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Review

Strategies for livestock wastewater treatment and optimised nutrient recovery using microalgal-based technologies

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ABSTRACT

Global sustainable development faces several challenges in addressing the needs of a growing population. Regarding food industries, the heightening pressure to meet these needs has resulted in increased waste generation. Thus, recognising these wastes as valuable resources is crucial to integrating sustainable models into current production systems. For instance, the current 24 billion tons of nutrient-rich livestock wastewater (LW) generated yearly could be recovered and valorised via biological uptake through microalgal biomass. Microalgae-based livestock wastewater treatment (MbLWT) has emerged as an effective technology for nutrient recovery, specifically targeting carbon, nitrogen, and phosphorus. However, the viability and efficacy of these systems rely on the characteristics of LW, including organic matter and ammonium concentration, content of suspended solids, and microbial load. Thus, this systematic literature review aims to provide guidance towards implementing an integral MbLWT system for nutrient control and recovery, discussing several pre-treatments used in literature to overcome the challenges regarding LW as a suitable media for microalgae cultivation.

Table

Main abbreviations		
Advanced oxidation processes (AOP)	Carbon: nitrogen ratio (C:N)	Nitrogen: phosphorus ratio (N:P)
Algal-bacterial symbiosis (ABS)	Cattle wastewater (CW)	Photo-Fenton (PF)
Ammonium nitrogen concentration (NH_4^+–N)	Chemical oxygen demand removal (CODr)	Poultry wastewater (PW)
Ammonia stripping (AS)	Constructed wetlands (CWS)	Total nitrogen removal (TNr)
Anaerobically digested livestock wastewater (ADLW)	Electrocoagulation (EC)	Total phosphorus removal (TPr)
Anaerobically digested swine wastewater (ADSW)	Fenton oxidation (FO)	Total suspended solids removal (TSSr)
Anaerobic digestion (AD)	Livestock wastewater (LW) Microalgae-based livestock wastewater treatment (MbLWT)	Swine wastewater (SW)

1. Introduction

The demand on food industries to meet the growing world food needs, primarily driven by global population growth projected to reach 10 billion by 2050, is particularly evident in the livestock industry. Currently, around 56 billion livestock animals are raised and slaughtered annually for human consumption worldwide, a figure expected to double by 2050 (López-Sánchez et al., 2022a). This increasing demand has considerable implications across the entire food supply chain, especially in terms of greenhouse gasses (GHG) emissions, water usage,

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and wastewater discharges associated with livestock farming.

Despite its importance, livestock farming significantly contributes to global GHG emissions, with animal manure accounting for about 37% of these emissions (Shakoor et al., 2021). Although the most significant demand for water in livestock production is attributed to livestock feed production – i.e., nearly 41% of total agricultural water use (Heinke et al., 2020), direct water consumption in the livestock sector equals 24% of the total freshwater consumed by the food and beverage industry, and up to 29% of that consumed by the agricultural sector worldwide (Oliveira et al., 2018; Svierzoski et al., 2021). Moreover, more than 24 billion tons of livestock wastewater (LW) are generated annually, most of which is discharged into the environment without effective nutrient control (López-Sánchez et al., 2022a). This high content of organic pollutants, nutrients, heavy metals, xenobiotics, and pathogens in LW leads to eutrophication and hypoxia in surface water bodies (Hu et al., 2020; B. Wang et al., 2022a), attributing 64–97% of the eutrophication potential globally to livestock farming (Garcia-Launay et al., 2018).

Eutrophication results not only in anoxic conditions and sediment accumulation in water bodies but also contributes significantly to methane (CH_4) release from anaerobic digestion, and nitrous oxide (N_2O) from denitrification of ammonium (NH_4^+) and nitrate (NO_3^-), which are more potent GHGs than carbon dioxide (CO_2) (by factors of 21 and 310, respectively) (Meerhoff et al., 2022; Moss, 2011). Simultaneously, climate change exacerbates these issues by increasing inorganic nutrient influx from land due to accelerated soil mineralization and reduced carbon storage efficiency in sediments. This process fosters the proliferation of invasive aquatic macrophytes and phytoplankton while intensifying deoxygenation at the waterbody surfaces (Meerhoff et al., 2022).

Consequently, controlling raw wastewater discharges is critical for environmental protection and water quality preservation, as water scarcity linked to pollution is expected to worsen in the coming years (Xinjie et al., 2019). Conventional LW treatments, such as intensive electromechanical systems (e.g., activated sludge processes), often require significant chemical inputs and contribute to indirect GHG emissions. Alternative low-cost treatment options like wastewater stabilisation ponds and anaerobic digesters, do not provide additional benefits for nutrient recovery besides wastewater reclamation or meet discharge quality standards (Rossi et al., 2022).

In response, the concept of a circular bioeconomy has emerged, focusing on sustainable production through innovative, closed-loop processes that utilise biological residues, such as LW, to reduce virgin resource consumption and generate high-value-added bioproducts (Leong et al., 2021). Biorefinery approaches in LW management can mitigate eutrophication and climate change impacts by recovering or recycling renewable carbon sources and nutrients, creating self-sustained business models and employment opportunities (Nagarajan et al., 2020).

Microalgae-based livestock wastewater treatment (MbLWT) has gained attention as a method that not only treats liquid effluents but also fixes atmospheric CO_2, storing carbon in biomass as various molecule products (e.g., pigments, proteins, lipids, carbohydrates, vitamins, and antioxidants), enhancing the cost-effectiveness of this technology (You et al., 2022). However, challenges for MbLWT exist, such as the high ammonium nitrogen concentration (NH_4^+–N) present in some LW that can inhibit microalgal growth by affecting the electron transfer of photosystem II, while high organic load and turbidity of LW hinder light penetration, affecting microalgal autotrophic growth (Ferreira et al., 2022; Liu and Hong, 2021). Additionally, microalgae can develop antagonist relationships with the native microbial communities due to nutrient competition (Aditya et al., 2022). Therefore, efficient treatments to condition LW for optimal microalgae growth are needed to make MbLWT a feasible process that reduces the negative environmental impacts of livestock farming.

This work aimed to critically analyse published literature to identify suitable pre-treatment processes for LWs, like flocculation-coagulation, ammonia stripping, UV radiation and biological treatments, contributing to better technical decisions for designing enhanced microalgae-based wastewater treatment systems optimised for maximum microalgae biomass growth and wastewater treatment (nutrient uptake). This review provides insights into the multifaceted challenges and opportunities associated with large-scale MbLWT technology implementation, and highlights the need for tailored approaches that consider factors like wastewater composition, microbial interactions, and variability of nutrient concentrations.

By consolidating these findings, this systematic literature review aids in advancing the scientific understanding of MbLWT systems. The primary contribution of this work lies in the comprehensive analysis of various pre-treatment alternatives highlighted in recent literature, serving as a guide for researchers aiming to implement this technology at a large scale and lay the groundwork for future research and innovation in the field.

2. Research methodology

This systematic literature review was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) methodology (Sohrabi et al., 2021). This approach was chosen to minimise bias, ensure consistency, robustness, and transparency in research, and demonstrate the review's quality. It enables readers to assess the strengths and weaknesses of the selected data and ensures the replicability of review methods.

2.1. Reference selection and data extraction

The methodology for this systematic review adheres to the structure depicted in Fig. 1, focusing on identifying, analysing, and assessing studies that have implemented pre-treatment of LWs prior to their use in MbLWT.

Scopus and PubMed served as the primary databases for compiling published journal articles, with the selection criteria covering research works published in the English language from 2018 onwards (last updated search conducted on 30/11/2024). Utilising the keywords illustrated in Fig. 1 and 246 journal articles were initially identified. A first screening based on a bibliometric analysis led to the exclusion of review papers, book chapters, and duplicates between the databases, reducing the sample to 157 journal articles. Subsequent screening, focusing on the journal paper title, further refined the sample to 136 articles. The third screening, involving a review of the abstracts, objectives, and methodologies, narrowed the selection to the journal articles that specifically applied pre-treatments before MbLWT. This process resulted in a final selection of 100 journal articles for comprehensive analysis.

2.2. Bibliometric analyses

The bibliometric analyses were conducted using the open-source R package 'bibliometrix' (Aria and Cuccurullo, 2017). This package was installed and activated within R Studio, from where the 'biblioshiny' tool was initiated to begin the analysis process. The data for these analyses were derived from the set of journal papers identified during the initial screening phase (excluding duplicates, including review papers and book chapters) to ensure a comprehensive scope of the research landscape.

3. Results and discussion

3.1. Bibliometric analysis

The time frame chosen for the bibliometric analyses was set to the last six years (2018–2023) to capture the most current information on

