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Uncovering the feasibility of using live Chlorella microbiomes in Domestic and Industrial Wastewater Treatment: Insights into Monoculture and Synergistic Mixed Co-Cultured System

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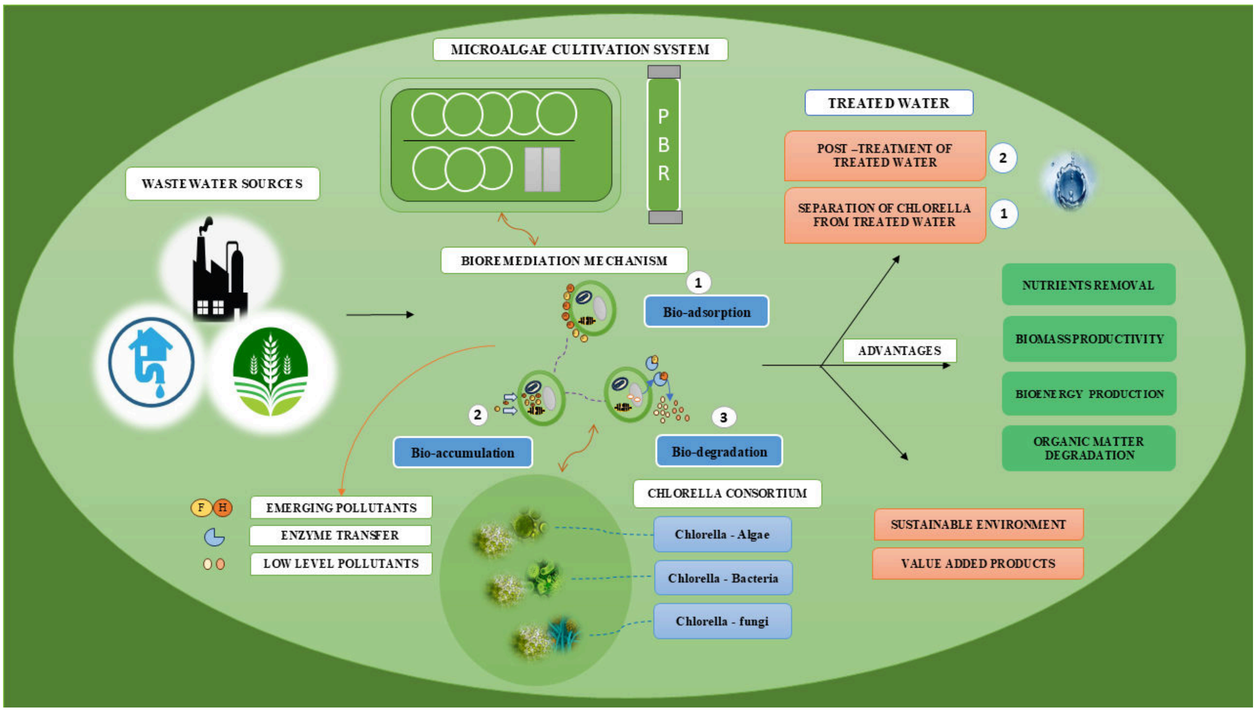
Abstract

In recent years, numerous innovative technologies have emerged for algal bioremediation aimed at achieving clean water. Given the diverse functionalities of algae, algal bioremediation presents a viable alternative to conventional wastewater treatment systems. This study specifically emphasizes the use of *Chlorella*-based algal remediation when integrated with other microbial cultures for clean water applications. Our research provides a thematic review of the real-time integration of *Chlorella* and its co-cultures with other heterotrophic microbes in the treatment of domestic and industrial wastewater. While many review articles discuss the role of various microalgae species in wastewater treatment generally, to the best of our knowledge, no comprehensive review has documented the use of live algal systems, specifically focusing on a consortium of *Chlorella* algae, *Chlorella* bacteria, and *Chlorella* fungi for pollutant removal in wastewater treatment. This review primarily investigates the mechanisms by which live algal cell consortia—both single cultures and co-cultures—remove biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrogen, phosphorus, and heavy metals from wastewater. Additionally, the review addresses important observations concerning the characteristics of consortia, optimal growth conditions, the interactions between algae and contaminants, and the use of molecular diagnostic techniques such as PCR, FISH, and metagenomics. Our findings indicate that heterotrophic systems consisting of *Chlorella* and bacteria demonstrate higher treatment efficacy compared to systems made up of *Chlorella* and fungi

Keywords: *Chlorella* -algae, *Chlorella* -bacteria, *Chlorella* -fungi, wastewater, co-culture, industry.

Graphical Abstract:



1. Introduction

Water is an essential natural resource for all living organisms, covering 71% of the Earth's surface. However, only 2.5% of this water is freshwater, while the remaining 97.5% is saltwater. In 2019, the World Health Organization and the United Nations Children’s Fund reported that 2.2 billion people lacked access to safely managed drinking water, and an additional 785 million people did not have access to basic drinking water. This situation is largely due to population growth and industrialization. A recent study by du Plessis [1] found that approximately 2 million tons of sewage and industrial and agricultural effluents are discharged into water bodies worldwide each day. This practice causes severe water pollution, which negatively affects water quality, ecosystems, and human health. Furthermore, chemical industries contribute to this issue by discharging about 70% of these effluents, which often contain toxic metals, dyes, inorganic compounds, antibiotic residues, and more. These toxic discharges further degrade water quality and lead to significant fluctuations in Chemical Oxygen Demand (COD) and Total Dissolved Solids (TDS) levels. Additionally, industrial pollution contributes to eutrophication in aquatic environments due to the high levels of phosphorus and nitrogen present in these discharges.

To address these issues, it is important to adopt a cost-effective and environmentally viable wastewater treatment system. Wastewater treatment plants are essential facilities that treat wastewater generated from municipal, industrial, and agricultural activities before releasing it into the environment or reusing it for beneficial purposes. The wastewater treatment plant primarily involves three methods to effectively remove pollutants including – physical, chemical and biological treatment methods. In addition to these conventional approaches, various advanced techniques are employed to treat different types of effluents in wastewater systems. These include membrane technology, thermal treatment, adsorption, electrodialysis, and photocatalytic degradation. However, these advanced treatment techniques have certain limitations, such as high energy consumption, cost issues, and demanding maintenance requirements. Further, these techniques frequently generate hazardous byproducts and excess sludge accumulation causing secondary contamination.

In general, biological wastewater treatment is environment-friendly and energy-efficient as compared to chemical treatment. In effluent treatment plants, biological treatment employs microorganisms to break down pollutants, primarily focusing on the removal of Biochemical Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) [2]. This micro-remediation technology is considered a promising tool in green technology, as microbes play a crucial role in the treatment as well as in the reclamation of wastewater. The microorganisms that are most commonly studied for biological wastewater treatment include bacteria, fungi, algae, yeast, and various other microbial groups. However, the application of these microorganisms for the degradation of toxic contaminants has mostly been investigated in laboratory settings, with less attention given to wastewater treatment facilities.

Among various microbial species, algae are valuable for wastewater treatment due to their diverse capabilities. Algae are broadly classified into two categories namely microalgae and macroalgae. Compared to macroalgae, microalgae are widely used in wastewater treatment. Microalgae are well-known for their high photosynthetic efficacy and fast growth rates, which make them a useful resource in various applications [3]. Microalgae plays a significant role in wastewater treatment by effectively removing nutrients, degrading organic and inorganic pollutants, and producing oxygen through photosynthesis. Consequently, research into the use of microalgae for reducing chemical oxygen demand (COD), biological oxygen demand (BOD), and nutrients such as phosphorus and nitrogen in wastewater is extensive [4]. The mechanisms through which microalgae remove pollutants include bio-adsorption, bio-accumulation, and bio-degradation [5].

Nowadays, most of the algae-based microbial wastewater remediation studies, primarily focus on the cultivation system that comprises - mono-culture and co-culture. In a mono-culture wastewater treatment system, single microalgae species are grown in a controlled environment such as a photobioreactor or pond. Microalgal species like *Chlorella* sp.[6], *Scenedesmus* sp.[7], *Arthrospira platensis* [8], *Nannochloropsis* sp.[9], *Dunaliella* sp.[10], *Anabaena* spp[11]., etc. are examples of monoculture systems used extensively in wastewater treatment. Most studies demonstrated the use of a monoculture system for the removal of BOD, COD, TN (Total nitrogen), TP (Total phosphorous), heavy metals and various other pollutants for wastewater from industrial, municipal, and agricultural sources [12]. However, while microalgal monoculture systems show promising potential for pollutant removal, they also encounter significant challenges. These challenges can lead to low productivity, deterioration of the culture, poor biomass quality, limited metabolic capabilities, vulnerability to environmental fluctuations, risks of contamination and disease, reduced nutrient removal efficiency, and a lack of redundancy and resilience.

To address the challenges in microalgal wastewater treatment, there has been a shift from monoculture-based remediation to co-culture systems. Co-culturing different algal species enhances performance efficiency by utilizing multiple metabolic pathways, which effectively degrades pollutants and improves overall system performance. The co-culture approach is gaining significant attention due to its metabolic adaptability and ability to thrive in diverse environmental conditions. Typically, a co-culture system involves the interaction of one microalga with other microorganisms, such as microalgae-algae, microalgae-bacteria, and microalgae-fungi associations. This symbiotic relationship between microorganisms leads to high biomass production and more effective pollutant degradation. For instance, co-culturing *Chlorella sorokiniana* with *Rhodotorula glutinis* showed high phosphate removal efficiencies of 93.48% with enhanced biomass and lipid productivity in agricultural wastewater

[13]. In another instance, the combination of *Chlorella pyrenoidosa* and *Aspergillus oryzae* in a co-culture system resulted in the effective removal of 98.6% Chemical Oxygen Demand (COD) and 99.8% Total Nitrogen (TN) from industrial wastewater. [14]. Recent studies indicate that co-culture systems are more sustainable and cost-effective for wastewater treatment compared to monoculture systems.[15]16].

To date, so many research and review articles have been published examining the performance of microalgae-based wastewater treatment systems. Most of these reviews offer valuable insights into the evaluation of microalgae in wastewater remediation. Some articles have also explored algal remediation in conjunction with resource recovery, sustainability, and environmental impacts. Examples can be found in references [17–44]. While most reviews have concentrated on monoculture algal remediation, few studies have investigated cocultured remediation. Specifically, there has been insufficient exploration into why *Chlorella* is effective at removing various pollutants from specific industrial wastewater treatment systems. Furthermore, comprehensive investigations into algal systems in wastewater and systematic reviews of molecular diagnostics in coculture systems are limited. To address this gap, our study aims to provide insights into how *Chlorella* and other microbial co-cultures remove toxic pollutants in cocultured systems for both domestic and industrial wastewater treatment, with a particular focus on molecular diagnostics.

This review focuses on the use of *Chlorella*, a microalgae species that has long been chosen for wastewater treatment worldwide due to its numerous benefits, including performance efficiency, sustainability, cost-effectiveness, and environmental friendliness. Notably, *Chlorella* offers several advantages, such as a high metabolic rate, which facilitates the efficient removal of pollutants like heavy metals, organic compounds, nitrogen, and phosphorus. Several specific review reports [37], [45], [46], and [47] have documented the effectiveness of the microalgal genus *Chlorella* in treating pollutants in wastewater, as highlighted in Table 1. However, topics like molecular diagnostics, scaling up for industrial applications, commercialization strategies, and addressing techno-economic challenges are sometimes overlooked or underemphasized. These elements are critical for transforming laboratory successes into viable, large-scale operations. and therefore, incorporated into the proposed review article. This would provide a more comprehensive view of the *Chlorella's* efficacy across various wastewater treatment systems.

We focus on a co-culture consortium system that includes *Chlorella* in combination with algae, bacteria, and fungi. This paper focuses exclusively on the use of live microalgae cultures for pollutant degradation, excluding any discussion of dead biomass. Furthermore, we provide an in-depth analysis of the mechanisms by which live *Chlorella* cells effectively remove pollutants from wastewater. Although several studies have addressed the degradation mechanisms of microalgae, there remains a significant gap in understanding the metabolic pathways involved in biodegradation, bioaccumulation, and bioadsorption for this algal species. In this context, our review highlights the importance of studying the biodegradation mechanisms of live *Chlorella* cells in the treatment of industrial and domestic wastewater effluents.

Table 1. List of review articles investigating *Chlorella*-based microalgae for wastewater treatment.

Short title	Key aspects discussed	Ref	Year
Effectiveness of *Chlorella* microalgae in removing organic contaminants from water bodies or wastewater.	▪ Role of *Chlorella* in removing organic contaminants and mechanism studies ▪ Overview of *Chlorella* in a single Monoculture system	[45]	2023
Use of the microalga *Chlorella vulgaris* for mercury bioremediation from wastewater and biomass generation.	▪ *C. vulgaris* efficacy in mercury removal and mechanism studies ▪ ▪ Biomass utilization for biogas generation	[46]	2024
Chlorella's Biology and Industrial Uses: Progress and Future directions.	▪ CO_2 fixation ▪ Greenhouse reduction and wastewater treatment ▪ Efficiency in nutrient removal ▪ Removal of contaminants using single culture for industrial and domestic wastewater treatment	[47]	2016
A review of algal and bacterial consortiums for wastewater purification and transformation into biodiesel, bioethanol, biohydrogen, biofertilizers, and animal feed.	▪ Microalgae – bacteria consortia treatment for wastewater treatment ▪ State of the art in nutrient recovery ▪ Studies on the utilization of algae for biodiesel, bioethanol, biohydrogen, and biofertilizers	[37]	2023

2. Mono-culturing and Co-Culturing of *Chlorella* and its interaction with pollutants

2.1 Single culturing system of *Chlorella* for degradation of pollutants

Chlorella, a green algae belonging to the Chlorophyta group is considered one of the best options for bioremediation. Some *Chlorella* species that have been used for pollutant remediation include *C. vulgaris*, *C. pyrenoidosa*, *C. sorokiniana*, *Chlorella zofingiensis*, *Chlorella regularis*, *Chlorella minutissima* etc [48].

Chlorella, being a photosynthetic species has the potential ability to absorb carbon dioxide and release oxygen, thereby increasing the dissolved oxygen levels in water. As a result, *Chlorella* is predominantly used in diverse environment applications. *Chlorella* in general not only increases biomass productivity but also possesses an ability to reduce COD, BOD, nitrogen, phosphorous, orthophosphate, heavy metals, toxic chemicals, micropollutants, suspended solids etc in both industrial and domestic wastewater sources [49]. With these multifaceted benefits, *Chlorella* species act as an effective tool for the wastewater treatment process.

For instance a study by Ganeshkumar et al [12] reported that the cultivation of *Chlorella sp. MM3* in mixed wastewater of piggery and winery resulted in the nutrient removal of 89% TN and 49% TP respectively. Similarly, in the study conducted by Alazaiza et al [14] *C. vulgaris* effectively removed 95% of Total nitrogen and 97% of Total phosphorus from sewage wastewater, with maximal biomass production. In a study led by Lv and team [50] two stages of microalgae-based treatment were used to remove nutrients from cow farm wastewater. The study employed *C. vulgaris* from five freshwater microalgal strains and removed 62.30%, 81.16%, and 85.29% of COD, TN, and TP respectively. In another study, investigations of Pathak and group [51] demonstrated that *C. pyrenoidosa* effectively removed 63% COD, 82% TN, and 87% TP from textile industry wastewater [51].

With further experimental analysis in a single culture system, Wang et al., [52] found that *C. vulgaris* JSC-6, carbohydrate-rich microalgae effectively treated piggery wastewater by removing 70-77% COD and 40-90% NH_3-N, depending on the wastewater dilution ratio. The highest removal percentage was achieved for 20-fold diluted wastewater. On the other hand, Zou et al proved that [53] *C. vulgaris* grew well in pretreated fresh pig urine, producing microalgal biomass and removing 98.20% of NH_4-N and 68.48% of total phosphorous in batch mode with an optimal hydraulic retention time of 7-9 days. In addition, a study by Ibarruri et al., [54] found that *C. protothecoides* showed a 94% and 62% efficiency in removing NH_3-N and PO_4-P from primary settled wastewater, indicating the potential of *Chlorella* sp.*,* efficiencies in wastewater treatment. **Table. 2** shows the treatment efficiency of *Chlorella* species in different types of wastewaters.

Table 2. Genus *Chlorella* in the degradation of various types of wastewater effluents

***Chlorella* sp.,**	**Source of Wastewater**	**Treatment efficiency (%)**			**References**
		COD	**TN**	**TP**	
Chlorella sp. *MM3*	Piggery and winery effluent	-	89	49	[12]
C. vulgaris	Sewage wastewater	-	95	97	[14]
C. vulgaris	Cattle farm wastewater	62.30	-	85.3	[50]
C. pyrenoidosa	Textile Industry effluent	63	82	87	[51]
C. pyrenoidosa	Swine wastewater	74.8	-	68.4	[55]
C. zofingiensis	Piggery wastewater	65.8–79.8	69.0–82.7	85.0–100.0	[56]
C.vulgaris	Palm oil mill effluent	50.5	-	84.0	[57]
C. pyrenoidosa	Pickle industry wastewater	84.67	85.82	92.46	[58]
C. pyrenoidosa	Piggery wastewater	86.8	85.2	84.3	[59]
C. vulgaris	Dairy wastewater	92	77	78	[60]

2.2 *Chlorella* – Algae consortium for wastewater treatment

The consortium system of *Chlorella*-algae establishes the symbiotic relationship between various algal species, mainly involving the *Chlorella* genus. This system leverages the complementary strength of different algal species to enhance resilience to environmental stress, biomass production and wastewater treatment. This microalgae consortium in wastewater treatment involves the cultivation of different algal species in the same system, which offers several advantages over monoculture algal systems. The advantage includes enhanced nutrient removal, resilience to environmental conditions, and good treatment efficacy. In the *Chlorella*-algae consortium, the interactions between different microalgal species create cooperative and competitive associations that facilitate metabolite exchange, biomass productivity and high nutrient removal efficiency [61]. However, these microalgal associations may also lead to the discharge of secondary metabolites known as allelochemicals. Allelochemicals are a group of organic compounds, produced by algae that shows adverse effects on co-cultured systems, inhibiting the growth or metabolic activities of pathogenic organisms. A study by Bacellar Mendes & Vermelho [61] reported that allelochemical production can be suppressed or improved by biotic and abiotic factors. Abiotic factors such as increased pH, nutrient starvation, low temperature, and light intensities boost allelochemical production, while extreme nutrient levels, low pH, increased temperature and light intensities restrict it. Biotic factors such as microbial concentration also affect allelochemical production. [62].

Several studies have confirmed the effectiveness of microalgal consortiums in wastewater treatment. For illustration, an experiment conducted by Hu et al.,[63] presented the growth characteristics and nutrient removal efficacy of microalgal co-culture, *C. vulgaris* and *Scenedesmus dimorphus* in landfill leachate. The work resulted in 0.266 g/L biomass production and removal of 81% COD, 80.1% NH_{4^+}-N, 72.1% TN and 86% TP respectively. The microalgae *C. vulgaris* and *Scenedesmus dimorphus* consortium system are highly effective in removing pollutants from wastewater, due to enzymatic activity and Chlorophyll (Chl) fluorescence parameters [14]. The enzyme involved in the consortium system involves SOD (superoxide dismutase) and CAT (catalase). These enzymes play a vital role in protecting microalgal cells from oxidative damage under stress conditions [64].

Similarly, Asmare and colleagues [65] carried out a study to investigate the remediation potential of *S. dimorphus* and *C. vulgaris* in dairy wastewater through a single and co-cultured system. It was found that co-cultivation of *S. dimorphus* with *C. vulgaris* achieved a degradation rate of 83.2%, 4.1% and 65.7% of nitrate equivalents (total concentration of nitrate-nitrogen) (NO_{3^-}-N) and 82.9%, 20.8%, and 84.6% of orthophosphate equivalents (PO_{4^-}-P) after 7 days of growth. From this co-culture system, it is observed that *S. dimorphus* produced higher biomass productivity and *C. vulgaris* presented improved chlorophyll accumulation and contributed to the removal of nutrients effectively from dairy wastewater effluents.

In addition, Oberholster and team [66] studied the use of *C. vulgaris* and *C. protothecoides* for the removal of nutrients and carbon-containing organic matter such as COD and TOC from domestic sewage wastewater. This study showed a removal efficacy of 35.4% TN, 74.4% TP, 22.2% Total organic carbon (TOC), 60% Chemical Oxygen Demand (COD), and 87% orthophosphate respectively. Furthermore, experiment conducted by Ndlela et al. [67] studied the removal of antibiotics using microalgal consortium, *C. protothecoides* and *C. vulgaris*. This study demonstrated that this microalgal consortium exhibited a removal efficacy of 77.3% ± 3.0 sulfamethoxazole and 43.5% ± 18.9 ofloxacin at a concentration of 10 ppb. In another study, the effective removal of 46.5% ± 5.3 sulfamethoxazole and 55.1% ± 12.0 ofloxacin, respectively is noticed at a concentration of 100 ppb [68]. The efficiency of a microalgal consortium system in wastewater treatment largely depends on the choice of microalgae-optimized environmental conditions [69]. **Table. 3** outlines the *Chlorella*- algae interactions and their treatment efficacy in different industrial and domestic wastewater.

2.3 *Chlorella* -bacteria consortium for wastewater treatment

Microalgae and bacteria interact with the ecosystem through various modes, namely - mutualism, commensalism, and/or parasitism. In microalgal culture, co-culturing algae and bacteria significantly improves algal growth, spore germination, disease resistance and morphogenesis, further making them valuable in biotechnological applications [70]. The microalgae–bacteria consortium in wastewater treatment presents a symbiotic interaction that degrades both organic and inorganic pollutants released from various effluents. This interaction occurs through the photosynthetic process, as microalgae produce oxygen, creating an aerobic environment beneficial to bacteria. Therefore, in an oxygen-rich environment bacteria thrive and break down organic and inorganic pollutants, producing carbon dioxide for microalgal consumption [71].

According to Tang et al., [72], microalgae and bacterial consortiums outperform mono-cultured systems for treating wastewater, due to their direct and indirect ecological interactions. The consortium system between microalgae and bacteria is compatible and provides an intricate interaction and substrate exchange. This interdependence and support provide robust growth, a stable environment and prevent pathogenic microbial invasion [73]. For instance, the symbiosis between *Chlorella* and bacteria showed predominant efficiency in treating wastewater both by enhancing biomass productivity and degrading organic and inorganic pollutants [74]. As a result, consortium systems of algae–bacterial communities have become increasingly prevalent in treating wastewater, by offering a promising solution for efficient and effective treatment processes.

Several studies have demonstrated the remarkable removal efficacy of *Chlorella*-bacterial co-culture systems. For instance, a study investigated by Nair & Nagendra [75] reported that *Chlorella pyrenoidosa* and nitrogen fixation bacteria were utilized to treat landfill leachate. The study inferred that 30% landfill leachate spiked municipal wastewater, effectively removed 81% Dissolved organic carbon (DOC), 70% TN and 89% orthophosphate and enhanced the biomass productivity of 2.8 g/L in the laboratory scale photobioreactor. It is noted that nitrogen-fixing bacteria are crucial for microalgal growth and also is a main source of protein synthesis [76]. Therefore, *C. pyrenoidosa* when co-cultured with nitrogen fixation bacteria assimilates nitrogen to increase the growth of microalgae and degradation efficacy.

In another study presented by Amini & Team [77] microalgae species *C. vulgaris* and activated sludge bacteria treated domestic wastewater in a semi-continuous photobioreactor with an inoculum ratio of 5:1,1:1 and 1:5. From the study it was identified that an inoculum ratio of 5:1 presented a maximum removal rate of 88% to 98% $N\text{-}NH_4^+$ and 84% to 89% $P\text{-}PO_4^{3}$ when compared to inoculum ratio of 1:1 and 1:5. Thus it is evident from the work that increasing the inoculum ratio of *C. vulgaris* – activated sludge bacteria, enhanced the removal efficiency of nitrogen [78]

In a study conducted by Ji et al., [79] *C. vulgaris*- *Bacillus licheniformis* with an algae-bacteria ratio of 1:3, removed 86.55% COD, 88.95% TDN and 80.28% TDP from synthetic wastewater. *C. vulgaris*- *B. licheniformis* co-existed in a consortium system and reached a c-di-GMP concentration of 3.85 μg/g, indicating their strong ability to quorum sense the signals. Consequently, the *B. licheniformis* grown in a consortium system led to the effective removal of COD, P and N nutrients and increased biomass productivity [80]. In another study led by Ferro and team [81], a removal efficacy of 49.5 ± 6.1% TOC, 55.7 ± 8.04% TN and 95.6 ± 3.6% TP are achieved from municipal wastewater with enhanced biomass productivity of 0.63 ± 0.03 g/L. The study utilized *C. vulgaris – Rhizobium sp*. Further, it revealed that nitrogen, phosphorus, and organic carbon were eliminated concurrently during mixotrophic development without the use of exogenous oxygen or carbon, lowering operational expenses.

Similarly, a work by Liu et al [82] investigated a co-culture system comprising *Chlorella sp* and *Acinetobacter sp.,* posing an effective removal efficiency of 93.01% COD and 98.78% TP, when treated in centrate wastewater. According to the study, it is reported that presence of *Acinetobacter sp.,* enhanced the growth of *Chlorella sp* and contributed to the removal of phosphorous at an initial concentration of 25 mg/L to 8.99 mg/L. This synergy allowed better growth conditions without intensive competition between two microorganisms. It is also reported that *C. sorokiniana and Chryseobacterium scophthalmus* degraded pollutants from the fermentation industry when cultured in an open suspended system. This consortium system achieved a removal efficacy of 80.3% TN, 46.7% TP and 63.7% COD at batch mode [83]. This is perhaps, due to *C. sorokiniana's* strong assimilation in absorbing nutrients accompanied by an effective removal of organic compounds by *C. scophthalmus*.

In consortium studies, the selection of suitable species plays a crucial role in optimizing the performance of the co-culture system. For instance, the microalgae *C. sorokiniana* prefers the Rhodobacteraceae and Rhizobiaceae families over Nitrosomonas and Dechloromonas. It has been reported that *C. sorokiniana*, when cultured with activated sludge from Rhodobacteraceae and Rhizobiaceae families, showed an effective removal efficacy of 98% nitrogen and 96% phosphate in just seven hours. In contrast, *C. sorokiniana* cultured with activated sludge from Nitrosomonas and Dechloromonas families showed a removal efficacy of 71.4% ammonia nitrogen in 14 days. Perhaps, it took 9 days to reach a steady state condition [84]. This comparison showed that each species has a distinct survival rate and metabolic capabilities. Therefore, selecting the right species for the wastewater treatment process provides high degradation of pollutants and biomass productivity. **Figure 1** illustrates the microalgae-bacteria interaction in wastewater treatment [85]. Various types of *Chlorella*–bacteria co-cultures and nutrient removal efficiencies are summarised in **Table 3.**

2.4 *Chlorella* - fungi consortium for wastewater treatment method

The microalgae-fungi consortium in wastewater systems forms a symbiotic relationship, namely mutualism, in which both microalgae and fungi benefit from each other's presence in wastewater [86]. In this system, microalgae perform photosynthetic activity and produce oxygen while fungi aid in the breakdown of organic and inorganic pollutants that are present in wastewater. As a result, fungi produce carbon dioxide which is utilized by microalgae for their respiration process. A study by Gururani et al., [87] reported that, in the microalgae-fungi consortium, heterotrophic fungi degraded natural organic pollutants released by microalgae, thereby acting as a natural wastewater treatment system.

Microalgae–fungi consortia are mostly utilized to remediate large molecular organic pollutants such as pesticides, medicines, detergents, and petro-alkanes, employing three mechanisms namely- biosorption, bioaccumulation, and biodegradation. Fungi, a heterotrophic organism largely rely on organic carbon as their energy source, making them more effective than the microalgal genus in removing chemical oxygen demand (COD) from wastewater [88]. In wastewater treatment, co-culturing microalgae with fungi enhances their efficacy in remediating wastewater [89]. As an illustration, how co-cultivation of *C. vulgaris* and *Penicillin geesteranus*, and *Aspergillus niger* and *Trichoderma reesei* effectively remove organic and inorganic pollutants in wastewater is demonstrated by Singh and Xu's team [90]

[91]. It is important to note that, the microalgae genus *Chlorella* showed higher biomass productivity and removal efficacy when they were grown with filamentous fungi.

A study by Wang et al. [92] reported that the co-cultivation system of *C. pyrenoidosa* and *A. oryzae* in potato starch wastewater (PSW) exhibited a synergistic effect, achieving a removal efficiency of 92.08% COD, 83.56% TN and 96.58% TP. Similarly, an experiment conducted by Qin & Team [93] utilized *C. vulgaris* and *Yarrowia lipolytica* to treat liquid digestate from the yeast industry. The co-cultured microalgae-fungi consortia have attained a removal rate of 79.23–80.38% COD and 80.25% NH_3 -N with biomass productivity of 1.23–1.56 g/L. With respect to experimental analysis, *C. vulgaris* and *Y. lipolytica* consortia showed faster cell proliferation, lower yeast cell size, and increased microalga chlorophyll content.

Yang et al. [94] in their investigation treated biogas slurry using microalgal species *C. vulgaris* and *Ganoderma lucidum.* This experimental analysis utilizes a photobioreactor with a light combination of red-blue with an intensity ratio of 5:5. and obtained a degradation rate of 82.32% ± 7.18% TN, 81.06% ± 7.06% COD and 82.98% ± 7.26% TP, respectively. Moreover, Kameoka and team found that GR24, a synthetic analog of strigolactones- a plant hormone involved in regulating growth and symbiosis- at a concentration of 10^{-9} M increases contact with microalgal cells that promotes the growth and bond between *C. vulgaris* microalgae- *G. lucidum* fungi. This technology proved to be effective in removing nutrients and COD in a co-cultured system, to purify biogas slurry [95][96]. From the study by Jiang et al [97], it is found that *Chlorella variabilis* and *G. lucidum* effectively removed contaminants present in synthetic wastewater, achieving a removal efficiency of 75.5% COD, 76.7% TN, 74.7% TP, with a biomass productivity of 0.89 g/L. However, it is also observed that *C. variabilis* and *G. lucidum* showed a sign of environmental stress, that is possibly linked to fluctuations in water quality.

Chlorella-fungal culture is extremely successful in degrading pollutants because the enzymes produced by fungus can degrade solid organics into low molecular weight nutrients that microalgae can absorb. In particular, the enhanced nitrogen uptake makes microalgae more susceptible to nitrogen assimilation, which increases the removal efficiency. **Figure 2** depicts the microalgae – fungi interaction toward heavy metal [87]. In accordance, various forms of *Chlorella* – -fungi co-culture and their treatment efficiency in different industrial and domestic wastewater efficacy were summarized in the **Table. 3**

Table. 3 *Chlorella* – algae, *Chlorella* – Bacteria and *Chlorella* -fungi i the degradation of various types of wastewater effluents

S. No	Source of Wastewater	Co-culture	Species	Removal efficiency	Way of cultivation	Process conditions	Ref
1	Dairy wastewater	Algae-Algae	*S. dimorphus – C. vulgaris*	NO_{3-} –N: 65.7% PO_{4}- –P: 84.6%	Batch	➢ Cultivation type – Photobioreactor ➢ light source - fluorescent lamps and eight LEDs. ➢ Temperature - 25°C ➢ Photoperiod - 12 hours	[65]

						➢ Inoculum added - 10%	
2	Municipal wastewater	Algae-Algae	*C.vulgaris-C. protothecoides*	TN: 35.4 % TP: 74.4 % TOC: 22.2 % COD: 60.0% PO_4^{3-}: 87.0%	Continuous	➢ pH 8.8 ➢ Temperature - 27 to 28°C ➢ Cultivation type - Photobioreactor	[66]
3	Municipal wastewater	Algae-Bacteria	*C.sorokiniana-activated sludge*	COD: 88% TN: 98% TP: 96%	Continuous	➢ Temperature - 25ºC ➢ Light Intensity - 2000 lux ➢ Culture medium – BG-11 ➢ 12h/12h light/dark ➢ Cultivation type – Flat plate photobioreactor	[84]
		Algae-bacteria	*C.vulgaris & Microcystis aeruginosa*	COD: 86.55% TN: 88.95% TP: 80.28%	Batch	➢ Light Intensity-120µmol m^{-2} s^{-1} ➢ Temperature - 28±1°C ➢ Culture medium – Microalgae - BG-11 / Bacteria - LB medium	[79]
		Algae – Bacteria	*C.vulgaris – Bacillus licheniformis*	COD: 86.55% TDP: 80.28%, TDN: 88.95%	Batch	➢ Light Intensity-120µmol m^{-2} s^{-1} ➢ Temperature - 28±1°C ➢ Culture medium – Microalgae - BG-11 / Bacteria - LB medium	
		Algae-Bacteria	*C.Sorokiniana – Activated sludge bacteria*	NH_3: 81% PO: 39% COD: 98%	Continuous	➢ Cultivation type – Membrane bioreactor ➢ Temperature - 30ºC ➢ Light intensity-120 µmol m^{-2} s^{-1}	[98]
		Algae – Bacteria	*C.vulgaris - Rhizobium sp*	TOC: 49.5 ± 6.1% TN: 55.7 ± 8.04% TP: 95.6 ± 3.6%	Continuous	➢ Cultivation type – laboratory-scale photo-sequencing batch reactor ➢ Temperature at 26 ± 2 ◦ C	[81]

		Algae bacteria	*C. vulgaris - Activated sludge bacteria*	TN: 98-100% TP: 92-100% COD: 94 – 96 %	Semi-continuous	➢ Medium – BG-11 ➢ Temperature - 25°C ➢ light intensity- 50μmol $m^{-2}s^{-1}$ ➢ Cultivation type – Tubular Photobioreactor supplying with 5% CO_2	[99]
		Algae – Bacteria	*C. vulgaris FACHB 30 - P. putida*	COD: 97%, NH_4^+ - N: 100% PO_4^{3-} -P: 100%	Batch	➢ Medium – BG-11 ➢ Temperature - 26 ± 2 °C ➢ All cultures are agitated with a magnetic stirrer at 140 rpm ➢ light intensity- 200 µmol m^{-2} s^{-1}	[100]
		Algae-bacteria	*C.vulgaris - Bacillus licheniformis*	TN: 88.82%, TP: 84.87% COD: 82.25% NH_4^+: 84.98%	Batch	➢ Initial Concentrations of B. licheniformis and C. Vulgaris • 1×10^5 cellmL^{-1} • 3×10^5 cellmL^{-1}. ➢ Temperature - 28 ± 1°C ➢ Medium – BG-11 ➢ light intensity - 120μmol m^{-2} s^{-1} ➢ pH – 7.14	[101]
		Algae-bacteria	*Chlorella sp. - Heterotrophic bacteria*	COD: 86.0 ± 2% TKN: 97.0 ± 3%	Batch	➢ Temperature - 22.8 C ➢ Cultivation type - Photo-sequencing batch reactor ➢ light intensity - 30 µmol m^{-2} s^{-1}	[102]
4	Stripped food waste permeates	Algae-bacteria	*C. sorokiniana- Anaerobic digestate effluent*	TN: 34% to 67%, NH_4^+-N: 65% to 97% COD: 60% to 14%	Continuous	➢ Indoor use of T5 growth lamps. ➢ Humidified air supplied - 100 ml/min. ➢ 16:8 light-dark cycle.	[103]

5	Soybean Processing industry	Algae-bacteria	*C.Pyrenoidosa - bacteria*	COD: 92.8% TN: 89.4%, TP: 98.2%.	Batch	➢ Medium – SWG and BG-11 ➢ Light intensity - 27 $\mu mol\ m^{-2}\ s^{-1}$. ➢ Light/dark ratio: 14:10. ➢ Temperature: 25 ± 0.5 °C. ➢ Intermittent shaking: 6–7 days ➢ pH 6.1 ± 0.5	[104]
6	Simulated fermentation	Algae-Bacteria	*C. sorokiniana- Stenotrophomonas acidaminiphila*	COD: 66.1% NH_4 -N: 77.8% TP: 24.6%	Batch	➢ Medium – BG-11 ➢ Fluorescent lamps - 150 $\mu mol\ m^{-2}\ s^{-1}$ ➢ Temperature of 28 ± 0.05 °C	[83]
		Algae – bacteria	*C. sorokiniana - Exiguobacterium aurantiacum*	COD: 51.6% NH_4 -N: 61.5% TP: 45.6%	Batch	➢ Medium – BG-11 ➢ Fluorescent lamps - 150 $\mu mol\ m^{-2}\ s^{-1}$ ➢ Temperature of 28 ± 0.05 °C ➢	
		Algae - bacteria	*C. sorokiniana - Chryseobacterium scophthalmus*	COD: 63.7% NH_4 -N: 80.3% TP: 46.7%	Batch	➢ Medium – BG-11 ➢ Fluorescent lamps - 150 $\mu mol\ m^{-2}\ s^{-1}$ ➢ Temperature of 28 ± 0.05 °C	
7	Artificial Wastewater	Algae – Bacteria	*C. sorokiniana - Pseudomonas H4*	COD: 60% TP: 72.8% NH_4 -N: 71%	Continuous	➢ Initial COD - 500 mg/L NH_{4+}-N - 25 mg/L ➢ PO_4^{3-}-P - 5 mg/L ➢ Temperature 25°C	[105]
8	Industrial wastewater	Algae – Bacteria	*C. pyrenoidosa - nitrogen-fixing bacteria Azotobacter beijerinckii*	COD: 20.8%, PO_4: 18.5% NH_4–N: 8.9%	Continuous	➢ Medium - BG11 ➢ Temperature- (25 ± 1) °C ➢ light cycle - 12 h:12 h ➢ light intensity of 9600 lux ➢ Inoculum density of *C. pyrenoidosa* and	[106]

						A. beijerinckii were 0.35 g/L and 1 × 10^8 cfu/mL	
9	Swine manure	Algae – Bacteria	*C. sorokiniana - activated sludge*	COD: 99%, TN: 86% TP: 75%	Continuous	➢ Cultivation type - 4.9 L enclosed tubular biofilm photo-bioreactor ➢ Temperature - 30 ◦C ➢ Light intensity – 10,000 lux	[107]
10	Piggery wastewater	Algae – Bacteria	*C. vulgaris - Exiguobacterium and B. licheniformis*	NH_{4+} - N: 84.4% TN: 78.3% TP: 87.2%, COD: 86.3%	Batch	➢ light intensity – 120 µmol m^{-2} s^{-1} ➢ Temperature 25 ± 1 ◦C ➢ ventilation rate - 0.3 L m1 ➢ Cultivation type – 1L columnar photobioreactor	[108]
11	Centrate wastewater	Algae – Bacteria	*Chlorella sp.- Acinetobacter sp.*	COD: 93.01% TP: 98.78%	Batch	➢ Algal cells density - 0.275 ± 0.025 g/L in 100ml. ➢ Light intensity - 120 ± 10 µmol m^{-2} s^{-1} ➢ Temperature - 25 ± 1 ◦C ➢ Relative humidity - 45 ± 3% at 200 rpm ➢ Cultivation type - Pilot-scale bioreactor	[82]
12	Vinegar production wastewater	Algae – Bacteria	*Chlorella sp - Bacillus firmus* and *Beijerinckia fluminensis*	COD: 22.1% TN: 20.0% TP: 18.1%	Batch	➢ Concentration of algae and bacteria - 1.0 × 10^5 cells/mL and 1% (v/v) or 10% (v/v). ➢ Temperature - 26 ◦C. ➢ Light intensity - 50 ± 10 µmol m^{-2} s^{-1} ➢ Cultivation type - 500 mL Erlenmeyer flasks	[109]

13	Potato processing plant	Algae – Bacteria	*C. sorokiniana - M. capsulatus*	TN: 67% TP: 43% COD: 91%	Batch	➢ Temperature - 30°C ➢ Light Intensity - 150 μmol m^{-2} s^{-1}, ➢ Gas flow rate - 0.5 L min^{-1} ➢ pH - 7.0 ➢ Cultivation type - Flat panel photobioreactor	[110]
14	Piggery wastewater	Algae – Bacteria	*C. vulgaris- R. sphaeroides*	NH_{4+} -N: 100% TN: 95% TP: 96% COD: 97%	Batch	➢ Temperature - 28 ±2 ◦C ➢ light intensity - 200 μmol m^{-2} s^{-1} ➢ Photoperiod - 12 h light/12 h dark	[111]
15	Domestic wastewater	Algae-bacteria	*C. variabilis TH03 - Wastewater bacteria*	COD: 64.7–90.7% TN: 85.1–96.8% TP: 99.7%–100%	Batch	➢ Temperature - 25.5–35 ◦C ➢ Maximum solar light intensity - 12670–107695 lux ➢ Flowmeter - 0.3 vvm ➢ Membrane filter - 0.22 μm	[112]
16	Synthetic drinking water	Algae-bacteria	*C. sorokiniana - denitrifier bacteria*	Nitrate: 65-70%	Batch	➢ Temperature - 25±3.5 °C ➢ Medium - Bold's Basal ➢ Reactor working volume – 1.5 l	[113]
17	Winery wastewater	Algae-bacteria	*C. sorokiniana - wastewater bacteria*	TN: 100% TP: 100%	Continuous	➢ Medium - N8, NH4 medium ➢ Light intensity - 10,000 lux ➢ Operating status - 16:8 light/dark cycle	[114]
18	Beer brewing factory	Algae-bacteria	*C. vulgaris MACC360 -Native bacteria from sludge*	TN: ~75% TP: ~75%	Batch	➢ Temperature - 30°C ➢ pH - 7.01 ➢ light intensity of 100 μmol m^{-2} s^{-1} ➢ Initial concentration of 10 g L^{-1} of COD ➢ Hydraulic	[115]

						retention time (HRT) - 10 days. ➢ Cultivation type – Photobioreactor	
19	Acid mine drainage	Algae-bacteria	*Chlorella sp. - sulfate reducing bacteria*	Cu:98% 95–99% metal in AMD	Continuous	➢ AMDs - 500-mL bottles. ➢ Temperature - 4°C ➢ Algae samples - 25-mL	[116]
20	Starch wastewater	Algae - Fungi	*C. pyrenoidosa - Aspergillus oryzae*	COD: 98% TN: 83.56% TP: 96.58%.	Continuous	➢ Biomass concentration - 0.5 g/L. ➢ light Intensity- 100 µmol m^{-2} s^{-1} ➢ Temperature 30°C ➢ pH - 7. ➢ Cultivation Type – Photobioreactor	[92]
21	Synthetic wastewater	Algae-fungi	*C. variabilis - Ganoderma lucidum*	(COD_{cr}): 75.5% TN: 76.7% TP: 74.7% NH_{3-} -N: 90.0%	Batch	➢ (16-h light/8-h dark cycle) ➢ Temperature - 25 ± 1 °C ➢ Light intensity – 200 µmol m^{-2} s^{-1}	[97]
22	Molasses wastewater	Algae-fungi	*C. vulgaris- Aspergilus.sp*	TN: 67.09% NH_3 -N: 94.72% COD: 70.68% TP: 88.39%	Batch	➢ Temperature - 30°C ➢ Aeration rate - 1 vvm ➢ Light intensity - 3000 lux ➢ pH 6.0 ➢ 100 g fungi biomass :1 L of microalgae culture ➢ Cultivation type – Bubble column photobioreactor	[117]
23	African catfish wastewater	Algae - fungi	*Chlorella sp - Aspergillus Niger*	TN: (>93%) NH_{4+}: 98.7% Orthophosphate: 92.2%	Continuous	➢ Temperature of 30 ± 2°C ➢ pH - 6.9±2 ➢ Medium - Bold's Basal	[118]

24	Municipal wastewater	Algae - fungi	*C. sorokiniana- Aspergillus niger*	TKN: 95.40% BOD: 81.78% COD: 83.67% TOC: 70.26%	Batch	➢ Medium - Bold's Basal ➢ Temperature - 25 ± 2 °C ➢ pH-6.0	[119]
25	Anaerobically digested swine wastewater	Algae - fungi	*C. vulgaris - Ganoderma lucidum*	COD: 79.74 ± 4.87 TN: 74.28 ± 6.13 TP: 85.37 ± 6.84	Continuous	➢ light intensity - 200 μmol m^{-2} s^{-1} ➢ Temperature - 25±1 °C ➢ pH 6.9 ➢ Medium – BG-11 ➢ Cultivation type - Photobioreactor	[120]
26	Yeast industry	Algae - fungi	*C. vulgaris - Yarrowia lipolytica*	COD: 79.23-80.38% NH_3 -N – 80.25%	Continuous	➢ Temperature: 25±1°C ➢ light intensity - 300±10μmol m^{-2} s^{-1} ➢ Medium – BG-11	[93]
27	Rice wine distillery wastewater and domestic wastewater	Algae - fungi	*C. pyrenoidosa - Rhodosporidium toruloides*	SCOD: 95.34 ± 0.07% TN: 51.18 ± 2.17% TP: 89.29 ± 4.91%	Continuous	➢ SCOD - 25,000 mg/L ➢ initial cell density - 2 × 10^7 cells/mL (yeast), 5 × 10^6 cells/mL (microalga) ➢ Temperature 30ºC ➢ Light Intensity 2000 lux ➢ pH 5.5	[121]

3. Inferring biodegradation mechanism and metabolic pathways of microalgae genus *Chlorella*

Microalgae genus *Chlorella*, a photosynthetic microorganism possesses the ability to tolerate or biodegrade various contaminants that are released from industrial, municipal and agricultural sectors. Microalgal cells are majorly composed of three bio-molecules that are responsible for binding various hazardous contaminants in wastewater - namely polysaccharides, natural proteins and lipids. In addition to these biomolecules, polymeric cell components such as exopolysaccharides, form a functional group that actively removes organic and inorganic contaminants by adsorbing them onto the surface of cells or within the cell matrices [122]. Further, microalgae employ various mechanisms such as biodegradation, bioaccumulation and biosorption to metabolize and detoxify contaminants that bind on cell surfaces [123].

3.1 Biodegradation

Biodegradation refers to the metabolic degradation of chemical substances or pollutants through the biological activity of living organisms [124]. Living organisms include microalgae, which efficiently degrade the pollutants within or outside of their cells via metabolic or catabolic reactions. Microalgal species are more specific and primarily dependent on the enzymatic process to degrade complete pollutants from aquatic ecosystems. Enzymatic activities such as hydrolysis, hydrogenation, hydroxylation, and glycosylation are employed to degrade pollutants.

Microalgal biodegradation methods are further classified into two main categories that include- intracellular and extracellular degradation [125]. Extracellular polymeric substance (EPS) is an important site for extracellular degradation, and it can be paired with cell-excreted enzymes to digest pollutants such as tetracycline, sulfamethoxazole, and bisphenol-A [126]. EPS-bound antioxidant enzymes help with this process. Furthermore, EPS can be utilized as a surfactant and emulsifier to boost pollutant bioavailability.

For instance, a study by Kiki et al. [127] reported that *C. vulgaris* effectively degraded azithromycin, clarithromycin, and roxithromycin in artificial wastewater with a removal efficiency rate of 78%, 76%, and 63% by providing 20 micrograms per drug. From this study, it is identified that *C. vulgaris* effectively biodegrades pharmaceutical contaminants in wastewater treatment. Gao et al., [128] in another study investigated the removal of sulfamethoxazole using *C. pyrenoidosa* which reported a biodegradation removal efficacy of 81%. Here biosorption and bioaccumulation accounted for 6.87% and 2.87%, respectively. In another instance, Song et al. [129] found that *Chlorella sp. UTEX1602* effectively removed the antibiotic thiamphenicol using biodegradation, bioaccumulation, and biosorption, with removal efficiencies of 82.6%, 0.5%, and 4.2%, respectively.

3.2 Bioaccumulation

Following the biodegradation mechanism, bioaccumulation is another dominant mechanism in wastewater remediation. Bioaccumulation is an active process by which substances including organic and inorganic pollutants accumulate inside the cell. This metabolic-dependent process requires energy and substrates for its action, enabling microalgae to efficiently absorb pollutants from their environment and store them within the cells [130]. Unlike biosorption, which binds the pollutants on the microalgal cell surface, bioaccumulation involves the binding of pollutants inside the microalgal cell [131]. Then, the bioaccumulated pollutants in microalgal cells induce the formation of reactive oxygen species (ROS), which play a vital role in microalgae metabolism by regulating growth, facilitating cell death, and aiding defense against pathogens [132]. However, the accumulation of pollutants can alter the sensitive stability of ROS production and scavenging processes within microalgae, leading to oxidative pressure and cell damage [133]. Moreover, excessive accumulation of pollutants can damage cells with the aid of altering protein and lipid levels, leading to cell death due to heightened oxidative ability. Bioaccumulation of any pollutants in aquatic systems is quantified by the bioconcentration factor. This factor determines the ratio of a contaminant's concentration within the adsorbent (microalgae cells) to its concentration in the surrounding medium [129].

Next comes the bioaccumulation process that occurs in multiple pathways, which include passive diffusion, passive-facilitated diffusion, or energy-dependent/active uptake across the cell membrane [134]. In passive diffusion, pollutants move from regions of high concentration to low concentration through the cell membrane, driven by concentration gradients. Passive-facilitated diffusion pathway assists carrier proteins or channels in facilitating the movement of pollutants throughout the cell membrane. In contrast to energy-dependent/active uptake mechanisms, it requires the expenditure of energy (e.g. ATP) to transport contaminants against the concentration gradients.

For instance, a recent study by Desiante et al. [135] reported that *C. vulgaris* can bioaccumulate levofloxacin (LEV) at concentrations of 1, 5, and 10 mg L-1, with a clearance rate of 27.77%, 12.92%, and 10.28%, respectively. Similarly, Hu et al. [136] found that *Chlorella* sp. efficiently removed 83% of atrazine with the aid of bioaccumulation from an initial concentration of 40 μg L−1. Both internal and external physical-chemical factors such as temperature, contact time, targeted pollutants concentrations etc affect bioaccumulation drastically. In addition, a study by Lv et al [137] reported that *Chlorella* sp*.,* possesses a strong ability to bioaccumulate polybrominated diphenyl ethers (PBDEs), persistent organic pollutants (POPs) when exposed to BDE-209 and BDE-47. The resultant half maximal effective concentration (EC50) of *Chlorella*- BDE-209 and BDE-47 were reported as 4090 $\mu g\ L^{-1}$ and 64.7 $\mu g\ L^{-1}$ indicating that BDE-47 has a strong inhibitory effect on microalgal cell growth compared to BDE-209 as shown in **Figure 3**.

3.3 Biosorption

Biosorption is another significant mechanism that contributes to pollutant removal [138]. Biosorption is a physical process influenced by the interactions between the extracellular properties (cell wall and EPS) and other chemical properties of pollutants. The term "sorption" refers to a non-metabolic process whereby emerging contaminants bind to the surfaces of microalgal cells. This allows pollutants in the aqueous phase to be adsorbed even after cell death on both living and non-living cells. Therefore, these sorption processes combined both electrostatic neutralization and hydrophobicity principles [139].

The cell wall and EPS in microalgae are primarily composed of a chemical group that binds with metal ions and adsorbs organic substances present in the contaminated water [140]. The chemical groups including carboxyl, amino, hydroxyl and sulfate carry a negative charge, that binds and attracts the positively charged metal ions through electrostatic interaction. Additionally, the amino and hydroxyl group forms a hydrogen bond and non-covalent interaction with the organic molecules, while the sulfate group enhances the microalgal cell wall's capacity to remove pollutants [141]. Due to the diverse chemical composition of the cell wall and EPS, microalgae-based technologies have a promising potential in environmental remediation. Furthermore, EPS is a complex organic compound released by microalgae, that predominantly consists of polysaccharides, lipids, proteins and humic substances [142]. These compounds not only contribute to pollutant removal but also protect the microalgal cells from severe environmental stress.

In an experiment conducted by Prosenc and team [143] biosorption mechanism of *C. vulgaris* was investigated. In the analysis, 28 organic pollutants (bisphenols, neonicotinoids, pharmaceuticals and related) were exposed to live microalgae biomass at concentrations ranging from 1 to 20 μg L−1 for a duration of 1 to 12 days. The study found that while the adsorption process was rapid, *C. vulgaris* possessed limited efficacy in removing pollutants. Typically, in a different work, Angulo et al., [144] used *Chlorella* sp. biomass and leftover biomass following lipid extraction to conduct two adsorption studies with the antibiotic cephalexin in wastewater. High rates of antibiotic removal were observed- roughly 71% and 82%, respectively. Furthermore, Embaby et al.,[145] demonstrated the removal of heavy metal uranium using biosorption with microalgal strain *C. sorokiniana*, with a maximum adsorption capacity of 188.7 $mg\ g^{-1}$. **Figure 4** illustrates the mechanism of microalgae in the removal of pollutants from wastewater [146].

4. Molecular diagnosis for monitoring of *Chlorella* detection, quantification, growth, and inhibitory activities

The use of advanced molecular diagnostic tools is at the forefront of developing robust microalgal strains to degrade contaminated wastewater. In the development of growing strong microbial strains for metabolizing wastewater, the use of advanced genetic diagnostic tools has to be prioritized. In addition, real-time monitoring of contaminants and microbial growth is indeed an important step in determining the optimal condition of microalgal culture containing wastewater [147]. Introspection of molecular diagnosis provides accurate analysis, quantification, and growth of microalgae. This microalgal monitoring approach provides insights into growth rate, growth phase, and detection of inhibitory activities in microalgal culture containing wastewater. Generally, the growth of microalgae is monitored via conventional methods. The conventional method involves manual cell counting (microscopic) [148], dry cell weight (DCW) [149], optical density measurement [150] and flow cytometry [151]. [152]. With further advancements in monitoring microalgae culture, molecular diagnostic techniques such as PCR, q-PCR, NGS, FISH and microarray, have emerged as promising tools to detect and identify the growth of microalgae [153]. These molecular tools are also used for monitoring the inhibitory activities with high sensitivity and specificity, to detect and quantify the microalgal interaction with inhibitors in culture medium. In the field of molecular diagnostics, PCR acts as a powerful qualitative method for detecting the presence or absence of microalgal cells in the culture. This method also enables the identification and quantification of microalgal culture via q-PCR/RT-PCR, with rapid analysis, sensitivity and specificity by utilizing the DNA sequence of microalgae.

For instance, Grivalsky and team [154] when experimented with mixed cultures of *C. vulgaris* (as a contaminant) and *Phaeodactylum tricornutum* (as the target) utilized PCR, and qPCR to detect contamination in the mixed culture of *C. vulgaris* and *P. tricornutum*. It was found that conventional optical microscopy was unable to detect *C. vulgaris* from the sample containing a cell ratio below 1:10^5, while other methods were able to detect the contaminant even at lower levels. Specifically, PCR/qPCR showed the highest sensitivity, with a detection limit of 75 cells/mL, regardless of *P. tricornutum*. Based on the findings, the author concluded that PCR/qPCR was the most effective method for early detection and quantification of microalgae in the commercial cultivation process. Further, q-PCR was employed to quantify the abundance of 28 microorganism sequences associated with microalgae *C. vulgaris* [155].

Fluorescence in situ hybridization (FISH) is another selective microscopic technique or molecular cytogenetic technique used for monitoring microalgae. For instance, EUB-338 I, II, and III probes tagged with fluorescein isothiocyanate (FITC) were used to analyze the interaction of *Azospirillum brasilense* and *C. sorokiniana* while probe Abras 1420 labelled with fluorochrome Cy3 probes detected bacteria present in the *C. vulgaris* that include *A. brasilense* [156]. With reference to FISH, molecular identification techniques like ITS (Internal Transcribed spacer) and 18S ribosomal DNA are used for detecting the *Chlorella* species [157]. