonication is the most used mechanical method for WAS disruption, as it does not require highly costly equipment (Des Soye et al., 2018; Kim et al., 2003; Shrestha et al., 2012). Conversely, hydrothermal and alkali treatments face higher costs, this being their main drawback. It should be noted that hydrothermal treatments can achieve similar effects with lower temperatures by increasing the contact time. Alkali treatments, apart from the higher cost associated with chemical treatments compared to mechanical or biological ones (Kim et al., 2009), have other drawbacks, including corrosion, odours and the need to neutralize the treated stream (Ruiz-Hernando et al., 2013).

Landfill leachate is also a stream with high COD and humic substances content. Two different strategies for humic acid precipitation have been reported: classic acidic precipitation (Iskander et al., 2019) achieved recoveries of 1.86 g per L of raw leachate, while a maximum recovery yield of 95.5% was obtained after coagulation by (3-aminopropyl)triethoxysilane. Unfortunately, initial concentration of landfill leachate in (Zhou et al., 2018) is not clearly stated, so no comparisons in

Table 4
Consulted studies about precipitation in farming and food-related sources.

Source	Compounds	Content	Pre-treatment	Precipitation method	Main results	ref
Potato fruit juice	Proteins	n/a	-	Thermal/acidic, acidic, $FeCl_3$, $MnCl_2$, ethanol, or $(NH_4)_2SO_4$ precipitation; and CMC complexation	Maximum recovery yields (%) by precipitation agent: Thermal/acidic, 90.2 acidic, 64.7 $FeCl_3$, 75.2 $MnCl_2$, 16.8 Ethanol, 55.2 $(NH_4)_2SO_4$, 98.8 CMC, 75.3	Waglay et al. (2014)
Dry-milled corn germ	Proteins	17.6-31.3% (% dry basis)	Wet milling, defatting, protein extraction in alkali water	Acid precipitation	Protein recoveries from 27.3% to 41.6%, depending on the source of origin.	Wilken et al. (2016)
Sorghum distillers grains	Proteins	n/a	Solubilisation in urea (8 M, 1:7.5 ratio), alkali treatment	Isoelectric precipitation (pH 4.5, addition of HCl 7M + Na_2SO_4 2wt%)	Maximum protein extraction yield of 70%.	Li et al. (2018)
Milled rice bran	Proteins	n/a	Soxhlet extraction with water	Sodium alginate and carrageenan precipitation	Maximum recovery yields of 95.3% of protein recovered with carrageenan and of 87.5% using alginate were obtained at 20°C.	Fabian et al. (2010)
Dehulled soybean flour	Proteins	24.18-24.95% (dry-weight basis)	Oleosome supernatant preparation in lab and pilot-plant scale	Distilled water and ethanol precipitation	Protein recovery yields: In lab scale, 48.4% (water) and 62.3% (ethanol). In pilot-plant scale, 49.6% (water) and 75.4% (ethanol).	Kapchie et al. (2012)
Jatropha curcas kernel and seed press cake	Proteins	23% (dry-weight basis)	Extraction with water; NaCl (0.1, 0.55 and 1.0 M); or NaOH (0.01, 0.055 and 0.1 M).	Acid precipitation	Better extraction and recovery yields with NaOH 0.055 M extraction. Maximum recovery yields of 69.6% (kernel) and 64.9% (seed press cake).	Lestari et al. (2010)
Lupinus angustifolius seeds	Proteins	34.8% (w/w)	Dehulling, NaCl extraction, filtration, pH adjustment	Precipitation with cold demineralised water	34.7% extraction yield	Sussmann et al. (2013)
Chicken drumsticks (skin-on bone-in)	Proteins	n/a	Homogenization, isoelectric solubilization	Isoelectric precipitation, pH 5.5, 10 min, 32-34°C or 4°C	51.9% extraction yield at 32-34°C; 29.7% at 4°C.	Tahergorabi et al. (2011)
Bovine and porcine lung	Proteins	323-649 g/kg lung	Mincing, alkali extraction	Isoelectric precipitation	Protein recoveries of 62.03% from bovine lung and 63.01% from porcine lung.	Lynch et al. (2018)
Egg white	Lysozyme	n/a	Dilution in buffer	Polyacrylic acid precipitation	Maximum recovery of 96%	Fisher and Glatz (1988)
Egg processing plant wastewater	Proteins, fats	1280-4313 mg/L (proteins), 1132-3892 mg/L (fats)	-	Lignosulfonate, CMC, bentonite, and $FeCl_3$ precipitation	Maximum recovery yields by precipitation agent: lignosulfonate, 90-95% (proteins), 92% (fats); CMC, 81-95% (proteins), 82-96% (fats); Bentonite, 90-95% (proteins), 90-96% (fats); Ferric chloride, 81-92% (proteins), 82-92% (fats).	Xu et al. (2001)
Mackerel whole fish	Proteins	14-16% w/w	Homogenization, ultrasound assisted extraction	Isoelectric precipitation	Maximum protein recovery yield of 74.3%	Álvarez et al. (2018)
Rainbow trout (*Oncorhynchus mykiss*) processing byproducts	Proteins	71.5% (dry-weight basis)	Trout mincing and homogenization with water, acidic (pH 2.5, 3.0) or alkaline (pH 12.0, 12.5, 13.0) solubilisation (aqueous phase)	Isoelectric precipitation (pH 5.5 for 10 min+ 1% beef plasma protein)	Maximum recovery yield of 90%	Chen and Jaczynski (2007)
Atlantic croaker (*Micropogonias undulates*)	Proteins	16.2% w/w	Blending, homogenization with water 1:9, protein solubilisation from pH 1.5 (acid-aided process) to pH 12.0 (alkali-aided process) in intervals of 0.5, centrifugation	Isoelectric precipitation (pH 5.5)	Maximum protein recovery yield of 78.7% after acid-aided process (pH 1.5).	Kristinsson and Liang (2006)
Channel catfish (*Ictalurus punctatus*)	Proteins	n/a	Grinding, homogenization with deionized water 1:9, protein solubilisation from pH 2.5 (acid-aided process) to pH 11 (alkali-aided process), centrifugation	Isoelectric precipitation (pH 5.5)	71.5% protein recovery after acid-aided process, 70.3% protein recovery after alkali-aided process.	Kristinsson et al. (2006)

terms of precipitation yields can be made. Protein recovery has also been studied for primary sewage sludge: Chishti et al. (1992) studied the use of several precipitating agents for protein recovery, ammonium sulphate being the most effective one. This conclusion agrees with that of García et al. (2017).

A summary of the studies consulted for this section, including source, precipitated compound(s), pre-treatment(s), precipitation method(s), and main results, is displayed on Table 2.

One of the main applications of proteins obtained by this kind of bulk extraction is animal feed. However, due to the presence of toxic components in sewage sludge, toxicity tests must be conducted to certify that this separation method generates edible, non-toxic proteins. Amongst the consulted works, only Hwang et al. studied the possibility of using precipitated protein for this purpose without further purification steps,

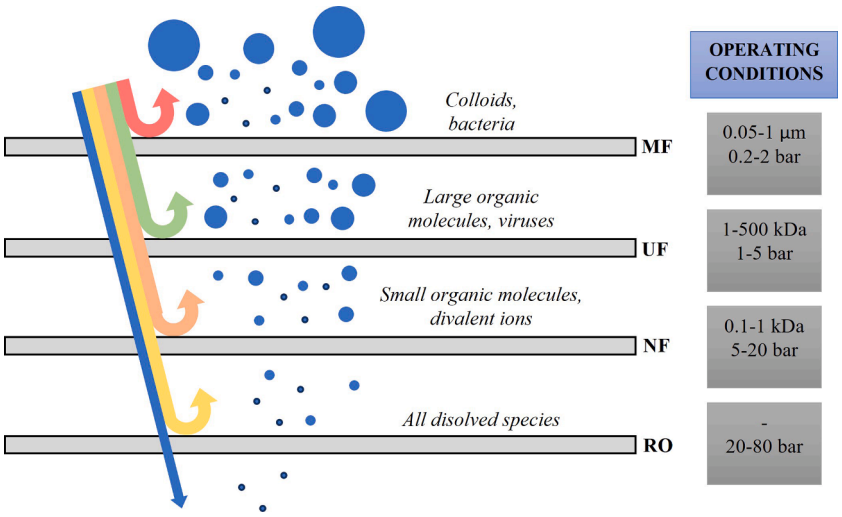

Fig. 2. Membrane pressure-driven processes (Cui and Muralidhara, 2010).

thereby concluding that its use as animal feed would be "technically feasible" (Hwang et al., 2008).

2.2. Precipitation in cell cultures and synthetic broths

Single-cell cultures are more easily comparable with WAS than the mixed-cell or pluricellular ones. Solubilisation of *Saccharomyces cerevisiae* and microalgae generate analogous biomolecules solutions. Protein recoveries reported by Boychyn et al. (2000) after ammonium sulphate and by Lorenzo-Hernando et al. (2019) after acidic precipitation are quite low (27.5% and 16.9% respectively). Besides, Boychyn et al. (2000) also achieved poor alcohol dehydrogenase (ADH) recoveries of 7.7% (see Table 3). However, these low yields could be attributed to unoptimized extraction methods (indeed, Lorenzo-Hernando et al. (2019) acknowledge that their study is still at a preliminary stage). For instance, Richardson et al. (1990) achieved maximum 100% ADH precipitation yield and near 80% of total protein precipitation yield from *S. cerevisiae* adding ammonium sulphate at 60% of saturation.

Pluricellular cultures were also tested for proteins or enzyme recovery, all of them consisting of *Aspergillus* sp. cultures (Costa et al., 2018; Nakkeeran et al., 2010; Netsopa et al., 2019, see Table 3 and Table A.1). Two methods previously untested in WAS were employed (ethanol precipitation and alginate affinity precipitation), achieving protein recoveries over 86% with ethanol precipitation. The high yields obtained with this method makes it appealing for application on WAS. As a starting point, it could be used ethanol concentrations from 78% to 92% (added dropwise), since they were reported as the most suitable ones, and it would be necessary the adjustment of the pH of WAS to obtain a slightly acidic one (5.5). The precipitation should be performed at low temperature (10-15°C) and for at least 3h.

2.3. Precipitation in farming and food-related sources

Information about biomolecules precipitation from several other sources is presented in Table 4 and Table A.2. Previous extraction is conducted in most of the studies to ameliorate protein purification. This strategy could be applied in WAS to solve the problem of protein-humic acid separation reported by García et al. (2017). Extraction methods used for this purpose were isoelectric (alkaline and acidic) solubilisation, sodium acetate buffer solution, water extraction and urea extraction.

As for the precipitation method, isoelectric precipitation was by far the most widely used. Other precipitation agents were ethanol, carboxymethylcellulose (CMC), ferric chloride, alginate, distilled water, polyacrylic acid, ammonium sulphate, manganese (II) chloride, carrageenan, a set of non-ionic and ionic surfactants, glacial acetic acid, lignosulfonate and bentonite. As seen in WAS, ammonium sulphate achieves high yields of protein recovery in all the analysed sources. Polyacrylic acid, sodium alginate and isoelectric precipitation also showed especially high yields of protein recovery (96%, 95.3% and up to 90%, respectively). Precipitating agents reported in those works should be studied for WAS to determine their eligibility in terms of cost and purification yield, taking as a starting extraction conditions the optimum ones used in them.

