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REVIEW

Integrating Microalgae Cultivation With Municipal Wastewater Treatment for Biofuel Production: A Semiquantitative Review

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ABSTRACT

This study evaluates the efficiency of municipal wastewater (MW)–based microalgae cultivation for biofuel production and wastewater treatment using a meta-analysis with random resampling and bias-corrected adjustments. Integrated systems achieved mean removal rates of 87.7% for total nitrogen, 86.2% for ammonium, 84.8% for total phosphorus and 81.5% for phosphate, with lower efficiencies for chemical oxygen demand (64.2%) and nitrate (75.3%). Biodiesel conversion efficiencies ranged from 12.4% to 97.5%, averaging 59.2% (95% CI: [43.3, 73.1]), whereas lipid content varied from 2.7% to 61.7%, averaging 29.1% [26.0, 32.1]. Mixotrophic cultivation and pretreated MW performed best under optimal conditions (22°C–25°C, high nutrient availability, low metal concentrations), whereas stress conditions enhanced lipid accumulation. However, most studies remain laboratory-scale, limiting real-world applicability. Key scale-up challenges include wastewater pretreatment, cultivation strategy, environmental stability, biomass harvesting and process integration. Addressing these challenges requires urgent full-scale validation in operational wastewater treatment plants.

1 | Introduction

Transitioning from fossil fuels to biofuels is essential for sustainable energy and environmental conservation. First-generation biodiesel sources competed with food supplies, whereas second-generation options struggled with low yields and high costs. As a third-generation feedstock, microalgae exhibit rapid growth rates, high lipid content and superior CO_2 fixation capacity (Ferreira et al. 2019; Greenwell et al. 2010). Under optimal conditions, microalgal biomass can double within 24 h (Doma, Abdo, et al. 2016; Kadir et al. 2018). Yet commercialisation remains hindered by high production costs, particularly for nutrient supply, harvesting and biomass processing (Chisti 2013).

Municipal wastewater (MW), a nutrient-rich mixture of nitrogen (N), phosphorus (P), organic carbon, solids, pathogens and trace compounds (Plöhn et al. 2021), emerges as a promising alternative feedstock. Although excessive nutrients in MW drive eutrophication (Umetani et al. 2023), they also act as an ideal 'liquid fertiliser' for microalgae (Abou-Shanab et al. 2013; Oswald and Golueke 1960). Coupling MW treatment with microalgae cultivation reduces input costs and yields dual benefits: pollution mitigation and valuable biomass production (Batista et al. 2015).

Recent studies confirm the potential of microalgae-based wastewater treatment and biofuel production systems (MWTBPS)

(Ardo et al. 2024; El-Sheekh et al. 2023; Fal et al. 2022). However, these systems still face major biological and technical challenges. The variable composition of MW can affect microalgal growth and biochemical profiles (Zhou et al. 2011), while harvesting, dewatering and pretreatment account for a large share of operational costs (Uduman et al. 2010). In addition, heavy metals, recalcitrant organics and pathogens in MW necessitate strict pre- or postcultivation treatment (Rawat et al. 2011).

Although several reviews have examined microalgae for effluent treatment and biomass production, none have provided a comprehensive synthesis specific to MW. Earlier efforts focused on individual aspects, such as biofuel type (Mehariya et al. 2021), limited wastewater types with only two studies on MW (Bhatia et al. 2021) or biodiesel processes without MW context (Arutselvan et al. 2022). A few recent summaries included older MW studies (Bora et al. 2024), but a statistical assessment of pollutant removal and energy conversion remains lacking, hindering practical application (Table S1). This gap underscores the need for a systematic review that organises MW characteristics relevant to microalgae cultivation and evaluates technical, biological and economic barriers together with feasible mitigation strategies.

Although earlier reviews have advanced understanding of microalgae for effluent treatment and biomass production, the integration of MW—given its high nutrient content and large volume—offers a particularly promising route for scaling up MWTBPS. Accordingly, the objectives of this review are to (i) provide a statistical synthesis of pollutant removal and energy conversion efficiencies (mean and 95% confidence intervals—CIs); (ii) examine cultivation conditions, key influencing factors, harvesting methods and MW pretreatment; and (iii) identify current challenges and future opportunities for optimizing MWTBPS performance.

2 | Methodological Review

2.1 | Scope of Review

This study employs a semiquantitative review approach (Pope et al. 2007), combining statistical analyses of pollutant removal efficiencies and biofuel yields with qualitative synthesis of recent advancements in microalgae-based MW treatment. The quantitative component involves extracting and harmonizing numerical data from eligible studies, followed by statistical summarisation (mean, bootstrap CI) to enable robust cross-study performance comparisons. The qualitative component integrates findings that cannot be directly compared numerically—such as operational challenges, cultivation techniques and emerging technological approaches—to provide context and practical recommendations for scaling up microalgae-based MWTBPS (Figure 1).

In MWTBPS, MW is first subjected to pretreatment—commonly sedimentation and filtration, and less frequently sterilisation—to improve cultivation efficiency by enhancing water quality, light penetration and biomass yield (Table S2). For example, *Chlorella vulgaris (C. vulgaris)* grown in settled wastewater removed 99.9% of N and P (Ahmad et al. 2013), whereas *Micractinium reisseri* in autoclaved influent achieved 0.41 ± 0.01 g/L biomass

FIGURE 1 | Schematic of the process for cultivating microalgae and producing biofuel in MWTBPS, illustrating a high-rate algal pond (HRAP) system as a representative configuration. Depictions of the St Helena and Hilmar advanced integrated wastewater pond systems located in northern California, USA, adapted from Craggs et al. (2014).

with 22.0% lipids (Abou-Shanab et al. 2013). Most studies (70%) applied filtration and sedimentation without sterilisation, as sterilisation can sometimes reduce lipid production, as observed for *Scenedesmus obliquus* in ultrasound-treated wastewater (Han et al. 2016).

Following pretreatment, microalgae assimilate N, P and other pollutants during cultivation, producing biomass rich in lipids, carbohydrates and proteins. This biomass can be converted into biodiesel via transesterification, bioethanol through fermentation, biomethane by anaerobic digestion, or biohydrogen via photobiological processes. Using secondary-treated wastewater or effluent mixed with anaerobic digestate can further improve settling efficiency and reduce energy costs for biomass separation (Cho et al. 2013; Eida et al. 2018). This integrated process simultaneously enhances wastewater treatment performance and generates renewable biofuels.

2.2 | Inclusion and Exclusion Criteria

Studies were included based on the following criteria: (1) use of MW for microalgal cultivation; (2) reporting of pollutant removal efficiencies, including COD, TN, TP, NH_4^+, NO_3^-, PO_4^{3-}; (3) provision of data on biofuel production, specifically biodiesel, biohydrogen, biomethane or bioethanol.

2.3 | Procedure

A comprehensive literature review was conducted on studies published between January 2014 (marking the initial phase of relevant research) and July 2024 (literature update during manuscript preparation and revision), focusing on microalgal cultivation in MW, nutrient removal efficiencies and biofuel production potential. Database searches were conducted primarily in Scopus, with additional targeted searches in Google Scholar to ensure broader coverage and capture studies not indexed in Scopus. Three keyword groups—'microalgae,' 'wastewater' and 'biofuel'—were applied using the search string detailed in Text S1.

The initial search, updated in July 2024, identified 436 articles. A multitiered screening process refined the selection—first by filtering titles and abstracts for relevant terms (e.g., municipal, domestic, urban), followed by full-text reviews to ensure alignment with the inclusion criteria in Section 2.2. Ultimately, 78 studies were selected for meta-analysis (Table S3; Table S4).

For comparative analysis, performance metrics included:

- Nutrient removal efficiencies for TN, TP, NH_4^+, NO_3^- and PO_4^{3-}.
- Organic matter removal efficiencies for COD and BOD.
- Heavy metal removal efficiencies (e.g., Mn, Fe, Cu, Pb).
- Biofuel yield indicators, such as lipid content (% dry weight), biodiesel conversion efficiency (%), biohydrogen yield (mL H_2/L or mL H_2/g VS), biomethane yield (mL CH_4/g VS) and bioethanol yield (g/L or mg/g biomass)

For the qualitative assessment, insights were synthesised on:

- Operational challenges (e.g., contamination, harvesting efficiency, wastewater composition variability).
- Cultivation techniques (e.g., open ponds, photobioreactors, pretreatment methods, microalgae–bacteria consortia)
- Future directions (e.g., integration with circular bioeconomy strategies, optimisation of conversion pathways, reduction of energy inputs).

2.4 | Data Analysis and Statistical Methods

Numerical data extracted from the selected studies were analysed to determine mean values, coefficients of variation (CV) and 95% CIs for pollutant removal efficiencies and biofuel yields. CIs were estimated using bootstrap resampling with 5000 iterations, applying the bias-corrected and accelerated (BCa) method to correct for bias, account for skewness and improve robustness against outliers (DiCiccio and Efron 1996; Lam et al. 2024).

Comparisons among pollutants and biofuel yield metrics were conducted descriptively by examining the magnitude of the means, the width of CIs and the degree of CI overlap. Outliers were identified using the interquartile range method and confirmed visually from distribution plots. Meta-analysis outputs were generated to summarise and visualise aggregated results across studies. The qualitative synthesis component integrated nonnumerical findings—such as system design features, cultivation methods, operational challenges and emerging technological approaches—using thematic content analysis (Thomas and Harden 2008) to complement and contextualise the quantitative results.

All statistical analyses were performed in R version 4.3.2 (R Core Team 2024). The primary packages used included *boot* for resampling (Canty et al. 2025; Davison and Hinkley 1997), *psych* for descriptive statistics (Revelle 2025) and *ggplot2* for visualisation (Wickham 2016).

2.5 | Study Scale and Wastewater Types

This review encompasses 78 studies spanning laboratory- to pilot-scales, with minimal evidence from full-scale WWTP applications. The vast majority were laboratory-based (70 studies, 83.1%), whereas only 8 studies (16.9%) involved pilot or semireal systems and none represented large-scale plants. This distribution suggests that technology is still in an early developmental stage and requires further validation under operational conditions. Regarding wastewater type, 53 studies (67.5%) employed real municipal or domestic wastewater, whereas 25 studies (32.5%) relied on synthetic, diluted, or pretreated wastewater (Tables S5 and S6). Although artificial media facilitate reproducibility and mechanistic insights, they cannot capture the variability and complexity of real effluents (Velásquez-Orta et al. 2024). In contrast, pilot-scale studies using raw, primary, or secondary wastewater illustrate both the opportunities and the challenges of translating laboratory findings into real-world applications.

3 | Microalgae-Based MW Bioremediation

3.1 | Nutrient Removal

The MWTBPS offers a promising and sustainable method for nutrient removal. A meta-analysis of 78 studies (Figure 2; Table S7) shows that TN removal is highly effective, with a mean efficiency of 87.7% [84.2%–90.4%] and low variability (CV: 12.9%). This reflects the reproducibility of total nitrogen (TN) metabolic pathways under diverse conditions: (i) NH_4^+ assimilation for biosynthesis, (ii) photosynthetically driven nitrification and (iii) heterotrophic denitrification supported by dissolved organic carbon (Abdelfattah et al. 2023). Together, these processes explain the stable TN removal compared with individual N forms, underscoring the practical potential of MWTBPS.

TP removal follows at 84.8% [80.0%–88.6%] with moderate variability (CV: 18.6%), influenced by factors such as species composition, pH, heavy metals, aeration and influent loading (Velásquez-Orta et al. 2024). NH_4^+ removal is also strong (86.2% [80.0%–90.4%]) but slightly more variable (CV: 18.9%) due to light–dark cycles. During photosynthesis, elevated pH shifts the NH_4^+/NH_3 equilibrium toward NH_3, whose toxicity inhibits growth and reduces assimilation efficiency (Azov and Goldman Joel 1982; Chai et al. 2021).