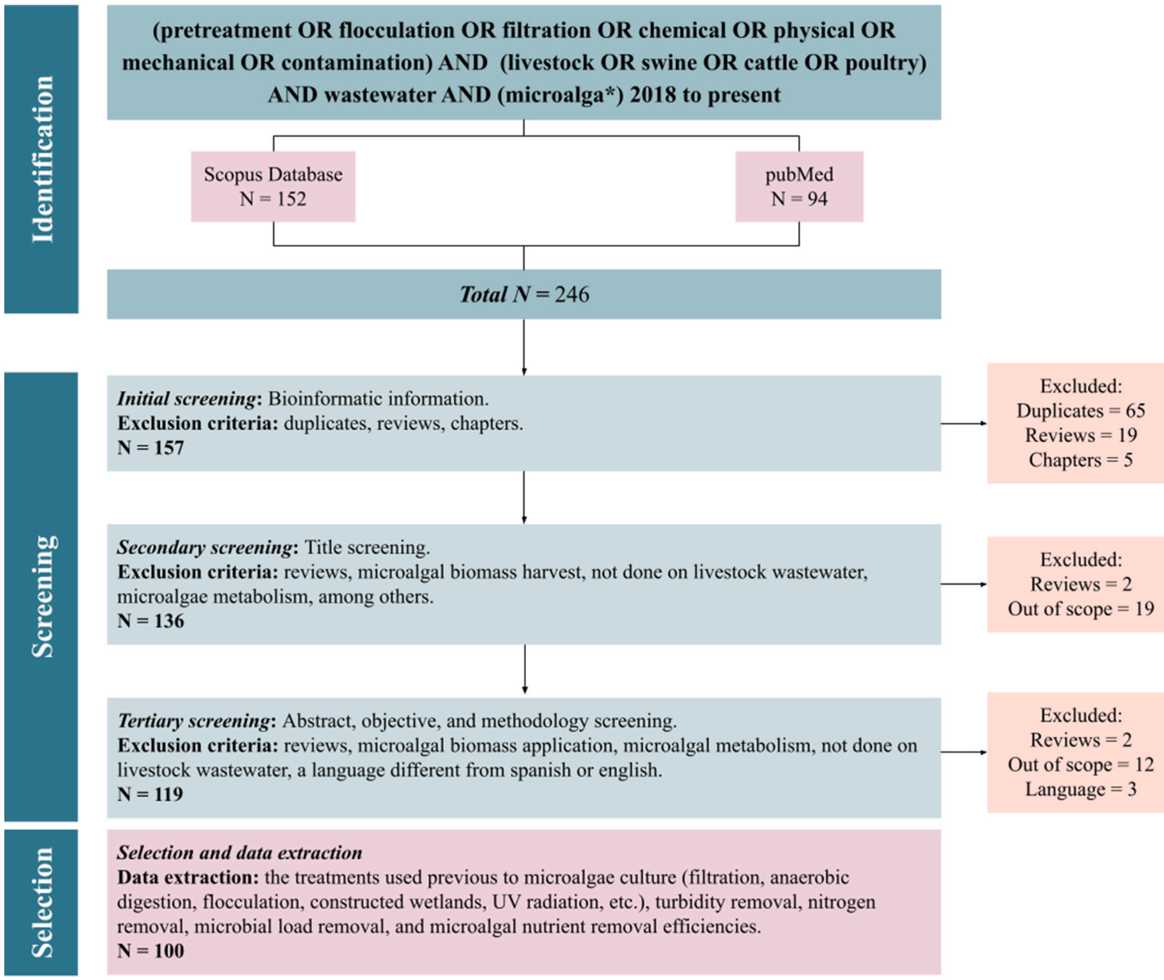

Fig. 1. Research methodology for data selection and data extraction.

Fig. 2. Trend topics in the optimisation of MbLWT. The position of the circle indicates the year with most publications per topic and each line defines the timespan that has been present.

the topic. Detailed information about these documents is presented in Table S1 in Supplementary Material.

MbLWT systems have gained recognition due to their potential application as an integrated biorefinery concept, in which the economic viability of such wastewater management approach is more financially attractive, encouraging livestock producers to invest in this technology (Cheng et al., 2020a). The attention towards MbLWT has notably increased in the past six years; from 16 research works published in 2018, the number nearly doubled to 27 journal articles in 2020, and escalated to 45 journal articles by 2022 (Figure S1 Supplementary Material), reflecting an overall annual growth rate of 11%. As of the last update of this work (30/11/2023), and in accordance with our search, exclusion, and inclusion criteria, the number of journal articles produced in 2023 has reached 33.

3.1.1. Trend topics

MbLWT-related trend topics have evolved over the past six years (Fig. 2). In 2018, the focus was primarily on understanding microalgae growth kinetics through culture conditions in bioreactors and using *chlorophyll a* as an indicator to measure biomass growth. From 2019 to 2022, research shifted towards microalgal biomass production, encompassing studies on metabolic nutrient pathways that support microalgae growth (i.e., algal phosphorus uptake) and potential products derived from this system (e.g., algal biomass, protein) (see Fig. 3).

The use of wastewater treatment to support microalgal biomass growth started to gain attention from 2019 onwards. Recent years have seen a surge in research aimed at optimising microalgal cultivation in wastewater, including the use of mixed cultures (bacterial consortia) to enhance the tolerance of microalgae to harsh and nutrient-competitive environments typical of non-axenic LWs (Liu et al., 2022; Lorentz et al., 2020; Marazzi et al., 2020; Mou et al., 2023).

In 2023, a trend is the focus on the "Chlorophyceae" class. Various microalgae strains have been investigated in the selected publications, with the *Chlorella* genus reported in 61% of these due to its outstanding performance over other microalgae genera (high adaptability to LWs, high biomass productivity, and efficient nutrient uptake ability) (Sun et al., 2019). Although previously categorised within the Chlorophyceae class, but recently, recent reports suggest its dispersion across both the Trebouxiophyceae and Chlorophyceae classes (Champenois et al., 2015). Thus, future trends might witness a shift in topic focus based on the classification, as 54% of the genera in the selected references belong to the Chlorophyceae class, while 67% are associated with Trebouxiophyceae, and 17% could be either class as the tested species were not always specified.

Finally, given the continuous rise in global water demand due to population growth, concerns regarding water scarcity are intensifying, posing significant challenges to global sustainable development, especially in the Global South. A balance between agricultural, domestic and industrial demand for fresh-water resources is critical. This not only secures water supply and aquatic ecosystem sustainability but also offers opportunities for both direct and indirect wastewater reuse (Baggio et al., 2021). Understandably, since 2021 "fresh water" has emerged as a key trend topic in MbLWT-related research, as it can make a significant contribution by producing a treated effluent that meets the needs of the future population (Fig. 2).

3.1.2. LW composition

Further analysis was conducted on the selected references (100 articles) to evaluate the most commonly applied treatment processes before microalgal culture. Regarding the type of LW, more than half of selected studies (65%) used swine wastewater (SW). Cattle wastewater (CW) or similar (cattle slaughterhouse wastewater, cattle manure, dairy wastewater) was utilised in around 22% of the total, with dairy wastewater (DW) constituting 39% of these group. Poultry wastewater (PW) and related (poultry slaughterhouse effluent, poultry litter) represented 18%, and 4% did not specify the type of LW.

The nutrient composition of LW varies significantly depending on its source, whether SW, PW, or CW (Table 1). For instance, Liu et al. (2022)

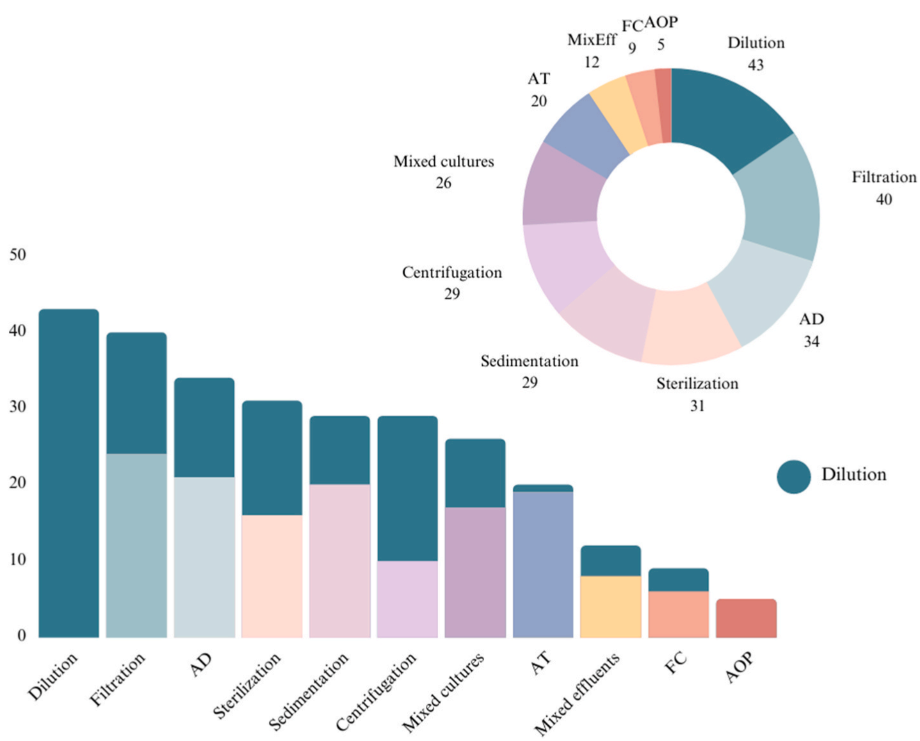

Fig. 3. Number of articles reporting pre-treatment processes and/or dilution previous microalgae cultivation. AD: anaerobic digestion; AT: aerobic treatments; MixEff: mixed effluents; FC: flocculation-coagulation; AOP: advanced oxidation processes. The blue in the bar graph represents the proportion that dilution was used in each pre-treatment. The percentages of the analysed pre-treatments in this section do not add up to 100% due to instances where some authors utilised multiple pre-treatments within a single study. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Composition of raw swine wastewater (SW), cattle wastewater (CW), poultry wastewater (PW), and dairy wastewater (DW), including nutrients, heavy metals, and emerging contaminants.

Nutrients (mg L^{-1})	SW	CW	PW	DW
Total Organic Carbon (TOC)	150–1918.9			
Chemical Oxygen Demand (COD)	50–130,800	650–700	2185–7313	3000–33,850
Biochemical Oxygen Demand (BOD)	1500–5000		1300–2500	2660–2940
Total Solids (TS)	70–73,950[c]	760	1400–5900	11–15
Total Nitrogen (TN)	270–7000		110–295	420–470
Ammonium (NH_4^+–N)	50–3400	22,218–22,498	123	295–800
Nitrate (NO_3^-–N)	0.002–19	3021–3061	0.2	
Nitrite (NO_2^-–N)	0.001–200			
Total Phosphorus (TP)	17–1400		44–69	28–250
pH	6.3–9.26	6.11	6.84–7.28	6.05–7.9
Turbidity (NTU)	120–160		650–950	10,600
Heavy metals				
Al	0.210			
B	0.342		0.200	
Ca			11.4–87.6	0.124
Co			0.013	0.003
Cr			0.020–4.40	0.033
Cu	0.500		0.160–0.459	0.029
Fe	0.728		0.300–4.0	2.42
K			104	
Mg			161–601	25.2
Mn	0.183		0.292–0.566	0.063
Mo			0.028	0.028
Na			1008	
Ni	0.281		0.021–0.085	0.039
Pb	0.281		0.007–2.10[a]	
Sc			0.006	0.005
Zn	0.500		1.00–1.14[a]	1.082
Emerging contaminants [b]				
Acetylsalicylic acid	>7.741 × 10^{-3}			
Doxycycline	1.091 × 10^{-3}			
Enrofloxacin	0.371 × 10^{-3}			
Estrone	<0.764 × 10^{-3}			
17-α-Ethinylestradiol	1.953 × 10^{-3}			
Marbofloxacin	0.014 × 10^{-3}			
Oxytetracycline	0.028–0.447 × 10^{-3}			
Penicillin G	0.038 × 10^{-3}			
Salicylic acid	39.575 × 10^{-3}			
Sulfadiazine	>0.0780 × 10^{-3}			
Sulfamethazine				0.0476
Sulfathiazole				0.0857

[a] The concentration is expressed in mg kg^{-1}, according to Hülsen et al. (2018).
[b] The concentration of emerging contaminants was obtained from (Portela-Monge et al., 2022), an external source of the selected references, as to the best of our knowledge, none of these reported the presence of emerging contaminants.
[c] Total suspended solids (TSS).

reported a SW composition with 8900 mg mL^{-1} of Chemical Oxygen Demand (COD), 6820 mg mL^{-1} of Total Nitrogen (TN) and 390 mg mL^{-1} of Total Phosphorus (TP). In contrast, Zhao et al. (2022) documented a SW composition with higher COD content (12,000 mg mL^{-1}), but lower TN (1700 mg mL^{-1}) and TP (80 mg mL^{-1}). Similarly, Wang et al. (2022) noted a CW composition with a COD and NH_4^+–N content of 10,347 and 5670 mg mL^{-1}, respectively, whereas Jain et al. (2022) reported 15 times lower COD content (674 mg mL^{-1}) and almost four times higher NH_4^+–N content (22,358 mg mL^{-1}).

Reports addressing the initial concentration of heavy metals in LWs were limited, and others focused only on microalgae uptake without specifying the concentrations in the raw effluent. The knowledge gap was more evident regarding emerging contaminants, mentioned only in two of the final screened references. Acknowledging the importance in supporting safer and more sustainable circular economy efforts, we included data on the concentration of heavy metals and emerging contaminants in LW (Table 1). Notably, information on the specific parameters of CW, PW, and DW was lacking, due to studies reporting effluent compositions post treatments like dilution, filtration, anaerobic digestion (AD), or others. Nevertheless, the data presented in Table 1 is compiled considering the original state of the raw effluent.

3.1.3. Reported pre-treatment strategies to reduce pollutant load in LW

Dilution was identified as the most common strategy to mitigate the detrimental impacts of raw LW on microalgae cultures, being reported in 47% of the selected studies. Filtration followed closely, being employed in 42% of the cases. Stationary screens and grills were often used to filtrate large solids, thereby diminishing sedimentation and solid accumulation in algal reactors (Q. Wang et al., 2021). The primary goal of filtration, as noted by most authors, was the removal of suspended particles, reducing the turbidity and organic matter. However, it also extracted nutrients and bacteria attached to particulate organic matter (Ran et al., 2021).

Additionally, 32% of the studies applied LW sterilisation for bacteria removal. Of these, 72% utilised autoclaving, while the remainder employed methods like filtration, UV radiation, or chemical treatments. Sedimentation and centrifugation were also prevalent pre-treatment steps for solid removal, reported in 30% and 31% of the references, respectively. Sedimentation is a low-energy process in contrast with centrifugation, although centrifugation has also been applied at full scale by some livestock producers (Wang et al., 2020a).

Regarding biological processes, anaerobic digestion (AD) was used in 35% of the studies for LW treatment. AD is a well-established technology with the added benefit of producing bioenergy (biogas) and a nutrient-rich effluent (digestate) with a lower organic load. Furthermore, 29% of the articles explored alternative biological processes using mixed cultures. Within this category, 52% focused on microalgae-bacteria consortia, 21% on microalgae-microalgae consortia, and 31% a combination of both. Aeration and air stripping treatments, primarily targeting NH_4^+–N concentration reduction, were employed in 20% of the studies, while 13% of the articles incorporated mixed effluents in their treatment processes.

Flocculation-coagulation and advanced oxidation processes (AOP), although less common, (9 and 5%, respectively), have shown promise in removing unconventional contaminants like antibiotic-resistant genes (L. L. Chen et al., 2021; Zhang et al., 2021). In addition, advanced hybrid treatment methods, including membrane bioreactor combined with reverse osmosis, ozonation, and membrane distillation, have been effective in reducing emerging contaminants, albeit detailed in other reviews (Parida et al., 2021).

3.2. Microalgae-based livestock wastewater treatment

The livestock industry supports the livelihood of 1.3 billion people worldwide, making it essential to establish integrated infrastructure facilities that transform waste into value-added sustainable products, encouraging industries to treat their wastes while mitigating climate change (Deb et al., 2022; López-Sánchez et al., 2023). Microalgal cultivation systems represent a promising and cost-effective solution for efficient bioremediation of waste streams. These employ mechanisms like biosorption, biodegradation, and photolysis and have the potential to be integrated into an active biorefinery approach for resource recovery and generation of valuable products. A key advantage of using microalgae in wastewater treatment is the recovery of essential nutrients such as carbon (C), phosphorus (P), and nitrogen (N) through biological uptake by microalgae.

P and N are essential for life, serving as irreplaceable elements in producing crucial biomolecules involved in cellular metabolism, including nucleic acids (DNA, RNA), lipids, energy transfer molecules (ATP, NADPH), and proteins (Wang et al., 2022b). Microalgal biomass cultivation requires access to these nutrients, which can be sourced cost-effectively from wastewater effluents. This not only leads to positive outcomes in wastewater remediation, but also allows the use of nutrient-rich harvested biomass in agricultural applications, such as fertilizers or supplements.

In MbLWT, total phosphorus removal (TPr) typically exceeds 90% (Table S3 in Supplementary Material), while total nitrogen removal (TNr) varies, often depending on the availability of specific chemical nitrogen species. Microalgae cultivated in LW prefer NH_4^+ over NO_3^- and nitrite (NO_2^-) (Cheng et al., 2020b). *Chlorella* sp. FACHB-8 and *Kirchneriella obesa* FACHB2104 cultured in tenfold diluted, unsterilised CW rapidly decreased NH_4^+–N contents (close to zero by the 14th day of cultivation), with 87% of the N present in CW being in the form of NH_4^+ (Wang et al., 2022c). Thus, given that microalgal biomass production depends on the N and P concentration, LW emerges as ideal nutrient source for microalgal cultivation.

Nutrient balance is crucial, as microalgal cell division and protein content in biomass diminish in environments deficient in N and P. For instance, an N:P mass ratio below 10 indicates both N and P are limiting nutrients; an N:P ratio between 10 and 40 supports stable biomass growth; and a N:P ratio above 40 points to P as the limiting nutrient (Huo et al., 2020; Huo et al., 2021). Conversely, nutrient excess can be toxic, inhibiting microalgal growth. When the chemical equilibrium shifts from NH_4^+ to NH_3, microalgal growth inhibition increases as NH_3 can diffuse freely through the cell membrane (Dinnebier et al., 2021). Also, the high organic matter content in LW, indicated by COD and total suspended solids (TSS), contributes to the high turbidity and chromaticity in the culture media, which can significantly restrict microalgae growth due to inadequate photon supply (Shao et al., 2022). Consequently, the high NH_4^+–N and organic content, and poor light penetration typically observed in LW hinder the application of MbLWT (Deb et al., 2022; Shao et al., 2022). However, adding appropriate pre-treatment processes to improve MbLWT performance (Fig. 4), as discussed in the following sections, could facilitate its large-scale implementations.

3.3. Decrease in organic load and turbidity

Turbidity is caused by suspended particles in the media, including very small colloidal particles and large flocs both of organic and inorganic origin, and microorganisms. For instance, poultry slaughterhouse wastewater has been reported to be heavily loaded with organic matter (1200–15,950 mg COD L^{-1}) (Njoya et al., 2019). Almost 35% of the total organic matter corresponds to large floating debris resulting from the agglomeration of grease and fat, while around 55% corresponds to suspended solids containing lipids, proteins, pathogenic microbes and others (Terán Hilares et al., 2021). LW generally exhibits high turbidity (Table 1), impeding light penetration and photosynthesis (Ferreira et al., 2021). Therefore, removing suspended matter is essential, especially when targeting microalgal biomass production (Shao et al., 2022).

Microalgae have effectively remove turbidity, colour and even odour from wastewater (Ummalyma et al., 2023). However, the characteristics of LW can be too extreme for some microalga to survive. To remove colour caused by organic matter, two main methods are employed: discolouration or biodegradation. Discolouration of wastewater merely reduces the intensity of colour, but treated water may still contain organic residues, fostering bacterial growth and thus competing with microalgae for nutrients. Biodegradation involves microorganisms (bacteria and microalgae) using organic matter as a carbon source. The extent of microbial biodegradation can be assessed by measuring reductions in BOD, COD, Total Organic Carbon (TOC), or other suitable surrogate parameters. In that sense, *Chlorella* ESP-6 has been reported to remove up to 98% of colour from undiluted cattle slaughterhouse wastewater while converting organic pollutants into simpler compounds with nearly 99% efficiency (Oktor, 2023). In another study, *Chlorella vulgaris* achieved turbidity removal efficiencies between 88 and 92% from DW due to the adsorption capacity of the surface of microalgal cells coupled with the assimilation of dissolved organic substances by microalgae (Wang et al., 2020b). However, in this case, the DW was diluted with clean water, which is not environmentally sustainable.

Conversely, there are instances where microalgae might contribute to an increase in the organic matter content of the effluent. Biological treatment using microalgae often require long retention times, mainly due to poor mixing conditions (e.g., wastewater stabilisation ponds). This can result in the accumulation of algal biomass sediments within treatment units. Consequently, an increase in organic matter concentration in the final effluent may occur due to bacterial decomposition of dead microalgal biomass after the fixation of the amended CO_2 through autotrophic metabolism. Additionally, microalgae cells can release organic carbon under low nutrient conditions, a common response in cultures with limiting levels of N or P (Acebu et al., 2022; Zhang et al., 2022).

Conventional solid–liquid separation processes can remove suspended organic matter from its soluble fraction. Such processes can be gravitational (e.g., sedimentation tanks), mechanical (e.g., filtration and centrifugation), or chemical (e.g., flocculants and coagulants) (Osabutey et al., 2023). These will be further discussed in the following sections.

3.3.1. Dilution of wastewater feedstock

Dilution, as noted in 47% of the selected references, stands as the simplest and most commonly used strategy to alleviate microalgae growth inhibition caused by high NH_4^+–N content, organic matter concentration, and poor light transmittance prior microalgae inoculation (Shao et al., 2022). Terán Hilares et al. (2021) observed complete COD removal (CODr) while cultivating *C. vulgaris* in 25% diluted poultry slaughterhouse wastewater (1:4 dilution ratio). In contrast, the

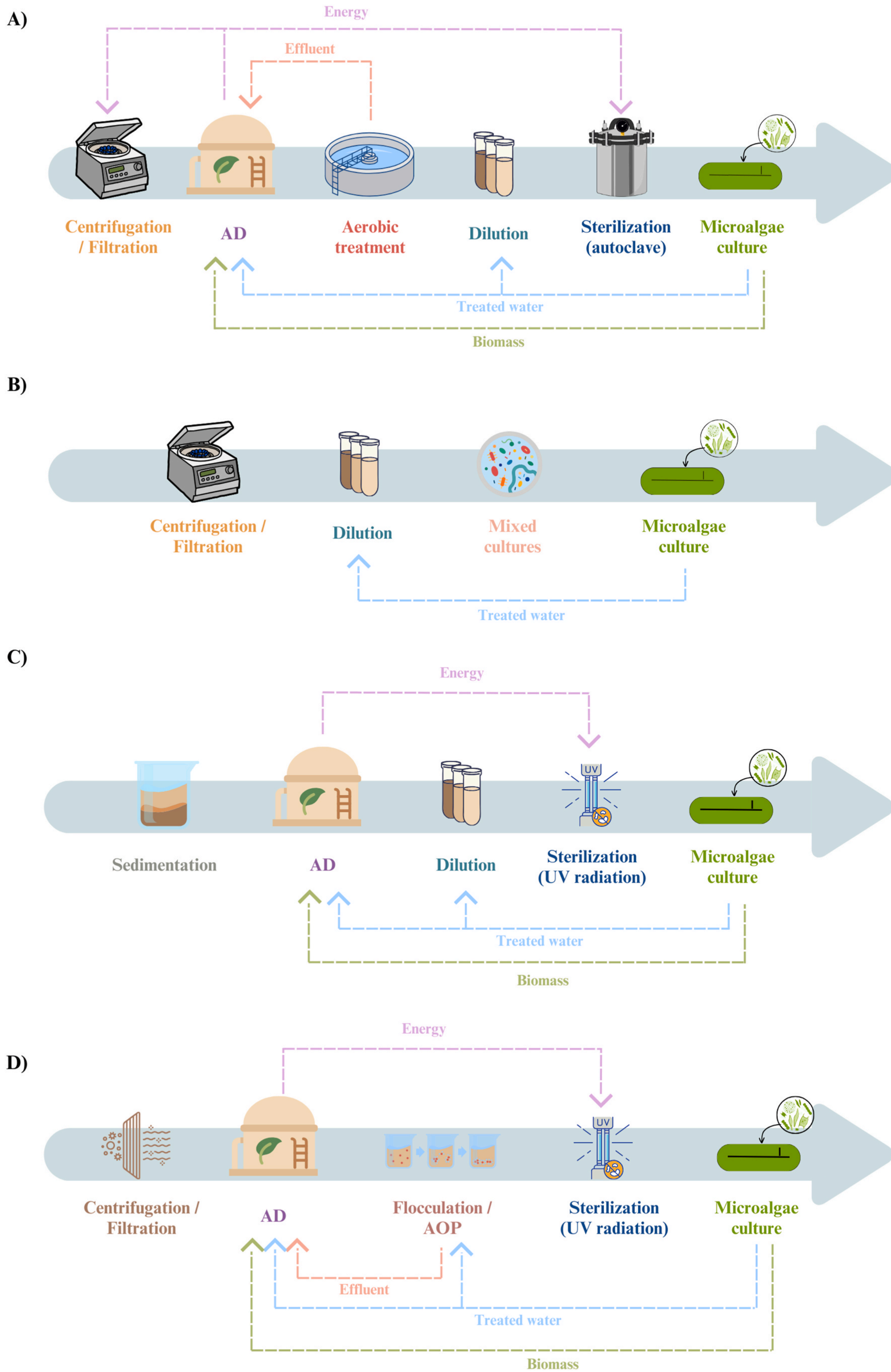

Fig. 4. Predominant treatment sequence of MbLWT systems in A) 2018, B) 2019 and 2022, C) 2020, D) 2021 and 2023.

biological treatment of undiluted effluent reached 81% CODr. Ran et al. (2021) investigated the growth of *C. vulgaris* FACHB–8 in anaerobically digested swine wastewater (ADSW) with dilution ratios of 1:1, 1:3, 1:5, and 1:7. They found that lower dilution ratios (1:1 and 1:3) impeded growth, likely due to the dark brown colour reducing light transmittance or toxicity of the highly-concentrated NH_4^+–N in ADSW. Although microalgal growth was initially slow in less diluted LW, cultures lasted longer as there was more nutrient availability in the media. However, dilution is not always proportional to pollutant removal. Wang et al. (2020b) cultured *C. vulgaris* in 1:5, 1:10, 1:15, and 1:20 diluted DW over seven days with an inoculum of 0.23 g L^{-1}, and the Total Kjeldahl Nitrogen removal (TKNr) and turbidity removal displayed no significant differences, while the TPr remained within a narrow range (52–65%).

Despite 47% of studies employing dilution to reduce the organic load, turbidity, or NH_4^+–N, this approach can sometimes affect microalgae uptake and impede pollutant removal. Moreover, dilution compromises the sustainability of the treatment, as it is not economically (increased costs due to larger volumes that need larger facilities) or environmentally (consumption of large amounts of freshwater) viable (Wang et al., 2021).

3.3.2. Anaerobic digestion (AD)

Anaerobic digestion (AD), an anoxic biological wastewater treatment process, is widely implemented to reduce the organic load of LW. It was the third most used process in the selected references. During AD, a gas mixture (biogas) and an effluent (digestate) are produced. The biogas, primarily composed of CH_4 and CO_2, can be used to produce energy. The effluent, known as digestate or anaerobically digested livestock wastewater (ADLW), requires further treatment before being reused or discharged due to its high content of organic matter, N, P, and pathogens (Huo et al., 2021). Wang et al. (2022a) conducted a full-scale microalgal electroactive wetland coupled with AD to treat SW. The AD achieved removal efficiencies of 77–89% CODr, 27–30% TPr, 26–73% NH_4^+–N removal, and 50–63% TNr. These efficiencies were temperature-dependent, with higher values in summer and spring, and lower in autumn and winter. However, since AD does not efficiently remove inorganic pollutants (TP and TN), a subsequent microalgal electroactive wetland was employed, significantly enhancing pollutant removal to 91–98% CODr, 67–95% TPr, 36–98% NH_4^+–N removal, and 72–95% TNr. Compared to a control system without microalgae, the AD combined with a microalgal electroactive wetland demonstrated superior pollutant removal, averaging about 10% higher (Wang et al., 2022a).

The organic matter in ADLW tends to be more bioavailable for microalgal growth due to anaerobic transformations producing volatile fatty acids (VFAs) like acetic acid, propionic acid, and butyric acid. Any surplus sugar compounds will also remain in the AD effluent, serving as a carbon source readily available for microalgae growth (Sun et al., 2019). Furthermore, AD facilitates access to other nutrients by converting complex organic compounds into ammonium and phosphates. However, due to the varying composition of the raw LW (Table 1), some ADLW can display more complex carbon compounds, including bacterial biomass, not readily available for microalgal metabolism. Therefore, AD is not always the best treatment to precede MbLWT, as the strength of ADLW is still very high. Gracida-Valdepeña et al. (2020) found that culturing *Chlorella* sp. in SW and ADSW yielded different results. Despite ADSW having almost 12 times lower COD concentration (376 mg L^{-1}) than in SW (4365 mg L^{-1}), AD led to a fourfold increase in NH_4^+–N concentration (570 mg L^{-1} in comparison with 136 mg L^{-1} present in SW). This resulted in a lower microalgal cell concentration when cultured in ADSW (<50 × 10^6 cells mL^{-1}) compared to SW (339 × 10^6 cells mL^{-1}). Similarly, microalgal treatment in SW resulted in higher CODr (91%) than ADSW (<40%), but NH_4^+–N and PO_4^{3-}–P removals were higher in microalgal treatment of ADSW (around 51% and 78%, respectively) than in SW (43% and 70%, respectively). However, the effectiveness of CODr in these cases was also attributed to bacterial oxidation, given the non-sterile media (Gracida-Valdepeña et al., 2020).

Despite the typically high NH_4^+–N concentrations in ADLW, less than half of the references applying AD (40%) used dilution in their studies. Some combined AD with other methods, like ammonia stripping, to reduce NH_4^+–N concentrations, a method to be discussed later.

3.3.3. Fenton and photo-Fenton oxidation

Fenton and photo-Fenton oxidation are advanced oxidation processes (AOP) primarily used to reduce the organic load and colour in wastewater, thereby enhancing light penetration for microalgal growth. These AOP techniques, also including ozonation and cold plasma, generate hydroxyl radicals ($OH^\bullet$) known for their high oxidation potential (E_{0ox} = 2.8 V) and ability to oxidise organic matter (Ferreira et al., 2022; J. C. Lee et al., 2021a). In these treatments, reactions involving iron (Fe^{3+}) and hydrogen peroxide (H_2O_2) are classified as Fenton-like reactions. H_2O_2 oxidises Fe^{2+}, acting as a catalyst to produce $OH^\bullet$ (Zhang et al., 2022). The process efficiency heavily depends on the H_2O_2:Fe ratio to ensure a complete reaction and total consumption of H_2O_2.

In photo-Fenton (PF) processes, photochemical reactions are driven by low-energy photons, quickly reducing oxidised Fe^{3+} to Fe^{2+} by UV–visible (UV–vis) radiation, allowing for cyclic regeneration. PF processes can operate under solar irradiation, with photons reacting rapidly and non-selectively to fully degrade organic contaminants and generate minimal solid waste. In addition, $OH^\bullet$ can oxidise NH_4^+ to NO_3^-, which is less toxic for microalgal growth (Ferreira et al., 2022). Therefore, Fenton oxidation (FO) coupled with biological treatment offers promise, enhancing wastewater biodegradability, de-colourisation, turbidity reduction, organic degradation, and pollutant removal (TN, TOC, TP, and COD).

AOP methods have been successfully applied to treat several types of wastewaters, including LW (Cui et al., 2021; Huo et al., 2021; J. C. Lee et al., 2021b). Ferreira et al. (2022) experimented with different pre-treatments applied to SW (electrocoagulation, ammonia stripping, PF, and constructed wetlands). PF emerged as the most effective treatment that most effective in reducing contaminant load, achieving 93% CODr, ~100% colour removal, and 99%TPr. However, excessive TPr might not favor microalgal growth, potentially leading to P deficiency and growth inhibition. Cui et al. (2021) cultured *Chlorella sorokiniana* SXAU–04 in PW treated with PF, but growth was hindered by H_2O_2. More than 20 mL L^{-1} H_2O_2 concentration significantly inhibited microalgal growth, with residual H_2O_2 concentrations reaching 35 mL L^{-1}. However, PF treatment conditions can be adjusted to increase the tolerance of microalgae. Microalgae thrived in pre-treated PF-PW with NH_4^+–N concentrations below 300 mg L^{-1} and H_2O_2 doses of 110–120 mL L^{-1} (Cui et al., 2021).

Although PO enhances pollutant removal compared to simple FO, introducing UV–vis radiation or any artificial light source represents extra costs that can account for an additional 35% in operating expenses (Ferreira et al., 2022; Hermosilla et al., 2009). Conversely, the use of H_2O_2 and UV radiation in PF processes can facilitate pathogen removal, potentially reducing costs associated with disinfection (Ferreira et al., 2022). Combining FO/PF with MbLWT offers promising economic and environmental benefits, particularly due to the absence of reported dilution in the selected references. However, this process is in its infancy, with only 5% of the selected references employing these methods. Thus, more research is necessary to comprehensively assess the potential and effectiveness of AOPs coupled with MbLWT.

3.3.4. Flocculation-coagulation

Flocculation-coagulation turns fine solid particles into large flocs, which gravitational or mechanical separation processes can remove, thus clarifying the media. This method is simple to operate and boasts high removal efficiencies for turbidity, chromaticity, COD, and TSS. However, only 9% of the reviewed studies implemented flocculation-coagulation, with three focusing on electrocoagulation (discussed below). Despite their limited number, these studies yielded effective results. Oliveira et al. (2018) compared raw poultry slaughterhouse wastewater with its flocculated counterpart, noting significantly lower TS, TSS, COD, and TP contents in the flocculated poultry wastewater. Besides, the biomass productivity of *Tetradesmus obliquus* (formerly *Scenedesmus obliquus* according to Oliveira et al. (2021)) in both types of wastewaters and Bristol medium showed no significant differences, with algal growth rates ranging from 0.08 to 0.13 g L^{-1} d^{-1} (Oliveira et al., 2018). Similarly, Zhou et al. (2019) achieved a biomass concentration of 3.30 g L^{-1} by cultivating *Chlorella* sp. in flocculated ADSW, contrasting with a peak of 0.50 g L^{-1} in raw ADSW, where biomass started declining after five days.

Several flocculants have been successfully applied, including simple inorganic salts (e.g., ferric sulfate, ferric chloride, and aluminium sulfate), inorganic pre-polymerised ferric or aluminium salts (e.g., polyaluminium chloride – PAC), synthetic organic polymer flocculants (e.g., polyacrylamide – PAM), and natural material–derived organic flocculants (e.g., chitosan and sodium alginate) (Wang et al., 2020a). The combined use of coagulants and flocculants enhances solid–liquid separation, with the coagulant initiating particle formation and the flocculant promoting their agglomeration (Osabutey et al., 2023). Osabutey et al. (2023) compared five flocculants (polyacrylamide polymer – PAM, Magnoflac, chitosan, and cationic starch) and one coagulant (ferric sulfate – $Fe_2(SO_4)_3$) on SW. PAM was the most effective after 30 min, achieving 90% TSS removal (TSSr) at pH 8 with a dosage of 193 mg L^{-1}, increasing to 93% TSSr after 24 h. $Fe_2(SO_4)_3$ displayed 64% TSSr at pH 6.5 with 2500 mg L^{-1} of dosage after 30 min, and 67% at 24 h; however, at pH 5, TSSr increased to 75% at 24 h. Chitosan, cationic starch, and Magnoflac displayed TSSr only apparent after 24 h, with 60% (pH 8, 500 mg L^{-1}), 78% (pH 8, 250 mg L^{-1}), and 72% (pH 8, 258 mg L^{-1}) TSSr, respectively. The pH was not modified for these three flocculants, but variations in pH could increase TSSr as evidenced by $Fe_2(SO_4)_3$. Despite PAM and cationic starch achieving higher TSSr, PAM was discarded due to environmental concerns regarding its slow degradation and ecotoxicity. Consequently, cationic starch (made from potato peel) and $Fe_2(SO_4)_3$ were chosen for further research. When microalgae were cultured in this flocculated SW, the lag phase was shorter (10 days) than that of the raw SW (19 days), with a similar cell concentration in flocculated SW (3.20×10^9 cell L^{-1}) than in untreated SW (2.29×10^9 cell L^{-1}) (Osabutey et al., 2023).

Inorganic flocculants and organic polymer flocculants are often used but can be costly, difficult to manage in terms of remaining sludge treatment, and lead to secondary pollution, limiting their large-scale applications (Gu et al., 2021; Terán Hilares et al., 2021). Thus, natural organic flocculants have emerged as an alternative, avoiding secondary pollution derived from residual metal ions or toxic polymeric monomers, such as acrylamide in PAM (Osabutey et al., 2023; Wang et al., 2020a). An acid precipitation method using H_2SO_4 for LW was explored, where acidification-induced precipitates trapped suspended and colloidal matter, reducing the organic matter and turbidity. This method allowed 80% CODr by precipitation and over 83% of residual CODr in microalgae cultivation using the corresponding supernatant. Additionally, turbidity dropped from 650 to 950 NTU to 98 NTU, with a notable reduction in microbial load due to the low pH values (4, 5, and 6) (Terán Hilares et al., 2021). Also, chitosan flocculation followed by fast centrifugation in DW achieved 90–93% TSSr. Chitosan destabilises negative surface charges of suspended colloids with its protonated amino groups, forming big flocs. An optimal chitosan dosage (683 mg L^{-1}) maximised CODr (73%), TKNr (59%), TPr (43%), and turbidity removal (93%). High turbidity removal minimally inhibited microalgal growth (*C. vulgaris*), resulting in a biomass density of about 1.20 g L^{-1}, and further CODr, TKNr, and TPr by 82, 90, and 83%, respectively, achieving a total process (flocculation and microalgal culture) efficiency of 95% CODr, 96% TKNr, and 91% TPr.

Industrial residue has been revalorised as a flocculant/coagulant to reduce the turbidity of LW. Biomass ash obtained from the combustion of biomass wastes in ceramic ovens, when added to CW, acted as a coagulant, reducing TSS and turbidity. This treatment increased pH to 12 or higher, promoting structural changes in proteins and other molecules, breaking micellar structures and reducing the solubility of many effluent components. After dilution and ash addition, the pH increased, resulting in visible progressive precipitation and clarification of the solution with 38% CODr. However, a 74% TNr indicated the need for N supplementation during microalgae culture. This high pH also helped to destroy microorganisms (e.g., bacteria), reducing contamination and competition with microalgae, as microalgae are more resistant to alkaline conditions (Viegas et al., 2021).

Although flocculation is a promising pre-treatment for MbLWT, careful selection of flocculants is crucial to avoid excessive TNr or inadequate NH_4^+–N removal. Contrary to Viegas et al. (2021), Oliveira et al. (2018) found double the NH_4^+–N content in poultry flocculated slaughterhouse wastewater compared to the raw effluent. While flocculation is a simple and effective method for removing turbidity, chromaticity, and COD, a thorough TN content analysis is crucial to avoid potential inhibition, whether due to excess or deficiency.

3.3.5. *Electrocoagulation*

Electrocoagulation (EC) is a process that destabilises pollutant charges through sacrificial metal electrodes (iron or aluminium) that releases cations, such as Fe^{2+} or Al^{3+}. These cations form hydroxides or poly-hydroxides with a great affinity to coagulate (Ferreira et al., 2022; Huo et al., 2021). In wastewater, pollutants aggregate and are separated from the solution either by sedimentation, aided by the heavier floc weight, or by flotation through microbubbles generated by water electrolysis (Huo et al., 2021). This treatment process enhances effluent filterability, removes COD, turbidity, TSS, and colour. In addition, EC can be easily coupled with AD without introducing exogenous pollutants, except for iron (Fe), which can stimulate microalgal growth as it is an essential element for almost all microorganisms (Huo et al., 2020). However, EC was used in only three articles identified through the systematic search, as it is an emerging technology recently applied more broadly in industrial and municipal wastewater, particularly for textile wastewater. Its application in agricultural wastewaters like LW is still underexplored, especially in combination with MbLWT.

The efficiency of EC treatment depends on the electrode type, electrode distance, current density, pH, electrolyte conductivity, chemical composition, temperature and other operational conditions. Ferreira et al. (2022) tested different electrodes to treat undiluted SW, finding that zinc (Zn) electrodes achieved the highest CODr (35–41%) and TSSr (9–20%). Conversely, aluminium (Al) and Fe electrodes, despite being widely used, increased the TSS content due to electrode corrosion (Ferreira et al., 2022). Therefore, further investigation is required regarding interactions between electrodes and complex media like LW, which could limit EC application.

External strategies have been implemented to enhance the efficiency of EC, such as H_2O_2–enhanced electro–Fenton. This method utilises spontaneously produced H_2O_2 during electrolysis at the electrode by reduction of oxygen (O_2), which initiates a Fenton reaction (named electro-Fenton reaction) when combined with iron salts. Huo et al. (2021) demonstrated that increasing concentrations of H_2O_2 (up until 300 mg L^{-1}) in ADSW treated with H_2O_2-enhanced electro-Fenton, significantly increased CODr (<20–37%), colour removal (<50–86%), TSSr (<30–61%) and TPr (10–92%). However, concentrations above 200 mg L^{-1} led to residual H_2O_2 accumulation, which could be prejudicial to microalgae. In this study, although *Chlorella* sp. grew in EC-treated ADSW, achieving a protein content of 54% in biomass (2.07 g L^{-1}), CODr, TPr, and colour removal were not efficient (−22, 34, and 49%, respectively), possibly due to cell damage and pigment leakage (Huo et al., 2021). Thus, while EC coupled with the Fenton reaction effectively removes solids and organic pollutants, the impact on subsequent microalgal culture requires further investigation.

A limitation of EC is its minimal effect on NH_4^+–N removal, as NH_4^+

exists as small molecules not easily flocculated (R.-F. R.-F. Chen et al., 2021). In contrast, negatively charged soluble P rapidly forms $AlPO_4$ with Al^{3+} and can be removed through precipitation (Huo et al., 2020). However, since N is not removed, the N:P ratio increases, which may not be optimal for microalgae growth, necessitating additional treatments to lower N concentrations.

EC has been reported as a reasonable, cost-effective LW treatment (Hellal et al., 2023; Yokus et al., 2023), although some authors have related an increase in voltage with an increase in CODr (Manjunath et al., 2023), which could be disadvantageous for economic aspects. Optimal conditions are crucial for economic feasibility, including specific pH, current, reaction time, and wastewater composition. However, most research is at the laboratory scale and does not reflect large-scale treatment costs. Therefore, pilot-scale economic evaluations are essential to determine the feasibility of EC for LW treatment, alongside further investigation into microalgae behaviour in EC-treated effluents.

3.4. Decrease in NH_4^+–N

Microalgae tolerance to high N contents is influenced by the microalgal strain and the form of N present in the media. Free ammonia (NH_3) can permeate across algal cell membranes and cause toxic effects that adversely impact growth. While NH_3 inhibition typically occurs above 61–96 mg NH_3 L^{-1} (Rossi et al., 2020), *C. vulgaris* has shown tolerance up until 220 mg NH_3–N L^{-1}, with a subsequent decrease in cell viability (61%) above this concentration (Zheng et al., 2019).

Despite NH_4^+–N toxicity hindering microalgae physiological activities (e.g., converting ATP to ADP), higher tolerance to NH_4^+–N is generally exhibited due to their preference for NH_4^+ as a nitrogen source (Chai et al., 2021). For instance, *C. vulgaris* has tolerated almost three times more NH_4^+–N concentration (625 mg NH_4^+–N L^{-1}) than NH_3–N (López-Sánchez et al., 2022a). Nevertheless, NH_4^+–N microalgae tolerance can change depending on a previous acclimation to higher concentrations. The Chlorophyceae class has shown a tolerance of around 128.72 mg NH_4^+–N L^{-1}, although some microalgae of this class (*C. thermophila* and *C. sorokiniana*) have tolerated up to 600 and 1400 mg NH_4^+–N L^{-1}, respectively (Table 2). Thus, screening for NH_4^+ tolerance is crucial for ensuring the technical feasibility of a specific microalgae strain.

Conventional N removal processes involve an aerated stage for nitrification (NH_4^+ oxidation to NO_3^-, via NO_2^-), followed by an anoxic step for denitrification (reduction of NO_3^- to nitrogen gas) (Svierzoski et al., 2021). However, another strategy to circumvent NH_4^+/NH_3 inhibition involves controlling the chemical equilibrium through pH adjustment, as the concentration of NH_3 is pH dependent and in equilibrium with NH_4^+ (Rossi et al., 2020). For certain microalgae, high NH_4^+ levels (up to 3000 mg NH_4^+–N L^{-1}) in ADLW are not problematic if pH is neutral (Q. Wang et al., 2021). Dinnebier et al. (2021) cultured *C. sorokiniana* in NH_4^+–N concentrations as high as 1300 mg N L^{-1} by keeping a neutral pH (7–7.5), while Ran et al. (2021) could not grow microalgae in digestate exceeding 20 mg NH_4^+–N L^{-1}. CO_2 injection can also assist in pH adjustment, as microalgal photosynthesis utilising CO_2 tends to increase pH, while NH_4^+–N absorption results in a pH decrease due to the release of H^+ ions (Ferreira et al., 2021; Ran et al., 2021). Thus, this section explores different strategies to mitigate microalgal growth inhibition caused by high NH_3/NH_4^+ concentrations commonly found in LW.

Table 2
Microalgae NH_4^+–N concentration tolerance reported in the selected bibliography.

Microalgae strain	NH_4^+–N tolerance concentration (mg L^{-1})	Reference
Didymogenes chengda	300	(Tang et al., 2023)
Chlorella sorokiniana	300 - 1400	(Cui et al., 2020; Jain et al., 2022; Q. Wang et al., 2021)
Chlorella thermophila	600	Jain et al. (2022)
Chlorella vulgaris	110–625	(Hülsen et al., 2018; Svierzoski et al., 2021)

3.4.1. Ammonia/air stripping

Ammonia stripping (AS) is an established method for reducing NH_3–N levels in wastewater. This process, known for its ease of operation, high efficiency, and stability, was utilised in 21% of the studies selected for this systematic review. AS operates by injecting air into wastewater with a pH adjusted above 8.5, leading to the volatilization of NH_3 due to its high volatility.

The effectiveness of NH_3 removal via AS strongly depend on airflow rate, pH, and temperature. Elevated temperatures (up to 80 °C) and high pH (10–11) enhance the stripping efficiency by promoting NH_3 formation. Typical airflow rates during AS range from 0.50 to 10 vvm (Ferreira et al., 2022). AS is considered highly efficient among various methods, particularly for wastewater with high NH_3 concentrations, as it does not generate extra sludge and uses relatively inexpensive alkaline reagents (e.g., NaOH, $Ca(OH)_2$, or even no reagents at all) (Abbà et al., 2023; Folino et al., 2020). However, it is crucial to maintain TN concentrations at levels where no deficiency nor excess inhibits microalgal growth. Zheng et al. (2018) noted that microalgae cultured in AS-pre-treated SW decreased cell viability and biomass concentration below or above 110 mg NH_4^+–N L^{-1}. At the optimal NH_4^+ concentration (110 mg NH_4^+–N L^{-1}), nutrient removal was maximised (96% CODr, 100% NH_4^+–N removal, 93% TNr and 91% TPr).

A main drawback of AS is the production of alkaline effluent, necessitating further treatment, although microalgae can grow in such alkaline conditions (Q. Wang et al., 2021). Additionally, fouling issues can affect stripping performance, leading to increased operational and maintenance costs (Ferreira et al., 2022). Lastly, as the release of NH_3 gas causes additional environmental adverse effects, an adsorbing unit containing an acid solution to capture the volatilised NH_3 is required (e.g., sulfuric acid to produce ammonium sulfate, which can be used as fertiliser). This step can also increase capital and operational costs, and complicates the circularity of the treatment (Ferreira et al., 2022; Q. Wang et al., 2021).

3.4.2. Constructed wetlands

Constructed wetlands (CWS) are engineered systems that emulate natural remediation processes. They incorporate wetland vegetation, soils, and associated microbial communities to treat wastewater in a controlled environment. Recognised for being economical and easy to maintain, CWS can convert N and P into bioenergy through microalgal-bacterial consortia (T. Wang et al., 2022b). However, of the 100 references identified in this review, only two studies employed CWS.

CWS facilitate using LW directly as microalgal growth media (without dilution) and effectively removes pollutants such as BOD, COD, and NH_4^+–N. Notably, nitrification within these wetlands converts NH_3 to nitrates due to the passive aeration, benefiting microalgae growth by reducing NH_3 levels (Ferreira et al., 2022). In the study of Ferreira et al. (2022), various pre-treatments (EC, AS, PF, and CWS) were examined. While AS and EC demonstrated effective nutrient removal, the microalgae *T. obliquus* could not grow on AS-treated or EC-treated SW. In contrast, microalgae grew successfully in CWS-treated SW, initiating growth after a 7-day lag phase and surpassing growth rates in Bristol medium and 1:20 diluted SW. Besides, *T. obliquus* achieved nearly complete NH_4^+–N removal (99.8%) in CWS-treated effluent, considerably outperforming the results in PF-treated effluent (37% NH_4^+–N removal), possibly due to the lower NH_4^+–N concentration in CWS-treated SW (~10% of that present in PF-treated SW) (Ferreira et al., 2022). This outcome proved the effectiveness of CWS in removing NH_4^+–N and enhancing microalgal growth and nutrient removal.

Despite their efficiency as a pre-treatment method for MbLWT, CWS require large areas, are susceptible to climatic variations, and their performance significantly depends on the plant species used. Additionally, the substrates used in CWS can be easily saturated and plugged (Fernández del Castillo et al., 2022; Verduzco Garibay et al., 2022).

3.4.3. Physical absorption/adsorption

Sorption processes involve several interactions (hydrogen bonding, electrostatic attraction, ion exchange and hydrophobic effects) between pollutants and sorbent surface (Masebinu et al., 2019). These processes are particularly adept at removing NH_4^+–N, and are promising for wastewater pollutant removal due to their high efficiency, operational simplicity, and low cost (He et al., 2018). However, applying sorption processes to LW remains underexplored, as only two studies of the selected references reported it.

Increasing pore volume and functional groups that adsorb NH_4^+ (e.g., carboxylic or lactonic), and decreasing sorbent's surface area are some strategies that enhances adsorption (Yu et al., 2020; Zhang et al., 2022). Mg–Fe-based hydrotalcite, zeolite, vermiculite, zero-valent iron, granular ferric hydroxide, activated carbon, and biochar are sorbents that have been developed (Zhang et al., 2022; Masebinu et al., 2019). Yu et al. (2020) utilised biochar for NH_4^+–N removal in SW, achieving 32% NH_4^+–N removal, 33% TNr, 25% TPr, and 26% CODr. However, due to the high residual nutrient concentrations in the treated SW, a subsequent 10-fold dilution was necessary to mitigate NH_4^+ toxicity for microalgae (*C. vulgaris* FACHB-30) (Yu et al., 2020).

Physical adsorption can be effectively combined with other treatment methods. Zhang et al. (2022) integrated iron–carbon micro-electrolysis treatment with physical adsorption using zeolite or vermiculite in SW. The combined treatment of iron–carbon micro-electrolysis, adsorption using vermiculite, and microalgae cultivation reduced NH_4^+–N levels from 56.73 to 1.33 mg L^{-1}. However, the iron-carbon micro-electrolysis introduced a considerable amount of iron ions (up to 45 mg L^{-1}), which could negatively impact subsequent microalgae cultivation and cause secondary environmental pollution. Furthermore, the hydrophilic nature of zeolite and vermiculite surfaces limited their capacity to adsorb organic contaminants, impacting CODr negatively (−0.04 and −1.27% CODr, respectively) (Zhang et al., 2022).

Some sorbents may produce solid waste that could potentially be used as fertiliser in agriculture (Yu et al., 2020). However, further research is warranted given the limited information on physical adsorption coupled with MbLWT, and the challenges associated with sorbents, such as environmental instability and recollection difficulties (He et al., 2018). Consequently, the research should concentrate on the interactions of sorbents with LW, and synergies between physical adsorption, microalgae culture, and circular bioeconomy.

3.4.4. Mixed effluents

Wastewater can be categorised into low strength (e.g, municipal, domestic wastewaters) and high strength (e.g., tannery effluent, central wastewater, carpet mill effluent, and LW). Low-strength wastewater, when used for microalgal culture, has insufficient nutrient supply such as N and P, which compromises biomass productivity. Conversely, high-strength wastewaters often contain nutrient overload, unsuitable for direct microalgal growth (Jain et al., 2022).

Mixing different types of effluents, as applied in 13 of the 100 studies reviewed, can balance nutrient levels, eliminating the need for freshwater dilution or synthetic media, and enhancing MbLWT sustainability. Among the treatments used in the selected references, centrifugation, filtration, and AD applied dilution in a higher proportion (68, 40, and 40%, respectively) than those using mixed effluents (31%). For instance, Cui et al. (2020) diluted PW with municipal wastewater (MW) at ratios of 1:3 and 1:5 PW:MW, resulting in significantly higher biomass production (188% 1:3 and 197% 1:5, respectively) than when cultured in BG11-medium, and achieved nearly complete nutrient removal (Cui et al., 2020). Similarly, Deng et al. (2018) achieved more than two times higher biomass yield (2.42 g L^{-1}) with ADSW diluted 3-fold with centrate wastewater (concentrated MW) in comparison with a previous study using ADSW diluted 3-fold with distilled water (1.05 g L^{-1}). Besides, at a ADSW diluted 6- and 8-fold with centrate wastewater no lag phase was observed for *C. vulgaris* (Deng et al., 2018).

The proportion of mixed effluents is crucial, as it influences microalgal growth and nutrient removal. López-Sánchez et al. (2022b) found that specific combinations of ADLW (ADSW, anaerobically digested PW (ADPW) and anaerobically digested CW (ADCW)) favoured different parameters of MbLWT. For instance, higher proportions of ADCW were positively correlated to TNr and CODr, while higher TPr efficiencies were positively correlated with higher ADCW and ADPW ratios. Furthermore, a mixture with a lower proportion of ADSW and equal proportions of ADPW and ADCW led to optimal results for *C. vulgaris* growth (3.61×10^7 cell mL^{-1}) and nutrient removal (85% TNr, 66% TPr, and 44% CODr).

Despite these advantages, mixing different wastewater types poses challenges, including the availability of complementary wastewater, potential transport costs, and introducing heavy metals or emerging contaminants from one wastewater type to another. These limitations restrict broader application, particularly regarding the implications of combining wastewaters with varying contaminant profiles and scenarios where industrial plants producing high-strength wastewater are distant from sources of low-strength wastewater (Cui et al., 2021; Zheng et al., 2019).

3.5. Decrease in competing microbiological communities

Biological contamination poses a significant challenge in microalgae cultivation and further large-scale MbLWT implementation, as the microorganisms present in LW compete with microalgae for nutrients (Aditya et al., 2022). Xinjie et al. (2019) identified 35 dominant genera of bacteria in ADSW, including pathogenic bacteria *Clostridium* spp., *Arcobacter* spp., *Escherichia* spp., *Chryseobacterium* spp., and *Pseudomonas* spp. However, the composition and abundance of these microorganisms changed depending on the LW treatment stage (Zheng et al., 2022). Therefore, incorporating additional treatments, such as UV radiation, is often necessary to address biological contamination in MbLWT systems.

Different strategies can be employed to mitigate biological contamination during MbLWT, such as acclimation of microalgal strains, pH adjustment, and high NH_4^+–N concentrations, which allow microalgae growth while inhibiting bacterial proliferation. Shayesteh et al. (2022) demonstrated this by culturing *Scenedesmus* sp. in anaerobically digested abattoir wastewater in an outdoor raceway pond without any invasive biological contaminants (fungi, protozoa, or other microalgae) throughout one year. This success was attributed to the acclimation and adaptation of *Scenedesmus* sp. combined with its tolerance to high NH_4^+ contents, high initial cell density and fast growth rates. Also, the pH contributed to biological contamination control, as in this study, it could reach up to 11 within three days. Thus, an 80% removal of the microbial population (aerobic bacteria, microalgae grazers, protozoa, mini-metazoa) from the culture was observed (Shayesteh et al., 2022). Therefore, the strategies used in the references selected from the systematic research aiming at eliminating or controlling the biological population in LW to enhance the efficacy of MbLWT are discussed below.

3.5.1. UV radiation

UV radiation is a simple and effective method for wastewater treatment, yet its application in LW is limited, as evidenced by only two of the 100 studies reviewed employing it. The high turbidity characteristic of LW hampers UV effectiveness, which relies on light penetration. Nevertheless, Svierzoski et al. (2021) experimented with cattle slaughterhouse wastewater treated with a biological system coupled with UV radiation, followed by microalgal cultivation. After UV irradiation for 5, 15 and 60 min, the total coliforms were reduced to 4000, 3000, and 400

CFU (100 mL)$^{-1}$, respectively. The study observed a linear relationship between the accumulated energy per volume unit and the reduction in CFU, indicating that total coliforms could be entirely removed with a UV-C dose of 79 kJ L^{-1}. Interestingly, UV-C irradiation affected nutrient concentrations, increasing TP and TN levels. However, further research is necessary to fully understand these nutrient variations post-UV treatment (Svierzoski et al., 2021). During subsequent microalgae cultivation using the biologically UV-treated effluent as a media, higher TNr (99%) was achieved, and 43% TP was removed, as microalgae faced reduced nutrient competition with other microorganisms.

Disinfection of effluents using UV light on LW is not commonly reported due to its high turbidity. Pre-treatment methods that reduce the turbidity of LW could make UV radiation a more viable option for biological decontamination. However, a research gap exists regarding disinfection methods suitable for large-scale applications. Of the 32 studies reporting sterilisation or disinfection for LW, nearly 72% used autoclaving, a technique not feasible for large-scale use. This gap underscores the need for further investigation into practical and scalable disinfection methods for LW treatment.

3.5.2. *Chemical sanitisers*

Chemical sanitisers are typically used for pathogen inactivation in wastewater treatment, yet only a small fraction (five out of 32) of the selected references using sterilisation/disinfection employed them. The chemicals employed for sterilisation purposes in these studies were sodium hypochlorite (NaClO) and trichloroisocyanuric acid (Xie et al., 2022; Zheng et al., 2021). NaClO has proven to be effective not only in pathogen reduction but also in CODr and NH_4^+–N removal. Dispersed-NaClO generates hypochlorous acid (HClO) and hypochlorite ion ($^{-}$ClO), reacting with organic matter and NH_4^+ (producing compounds that eventually convert into N_2) (Lv et al., 2018). Sun et al. (2019) added NaClO to sterilise ADSW for *C. vulgaris'* culture. The NaClO solution, containing 130 mg of available chlorine per litre, was applied for 12 h and subsequently neutralised with $Na_2S_2O_3$. Also, high air flux was used post-sterilisation to eliminate residual active chlorine and reduce NH_4^+–N levels (Sun et al., 2019).

However, using chemical sanitisers requires caution, as bacteria can develop resistance to these chemicals, and residual concentrations could adversely affect microalgae (Xinjie et al., 2019). An alternative approach is pH modification (acid or alkaline pre-treatment). Bacterial growth is optimal between pH 6 and 8.5, and deviations above nine or below six can inhibit bacterial proliferation (Terán Hilares et al., 2021). Musetsho et al. (2021) lowered the pH of poultry litter extract to two using HCl 5 M, achieving a significant bacterial load removal (99.99%) and 41.18% TSSr. However, this method slightly increased NH_4^+–N concentration (8.33% increase) and a further pH adjustment was needed for microalgal cultivation. Thus, despite its effectiveness, the economic and environmental impacts of pH adjustment, including the extensive use of chemicals for pH regulation and increased GHG emissions (acidic pH liberates CO_2), limit the practicality of this approach in large-scale applications (Arzate et al., 2019).

3.5.3. *Mixed cultures*

Research on mixed cultures, applied in 29% of the reviewed references, focuses mainly on bacteria-microalgae co-culture (52%), called algal-bacterial symbiosis (ABS) systems. Some of these articles explore microalgae-microalgae interactions (21%), while 28% of the studies investigate both ABS and microalgae-microalgae interactions, and a single article studied plant-microalgae interactions (Xinjie et al., 2019).

In ABS, microalgae use NH_4^+ and CO_2 produced from bacterially-degraded organic compounds in photosynthesis, generating oxygen for bacterial growth and organic matter oxidation (Yu et al., 2020). Similarly, heterotrophic bacteria can supply vitamin B as organic cofactors or produce siderophores to fix iron, which the microalgae can use. This symbiotic interaction can reduce aeration energy consumption and increase treatment efficiency, microalgal cell growth and tolerance to harsh conditions, such as high NH_4^+–N concentration in LW (Cui et al., 2020; Gu et al., 2021).

Microalgae typically exhibit lower CODr and can even increase COD concentrations due to abiotic stress (Huo et al., 2021). However, ABS can enhance nutrient removal, as seen by Liu et al. (2022), who achieved a 160–327% increase in CODr compared with the groups without indigenous bacteria. Even TNr and TPr were increased by 24% and 31% compared with the pure microalgae group (Liu et al., 2022). Similarly, Yu et al. (2020) observed higher CODr in ABS (99%) than solely *C. vulgaris* (88%) when cultured in SW.

Balancing microalgal and bacterial loads is critical in ABS systems to avoid microalgae displacement and optimise nutritional exchange. Chen et al. (2022) found that specific inoculum concentrations of microalgae (0.20 g L^{-1}) and bacteria (0.15 g L^{-1}) yielded the best performance in terms of biomass productivity (0.56 g L^{-1} d^{-1}) and nutrient removal (85% CODr, 94% BODr and 87% TNr). Interestingly, bacterial competition with microalgae for nutrients can stimulate lipid accumulation in microalgae, which is beneficial for biofuel applications, though this interaction requires further research to avoid later microalgae displacement (Luo et al., 2019).

Exploring suitable ABS configurations is another strategy to enhance conventional biological wastewater treatment methods (Li et al., 2022). For instance, different bacterial strains show varying degrees of nutrient removal. Chen et al. (2022) observed that *C. sorokiniana* cultured with *Acinetobacter lwoffii* in SW achieved lower CODr and TNr (61% and 69%, respectively), than when cultured with *Bacillus thuringiensis* (71% CODr and 78% TNr). Also, depending on the treatment phase, the dominant bacterial genera can change to influence microalgal growth or nutrient removal. *Stenotrophomonas* sp. (Gammaproteobacteria) can promote the growth of the microalgae, while *Comamonas* sp. (Proteobacteria) and *Sphingobacterium* sp. (Bacteroidetes) are active in NH_4^+–N removal (Montaño San Agustin et al., 2022).

Therefore, comprehensive monitoring of microbial communities and specific biochemical indicators, such as quantum yield, enzyme activities, and sequencing, is essential to understand these interactions and validate the feasibility of ABS systems in LW treatment.

4. Selection of an integral MbLWT system

The development and large-scale implementation of clean, effective, and renewable technologies for biorefinery product acquisition (treated water, microalgal biomass with several applications, biogas, and fertilisers) represent a significant challenge for scientists. Simultaneously, it stands as a priority for local systems and industry stakeholders (Zieliński et al., 2018). Consequently, the forthcoming sections are designed to guide the development of large-scale MbLWT systems.

4.1. *Evolution of treatment application trend over time (2018:2023)*

Over the years, distinct trends in wastewater treatment have emerged. Sterilisation was frequently reported in the reviewed references from 2018, with six out of 11 using this method, predominantly via autoclaving, and two using NaClO. Other commonly used treatments included filtration, dilution, AD, centrifugation, and aerobic treatments. Thus, the predominant treatment sequence in 2018 would involve filtration or centrifugation for solid removal, organic load reduction via AD, aerobic treatment for NH_3/NH_4^+ removal, dilution to reduce turbidity, sterilisation via autoclaving (NaClO addition could also be considered) (Fig. 4A).

In 2019, the focus shifted slightly, with a decreased use of AD (three out of 12 articles) and an introduction of mixed cultures, indicating a possible shift from sterilisation to robust consortia for microbial control. Then, the treatment sequence for 2019 involves filtration or centrifugation, dilution for organic load, turbidity and NH_3/NH_4^+ mitigation, and mixed cultures (Fig. 4B). By 2020, AD regained prominence, appearing in seven of the 14 articles published that year, while sedimentation emerged as a notable treatment (reported in five articles). Sterilisation and dilution were also frequently used (in six articles each), although one of the articles applied UV radiation as an alternative to autoclave, enhancing the scalability of the system. Thus, the revised pre-treatment train in 2020 could involve sedimentation for solid removal, followed by AD, dilution for turbidity and NH_3/NH_4^+ mitigation, and UV radiation (Fig. 4C).

In 2021, there was a noticeable decrease in dilution usage, with only five of the 18 articles employing it. This year also saw an increase in the use of flocculation and AOP, suggesting a shift towards more efficient turbidity and NH_4^+–N control methods. An alternative treatment train without dilution could include sedimentation or filtration, AD, flocculation or AOP to address turbidity and NH_4^+–N concentration and UV radiation (Fig. 4D). The trend continued in 2022, focusing on mixed cultures, as seen in 12 of the 28 articles. However, this year also saw the introduction of various treatments, such as hydrodynamic cavitation, CWS, iron-carbon micro-electrolysis, and adsorption methods. Thus, 2022 exhibited a similar trend as 2019 (Fig. 4B), with the addition of sporadic non-conventional treatment reports. By 2023, sterilisation was less commonly reported, with only three instances out of 17 articles, and the use of NaClO and UV radiation was noted. Additionally, flocculation, mixed effluents, and AOP appeared in a single article each, indicating a trend towards diversified treatment approaches. Hence, the prevailing trend in pre-treatment strategies for LW treatment in 2023 closely aligned with the patterns observed in 2021 (Fig. 4D).

Most reported treatment technologies were applied sequentially rather than in parallel (S. A. Lee et al., 2021a; Svierzoski et al., 2021). However, synergies between operational units should be considered in system design. For instance, biogas from AD could be utilised for energy-intensive processes (centrifugation or UV radiation), while effluents with less organic and NH_4^+–N load can be recirculated to avoid freshwater dilution. Additionally, beyond its value-added applications, microalgal biomass can be integrated into AD to enhance biogas quality.

4.2. Decision-making process, selection and design of treatment systems

Designing an effective wastewater treatment system requires meticulously evaluating various alternatives, configurations, and technologies. This process is crucial to meet specific goals such as maximising efficiency, reducing costs, or adhering to environmental standards. Although Table S3 of the Supplementary Material compiles microalgal nutrient removal efficiencies from various studies, pinpointing the best pre-treatment method remains challenging. This difficulty arises due to diverse conditions (e.g., pH, light intensity, aeration, agitation, temperature, inoculum size), dosages (e.g., flocculants, UV irradiation), scales (ranging from bench-scale to large-scale), timespan (from a few days to year-round), contextual factors (e.g., available area, materials, climate, budget), and differing compositions of LW.

Researchers often select treatment methods based on perceived benefits and focus on optimising those methods. For instance, Zheng et al. (2022) developed a large-scale SW purification system that included solid-liquid separation, AD, flocculation, facultative anaerobic and aerobic pools, an oxidation pond, and a final disinfection pool, with microalgae cultivation occurring in the anaerobic, aerobic, and oxidation stages. However, despite research on MbLWT spanning over four decades (Dilov et al., 1979), only a small percentage (10%) of the studies reviewed involved large-scale microalgae cultivation. Various approaches have been explored, including Techno-Ecological Synergy (TES) Design implementation (Aleissa and Bakshi, 2021; Gopalakrishnan and Bakshi, 2018), which integrates ecosystems as unit operations in the process design (capacity of ecosystems to support the proposed activities). However, the limited application of large-scale MbLWT systems highlights the need for more comprehensive research to improve the design and effectiveness of these systems.

To aid in the complex decision-making process and selection of treatments preceding MbLWT, Table 3 highlights the advantages and

Table 3
Advantages and disadvantages of the reported treatments preceding MbLWT.

Pre-treatment	Advantages	Disadvantages	TRL[a]
Dilution	Simultaneously alleviate the inhibition of microalgae growth induced by high NH_4^+–N content, organic concentration, and poor light transmittance before microalgae inoculation. Support better growth during the first days. It is a simple alternative with low operational costs if the resource is available.	Do not support longer microalgae cultures as less nutrients are present in the media. Dilution is not always proportional to pollutant removal. It is not economically or environmentally viable at a large scale. May increase the overall volume of wastewater. Compromises freshwater resources.	6
Sedimentation	Efficient at removing settleable solids, suspended particles and larger colloidal particles, thus, reducing turbidity. Simple and low-cost. Easily scalable.	May have high retention times. It has a in limitation removing fine particles. Production of sludge which requires further treatment or adequate disposal. Large-space dependency. Regular maintenance is needed to prevent accumulation of the sludge.	9
Anaerobic digestion	Widely applied worldwide at large scale. Efficient organic load removal. Can improve MbLWT by increasing COD bioavailability. Production of biogas for energy generation, improving the economic feasibility.	Generates a residual digestate with high inorganic content that needs further treatment. Can contain more N_4^+–N concentration than the raw effluent. Longer processing times. Requires a specialised infrastructure.	9
Advanced oxidation processes	Efficiently reduces the colour and turbidity. Promotes organic degradation and complex pollutant removal. Promotes wastewater biodegradability. Promotes pathogen removal.	Scarce evidence of successfulness when coupled with MbLWT. Residual H_2O_2 could inhibit microalgae growth. Could remove a significant TP concentration. Extra costs if light (e.g., UV light) is applied. Possible high energy consumption. Requires advanced equipment and skilled operators. May generate harmful waste.	4–5
Electrocoagulation	A clean, reliable and cost-effective wastewater treatment process, generally used to remove COD, turbidity, TSS, and colour. Can work without chemical additives. The solution filterability is enhanced. Easily scalable for different volumes.	TSS could increase due to electrode corrosion. More investigation is needed regarding the interactions between the electrode and complex media (LW). Could hinder microalgae application. Could present an unbalance in N:P ratio. Limited organic load removal. Requires high electrical inputs.	4

(*continued on next page*)

Table 3 (*continued*)

Pre-treatment	Advantages	Disadvantages	TRL[a]
Flocculation-Coagulation	Simple to operate and cost-effective. High turbidity, chromaticity, COD, and TSS removal. Can be easily coupled with MbLWT.	Possible higher NH_4^+–N content. Could require chemical additions. Produces a sludge that require special treatment for disposal or reutilisation.	9
Air stripping	Easy operation, high efficiency, and process stability. Best for wastewaters containing high concentrations of NH_3. The operation requires simple equipment. Can be easily combined with other treatments.	Results are not always consistent in literature. Requires an input of alkali. Produces an alkaline effluent. Could display high operation and maintenance costs (fouling problems). Adjacent unit to dissolve the free ammonia is needed. Some contaminants are not degraded but transferred to the air.	9
Physical adsorption	Can enhance C:N ratios. Sorbent can be made from different biomass. Relatively easy techniques can be applied to optimise the treatment.	Could generate solid wastes. Sorbents could present instability in environment, difficulty in recollection, and high expense depending on the sorbent used. Efficiency depends on contact time and adsorbent properties.	4–5
Mixed effluents	Can balance nutrient content. Can avoid dilution with freshwater. Improves microalgal lag growth phase.	Difficulty for finding complementary wastewater. Potential transport cost (plants producing high-strength wastewater are distant from sources of low-strength wastewater). Potential introduction of heavy metals or emerging contaminants from one wastewater type to another. Hard to standardise due to the variation of the effluents.	5–6
Mixed cultures	Higher nutrient removal, particularly CODr. Higher tolerance to harsh environments. Higher biomass concentration. Can take advantage of synergies between strains. Reduction of the lag phase.	One species can take over the other. Nutrient competition. Generation of extracellular metabolites that inhibit other microorganisms.	4–6

MbLWT: microalgae-based livestock wastewater treatment; COD: Chemical Oxygen Demand; TSS: Total Suspended Solids; CODr: Chemical Oxygen Demand removal; C:N ratio: Carbon:Nitrogen ratio; N:P ratio: Nitrogen:Phosphorus ratio.

[a] Given that the articles did not explicitly mention TRLs, its inclusion was determined based on the contextual information provided by each author.

disadvantages of the principal treatments identified in this systematic search. Additionally, Technology Readiness Levels (TRLs) were also added in Table 3 to allow consistent comparison regarding the maturity level of each pre-treatment towards the full economic operation.

5. Conclusions and perspectives

Microalgae-based livestock wastewater treatment technology holds significant promise as a solution to contemporary environmental and resource challenges. However, its practical application is hindered by factors inherent in livestock wastewater, such as turbidity, NH_4^+–N concentration and biological contamination. Centrifugation, sedimentation, filtration, advanced oxidation processes, and flocculation have proven effective for turbidity removal. Anaerobic digestion is widely employed for organic matter reduction and aerobic treatments for NH_4^+–N removal. Additionally, autoclaving and UV radiation reduce microbial load before MbLWT, complemented by strategies like algal-bacterial symbiosis systems to prevent microalgae displacement.

Designing an integrated system with synergies between operational units becomes crucial to the progression of MbLWT technology towards large-scale implementation, as well as standardising comparison parameters, such as Technology Readiness Levels, to facilitate informed decision-making among various technologies within the circular economy framework. While technical and logistical challenges remain, ongoing research and development are crucial to unlocking the full potential of MbLWT for wastewater management and valuable biomass production. Its advancement will contribute to a sustainable future, integrating environmental stewardship with resource recovery and management.

CRediT authorship contribution statement

Ana Laura Silva-Gálvez: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Anaid López-Sánchez:** Writing – original draft. **Miller Alonso Camargo-Valero:** Writing – review & editing, Supervision, Funding acquisition. **Franja Prosenc:** Writing – review & editing, Supervision, Writing – review & editing, Supervision. **Martín Esteban González-López:** Validation, Supervision, Methodology, Formal analysis, Conceptualization. **Misael Sebastián Gradilla-Hernández:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

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

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

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