Protein extraction from dried WAS with hot alkaline water should be studied, since it is a simple way of separating protein from the matrix prior to its precipitation according to Wilken et al. (2016). The extraction yields obtained by these authors were not as high as it would be desirable (from 27.3 to 53.7% protein recovered, depending on the sample). However, this technique has been studied with a simpler substrate (defatted corn flour), therefore, the achievable extraction yield and purity of the protein obtained from WAS may be different.

Few studies have obtained protein recovery yields higher than those reported by Pervaiz and Sain (2011), in which 92% recovery was attained by alkali treatment and acidic precipitation at pH 3.0, and García et al. (2017), who achieved 87% recovery after TH pre-treatment and ammonium sulphate precipitation. Therefore, without considering the difficulties of the co-precipitation of proteins and humic acids, it seems that the matrix of WAS has not a remarkable effect on the protein extraction yield.

3. Membrane filtration

The use of membranes allows for the selective separation of stream components. Depending on the membrane characteristics, this separation may be driven by the molecular size, charge, concentration, chemical-physical properties, etc. (Saleh and Gupta, 2016). The most standardised membrane technologies are pressure-driven processes (Baker, 2012). Depending on the molecular weight cut-off (MWCO), i.e., the molecular weight at which 90% of the macromolecular solute is rejected by the membrane (Singh, 2014), this technology is denominated microfiltration (MF), UF, nanofiltration (NF) or reverse osmosis (RO) filtration (Fig. 2).

Membranes can be produced in a broad range of materials (ceramic, polymeric) and geometries (commonly flat, spiral wound, tubular or hollow fiber (Berk and Berk, 2009)) which determine operational characteristics, such as flux, lifespan, or selectivity (Gohil and

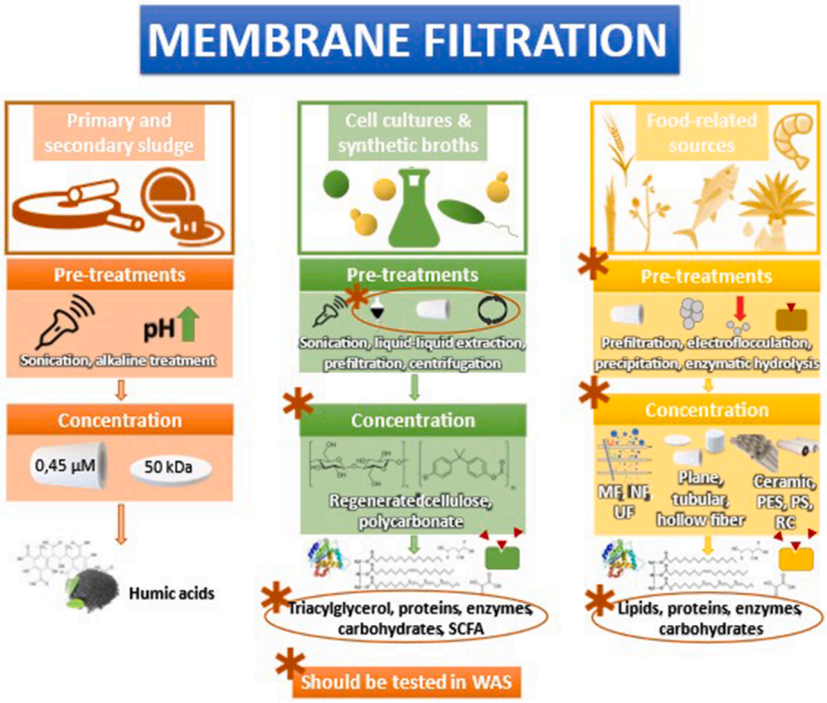

Fig. 3. Uses of membrane technology for the recovery of added-value biomolecules.

Choudhury, 2018; Kanani et al., 2010).

The fact that it is a clean technology with low energy costs, able to replace other conventional processes and couple it with other technologies as a hybrid process, has aroused great interest, finding application in a broad range of industries, such as food, agriculture, medicine, pharmacy, energetics, soil, or water technologies, among others (Saleh and Gupta, 2016).

The main drawback of this technology is membrane fouling. Membrane fouling is caused by physical, chemical or biological interactions between the foulants and the membrane, which eventually leads to a loss in membrane permeability and/or selectivity (Li and Chen, 2010). Understanding fouling mechanisms and foulants nature is critical to correctly deal with this major issue (Guo et al., 2012). This phenomenon occurs differently depending on the technology used. NF and RO have smaller pores, so the fouling will be more severe and they will normally require some sort of pre-treatment before operation, especially if working with highly contaminated streams (SAMCO, 2019), thus increasing the operating cost. These membranes with smaller pores demand higher pressures to filter the target stream while delivering lower fluxes of permeate, which results in a higher operating cost per litre of permeate obtained (SAMCO, 2017a, 2017b). In turn, these technologies allow to retain and concentrate molecules (or even ions) with smaller MWCO. Thus, a decrease in pore size is only justified when aiming to concentrate smaller biocompounds.

The use of membrane technology for the recovery of added-value biomolecules has been mainly studied as a means of concentration of a broad range of compounds from primary and secondary sludges, cell cultures, synthetic broths and food-related sources (Fig. 3).

3.1. *Membrane separation in WAS, cell cultures and synthetic broths*

Revalorization of WAS is mainly focused on the recovery of nutrients as ammonia or struvite through dialysis, NF or RO (Xie et al., 2016). Few works are focused on humic and fulvic acids, which are the only larger biomolecules whose recovery by membrane filtration has been studied (Table 5). The aim was not to purify the humic acids, but to concentrate them in order to obtain a fertilizer. Only four papers have focused on humic acid recovery from secondary sludge employing membrane separation, three of them being from the same first author. These three similar works aim to dewater sludge via alkaline treatment and concentrate humic acids by means of membrane filtration. To this end, Li et al. (2009) concentrated humic acid by 20-fold with a UF membrane (MWCO is not specified in the study), obtaining a more nutritional sludge humic fertilizer than other sludge fertilizers, and with a lower content of heavy metals. However, Li et al. (2014b) achieved a humic acid concentration far from the standard set by the Chinese Ministry of Agriculture (4.9 g/L vs 30 g/L required by the standard), so further treatment steps are needed to increase it by seven-fold. In this study, retentions of 96% for proteins, 63% for polysaccharides, 48% for nucleic acids, and 21% for fats were obtained. Nevertheless, the separation of these compounds was of secondary importance, for their concentrations (213.5, 760.7, 330.0, and 415.0 mg/L for protein, polysaccharide, fat, and nucleic acids, respectively), which were significantly lower than that of humic acids, were considered as an add-on to humic acid fertilizer composition. These results were validated by the same authors in another study (Li et al., 2014a).

On the other hand, Wei et al. designed an integrated process to recover proteins and humic acids from sewage sludge, which has already been partially discussed in the precipitation section. They studied recoveries at different pH values ranging from 1.0 to 4.0. pH values higher than 2.0 greatly hampered humic acids recovery, so this value was chosen as the most suitable in terms of yield and cost. They reported a humic acid recovery of 124.4 mg/g volatile suspended solids (VSS).

With regards to filtration characteristics, the studies reported by Li et al. (2009)and Wei et al. (2016) on the one hand, and Li et al. (2014b) and (2014a) on the other hand, used the same type of membranes. In both the first two studies, a tubular ceramic membrane with a mesh size of 0.45 µm, which falls more in the range of MF, was used, although both authors claim that they ultrafiltered the sludge. Otherwise, polysulphone (PS) flat sheet membranes were tested more thoroughly in Li et al. (2014b), (2014a) works, where MWCO of 1, 10, 30, and 50 kDa were tested. The 50 kDa membrane proved to be the most suitable regarding balance between dissolved organic carbon ratio of the retentate solution and the membrane flux.

Table 5
Consulted studies about membrane separation in WAS.

Source	Compounds	Content	Pre-treatment	Filtration conditions	Main results	Ref
Thickened activated sludge	Humic acids	24% w/w organic matter	Alkaline treatment, dewatering, pre-filtering with membrane with mesh size of 0.45 μm.	Porous tubular membrane Membrane area: 0.35 m^2 Transmembrane pressure (TMP): 1.0 MPa	20-fold concentration of humic acid.	Li et al. (2009)
Activated sludge	Humic acids	1199.1-1456.1 mg/L	Ultrasonication, protein precipitation	Porous tubular ceramic membrane TMP: 1.0 MPa. Mesh size: 0.45 μm	Maximum recovery rate of humic acid during the co-recovery of 154.1 mg/gVSS at pH 1.	Wei et al. (2016)
Activated sludge	Humic acids	1467.3 mg/L	Alkaline treatment, centrifugation	Flat sheet membranes MWCO: 1, 10, and 50 kDa TMP: 0.2 MPa.	3 to 3.5-fold concentration of humic acid (4239 mg/L).	Li et al. (2014b)
Mixed primary and secondary sludge	Humic and fulvic acids	1913.8-2535.1 mg/L (humic acids), 238.8-316.3 g/L (fulvic acids)	Alkaline treatment, centrifugation	Flat sheet membranes MWCO: 1, 10, 30 and 50 kDa TMP: 0.2 MPa. Volume concentration ratio (VCR): 5.	Membranes with MWCO of 30-50 kDa are more appropriate than those with lower MWCO for the recovery of macromolecular organic substances.	Li et al. (2014a)
Microalgae	Triacylglycerol, proteins and carbohydrates	251 mg/g dry cells (triacylglycerols), 302 mg/g dry cells (proteins), 83 mg/g dry cells (carbohydrates).	Cell disruption by ultrasonication, pigment and triacylglycerol extraction in organic solvent	Membranes: Al_2O_3, 600 kDa, tubular; TiO_2, 60 kDa, tubular (ceramic); PS, 600 kDa, plane; RC, 100 kDa, plane; RC, 30 kDa, plane (polymeric) plane membrane filtration area 12.56cm2, tubular membrane: filtration area 18.68cm2 TMP: 0.4 bar T: 25°C VCR: 2	Membrane used for fractionation was RC, 30 kDa, plane. Recovery of carbohydrates (88%) and proteins (68%) in the retentate. Recovery of triacylglycerol in the permeate (recovery factor of 60%, purity of ~70%).	Djamai et al. (2019)
Solid-state cultures of *Aspergillus carbonarius*	Enzymes (polygalacturonase)	2450 U/mg	Extraction, alginate affinity precipitation	Integrated membrane processing 3 membranes studied: MF: Hydrophilic amphoteric nylon membrane, pore size 450 nm UF: Hydrophilic plane membranes with PS (MWCO of 50 kDa) and PES (MWCO of 10 kDa) as active layer/coating and polypropylene as support layer, effective area of 15 cm2 TMP: 0.1 MPa (MF); 0.5 MPa (UF) T = 25 °C VCR: 10	80% cumulative recovery of polygalacturonase after integrated process, 72% of protein, 99% of carbohydrates	Nakkeeran et al. (2010)
Fermentation broth	Enzymes (surfactin)	596 mg/L	Centrifugation	Two-step UF, with (1st mode) and without (2nd mode) cleaning the membrane between UF steps. Membranes tested: RC, 10 kDa, effective area 50 cm2 PES, 10 kDa, effective area 50 cm2 TMP: 1.5, 2.0 and 2.5 bar (1st mode), 2.0 bar (2nd mode) Room temperature	Total recoveries of 94% for PES 10 kDa and 92% for RC 10kDa after UF-2. Applied TMP or mode of filtration had no significant effect in the selectivity of filtration. PES membrane showed higher recovery and similar purity compared to RC.	Isa et al. (2008)
Fermentation broth	Succinic acid	n/a	UF preclarification Ion exchange	NF Polyamide (active layer) and PS flat sheet membrane, 150-300 Da, effective area 155 cm2 TMP: 1.6 MPa T: 25°C ± 2°C. Flow rate: 160 L/h	Retention of succinic acid of 92%	Antczak et al. (2018)

(*continued on next page*)

Table 5 (*continued*)

Fermentation broths	Lactic acid	84.30-86.40 g/L	Precipitation, centrifugation	Protein removal from the cell-free broth by sequential UFs (30 kDa, 5 kDa, and 1 kDa). Lactic acid separation and concentration by in-series RO.	Recovery of 100% of lactic acid with 97% purity.	Phanthumchinda et al. (2018)
Humic acid solutions	Humic acids	n/a	-	Polymeric membranes tested: YM2 (flat hydrophilic, 1 kDa, 45 cm2), PM10 (flat, lipophilic, 10 kDa, 45 cm2), YM100 (flat, hydrophilic, 100 kDa, 45 cm2), H10P3-20 (hollow fiber, PS, 3 kDa, $9\bullet10^{3}$ ^{cm2}).	Retentions of 80-90% for humic acid, 60-70% for fulvic acid and 40-70% for calcein.	Küchler and Miekeley (1994)

Based on these studies, the use of UF with low MWCO, between 1 and 10 kDa, has the advantage of obtaining slightly higher retentions (up to 79%) than those attained with higher MWCO, from 30 to 50 kDa (~70%). This indicates that humic acids mainly present a MW higher than 50 kDa. Thus, the assessment of NF and RO for the recovery of this macromolecular compound is considered not necessary. Besides, the membrane fluxes decrease significantly when MWCO of 1 and 10 kDa were used, the values being from 3 to 4 times lower than those obtained with MWCO of 30 and 50 kDa. Therefore, low MWCO has the disadvantage of operational efficiencies, since the increase in the humic acid retention does not justify the significant decrease in membrane flux. Recovery of triacylglycerol, proteins and carbohydrates from a similar source (microalgae) was reported by Djamai et al. (2019). In their study, several membranes were tested, and it was disclosed that regenerated cellulose (RC) membrane (30 kDa) was less prone to fouling. Dumay et al. (2008), who studied the concentration of lipids and peptides from surimi manufacturing washing waters, also concluded that recovered cellulose (10 kDa) was the best performing membrane among those studied, due to its higher efficiency and regenerability. Isa et al. (2008) also found that polycarbonate membrane was easier to clean compared to polyethersulphone (PES) membrane when purifying surfactin from fermentation broth, although it showed lower recoveries.

Taking into account these studies, UF can be considered the suitable membrane technology for the recovery of proteins, carbohydrates and lipids since yields between 70 and 98% were obtained. Polymeric membranes were found to be more adequate than ceramic ones since they allowed to obtain higher biocompound yields. Besides, membrane fouling can also be controlled as function of the membrane material selected. In this sense, the use of regenerated cellulose showed the advantage of the better regenerability and less susceptibility to fouling than other polymeric materials tested, including PES, polyacrylonitrile and polyvinylidene fluoride. Regarding MWCO, the most appropriate varied from10 to 40 kDa in order to reach a compromise between recovery yields and flux decrease. It should be noted that with a MWCO of 3 kDa, the flux was half the one obtained with 10 kDa and the improvement in recovery was minimum. For this reason, the analysis of NF and RO is considered not needed since the increase in the recovery would not justify the greater fouling and the corresponding decrease in flux.

Although the literature about solubilised/liquefied WAS is very limited, its high content in natural organic matter is expected to cause difficulties in membrane operation, in terms of flux performance and fouling, since it is reported as the main foulant in surface waters (Fane et al., 2006). Therefore, it will be necessary to establish a cleaning strategy to lessen the organic matter effect and enhance productivity. To that end, physical cleaning should be used to reduce membrane fouling and the frequency of chemical cleaning, which extends membrane lifetime and decreases operating costs. Backwashing, backpulsing, crossflushing, sponge ball cleaning and air sparging are the most widely employed methods of physical cleaning (Gao et al., 2019). It was reported that backpulsing cause the detaching of the trapped foulants and backwashing remove them from membrane surface and pores (Fraga et al., 2017). Thus, the combination of backpulsing and backwashing can provide a synergistic effect on membrane cleaning (Fraga et al., 2017; Hau and Leung, 2016).

3.2. *Membrane filtration in other industries*

Although the studies conducted on WAS only contemplated the recovery of humic acids through UF, the works which employed other source streams report a broader range of potential target biomolecules. Proteins, enzymes, fats and carbohydrates (apart from humic acids) have been successfully recovered from different vegetal and animal wastewaters (Table 6 and Table A.3).

Carbohydrates, proteins and humic acids were all recovered in the retentate. The role of membrane filtration in the recovery of these molecules from WAS could be to concentrate them prior to a subsequent purification step, or the fractionation of the stream using membranes of different MWCO to obtain solutions concentrated in the different species.

Due to the particular characteristics of the streams reviewed in this sub-section, some of the pre-treatments used may not be applicable to WAS filtration (e.g., as Dumay et al. work with a sludge consisting mostly of proteins generated during surimi manufacturing, they perform a hydrolysis with proteases prior to a subsequent UF). On the other hand, several pre-treatments here reviewed may serve as a pre-treatment for reducing membrane fouling and improving the efficiency of this operation, namely centrifugation, prefiltration, protein extraction or electroflocculation.

As the industrial application of centrifugation is hindered by its high cost (Najjar and Abu-Shamleh, 2020), its use as a pre-treatment stage before WAS filtration would not be competitive against prefiltration. Prefiltrations, either surface or depth filtrations (with sand and stone beds), as tested by Wu et al. (2007) and Mohammad et al. (2009), are aimed to remove larger suspended residues, so membranes with lower cut-off would be more adequate as those tested by Li et al. (2009). Since only one mesh size has been evaluated in WAS, the cut-off size of the pre-filtration membrane could be optimised. This pre-filtration is not considered a high energy-consuming stage, as the temperature and pressure set for it can be maintained during subsequent filtrations.

As discussed in the previous section, an aqueous protein extraction from dried WAS together with its concentration by membrane filtration could be a feasible way of fractionating the sludge. Thus, proteins would be recovered before concentrating them through membrane filtration and then subsequent precipitation could be applied.

Electroflocculation can also be used as a low-cost pre-treatment prior

Table 6
Consulted studies about membrane separation in other industries.

Source	Compounds	Content	Pre-treatment	Filtration conditions	Main results	Ref.
Barley (*Hordeum vulgare*)	Enzymes (β-glycosidases)	82.23 U/mL (β-galactosidase), 40.64 U/mL (β-glucosidase)	Preparation of enzyme extract through extraction in 0.1 M, pH 6.0 ammonium acetate buffer.	Plane PES membrane, 100 kDa, effective filtration area of 50 cm2. 2 modes of operation: 1) Concentration followed by diafiltration, 2) diafiltration followed by concentration TMP: 0.6 bar T: 25 ± 2°C VCR: 3	Best results: Mode 1) β-glucosidase was purified 4.38-fold and concentrated from 40.64 to 111.87 U/mL. Mode 2) β-galactosidase was purified by 4.56-fold and concentrated from 82.23 to 236.03 U/mL.	Hemavathi and Raghavarao (2011)
Palm oil mill effluent	Proteins, carbohydrates	12.9 g/L (proteins), 28.9 g/L (carbohydrates)	Prefiltration through stones and sand filter beds, and surface filtration with paper of 8 μm pore size.	PS plane membrane, 20 kDa, effective membrane area15.2 cm2 TMP: 0.8MPa T: 25 °C (±2 °C).	Recovery of protein and carbohydrate up to 61.4% and 76.4%, respectively	Wu et al. (2007)
Palm mill oil effluent	Proteins	91.4 g/L	Physical pre-treatment processes (depth and surface filtrations) and MF process.	3 membranes tested: PS UF membrane, 20 kDa PES membranes, 10 and 2 kDa TMP: 1-10 bar. T: 25 °C (±2 °C).	Best performance at highest MWCO and TMP (20 kDa, 10 bar) Maximum reduction of total suspended solids, turbidity, chemical oxygen demand, total dissolved solids of 98.3%, 96.2%, 82.0%, 41.2%, respectively. Maximum protein recovery of 98.3%, 96.2%, 82.0%, 41.2% and 78.0%, respectively.	Mohammad et al. (2009)
Isolated soy protein production wastewater	Proteins	1870 mg/L		3 monotubular ceramic membranes: 5, 20 and 50 kDa; membrane area of 0.0047 m^2. TMP: 1-8 bar Crossflow velocity (CFV): 2.4 m/s T: 25°C	Best results with 5-kDa membrane, showing the least reduction in permeate flux over time and the highest retention percentages of protein (52%).	Cassini et al. (2010)
Poultry processing wastewaters	Proteins	20.15 g/L	MF	2 membranes used: RC, spiral-wound membranes, 3 and 30 kDa, area of 0.92 m^2. TMP: 200 ± 15 kPa CFV: 2.5 ± 0.2 m/s. T: 20°C	Total recovery of soluble proteins as well as the average degree of concentration amounted to 84% and 9.3, respectively using crossflow filtration.	Białas et al. (2015)
Marinated herring (*Clupea harengus*) brine	Proteins and fatty acids	6.99% w/w (proteins), 0.96% w/w (fatty acids)	Electroflocculation, prefiltration	Tubular SiC membrane, pore size 0.040 μm, filtration area 0.09 m^2 TMP: 2-2.6 bar T: from 5-7 °C to 24-26 °C CFV: 2 m/s	75-82% (<62.7 mg/mL) of the protein and 75-100% of the fatty acids retained	Gringer et al. (2015)
Fish meal effluent	Proteins and oil	1.2-8.1 g/L (proteins), 0.39-20.5 g/L (oil)	MF	Mono-tubular mineral (thin deposit of ZrO-TiO, on a carbon support) membrane, 15 kDa,. Surface area 0.0226 m^2. TMP: 4 bar CFV: 4 m/s T: 20°C	Maximum retentions of 40.0% and 26.0% for oils and proteins, respectively. Lower protein rejections after concentration to VCR=17 (11.5%).	Afonso and Bórquez (2002)
Washing water from surimi manufacturing	Proteins and lipids	26.5 g/L (proteins), 1.8 g/L (lipids)	Centrifugation, enzymatic hydrolysis	Polymeric plane membranes tested, membrane surface 100 cm2. Materials: PES, polyacrylonitrile, polyvinylidene fluoride and RC. MWCO: 3, 10, 40, 50, and 100 kDa. TMP: 0-3 bar T: 15°C CFV: 1.7 m/s	10 kDa RC membrane had the highest performance and was further evaluated. COD reduced by 75%. Recovery rates of 98.0% for lipids and 79.9% for proteins with RC membrane (10 kDa). VCR of 3.8	Dumay et al. (2008)

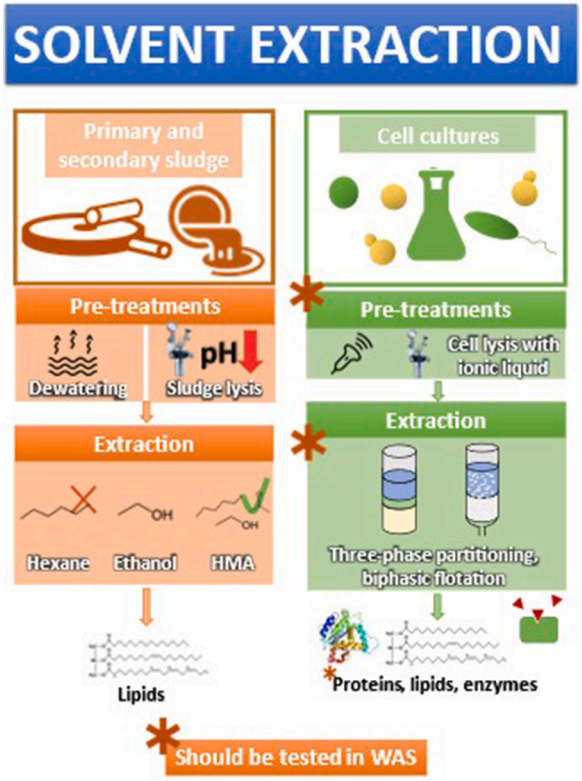

Fig. 4. Uses of solvent extraction technology for the recovery of added-value biomolecules.

to sludge filtration, with the aim of reducing membrane fouling. It has yet to be tested in WAS to determine if this treatment is more cost-effective than prefiltration or centrifugation (Gringer et al., 2015).

In-depth studies on membrane performance should be conducted to test different materials (only PS membranes have been tested within the UF range) and geometries (hollow fiber, tubular…), as well as to characterize and model fouling and to optimize the operating conditions in order to maximize permeate fluxes. The suitability of other pre-treatments, such as the WO discussed in section 2, should also be evaluated.

4. Solvent extraction

Solvent extraction mechanism relies on the difference of solubility of a solute between two immiscible or lowly miscible solvents. During this operation, a solvent with higher solubility is put in contact with the phase where the solute is initially present, and so the solute migrates to the solvent with higher solubility (Clarke, 2013). Depending on the state of aggregation of the two phases, the solvent extraction is named solid-liquid extraction if the solute is contained in a solid matrix and extracted with a liquid solvent, or liquid-liquid extraction if both the matrix and the solvent are in liquid state. Solvent extraction presents advantages, such as better separation effect than precipitation, higher selectivity and mass transfer velocity than ion exchange, or lower energy consumption and easier large continuous operation than distillation (Chen and Wang, 2017). A correct choice of the solvent is critical in the suitability of the operation and must take into account factors, such as the distribution coefficient, the immiscibility with the contrary liquid phase, the ease of recovery and cost (Sprakel and Schuur, 2019).

Besides the conventional methods of liquid-liquid extraction, new methods combining solvent extraction with other technologies have been developed (Chen and Wang, 2017), mainly liquid biphasic flotation systems, a bubble-assisted, gentler and greener L-L extraction technique (Khoo et al., 2020; Leong et al., 2019). These technologies have not yet been studied on WAS, but only on other cell cultures (Fig. 4).

4.1. Solvent extraction on WAS

Solvent extraction on WAS has been studied exclusively for the recovery of lipids (Table 7). In all the consulted literature, pre-treatments, such as centrifugation, sun drying, oven drying, or pressure filtration were conducted in order to dewater the WAS to some extent prior to the extraction. Besides these pre-treatments, the influence of acidification or sub-critical water pre-treatments was also studied. The work of Olkiewicz et al. (2014) analysed the influence of the drying method and acidification in lipid extraction efficiencies from primary, secondary and blended sludges. $MgSO_4 \bullet H_2O$ drying, oven drying at 105 and 70 °C, freeze-dryer drying, fume hood drying and sun drying methods were tested, the $MgSO_4 \bullet H_2O$ being the method that achieved the best moisture removal and lipid yield, thereby disclosing that moisture levels directly affect the extractability of the lipids. As for the acidification pre-treatment, it increased lipid extractability in all cases, although in WAS the resulting improvement was low (from 5.1to 6.3% of lipid yield). Huynh et al. (2010) studied the effect of sub-critical water pre-treatment of powdered and oven dried WAS (which was also mixed with diatomaceous earth to improve the solvent flow through the sample) on lipid extraction efficiency, using hexane as the solvent. Sub-critical water pre-treatment increased four times the amount of extractable neutral lipids.

Hexane, methanol and acetone were employed as solvents for lipid extraction. The more exhaustive comparison between these three solvents was carried out by Dufreche et al. (2007). In this study, hexane, methanol, and a 60% hexane/20% methanol/20% acetone mixture (HMA) were tested as solvents at 10.3 MPa and 100 °C for 1 h. The extraction yields achieved with pure hexane were really small (1.94%), and 10 times lower than those achieved with pure methanol (19.39%) and HMA (21.96%). Such low yields were reported by all the authors that worked with hexane: Olkiewicz et al. (2014) obtained a maximum yield of 6.3% after 9 extraction stages at room temperature, with 1:1 ratio and for 20 min; Huynh et al. (2010) achieved a maximum yield of 7.87% with a Soxhlet extraction; Melero et al. (2015) reported negligible yields (less than 1%) at ~65 °C during 2.5 h. Methanol gave better results as reported by Dufreche et al. (2007) (19.39%, as mentioned above), but gave lower yields based on the studies by Melero et al. (2015), with only 2.1% at ~65 °C during 2.5 h; and by Revellame et al. (2011), with a maximum yield of 3.93% at 75°C, using a solvent ratio of 30mL methanol:g sludge and 10% (volume) of catalyst concentration.

Apart from the abovementioned chemical or hydrothermal pre-treatments, WAS dewatering has been studied by several authors before solvent extraction. Sludge dewatering is the bottleneck of WAS management, and many efforts have been made towards developing cost-effective methods that significantly reduce the amount of water on WAS. Due to its particular structure, it is estimated that only 15–30% of WAS water content can be removed with mechanical methods, such as decantation (Wang et al., 2009). Direct thermal drying of sludge has been implemented at industrial level, although the very high energy demand of this technology has restricted its use (Wang et al., 2009). Nonetheless, new drying methods, such as microwave drying (Kocbek et al., 2020), have been promisingly researched.

4.2. Solvent extraction on cell cultures

The study of solvent extraction of biomolecules from microorganisms is better developed for microalgae (*Arthrospira platensis, Chlorella vulgaris, Chlorella pyrenoidosa, Chlorella sorokiniana* and *Nannochloropsis gaditana*) and bacteria (*Burkholderia cepacian*) than for WAS. Most of the reviewed papers focused on the recovery of proteins (9 to 6 that study the recovery of lipids), although two thirds of these works reported on liquid biphasic flotation (Table 8). For the extraction of lipids, n-hexane

Table 7
Consulted studies about solvent extraction on WAS.

Source	Compounds	Content	Pre-treatment	Extraction conditions	Main results	Ref.
Primary, thickened secondary and blended sludges	Lipids	26.3% dry-weight basis (primary sludge), 7.7% dry-weight basis (WAS), 21.1% dry-weight basis (blended sludge)	Acidification and drying or acidification.	Solvent: hexane. 2 methods: a) Soxhlet extraction of dried sludges. b) Sequential 9-stage liquid-liquid extraction with mechanical agitation at room temperature.	Primary sludge: a) 26.3% recovery yield. b) Maximum recovery yield of 29.6% (dry weight basis) with sludge to hexane ratio 1:2. Secondary and blended sludges: Absolute yields of 19.1% for blended sludge and of 6.3% for secondary sludge after 9 extraction stages (conditions: 1:1 sludge to hexane volume ratio, each stage extraction time — 20 min.)	Olkiewicz et al. (2014)
Dewatered activated sludge	Lipids	2.10-7.87% (w/w)	Sub-critical water pre-treatment.	Soxhlet extraction. Solvent: hexane.	Oil recoveries of 2.10% and 7.87% (dry weight basis) without and with sub-critical water pre-treatment, respectively.	Huynh et al. (2010)
Secondary sewage sludge	Lipids	n/a	Dewatering by centrifugation or pressure filtration and posterior Hydromatrix addition.	Extraction at 10.3 MPa and 100 °C for 1 h. Different solvents used: 1. 60% hexane/20% methanol/20% acetone (HMA) (same mixture three times); 2. Pure methanol followed by pure hexane (MH); 3. Pure hexane (single extraction); 4. Pure methanol (single extraction). Solvent to solid ratio 40:1 g/g	Gravimetric yields of oil in grams of oil per gram of dry sludge: 1. HMA extraction 1 yield=21.20%, total yield (27.43 ± 0.98)%. 2. Pure methanol extraction yield: 19.39%, total yield (21.96 ± 2.28)% 3. Pure hexane extraction yield: 1.94%. 4. Pure methanol extraction yield: (19.39 ± 3.20)%.	Dufreche et al. (2007)
Primary and secondary sewage sludge	Lipids	n/a	Dewatering by settling and centrifugation, oven drying and milling into powder	Tested solvents: n-hexane, methanol. Extraction time: 2.5 or 4 h. Sewage sludge to solvent ratio: 10 g:100 mL or 10 g:150 mL. T~65°C	No substantial differences at different extraction conditions of time and sewage sludge to solvent ratio. Lipid extraction yields (based on the starting dry sludge): Primary sludge, n-hexane: 7.4 wt% Primary sludge, methanol: 13.6wt% Secondary sludge, methanol: 2.1wt %	Melero et al. (2015)
Activated sludge	Biodiesel (in-situ transesterification)	n/a	Gravity-settling overnight, followed by centrifugation	Optimization of conditions: T from 45 to 75 °C; methanol to sludge (solids) ratios from 5 to 30 mL/g; catalyst concentrations from 1 to 10% (based on volume of methanol).	Maximum yield of 3.93 ± 0.15 wt% at 75°C, 30 mL/g methanol:sludge ratio and 10% volume of catalyst concentration.	Revellame et al. (2011)
Primary sludge	Lipids, proteins	n/a	Oven drying	Solvents: [C_4mim][$MeSO_4$] and [P($CH_2OH)_4$]Cl. Conditions: 1 g TS equivalent to 10 cm3 ionic liquid ratio, 100 °C, 24 h, with stirring.	Higher yields obtained with raw sludge. Extraction yield of 26.9 ± 1.0 (g/100g dry sludge) obtained with [C_4mim][$MeSO_4$] and 27.6 ± 0.6 with [P($CH_2OH)_4$]Cl vs 27.2 ± 0.4 obtained with standard Soxhlet method. Protein extraction of 16.6 ± 1.2 g/100g dry sludge) with [P($CH_2OH)_4$]Cl.	Olkiewicz et al. (2015)

was used after cellular lysis with ionic liquid (Lu et al., 2019) or steam explosion (Lorente et al., 2017). Ionic liquids ([C_4mim][$MeSO_4$] and [P($CH_2OH)_4$]Cl) were scarcely used as extractants, as only (Olkiewicz et al., 2015) employed them for the recovery of proteins and lipids from primary sludge (as mentioned above, Lu et al. (2019) used [BMIM]Cl to lysate the cell prior to extraction with n-hexane). This technique has not been tested as a lysis pre-treatment for WAS; nonetheless, ionic liquids are from 2 to 8-fold more expensive than regular organic solvents, and some of them are toxic, preventing the recovered biocompounds from being subsequently used for nutritional purposes or as animal feed.

Regarding more novel techniques, one study (Chew et al., 2019) dealt with a microwave-assisted three-phase partitioning, employing t-butanol as solvent and completing the three-phase system with ammonium sulphate. As for biphasic flotation extractions, several combinations of solvents were assayed, all of them obtaining high yields except for Sankaran et al. (2018b), which only achieved 23% protein recovery yield.

In the light of these data, it can be deduced that hexane is not a suitable extractant for lipids from secondary sludge, whereas methanol provides higher rates only under harsh conditions (~100 bar and

Table 8
Consulted studies about solvent extraction on cell cultures.

Content	Source	Compounds	Content	Pre-treatment	Extraction conditions	Main results	Ref.
	A. platensis (spirulina)	Proteins	n/a	Sonication, manothermosonication	Solvent: sodium phosphate buffer (0.1 M; pH 7.0). Ratio biomass/solvent 1:20 (g/g), pressure 1 or 3 bar, T 10 or 30 °C, ultrasonic intensity 20 or 60 W/cm^2	Maximum protein recovery yield of 28.42 ± 1.15 g/100 g DW achieved with 2 bar, 24°C and ultrasound intensity of 55 W/cm^2.	Vernès et al. (2019)
	C. vulgaris	Proteins	n/a	-	Microwave-assisted three phase partitioning (MWTPP): ammonium sulphate-t-butanol (solvent)-protein solution Conditions for MWTPP (optimized): 30%w/w ammonium sulphate; 0.5%w/w microalgae solution; 1:1 vol ratio; 120 s microwave time, 80% duty cycle; 100W	Yield of protein: 63.2%. Separation efficiency: 67.2%	Chew et al. (2019)
	C. pyrenoidosa sludge	Lipids	10.3% (dry-weight basis)	Lysis with [BMIM]Cl.	2-step extraction: n-hexane in 2:1 ratio for 30 minutes; ethyl alcohol 2:1 ratio.	The average lipid yield of 89.3% using the recycled [BMIM]Cl.	Lu et al. (2019)
	N. gaditana microalgae	Lipids	22.2% (dry ash free-weight basis)	Steam explosion (150 °C, 5% sulfuric acid), optional prefiltration with 5000 Da membrane	Solvent: n-hexane, ratio 1:1. Experimental conditions; 60°C, 2h, with stirring.	Pre-treatment with prefiltration was preferred. 17.6% lipid recovery (w/w, DAF of untreated microalgae basis, 79% of the total lipid).	Lorente et al. (2017)
	C. vulgaris FSP-E strain	Proteins	n/a	Sugaring out, sonication	Liquid-liquid flotation using glucose and acetonitrile	Lab scale: 86.38% efficiency and 93.33% yield at 0.6% biomass concentration, 200 g/L of glucose concentration, 100% acetonitrile concentration with 5 min of 5s ON/10s OFF pulse mode and at a flow rate of 100 cc/min. Large scale: 85.25% efficiency and 92.24% yield.	Sankaran et al. (2018a)
	B. cepacia	Enzyme (lipase)	n/a	-	Liquid-liquid flotation using Triton X-100 and xylitol	Average lipase separation efficiency and yield of 86.46 and 87.49% with 25% w/w of xylitol concentration, 15% (w/w) Triton X-100, 80% w/w of crude lipase, 4 mL of top phase, 35 mL of bottom phase, pH 7 and 15 min of flotation time.	Sankaran et al. (2018c)
	C. sorokiniana CY-1 strain	Proteins	57% (dry-weight basis)	-	Liquid-liquid electric flotation using 1-propanol and dipotassium hydrogen phosphate	23.41% recovery and 173.08% separation efficiency with 60% (v/v) of 1-propanol as top phase, 250 g/L of dipotassium hydrogen phosphate as bottom phase, crude microalgae loading of 0.1 g, air flowrate of 150 cm3/min, flotation time of 10 min, voltage of 20 V and electrode's tip touching the top phase of LBEF.	Sankaran et al. (2018b)
	C. sorokiniana CY 1	Proteins	n/a	-	Liquid-liquid flotation using glucose and acetonitrile assisted by ultrasonication	81% yield with 200 g/L glucose as bottom phase with volume ratio of 1:1.25, 10 s of resting time for ultrasonication, 5 s of ultrasonication in pulse mode and 0.25 g of biomass (dry weight basis).	Chia et al. (2019)
	C. vulgaris FSP-E	Proteins	>70%	-	Sugaring-out assisted liquid biphasic electric flotation using sugar and acetonytrile	Separation efficiency of 73.999% separation efficiency and 69.665% yield with 0.05 g of microalgae biomass, 15 V of DC current supply with tip of the electrode at the bottom phase, 300 g/L glucose and CAN concentration of 100%, air flowrate of 150 cm3/min, flotation time of 15 min.	Koyande et al. (2019)
	C. sorokiniana CY-1	Proteins	33.70% w/w	-	Liquid biphasic flotation with the aid of ultrasonication using ammonium sulphate and propanol	97.44% separation efficiency and 88.86% yield with 250 g/L ammonium sulphate, 60% (v/v) 2-propanol, 1.0 VR, initial, 20 g/	Phong et al. (2017)

(*continued on next page*)

Table 8 (*continued*)

				L crude biomass load, 4 mm^3/ min air flowrate and 10 min of flotation time.		

Table 9
Consulted studies about solvent extraction on animal and vegetal sources.

Source	Compounds	Content	Pre-treatment	Extraction conditions	Main results	Ref.
Moringa oleifera seeds	Proteins, lipids	n/a	Drying, hulling and grinding	Aqueous extraction at 30°C for 2 h with ethanol and petroleum ether (sample to solvent ratio 8:10).	33% protein recovery, 22.3% lipid recovery	Chen et al. (2019)
Acacia tortilis seeds	Proteins	37.5%	Grinding, defatting	2 extractions: 95% ethanol extraction and 1 M NaOH extraction with sample to solvent ratio 1:20 (w/v), room temperature, 1 h (EtOH) or 2 h (NaOH) at pH 11.	Yield of protein: 63.2%. Separation efficiency: 67.2%	Embaby et al. (2018)
Grass pea flour	Protein	26.16%	Defatting, sieving	Extraction with 1 M NaOH with sample to solvent ratio of 1:15 (w/v) at room temperature and pH 9.96	14.25% recovery	Feyzi et al. (2018)
Mung bean	Proteins	23.73% (dry-weight basis)	Hulling, grinding, sieving	Extraction with KOH with sample to solvent ratio of 20 mL/g at 40 °C and pH 9.1	77.32% yield	Du et al. (2018)
Defatted peanut meal	Proteins	53.02%		Extraction with deionized water, 0.2 M KOH, NaOH, 0.2 M NaCl or $NaHCO_3$, with sample to solvent ratio of 1:20 (w/v) for 1 h at 52°C and pH 9-10	85.2% protein yield obtained using 0.2 M NaOH	Uddin et al. (2018)
Defatted rice bran	Protein	16.75% (dry-weight base)	-	Extraction with 1 M NaOH with sample to solvent ratio 6:34 (w/ v) for 300 min at 52°C and pH of 10	34.51% recovery	Bernardi et al. (2018)
Perilla frutescens flour	Lipids	40.06%	-	Extraction with liquefied n-propane at 40°C and 8 MPa for 80 min	34.78% yield	Da Silva et al. (2015)
Sesame seed	Lipids	52.6% (w/ w)	Drying, milling	Extraction with liquefied n-propane at 60°C and 12 MPa for 55 min	34.1% yield	Corso et al. (2010)
Sunflower seeds	Fatty acids	53.4% (w/ w)	Grinding	n-Butane at 40°C and 370 kPa	36.9% yield, solvent-free and food grade	Rapinel et al. (2017)
Chicken liver	Proteins	-	Blending, degreasing, drying	Alkaline (0.80% NaOH, sample to solvent ratio 1:70 [w/ v], 50°C, 5h) and ultrasound-assisted alkaline (0.80% NaOH, 40°C, on-time 2s, off-time 3s pulses, 24 kHz, 300W) extractions	67.6% and 43.5% yield for ultrasound-assisted and alkaline extractions, respectively.	Zou et al. (2017)
Mackerel fish	Proteins	14-16% (w/ w)	Blending	Acidic, alkaline (HCl or NaOH 0.1-0.4 M, sample to solvent ratio 1:10, 4°C, 10 min) and ultrasound-assisted (HCl or NaOH 0.1 M, sample to solvent ratio 1:10, 4°C, 750W, 20 kHz [5s on-5s off], 60% amplitude, 10 min) extractions	94.71% yield with ultrasound assisted NaOH 0.1 M extraction, vs 74.25% recovery with NaOH 0.4 M	Álvarez et al. (2018)
Egg yolk	Lipids	58.26% (w/ w)	Spray-drying	Subcritical fluid-propane extraction (solid-liquid ratio of 1:9 (g/mL), 40°C, 120 min)	63.88% extraction yield	Su et al. (2020)

100 °C). The efficiency of other solvents should be tested, whether they are traditional ones, such as chloroform; or novel ones, such as ionic liquids. An optimization of the extraction conditions should be conducted in addition to a hydrolytic pre-treatment that unbinds fats from protein, carbohydrate and/or minerals, since it has been reported that these bindings prevent solvent alone to fully recover lipids from WAS (Luthria and Anderson, 2004). Other hydrolytic or disruptive pre-treatments should also be studied, such as steam explosion, lysis with ionic liquid, sonication, TH, or WO. Also, the extraction of other biomolecules, such as proteins or humic acids, should be explored, as well as the application of newer technologies, such as biphasic flotation to reduce costs and energy expenditures.

Results reported by Huynh et al. (2010) showed that the physico-chemical characteristics of WAS hindered the lipid extractability during a S-L extraction, as the lipids are mainly embedded in the membrane cell. Accordingly, a previous cell lysis pre-treatment (as sub-critical water treatment in the case of Huynh et al.) can significantly improve the extraction yield. It can also be seen that neither proteins (in algae) nor humic acids (in landfill leachate) affect the extractability of oils and lipids during a L-L extraction, so this technique is also expected to be efficiently applicable in lipid extraction from WAS. It remains uncertain if humic acids could interfere with proteins during three-phase partitioning or other extraction methods, and research is needed at this point to clarify the feasibility of protein extraction from WAS in an efficient and selective way.

4.3. Solvent extraction from animal and vegetal sources

Recent research in solvent extraction from vegetal sources has paid more attention to protein recovery (Table 9). In this sense, ethanol and alkaline solutions were studied as solvents. As the yields obtained highly differ depending on the source, aqueous protein solution should be tested in WAS to determine its feasibility. However, in the study by Uddin et al. (2018), in which different aqueous solvents for protein extraction were evaluated, the highest recovery yield (85.2%) was achieved with NaOH solution, this being in accordance with the results obtained by Álvarez et al. (2018).

On the other hand, extraction with liquefied gas has been recently tested as a green extraction method (Chemat et al., 2020). However, the application of this technology in an integrated process may be hindered by the need of pressurizing, which can increase the total cost of the operation.

Research on solvent extraction from animal sources is scarce, and the literature is focused, as with vegetal sources, on S-L extraction. Thus, those procedures would only be applicable to dried sludge. Aqueous alkaline protein extractions have been tested with high yields, reaching a 94.71% recovery with NaOH 0.1 M and ultrasound assistance (Álvarez et al., 2018). Alternatively, lipid recovery from egg yolk by subcritical fluid-propane extraction has been studied, although the high cost and complexity of the operation is not presumably competitive against more standarised extraction methods.

Table 10
Main characteristics of physical and chemical adsorption. Adapted from Hu and Xu (2019).

	Adsorption categories	
	Physical adsorption	*Chemical adsorption*
Adsorption force	Van der Waals forces	Chemical bond force
Selectivity	Non-selective adsorption	Selective adsorption
Adsorption layer	Single or multiple layers	Single layer
Adsorption heat	Low	High
Adsorption rate	Fast	Slow
Stability	Unstable	Stable

5. Adsorption

Adsorption is a separation method where a solid or liquid surface is used to retain specific components from a feed solution, which can be later recovered by desorption (Hu and Xu, 2019). The adsorption forces can be either physical or chemical, their respective characteristics being presented in Table 10.

The nature of the adsorbent can be very varied. Activated carbons, which can be produced from any carbonaceous material (Saleem et al., 2019), are the most widely used adsorbents in wastewater treatment (Hu and Xu, 2019), along with other adsorbents, such as inorganic materials (activated alumina, silica gel…) or ion-exchange resins (Crini et al., 2019).

Adsorption can be performed in different configurations, namely in batch (Eregowda et al., 2020; Reyhanitash et al., 2017) or by chromatography, either in an expanded bed (Hansson et al., 1994; Johansson et al., 1996; Strætkvern and Schwarz, 2012) or in a packed column (Li et al., 2013).

Adsorption technology can specifically recover different compounds of interest by applying different adsorbents and elution conditions, being a valuable method in purification processes. Drawbacks of this technique include the high cost of the adsorbent and the lack of research in complex wastewaters (Hu and Xu, 2019).

Adsorption technology as a means of waste valorization has been studied in a broad range of both synthetic and real sources, but not yet in WAS (Fig. 5).

5.1. Adsorption on WAS

As far as we know, no literature about recovery of biomolecules from WAS hydrolysates by adsorption has been published. SCFAs, carbohydrates and proteins could be recovered by this technique, although it has been seen that protein and humic acid are difficult to separate due to their similar adsorption affinities.

Regarding the application of adsorption chromatography, expanded bed adsorption is the most suitable for WAS, as it allows to work with unclarified streams, in opposition to packed columns (Barnfield Frej et al., 1994).

The adsorption step usually goes with the chromatography step to achieve valuable protein recovery. As future steps in WAS research, it would be necessary to characterise the proteins present in the hydrolysate and study if there are any valuable ones that justify their purification by means of this high-performance, more expensive separation and purification method.

5.2. Adsorption in other industries

Works regarding the recovery of proteins and SCFAs are prevalent in the existing literature, while only one study regarding the recovery of carbohydrates via adsorption techniques (Westerberg et al., 2012) has been found, and none recovering humic acids. Depending on the biomolecules to be recovered, the nature of the source varies: protein recovery has only been studied from real sources, namely microorganisms (Barnfield Frej et al., 1994; Bierau et al., 2001; Hansson et al., 1994; Johansson et al., 1996) and food-related sources (Li et al., 2013; Strætkvern and Schwarz, 2012); whereas SCFAs were almost evenly extracted from synthetic (Eregowda et al., 2020; López-Velandia et al., 2014; Reyhanitash et al., 2017; Suescún-Mathieu et al., 2014; Yousuf et al., 2016) and from real sources (Da Silva and Miranda, 2013; Karp et al., 2018; Talebi et al., 2020; Table 11).

Protein recovery has been studied exclusively by adsorption chromatography, mainly of the expanded bed type, which, as stated above, would be the most recommendable configuration dealing with a complex matrix as solubilised WAS. The literature focus on the recovery of single specific valuable proteins rather than on separating the bulk protein present on the feed. The highest protein recoveries (95%), both at lab and pilot scale, were achieved with the ion exchange resin STREAMLINE DEAE as stationary phase (Barnfield Frej et al., 1994). However, it is important to note, that these recovery yields vary depending on the target protein. For instance, while using the same resin (STREAMLINE DEAE) and starting from the same source (*E.coli* culture), the extraction yields of recombinant ZZ-M5 (Hansson et al., 1994) and modified *Pseudomonas aeruginosa* exotoxin A (Johansson et al., 1996) proteins differed from 93% to 79%, respectively. Resins are the most widely used stationary phase. Furthermore, the only adsorbent which is not a resin (i.e., collagen fibre) is used in packed bed adsorption

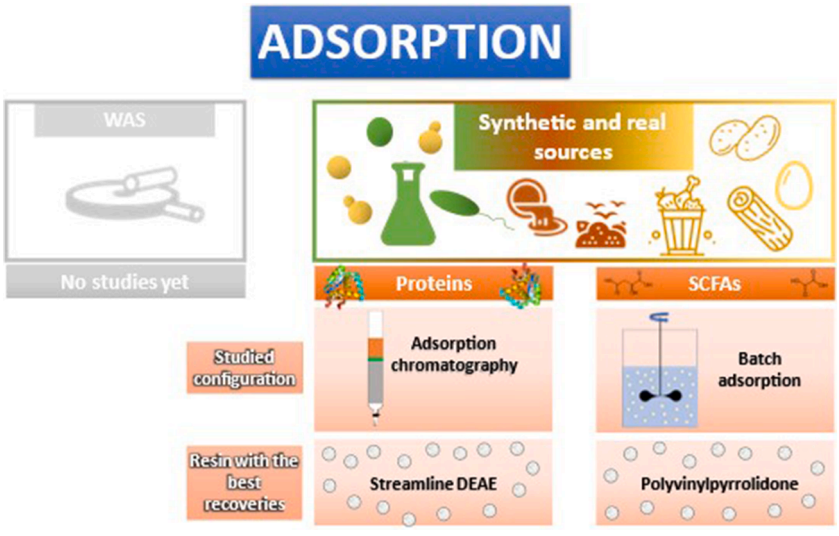

Fig. 5. Uses of adsorption technology for the recovery of added-value biomolecules.

Table 11
Consulted studies about adsorption.

Source	Compounds	Content	Pre-treatment	Extraction conditions	Main results	Ref.
Escherichia coli culture	Protein (recombinant protein ZZ-M5)	550 mg/L fermentation broth	-	Expanded bed adsorption chromatography with ion exchange resin (STREAMLINE DEAE) Online 1:1 mixing with loading buffer. pH adjusted to 5.5 to adsorb target protein. Elution with 0.5 M NaCl.	16-fold size reduction 93% protein recovery 99.6% OD reduction	Hansson et al. (1994)
E. coli culture	Protein (modified *Pseudomonas aeruginosa* exotoxin A)	1.95 g/L	Cell disruption by osmotic shock	Expanded bed adsorption chromatography with ion exchange resin (STREAMLINE DEAE) Column washing with 50 mM Tris buffer pH 7.4. Elution with 20 mM Tris buffer, pH 7.4 containing 0.5 M NaCl.	79% protein recovery, 92.5% volume reduction	Johansson et al. (1996)
Potato juice	Protein	10.1 g/L	Homogeneization, centrifugation to remove starch	Expanded bed adsorption chromatography with resin modified with a mixed mode ligand. Column washing with 10 mM citric acid/citrate pH 4.5; elution with 20 mM sodium hydroxide, pH 12.	Total protein yield: 54.0 ±9.8% Esterase yield: 80.5 ±11.4%	Strætkvern and Schwarz (2012)
Chicken egg white powder	Enzyme (lysozyme)	n/a	-	Packed bed adsorption chromatography with collagen fiber adsorbent Elution with pH 7.5 buffer and pH 7.5 buffer containing 0.6 M NaCl subsequently	93.6% purity obtained with a mass recovery of 86.7%	Li et al. (2013)
E. coli homogenate	Protein (annexin V)	n/a		Expanded bed adsorption chromatography with ion exchange resin (STREAMLINE DEAE) Washing with 30 mM ammonium acetate, pH 5.5, 3 mS/cm; Elution with 30 mM ammonium acetate containing 250 mM NaCl, pH 5.5, 28 mS/cm	Recovery of approximately 95% at both lab scale and pilot scale	Barnfield Frej et al. (1994)
Waste brewers' yeast	Enzyme (glyceraldehyde 3-phophate dehydrogenase)	5.9 U/mL	Wet-milling, filtering	Expanded bed adsorption dye-ligand affinity chromatography (Cibacron Blue 3GA immobilised as a pseudo-affinity ligand upon Macrosorb K6AX)	Purification factor of 3.9	Bierau et al. (2001)
Fermentation broths	Propionic acid	0.5-50.0 g/L		Batch adsorption with weak base resin (Purolite A133S) and activated carbon (Carbomafra 119) tested as adsorbents Water, ethanol and n-propanol tested as eluents	64% recovery with resin and n-propanol	Da Silva and Miranda (2013)
Synthetic carboxylic acid solutions	Acetic, propionic and butyric acids	acetic acid 16% v/v, propionic acid 2% v/v, butyric acid 2% v/v	-	Batch adsorption with activated carbon and modified activated carbon from watermelon shells tested as adsorbents.	Adsorption efficiencies of 71%, 70% and 63% for acetic acid, propionic acid and butyric acid with modified activated carbon; and of 32%, 30% and 27% for the respective acids with activated carbon.	López-Velandia et al. (2014)
Fermentation broths	Succinic and propionic acids	38.7 g/L (succinic acid), 35.6 g/L (propionic acid)	Pre-filtering, chromatography with cation exchange resin (DOWEX G-26 resin) to remove cations, activated carbon treatment	Packed bed adsorption chromatography with PVP resin (Reillex 425) Elution with methanol for succinic acid and acetone for propionic acid.	Loading capacity of 106 mg of succinic acid/g dry PVP. 4 BV of methanol needed to completely desorb succinic acid. Loading capacity of 85 mg of propionic acid/g dry PVP. 2.5 BV of acetone required to fully desorb propionic acid.	Karp et al. (2018)

(*continued on next page*)

Table 11 (*continued*)

Synthetic carboxylic acid solutions	Acetic, propionic and butyric acid	acetic acid 16% v/v, propionic acid 2% v/v and butyric acid 2% v/v		Batch adsorption with activated carbon from sugarcane bagasse. Desorption tested by sonication, heating, sonication followed by heating and heating followed by sonication.	Highest individual VCA adsorption percentages of 60, 48 and 21%. Highest VCA desorption percentage obtained by sonication (38.02%), then by heating (34.68%)	Suescún-Mathieu et al. (2014)
Dark fermented synthetic food waste	Lactic, acetic and butyric acids	11.6 g/L (lactic acid), 6.6 g/L (butyric acid), 2.8 g/L (acetic acid)		Batch adsorption with weakly basic anion exchange resin (Amberlite® IRA-67) and activated carbon (Norit® type Darco®)	Adsorption of 73% and 63% of carboxylic acids by resin and activated carbon, respectively. Desorption was not studied.	Yousuf et al. (2016)
Synthetic fermented wastewater	Lactic, acetic, propionic and butyric acids	All acids at 0.25% wt		Batch adsorption with four types of polystyrene-divinylbenzene-based resins (Lewatit VP OC 1065 [primary amine], Amberlite IRA96 RF [secondary amine], Amberlite IRA96 SB [tertiary amine], and Lewatit VP OC1064 MD PH [nonfunctionalized]). Desorption by a temperature-profiles evaporation and stripping with N2.	Batch capacity of 9.7 g of lactic acid, 12.5 g of acetic acid, 26.5 g of propionic acid and 65.2 g of butyric acid per kg of nonfunctionalized adsorbent. After desorption, butyric acid was obtained with purities of up to 91 wt%. The other VFA could not be effectively concentrated.	Reyhanitash et al. (2017)
Synthetic carboxylic acid solution	Acetic, propionic, isobutyric, butyric, isovaleric and valeric acids	n/a		Batch adsorption with two anion exchange resins (Amberlite IRA-67 and Dowex optipore L-493). Desorption with NaOH solution.	Selective recoveries of > 85% for acetic acid and of ~ 75% for propionic acid.	Eregowda et al. (2020)
Fermented landfill leachate	Acetic and butyric acids	3.28 g/L (acetic acid), 1.12 g/L (butyric acid)		Batch adsorption with activated carbon (Bendosen C1570-5330341) Vortex, water bath sonicator, probe sonicator, and shaker studied as desorption methods. Deionized water, isopropyl alcohol and ethanol studied as desorbents.	Adsorptions of acetic and butyric acid of 88.94% and 98.53% respectively, with activated carbon and shaker. Selective recovery of 89.1% (2.54 g/L) of acetic acid with deionized water; 67.8% (0.71 g/L) of butyric acid recovery by ethanol.	Talebi et al. (2020)
Hot-water-extracted spruce wood	Saccharides (galactoglucomannans)	79%	Filtration, fixed-bed adsorption chromatography with hydrophobic polymeric resin (Amberlite XAD-16).	Fixed-bed adsorption chromatography with a phenylic reversed-phase analytical chromatographic column (XBridge Phenyl 5 μm). Elution with acetonitrile.	The upgraded GGM fraction contained about 1.5% aromatics. Polymeric xylan was accumulated in the GGM fraction. As products, 88% of upgraded hemicelluloses recovered.	Westerberg et al. (2012)

chromatography to separate lysozyme from a much simpler source (egg white), so towards the use of this adsorbent with solubilised WAS, a prior separation/clarification step should be regarded.

In respect of the adsorption of SCFAs, all the consulted studies but one performed batch adsorptions as the preferred configuration for the separation of the target compounds. The most studied species of SCFAs were propionic, acetic and butyric acids. In this case, the adsorbent aimed to capture all the SCFAs present in the feed and not just one specific species, like in protein adsorption. Thus, batch adsorption can be used as a straightforward means to extract SCFAs from solubilised WAS. Furthermore, as these batch adsorptions have been tested, it is reasonable to think that no major difficulties should be encountered. Nevertheless, studies on the adsorption of SCFAs from WAS should be performed before implementing this technique in a functioning process.

Several adsorbents have been tested, mainly activated carbons and different resins. In both papers where weak base resin and activated carbon were compared, the first adsorbent showed higher affinity for all SCFAs (propionic, acetic, butyric and lactic), although desorption was not studied. Polyvinylpyrrolidone (PVP) was the resin that achieved the highest recoveries, fully adsorbing and desorbing propionic and succinic acids under adequate conditions (using enough adsorbent/ bed volumes of elution solution).

Cell lysis by osmotic shock or wet bead milling appear to be applicable pre-treatments for WAS solubilisation reviewed in this section. Cell lysis can be achieved by osmotic shock by suddenly reducing the osmotic pressure around the cell, i.e., changing the cells from a highly saline medium to a low saline one (Johansson et al., 1996). This pre-treatment has not yet been tested on WAS; however, due to the great dewaterability difficulties that this sludge poses, it is reasonable to think that other methods, such as sonication or hydrothermal treatments may be more efficient in breaking the floc structures of the sludge.

Bierau et al. (2001) tested wet bead milling (mechanical disruption of the cell suspension by fine particles) as a pre-treatment for cell liquefaction, although the degree of solubilisation was not indicated in the article. Milling as a pre-treatment on WAS has not been thoroughly studied, and never towards the recovery of biocompounds. Its main drawbacks include lower solubilisation rates compared to the other reviewed pre-treatments and high energy consumption rates (Khanh Nguyen et al., 2021).

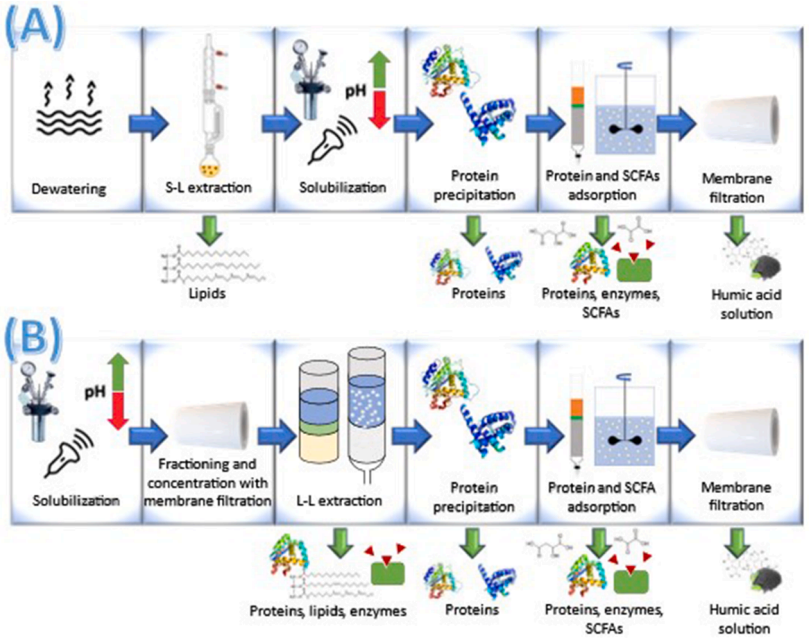

Fig. 6. Proposed integrated methods for WAS revalorization.

6. Conclusions and knowledge gaps

The revalorization of WAS through the recovery of added value biocompounds has been unevenly studied, depending on the target biomolecule and the technology employed for its obtention. In this sense, besides the recovery of inorganic compounds, such as phosphorus or nitrogen, the main biocompounds recovered were: (i) lipids by solvent extraction to obtain biofuel, (ii) humic substances using membrane filtration to produce also fertilizers, and (iii) proteins, although at lesser extent, through precipitation to use as animal feed or wood adhesive.

Considering the revalorization studies of other wastes with similar characteristics to that of WAS reviewed in this work, several research lines towards optimizing the process of WAS revalorization can be opened, including: (i) the use of ethanol or alginate as precipitation agents for proteins; (ii) the application of membrane technology for the concentration and further recovery of enzymes, proteins, lipids, carbohydrates and SCFAs; (iii) the use of state-of-the-art solvent extraction techniques (biphasic flotation or three-phase partitioning), to extract more efficiently not only lipids, but also proteins; (iv) the application of adsorption technology, either through adsorption chromatography to recover high-value target proteins, or batch adsorption to recover SCFAs. It should be noted that, prior to the use of adsorption chromatography to recover proteins, it is necessary to perform a proteomic characterization of WAS to determine the presence of the high-value ones, otherwise the use of this technology would not be justified, since it is a highly selective technique. Besides, specific protocols should be developed based on the physical-chemical properties of the target proteins.

The results gathered in this review show that the revalorization of WAS through an integrated process involving the recovery of bulk and specific proteins, lipids, SCFAs, and humic acids can be feasible, although more investigation is required to prove the extent of its effectiveness.

The effective recovery of carbohydrates has yet to be investigated, as reviewed studies tackling this matter were only focused on their concentration by membrane technology.

Two different integrated methods for the recovery of lipids, proteins and humic acids from WAS are proposed below based on the different reported technologies (Fig. 5):

(A) First, WAS would be dewatered. Dewatering WAS would both allow to extract lipids by a S-L extraction and concentrate the sludge for next purification steps. WAS would then be solubilised (the optimal solubilisation method among those reviewed in this paper should be determined in further studies). Bulk proteins would then be recovered by precipitation methods. After precipitation, high-value proteins, as well as SCFAs, would be recovered by either adsorption chromatography or batch adsorption. Finally, a concentrated humic acid fertilizer would be obtained by membrane filtration.

(B) WAS would be solubilised in the first place and concentrated and fractionated afterwards by membrane filtration. Lipids would then be recovered by either three-phase partitioning or by liquid biphasic flotation. Proteins and enzymes could also be recovered by this technique. From this point, the rest of the purification steps would be as detailed above (precipitation, adsorption and membrane filtration).

Regarding the preference of integrated method A or B, it should be noted that it is needed to perform a sub-critical water pre-treatment before S-L extraction in method A in order to improve the recovery of neutral lipids significantly. This pre-treatment is carried out at similar operating conditions (temperatures and pressures) than those used in WAS solubilisation stage by means of hydrothermal treatments (TH or WO). Therefore, the proposed method B could be preferable since WAS solubilisation stage is applied first, thus avoiding the sub-critical water pre-treatment. Besides, membrane filtration is employed for fractioning and concentration of biocompounds in method B. The use of this technique is highly convenient for three reasons: (i) the production of two valuable streams that can be purified: retentate and permeate, (ii) the improvement of the subsequent purification steps due to the high proportion of the biocompounds in each of these streams and (iii) its low energy consumption.

The main difference between the recovery efficiencies of the two proposed methods would lay on the solvent extraction step for the recovery of lipids: higher yields have been reported with L-L extraction, with recoveries between 6.3 and 7.87 g of lipids per 100 g of dry sludge (Huynh et al., 2010; Olkiewicz et al., 2014), while recoveries of only 2 to 4 g per 100 g of dry sludge were reported after S-L extraction (Melero et al., 2015; Revellame et al., 2011). As for the rest of the process,

recoveries are expected to be similar: up to 92% of protein can be recovered by precipitation (Pervaiz and Sain, 2011), and SCFAs are expected to be fully recovered by batch adsorption with PVP or with polystyrene-divinylbenzene-based resins applying optimised WAS: resin ratio and bed volumes of recovery solution. Finally, humic acids could be concentrated up to 20-fold by membrane filtration, with a recovery yield of 88.6% according to Wei et al. (2016).

More investigation has yet to be conducted before this integrated process can be effectively implemented, this being essential to turn the WAS residue into a renewable source in the context of circular economy.

The application of the proposed integrated methods to other waste streams will imply a thorough evaluation of the characteristics and composition of those streams in order to determine which steps/stages will be required.

Declaration of Competing Interest

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

Acknowledgments

The authors are grateful for the financial support from the Spanish Ministry of Science, Innovation and Universities (MCIU) through the project RTI2018-094218-B-I00 and FEDER funds from European Union and from the Employment, Industry and Tourism Office of the Principality of Asturias (Spain) through the project AYUD/2021/51041. The author Daniel Núñez thanks the Principality of Asturias, Spain, for their financial support through the Severo Ochoa scholarship no BP19-093.

Appendix A

Table A.1
Additional consulted studies about precipitation in cell cultures and synthetic broths.

Source	Compounds	Content	Pre-treatment	Precipitation method	Main results	ref
A. niger solid-state fermentation broths	Proteins, enzymes (xylanases)	4.15 UI/mL (xylanases), 230 mg/L (proteins)	Enzyme extraction by sodium acetate buffer solution (pH 4.5), subsequent filtration and centrifugation	Ethanol precipitation	Maximum recoveries of 86.2% of protein and of 64.4% of xylanase activity	Costa et al. (2018)
Solid-state cultures of *Aspergillus carbonarius*	Enzymes (polygalacturonase)	80 U/mL	Extraction in acetate buffer	Alginate affinity precipitation	-	Nakkeeran et al. (2010)
Bovine serum albumin, lysozyme and trypsin inhibitor solutions	Proteins, enzyme (lysozyme)	n/a	-	Non-ionic (Triton X-100, Tween 85 and Brij 30) and ionic (TOMAC and DODMAC solutions) surfactant precipitation	No precipitation observed for lysozyme. A maximum of 94.2% of bovine serum albumin precipitated with TOMAC at pH 9.0. A maximum of 58% of trypsin inhibitor precipitated with TOMAC at pH 6.2.	Ward et al. (2016)

Table A.2
Additional consulted studies about precipitation in farming and food-related sources.

Source	Compounds	Content	Pre-treatment	Precipitation method	Main results	ref
Potato fruit juice	Proteins	13 g/L	Concentration by low-temperature evaporation	Ethanol precipitation	62% of protein precipitated. 12% of the total protein recovered by resolubilization from the precipitate.	Taskila et al. (2017)
Simulated potato processing plant waste effluent	Proteins	24 g/L	-	Precipitation by CMC complexation (tested conditions: pH 1.0-6.0; T=25°C; NaCl 0.05, 0.1, 0.2 and 0.3 N; 0.05, 0.1, 0.2 and 0.3 CMC/protein ratio)	pH 2.5-3.5, CMC/protein ratio from 0.05:1 to 0.1:1. Optimal results were obtained using CMC with a degree of substitution of 0.85-0.95, CMC: protein ratio of 0.05, NaCl from 0.0 to 1.0%, and pH 1.5-4.0. The complex obtained, which was easily separated, contained 76.6% protein and 17.6% CMC.	Gonzalez et al. (1991)
Rice processing liquors	Proteins	7.7-13.0 g/L	-	Acid precipitation	Protein recovery yields of 47.7% and 30.5% for rice starch steep and sorter liquors, respectively.	Hegazi et al. (1973)
Coriander fruit (defatted whole fruit, dehulled seed, press cake from dehulled seed, steam-distilled dehulled seed, and press cake from	Proteins	13.0% (dry-weight basis)	Defatting, dehulling	Alkali solubilization-acid precipitation	Proteins recoveries of 41.9 ± 4.6 (defatted whole fruit), 39.6 ± 0.1 (dehulled seed), 38.1 ± 0.0 (press cake from dehulled seed), 26.2 ± 0.2 (steam-distilled dehulled seed) and 29.3 ± 0.9 (press cake from steam-distilled dehulled seed meal).	Hojilla-Evangelista and Evangelista (2017)

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Table A.2 (*continued*)

steam-distilled dehulled seed meal)						
Dissolved hair	Proteins	n/a	-	Glacial acetic acid precipitation	Maximum protein recovery yield of 68%.	Feairheller et al. (1972)
Antarctic krill (*Euphasia superba*)	Proteins	76.54% (dry-weight basis)	Homogenization with deionized water 1:3 (1-4 °C), isoelectric solubilisation (pH 2.0, 2.50, 3.0, 12.0, 12.50 and 13.0), centrifugation	Isoelectric precipitation (pH 5.5)	Maximum protein recovery yield of 50% after solubilisation at pH 2.	Chen et al. (2009)
Silver carp (*Hypophthalmichthys molitrix*)	Proteins	52.4% w/w	Grinding, homogenization with water 1:6, isoelectric solubilisation (acidic (2.0 and 3.0) and basic (11.5 and 12.5) pH), centrifugation.	Isoelectric precipitation (pH 5.5, 10 min)	Maximum protein recovery yield of 660 g/kg after solubilisation at pH 12.5.	Taskaya et al. (2009)

Table A.3
Additional consulted studies about membrane separation.

Source	Compounds	Content	Pre-treatment	Filtration conditions	Main results	Ref.
Alfalfa juice	Proteins	21 g/L	Prefiltration	MF (0.2 μm) and UF (20 kDa) with three different membranes Filtration modules: Dead-end filtration using amicon cell (DA) effective membrane area 31.7 cm2, Dynamic cross filtration using disk module (CRDM) effective area 176 cm2, Dead end filtration using rotating disk module (DRDM) TMP: 0.5-3 bar T: 35 °C Full recycling and concentration (VCR = 6) tests.	Productivities (L/m^2 h bar) MF DA 0.48 DRDM 4.73 CRDM 6.06 UF DA 0.43 DRDM 1.42 CRDM 1.70	Zhang et al. (2015)
Skim milk	Carbohydrates (lactose)	46 g/L	MF	UF (concentration step) PS membranes, 10 kDa, membrane area 0.47 m^2. TMP: 1 bar T: 25°C NF Spiral wound thin film composite polyamide Membrane, 180 Da, membrane surface 1.5 m^2 TMP: 8 bar T: 25°C ± 1°C VCR = 4.	UF Concentrated feed solution contained 4.6 ± 0.3% lactose, negligible amounts of proteins and a relatively high content of mineral salts. NF Lactose rejection = 92% Ash content in the feed reduced from 0.36 ± 0.04 (%w/v) to 0.108 ± 0.02, purity higher than 90%.	Rinaldoni et al. (2009)
Tuna protein hydrolysate	Proteins in the range of 1-4 kDa	72 g/L		Six-stage (3 UF and 3 NF) cascade Membranes tested: UF 3 channel tubular ceramic membrane, 8 kDa, surface area 155 cm2 NF PES membrane, 1 kDa TMP: 2 bar (UF), 10 bar (NF). CFV: 3 m/s (UF), 1.25 m/s (NF) T: 25 °C	Product purity of 49.3% with a process yield of 62.6%	Abejón et al. (2016). Saidi et al. (2013)
Herring marinade	fats, proteins, amino acids, salt, acetic acid and water	10.6 wt% dry matter, 3.9 wt% protein, 0.22 wt% fat, 9.0 wt % NaCl, 2.0 wt% acetic acid.	Mechanical fat removal, sieving	Sequential filtration, employed membranes, MWCO, CFV (in kg/min) and TMP (in bar) (all experiments conducted at room temperature): Fluoro polymer, 0.2 μm, 5 CFV, 0.9 TMP. PS, 50 kDa, 5 CFV, 7.8 TMP. Fluoro polymer, 20 kDa, 5.5-6-6 CFV, 5.9-6.0 TMP. Composite fluoro polymer, 10 kDa, 6.5 CFV, 5.4-6.3 TMP.	The 50 kDa stage produces a protein concentrate (>17 kDa). NF produces a retentate containing sugars, amino acids and smaller peptides and a NF permeate containing salt and acetic acid ready for reuse. Proteins are concentrated 30-fold, while amino acids and smaller peptides are concentrated 11-fold.	Søtoft et al. (2015)

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Table A.3 (*continued*)

				Fluoro polymer, 1 kDa, 6.5 CFV, 4.2-6.7 TMP. Polyamide on polyester, reject >98% $MgSO_4$ (2000 ppm, 9 bar, 25 °C), 6.5 CFV, 26.4-29.1 TMP.		
Shrimp waste	Protein (chitin)	60 g/L		3 tubular membranes tested: MF membrane, 0.1 μm 2 NF membranes, 450 and 300 Da, filtration surface area 1.68 m^2 and 1.75 m^2, respectively. TMP: 1.5 bar (MF), 3 bar, (NF 450), 5 bar (NF <300). CFV: 3.5 m-s, T: 70 °C	Increase of recovered proteins in the concentrate between 7 and 16%, can be achieved in the concentrate stream by reducing the chosen MWCOs. Concentration from 60 g/L to 156 g/L with NF membrane	Nguyen et al. (2016)
Cocoon cooking wastewater, refining wastewater and mixed wastewater from silk reeling	Protein (sericin)	n/a	-	4 hollow fiber PSF membranes tested MWCO: 6, 20 and 100 kDa for UF, and of 95.00% $MgSO_4$ rejection for NF. TMP: 0.7 MPa T: 20-60°C	Higher retentions of 97.1%, 97.2% and 98.1% for cocoon cooking wastewater, refining wastewater and mixed wastewater, respectively. More than 86% sericin protein recycled after the treatment by optimized UF–NF combination process.	Li et al. (2015)
Cocoon cooking wastewater	Protein (sericin)	5510–9883 mg/L	Precipitation, MF	Polymeric membranes tested: MF: cellulose, 20-25 μm; cellulose, 8 μm; glass fiber, 1.6 μm; glass fiber, 1 μm. All membranes effective area 10 cm2 UF: PES, 20 kDa; PES, 5 kDa; Thin film, 1 kDa. All membranes effective area 44 cm2 NF: Thin film, 190 Da, effective area 44–36 cm2; thin film, 100 Da, effective area 44–72 cm2. TMP: 200 kPa (UF), 500 kPa (NF). Flow rate: 0.03 m^3/h T: 18–25 °C	UF: partial recovery from 37 to 60% of sericin polypeptides. NF: maximum sericin recovery of 94–95%, containing all molecular weight fractions.	Capar et al. (2008)

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