In contrast, NO_3^- removal is lower (75.3% [67.8%–81.3%]) and more variable (CV: 24.7%), reflecting challenges in uptake. In mixed-N environments, microalgae preferentially use NH_4^+ before switching to NO_2^- or NO_3^-, because NH_4^+ assimilation requires less energy and more directly supports amino acid synthesis (Abou-Shanab et al. 2011; El-Sheekh et al. 2023; Kadir et al. 2018). PO_4^{3-} removal averages 81.5% [73.4–87.6%] with high variability (CV: 27.1%), indicating the need for further optimisation.

Overall, the lower and more variable performance of NO_3^- and PO_4^{3-} underscores the need for improved processes. Future research should focus on strategies such as high-affinity strains, multispecies cocultivation, algae–bacteria consortia, or engineering solutions to enhance nutrient uptake.

Figure 2 highlights the wide range of NH_4^+ and PO_4^{3-} removal efficiencies, with notable outliers. The data also show that many studies reported ~100% removal of PO_4^{3-}, TN, TP and NH_4^+ (Figure 2; Table S6), such as Woertz et al. (2009), Ahmad et al. (2013), Yang et al. (2016), Fal et al. (2022), Debeni Devi et al. (2023) and Umetani et al. (2023), demonstrating that MWTBPS can achieve complete nutrient removal under certain conditions.

Microalgae nutrient assimilation and accumulation mechanisms underpin the high removal efficiency of MWTBPS. They require substantial N and P for growth and biomass synthesis: N (1%–10% of biomass) regulates lipid content, whereas P supports ATP, phospholipids and nucleic acids (El-Sheekh et al. 2023; Fal et al. 2022). Beyond growth needs, microalgae can accumulate excess P via luxury uptake, storing it as polyphosphate granules that sustain growth under P limitation (Umetani et al. 2023). These traits allow MWTBPS to handle fluctuating wastewater loads while producing valuable lipids, carbohydrates and proteins. High removal efficiencies of TN, TP and NH_4^+ (>80%) demonstrate its sustainability, surpassing conventional treatment technologies, which typically achieve only 60%–80% under optimal conditions (Guimarães et al. 2016; Meng et al. 2024).

Nonetheless, variability in TP and NH_4^+ removal highlights the need for optimisation, for example, by adjusting hydraulic retention time, light intensity and nutrient loading (Debeni Devi et al. 2023; Yang et al. 2016). Cocultivation of different microalgal groups or microalgae–bacteria consortia may also improve nutrient removal through synergistic interactions (Kadir

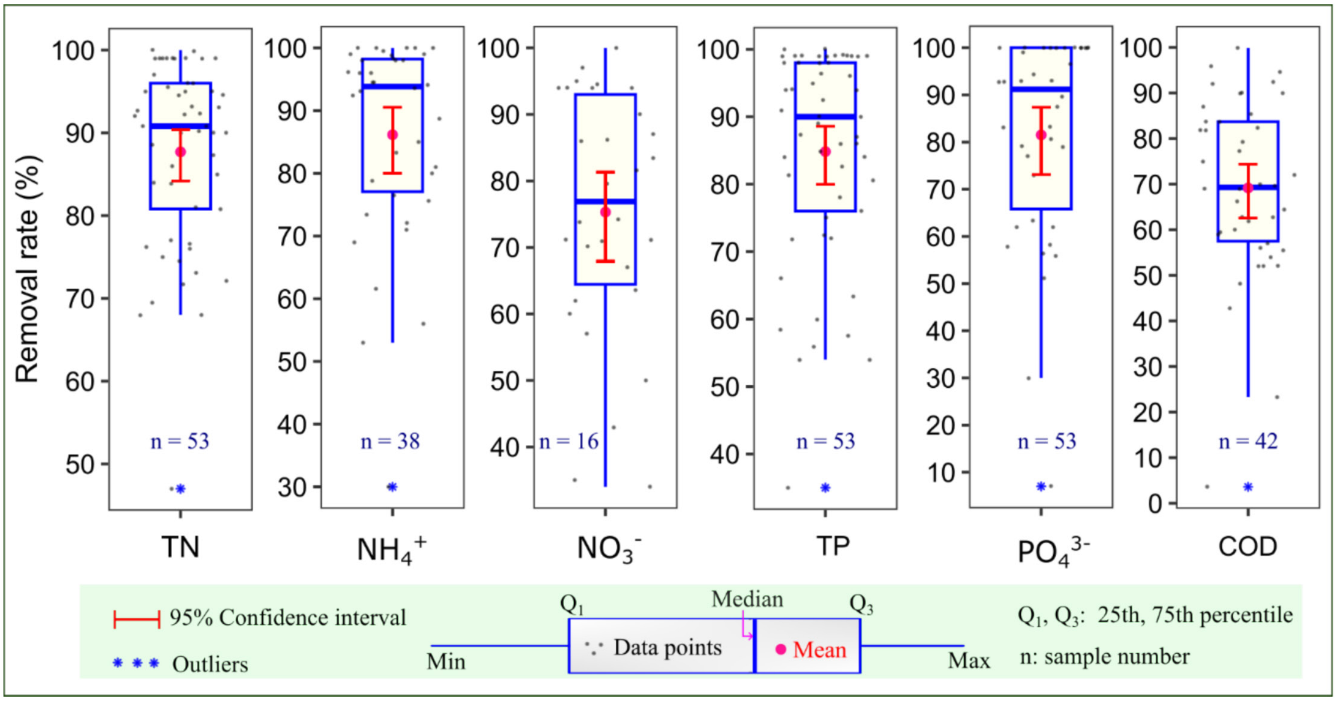

FIGURE 2 | Removal efficiency of water parameters of the MWTBPS.

et al. 2018), although performance differences among groups remain modest, likely due to species-specific variation in growth and uptake capacity (Arcila et al. 2023; Debeni Devi et al. 2023; Doma, Abdo, et al. 2016).

3.2 | Organic Matter Removal

The MWTBPS demonstrates capacity for organic matter treatment, assessed by COD and biochemical oxygen demand (BOD). Initial COD and BOD concentrations varied widely (11.2–1800.0 mg/L and 6.9–215.0 mg/L, respectively), reflecting the diverse pollution levels of MW sources (Table S7). This variability highlights the challenge of standardizing MWTBPS performance across different wastewater types (raw, primary effluent, secondary effluent and sludge supernatants).

Organic matter removal is the lowest among the studied pollutants, with a mean COD removal of 69.2% [62.3%–74.6%] and high variability (CV: 28.5%). Limited COD removal is linked to the autotrophic metabolism of microalgae, which preferentially use CO_2 rather than organic carbon (Arcila et al. 2023). CO_2 consumption raises pH and alters chemical oxidation, whereas microalgae also release organic compounds (e.g., glycolic acid) during the stationary phase, leading to a 'secondary organic load' and secondary COD, particularly under long-term operation (Doma, El-Liethy, et al. 2016; Kang et al. 2015; Lv et al. 2017). Moreover, excessive algal growth and biomass degradation can further increase COD when harvesting is inefficient (Batista et al. 2015; Doma, El-Liethy, et al. 2016).

These findings suggest that MWTBPS is more effective for removing nutrients (N, P) than for eliminating complete COD. To meet the dual goals of wastewater treatment and biomass production, integration with an initial COD-reduction stage is recommended. Improvements should focus on (i) pretreatment to lower organic loads, (ii) optimizing operations to minimise secondary organic release and (iii) developing efficient biomass harvesting technologies.

3.3 | Heavy Metals Removal

Heavy metals are common pollutants in MW, primarily originating from industrial and human activities. Although some heavy metals are essential trace elements required for enzymatic processes and cellular metabolism, microalgae can efficiently assimilate them even when concentrations exceed physiological requirements, with an average removal efficiency of more than 40%. High removal efficiencies of copper (Cu), iron (Fe), manganese (Mn) and lead (Pb) (all >85%) have been consistently reported across multiple studies (El-Sheekh et al. 2016; El-Sheekh et al. 2023; Mittal and Ghosh 2023; Sharma et al. 2020; Singh and Singh 2024; Tripathi et al. 2019), as summarised in Figure 3. In addition to these elements, other heavy metals such as chromium (Cr), nickel (Ni), cadmium (Cd), cobalt (Co) and zinc (Zn) have also been experimentally removed by microalgae.

This high performance reflects the unique mechanisms of microalgae, whereby metals are not only utilised for physiological functions but also accumulated and sequestered intracellularly or bound to the cell surface, while simultaneously activating biological pathways to cope with metal-induced stress (Priyadarshani et al. 2011; Sarma et al. 2024; Xiao et al. 2023). Research on intracellular mechanisms that explain how microalgae respond to heavy metal stress will support the development of strategies to achieve dual objectives in MWTBPS (Danouche et al. 2021; Tripathi and Poluri 2021; Xiao et al. 2023). Moreover, many microalgal species can maintain growth rates even under high metal-enriched conditions and other toxic compounds, reflecting their remarkable tolerance and adaptability (Duque-Granda et al. 2019; Kadir et al. 2018).

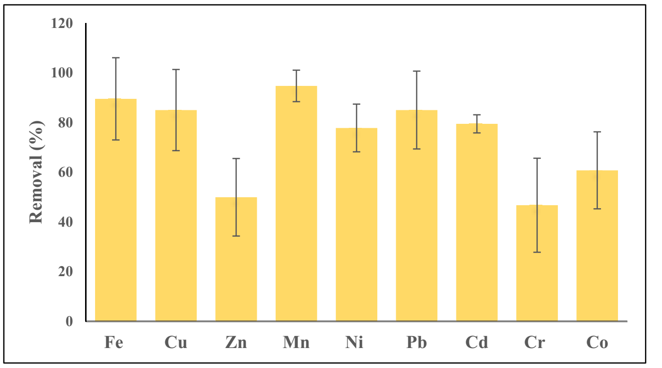

FIGURE 3 | Effective removal of heavy metals by microalgae cultivated in MW with updated average values derived from various studies.

However, the application of microalgae in heavy metal remediation still faces several challenges, including the selection of suitable species, optimisation of culture conditions, biomass harvesting and disposal of treated products (Sarma et al. 2024). Effective removal can be enhanced through pretreatment or integration with complementary technologies, enabling microalgae to maximise their biological potential while mitigating the challenges of complex wastewater matrices such as MW.

4 | Biofuel Production From Microalgae Cultivated in MW

4.1 | Biodiesel Production

Biodiesel is the most prominent bioproduct derived from microalgae, with yield depending on microalgal growth rate and lipid accumulation. Lipids typically make up 20%–60% of microalgal dry weight, reaching 80% under stress conditions (El-Sheekh et al. 2023). In MWTBPS, lipid content ranges from 14.7% to 23.1% (Nayak et al. 2016a). The results indicate that while MWTBPS supports microalgal growth, it is not optimal for lipid accumulation, as the stress conditions required are difficult to maintain under fluctuating influent composition and loading (Abdelfattah et al. 2023; Minhas et al. 2016). Although stronger stress can enhance lipid content, it concurrently suppresses growth (Zhu et al. 2022); thus, balancing biomass production and lipid accumulation is critical for the economic feasibility of biodiesel production (Figure S1).

Biodiesel conversion efficiency across microalgae species in MWTBPS varies widely (Table 1; Figure 4). *Chlorella* sp. exhibits

TABLE 1 | The efficiency of lipid conversion to biodiesel of certain microalgae species in MW.

Microalgae types	Lipid extraction method	Lipid content (% dry weight biomass)	Lipid productivity/ yield	Biodiesel conversion efficiency (%)	References
C. vulgaris	SE	42.5 ± 2.3	—	93.5	(Ahmad et al. 2013)
C. vulgaris	SE	32.7	—	74.4	(Lam et al. 2017)
C. vulgaris	SE	27.1	19.3 mg/L/d	—	(Ryu et al. 2014)
Chlorella pyrenoidosa	Co-extraction	41.1	—	12.4	(Ding et al. 2020)
Chlorella sorokiniana	SE	36.9	—	—	(Fal et al. 2022)
Scenedesmus acutus	SE	28.3	—	87.7	(de Alva et al. 2013)
S. obliquus	Folch method	17.9	24.7 mg/L/d	—	(Han et al. 2019)
Monoraphidium braunii	SE	42.0	5.3 mg/L/d	—	(El-Sheekh et al. 2023)
Arthronema sp.	Sonication method	32.1	180.0 mg/L	—	(Maheshwari et al. 2020)
Chlorococcum sp.	Soxhlet	30.5	25.0 mg/L/d	—	(Morsi et al. 2023)
Consortium	SE	38.0	70.0 mg/L/d	68.6	(Khalekuzzaman et al. 2021)
Consortium	Bligh and Dyer method	5.0 ± 2.4	—	70.6	(Doma, Abdo, et al. 2016)
Consortium	Bligh and Dyer method	2.7 ± 1.5	230.0 mg/L	88.9	(Leong et al. 2020)
Consortium	Bligh and Dyer method	26.2 ± 0.6	—	82.1 ± 3.9	(Soydemir et al. 2016)
Consortium	Bligh and Dyer method	46.2	—	—	(Debeni Devi et al. 2023)
Consortium	Folch method	45.0	18.7 mg/L/d	—	(Abdelaziz et al. 2014)
Consortium	SE	17.5	—	15.1	(Ahmad et al. 2012)
S. acutus	Ultrasonication + SE	49	—	—	(Angioni et al. 2018)
C. vulgaris	SE modified method	22.0	—	—	(Arbib et al. 2014)
Scenedesmus sp.	Bligh and Dyer method	20.5	8.6 mg/L/d	—	(Baldev et al. 2021)
C. vulgaris	Bligh and Dyer method	27.3	18.0 mg/L/d	—	(Cabanelas et al. 2013)
Botryococcus terribilis	Bligh and Dyer method	25.0	35.0 mg/L/d	—	(Cabanelas et al. 2013)
Chlorella sp.	Fluorescence staining method + microwave	35.3	—	—	(Hallenbeck et al. 2014)
Golenkinia sp.	Bligh and Dyer method	38.0	325.0 mg/L	—	(Hou et al. 2016)
Consortium	Bligh and Dyer method	26.4 ± 0.6	—	17.4	(Jaiswal et al. 2022)
Scenedesmaceae 7	SE	47.0	—	—	(Jämsä et al. 2017)

(Continues)

Microalgae types	Lipid extraction method	Lipid content (% dry weight biomass)	Lipid productivity/ yield	Biodiesel conversion efficiency (%)	References
Chlorella sp. *JK2*	Bligh and Dyer method	27.1	—	—	(Kang et al. 2015)
Scenedesmus sp.	Bligh and Dyer method	26.1	—	—	(Kang et al. 2015)
Consortium	Bligh and Dyer method	31.0	—	—	(Katam and Bhattacharyya 2020)
C. vulgaris	Ultrasonication	26.0	—	53.9 ± 9.6	(Kialashaki et al. 2019)
Consortium	SE	23.3 ± 1.3	103.0 mg/L/d	—	(Kim et al. 2014)
Scenedesmus dimorphus	Folch method	35.0*	—	—	(Kudahettige et al. 2018)
C. sorokiniana	Bligh and Dyer method	21.1	—	—	(Lee et al. 2023)
Consortium	Bligh and Dyer method	22.2 ± 1.1	242.0 mg/L	—	(Leong et al. 2019)
Chlorella kessleri	One-step extraction–transesterification method	25.9 (volatile suspended solids)	—	25.9	(Li et al. 2011)
Chlorella protothecoides	One-step extraction–transesterification method	19.5 (volatile suspended solids)	—	19.5	(Li et al. 2012)
Chlorococcum sp.	Bligh and Dyer method	30.6*	90.0 mg/L/d	—	(Mahapatra and Ramachandra 2013)
Tetradesmus obliquus	Bligh and Dyer method	10.0 ± 0.5	—	57.0	(Miyawaki et al. 2021)
Consortium	Bligh and Dyer method	35.2 ± 0.6	—	43.0	(Naaz et al. 2019)
Scenedesmus sp.	Bligh and Dyer method	23.1	14.2 mg/L/d	—	(Nayak et al. 2016a)
Consortium	Bligh and Dyer method	14.0 of ash-free dry weight	—	44.5 ± 4.7	(Roberts et al. 2013)
Consortium	Bligh and Dyer method	31.3 ± 0.1	18.1 mg/L/d	—	(Sharma et al. 2020)
Botryococcus sp.	SE: Axelsson and Gentili described	61.7	—	—	(Shen et al. 2017)
Consortium	Bligh and Dyer method	34.8	—	—	(Silambarasan et al. 2021)
Scenedesmus sp.	Folch method	30.5	19.0 mg/L/d	97.5	(Thangam et al. 2021)
Chlorella pyrenoidosa	Bligh and Dyer method	43.3	130.0 mg/L	—	(Wang et al. 2023)
Scenedesmus quadricauda	SE	21.8	6.3 mg/L/d	—	(Yang et al. 2018)

(Continues)

TABLE 1 | (Continued)

Microalgae types	Lipid extraction method	Lipid content (% dry weight biomass)	Lipid productivity/yield	Biodiesel conversion efficiency (%)	References
C. vulgaris	Folch method	30.0	—	—	(Zayadan et al. 2017)
Chlorella sp.	Folch method	33.5	50.8 mg/L/d	—	(Zhou et al. 2011)
Auxenochlorella protothecoides	Folch method	28.9	77.7 mg/L/d	—	(Zhou et al. 2011)
Botryococcus braunii LEM 14	SE	36.1	—	—	(Sydney et al. 2011)
Desmodesmus subspicatu	Bligh and Dyer method	20.3	—	—	(Kiani et al. 2023)
Scenedesmus sp.	SE	18.0	—	—	(Kumar Gupta et al. 2018)

Notes: —: data unavailable; SE: solvent extraction; *: under stress conditions.
Consortium: The simultaneous cultivation of two or more microalgal species in a single culture environment.

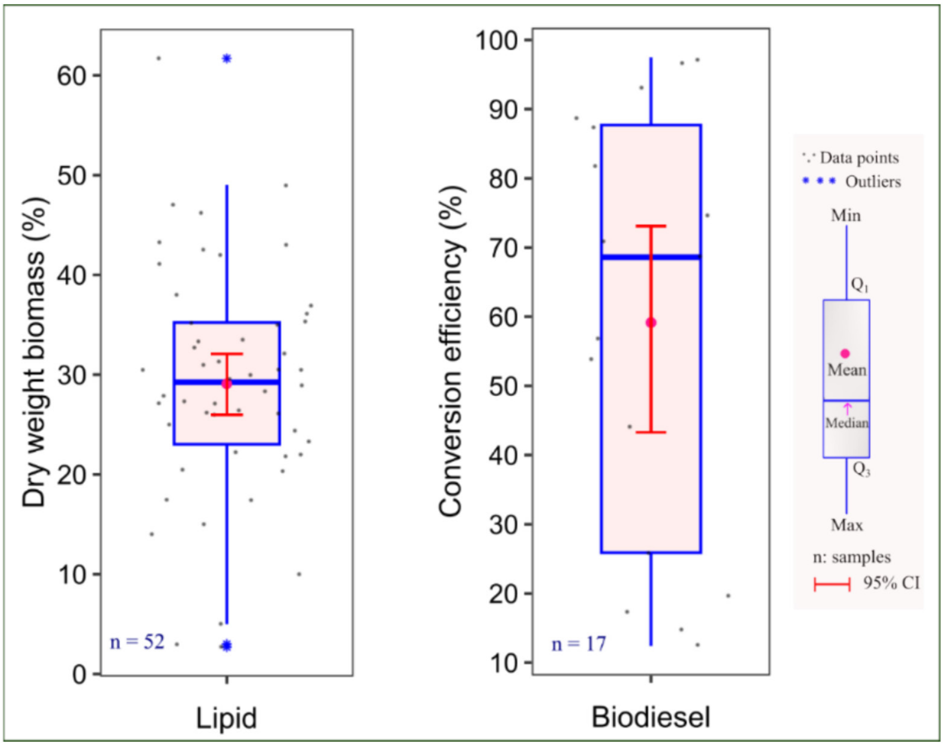

FIGURE 4 | Distribution of lipid and biodiesel content produced by MWTBPS.

the highest lipid content, although values range from 2.7% to 61.7%, with a mean of 29.1% [26.0, 32.1] (CV: 39.6%). Similarly, biodiesel conversion efficiency ranges from 12.4% to 97.5%, with an average of 59.2% [43.3, 73.1] (CV: 52.6%), highlighting species- and condition-dependent variability (Table S8). A high coefficient of variation reflects both interspecific physiological differences among microalgae and the difficulty of controlling fluctuating environmental factors, indicating that MWTBPS remains insufficiently stable and entails potential risks when scaled up to industrial applications. Optimizing cultivation and species selection is crucial for maximizing biodiesel yield in MWTBPS applications.

Microalgal consortia generally have lower lipid concentrations than monocultures (Table 1), but competition within consortia can stimulate lipid synthesis (Debeni Devi et al. 2023). For instance, *Chlorella* sp. in consortia accumulated 34.8% lipids, compared to 22.3%–29.5% in monoculture (Silambarasan et al. 2021). Resource competition and inhibitory compounds may reduce overall growth and lipid accumulation, whereas natural selection can favour lipid-rich strains (Olguin 2012). This provides an essential insight for the strategy of 'evolutionary engineering'—harnessing natural selective pressures to develop populations with enhanced and more stable lipid accumulation.

TABLE 2 | Conversion of microalgal biomass from MWTBPS into other biofuels.

Biofuel type	Microalgae types	Cultivation medium	Conversion method	Yield/efficiency	Remarks	References
BioH_2	*C. vulgaris*, *S. obliquus*, Consortium C—ConsC	MW (after primary treatment)	Dark fermentation with *Enterobacter aerogenes*	56.8 mL H_2 g^{-1} VS (*C. vulgaris*); 40.8 mL H_2 g^{-1} VS (*S. obliquus*); 46.8 mL H_2/gVS (ConsC)	Higher than sludge-based; limited by O_2 sensitivity and contamination	(Batista et al. 2015)
	C. vulgaris, *S. obliquus* (immobilised in alginate beads)	Artificial wastewater mimicking MW	Two-stage process: photosynthetic growth → sulfur deprivation and anaerobic dark incubation under different light wavelengths	60.4 mL H_2 L^{-1} (*C. vulgaris*); 128 mL H_2 L^{-1} (*S. obliquus*)	Light condition strongly enhances yield; purple light reduced biomass but enhanced lipid and H_2 production	(Ruiz-Marin et al. 2020)
Biomethane	*C. kessleri*, *C. vulgaris*, *Nannochloropsis oculata*	MW (centrate, after primary treatment; diluted with wastewater or seawater)	Anaerobic digestion	415 ± 2 mL CH_4 g^{-1} VS (*C. vulgaris*); 346 ± 3 mL CH_4 g^{-1} VS (*C. kessleri*)	Highlight the dual potential; species-specific limitations	(Caporgno, Trobajo, et al. 2015)
	Consortium	MW (HRAP after primary treatment)	Microwave pretreatment + anaerobic digestion	Untreated: 172 mL biogas/gVS (~68% CH_4); pretreated: 194–307 mL biogas/gVS (12%–78% increase), initial biogas rate ↑ 27%–75%	Microwave pretreatment markedly enhanced methane yield, but high energy input	(Passos et al. 2013)
	Consortium	MW (after primary treatment HRAP system)	Anaerobic digestion	347.9 ± 3.2 mL CH_4 g^{-1} VS	Longer HRT improved methane yield and nutrient removal	(Arcila and Buitron 2016)
	Consortium	MW (tertiary treatment, Algarve WTP, Portugal)	Anaerobic digestion (mesophilic 35°C, with/without thermo-acid hydrolysis)	Max biogas: 311 mL/gV; methane yield: 252 mL/gVS	Tertiary-consortia biomass delivered moderate methane yields but net energy saving	(Barros et al. 2022)
	C. vulgaris, *Aphanizomenon ovalisporum*, *Anabaena planctonica* (cyanobacteria)	MW (raw, from WTP Narbonne, France)	Anaerobic digestion	*C. vulgaris*: 184.8 mL CH_4/gCOD; *A. ovalisporum*: 218.2 mL CH_4/gCOD; *A. planctonica*: 261.6 mL CH_4/gCOD	Lower yield under alkaline conditions	(Mendez et al. 2016)
	Consortium	MW (cultivation) + piggery slurry (co-digestion)	Anaerobic digestion	Mono-digestion microalgae 408 ± 34 mL CH_4 g^{-1} VS; Co-digestion 60:40 (slurry/algae): 355 ± 27 N mL CH_4/gVS	Co-digestion improved methane yield	(Tsapekos et al. 2018)

(Continues)

TABLE 2 | (Continued)

Biofuel type	Microalgae types	Cultivation medium	Conversion method	Yield/efficiency	Remarks	References
Bioethanol	*N. oculata*, *Tetraselmis suecica*	Treated MW (Istanbul WTP, mixed with seawater at 25–100%)	Alkaline pretreatment + fermentation with *Saccharomyces cerevisiae*	*N. oculata*: up to 3.68% ethanol yield (75% W, 48 h); *T. suecica*: up to 7.26% ethanol yield (control, 48 h), 6.52% carbohydrate at 100% W	Higher carbohydrate accumulation capacity in *T. suecica*	(Reyimu and Özçimen 2017)
	Nannochloropsis gaditana	MW (0%, 30%, 60%, 100% mixed with f/2 medium)	Alkaline hydrolysis + fermentation with *S. cerevisiae*	Carbohydrate: 17.7% dwt (30% WW), 14.3% (control), 12.0% (60% WW), 9.3% (100% WW); ethanol yield: 94.3 ± 5.5 mg/g biomass (30% WW, highest), 89.0 ± 4.0 mg/g (control), 85.7 ± 1.9 mg/g (60% WW), 70.3 ± 2.4 mg/g (100% WW	30% MW optimised growth and carbohydrate accumulation, yielding maximum ethanol	(Onay 2018)
	S. obliquus	MW (after primary and secondary treatment, Mexico City WTP) + glucose supplementation (10 g/L)	Nonsterile heterotrophic cultivation; crabtree-positive yeast fermentation	Peak ethanol: 2.5 g/L after 48 h		(Walls et al. 2019)
	Scenedesmus sp.	MW (primary settling tank, Madurai, India)	Acid hydrolysis (2.5 N H_2SO_4, 100°C, 2 h) + fermentation (*S. cerevisiae* NCIM3288, 72 h)	Reducing sugars: 11.2% dwt; ethanol yield: 10.48 g/L	Biodiesel–bioethanol recovery from the same wastewater-grown biomass	(Thangam et al. 2021)
	Scenedesmus sp.	MW (secondary effluent, Mexico City WTP)	Acid hydrolysis (H_2SO_4, 85°C–90°C, 120 min) + fermentation with *Candida* sp. (wild-type) and *S. cerevisiae*	Hydrolysate sugars: 5.0 ± 0.3 g/L (glucose 83.7%); ethanol yields: 2.2 g/L (*Candida* sp., 85.8% conv.), 2.1 g/L (*S. cerevisiae*, 81.7% conv.);		(Romero-Frasca et al. 2021)
	Multiple types	MW (secondary effluent, UAE)	Hydrolysis + fermentation	Carbohydrate: up to 32% dwt (under N-starvation); ethanol ~95 mg/g biomass (estimated)		(Dębowski et al. 2025)
	Scenedesmus sp.	MW (IIT Kharagpur, India) + flue gas CO_2 (2.5%, 5%, 10%)	Hydrolysis–fermentation	Carbohydrate: 10.4%–21.9% dwt; Max productivity: 20.2 mg/L/d (10% CO_2 in PBR)		(Nayak et al. 2016b)
BioABE (acetone–butanol–ethanol)	Multiple types	MW (Logan City Wastewater Lagoon System, USA)	Dilute acid hydrolysis (H_2SO_4) + Fermentation with *Clostridium saccharoperbutylacetonicum*	Optimal (1.0 M H_2SO_4, 120 min, 80°C–90°C): 166.1 g sugars/kg algae; 5.23 g/L ABE (3.74 g/L butanol, 0.96 g/L acetone, 0.53 g/L ethanol)		(Castro et al. 2015)

Notes: The values presented in the table are average values; —: data not available.
Consortium: The simultaneous cultivation of two or more microalgal species in a single culture environment.
Multiple types: the separate cultivation of individual microalgal species under identical conditions, with data used to calculate average values.
Abbreviations: WTP: wastewater treatment plant; HRAP: high-rate algal ponds.

TABLE 3 | Summary of key challenges and corresponding future perspectives in microalgae-based biofuel production.

Challenges	Future perspectives
Heavy metals and toxic substances	Develop robust microalgae (e.g., *Chlorella*, *Scenedesmus*); optimise mixotrophic and photobioreactor systems
Energy-intensive harvesting	Use natural flocculants, centrifugation, flotation, solvents and supercritical fluids
Harmful microorganisms and solids	Apply wastewater pretreatment; sedimentation, filtration and sterilisation
Lipid extraction and biodiesel quality sensitivity	Implement co-extraction and optimised pretreatment (e.g., sedimentation, filtration, enzymatic treatment and ultrasound)
Limited research on biohydrogen and ethanol production	Expand species research; address technical challenges

Fatty acid composition is key to biodiesel quality, influenced by chain length and saturation (El-Sheekh et al. 2023). Microalgae cultivated in MW contain 91% C16–C18 fatty acids (Arcila et al. 2023), with *Chlorella sorokiniana* showing 63.2% C16–C18 (Fal et al. 2022), ideal for biodiesel. In addition to the high C16–C18 content, cultivation conditions such as nutrient stress, light intensity, or temperature can be modulated to enhance fatty acid saturation and elongation (Kudahettige et al. 2018; Minhas et al. 2016), thereby optimizing fuel properties such as cetane number and cloud point. Additionally, microalgal biodiesel exhibits desirable properties, including improved cold-start efficiency, reduced white smoke emissions and high oxidative stability. These advantages suggest that microalgae can not only serve as an alternative but may also surpass conventional biodiesel feedstocks in fuel quality. Moreover, the uniform fatty acid composition of microalgae cultivated from MW reduces fuel quality variability compared to plant oils and offers opportunities for integration with biorefinery strategies to valorise lipid- and protein-rich by-products, thereby enhancing the overall economic efficiency of the production chain (Umetani et al. 2023).

4.2 | Other Biofuels

The multifunctional bioenergy potential of MWTBPS is highlighted by the fact that its biomass can be valorised into biohydrogen, biomethane and bioethanol (Table 2). $BioH_2$ is promoted under nutrient stress and by algae–bacteria consortia, but remains constrained by oxygen sensitivity and transient yields (Batista et al. 2015; Iqbal et al. 2022). Biomethane recovery demonstrates high stability, yielding 200–415 mL CH_4 g^{-1} VS, with freshwater strains such as *C. vulgaris* performing comparably to sludge digestion (Lakaniemi et al. 2011). A key advantage of biomethane lies in its ability to valorise residual biomass remaining after lipid/biodiesel production, thereby enhancing the overall efficiency of anaerobic systems (Cabanelas et al. 2013; Caporgno, Taleb, et al. 2015). Moreover, N stress can redirect metabolism toward carbohydrate accumulation, supporting ethanol fermentation with reported titers of 2–7 g/L (Onay 2018; Reyimu and Özçimen 2017; Romero-Frasca et al. 2021; Walls et al. 2019). Under such conditions, carbohydrate contents may rise to 22%–43% dry weight (Batista et al. 2015; Ho et al. 2012), reinforcing the integrative potential of methane and ethanol pathways in MWTBPS. Table S9 summarises the advantages and disadvantages of different energy conversion methods for microalgal biomass in MW, highlighting the trade-offs between efficiency, cost and operational complexity. A comparative overview of conversion methods highlights trade-offs among yield, cost and operational feasibility, necessitating case-specific optimisation (Table S9).

5 | Challenge, Potential and Future Perspective

Combining microalgae cultivation in MW for pollution treatment and biofuel production is sustainable but faces significant technical and economic challenges (Table 3). The main technical barriers include the high energy demand for biomass harvesting (Kadir et al. 2018), lipid extraction (Yang et al. 2011), the presence of harmful microorganisms and suspended solids, fluctuations in wastewater composition and the low extraction efficiency due to the complex structure of microalgal cell walls (Lee et al. 2017). In addition, the production of other biofuels beyond biodiesel, such as biohydrogen and bioethanol, remains limited to the laboratory scale, with a lack of comparative data across species and methods, insufficient assessments of scalability and particularly a scarcity of studies addressing the dual objective of MW treatment and biofuel production (Iqbal et al. 2022; Ruiz-Marin et al. 2020). Nevertheless, the potential of MWTBPS is considerable, as MW provides a low-cost nutrient source and stress conditions that are highly favourable for lipid accumulation (El-Sheekh et al. 2023). In addition, residual biomass after lipid extraction can be utilised for biomethane and biohydrogen production, thereby enhancing the overall value within an integrated biorefinery framework (Caporgno, Taleb, et al. 2015). Moreover, when properly operated, MWTBPS contribute to CO_2 emission reduction. Several future directions for MWTBPS can be proposed, including:

- *Biological optimisation*: strategies of selection, evolution or genetic modification can create microalgal strains capable of withstanding stress, adapting well to wastewater variability, accumulating high lipid content and altering cell wall structures, thereby making the extraction process less energy-consuming.

- *Consortia development*: Evidence suggests that cocultivation of microalgae with other microorganisms can improve nutrient removal, enhance system stability and reduce harvesting costs via bio-flocculation.
- *Advances in cultivation technology and optimisation*: Findings from pilot- and semipilot-scale studies indicate that optimizing operational parameters, developing next-generation photobioreactors and implementing hybrid HRAP–PBR systems to balance cost and efficiency at large-scale are promising, laying the foundation for transition from pilot and semipilot setups to full-scale applications.
- *Diversification of bioproducts*: Beyond biodiesel, other biofuels can be derived from the same biomass within a biorefinery framework. Comparative studies on conversion methods (dark fermentation, bioelectrochemical processes, thermochemical conversion) and assessments of scalability are urgently needed.
- *Economic–environmental assessment*: Applying life cycle evaluation and techno-economic analysis will be crucial to demonstrate the feasibility and long-term sustainability of MWTBPS.

6 | Conclusions

This study confirms the feasibility of microalgal technology for MW treatment and biofuel production, offering a sustainable alternative to synthetic environments. High removal efficiencies were observed for TN, NH_4^+, TP and PO_4^{3-}, whereas COD and NO_3^- showed lower efficiencies. Biodiesel conversion varied widely (12.4% to 97.5%), averaging 59.2%, with lipid content averaging 29.1%, reflecting species- and condition-dependent outcomes. Mixotrophic cultivation in pretreated or primary/secondary-treated MW performed well under optimal conditions (22°C–25°C, high nutrient levels, low metal concentrations), with stress conditions enhancing lipid and carbohydrate synthesis. Despite its potential, challenges remain, including energy-intensive processes, scalability limitations and the need for optimised cultivation. Future research should focus on low-energy technologies, innovative biofuel pathways and integrated wastewater pretreatment strategies.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Fig. S1** The process of converting microalgal biomass into biodiesel. **Table S1:** Overview of main characteristics of closed reviews: wastewater treatment and biofuel production using microalgae. **Table S2:** Summary of microalgae cultivation in pretreated MW. **Table S3:** The change in the number of studies during the screening process. **Table S4:** List of studies used in this work. **Table S5:** Distribution of wastewater types across laboratory and pilot/real-scale studies (n = 78). **Table S6:** The pollutant removal capacity of the MWTBPS from reviewed studies. **Table S7:** Summary of results on the pollutant removal of microalgae cultivated in MW. **Table S8:** Summary of the efficiency of lipid conversion to biodiesel of certain microalgae species grown in MW. **Table S9:** Advantages and disadvantages of the energy conversion methods of microalgae biomass grown in MW. **Text S1:** Keywords and criteria for searching and selecting research papers.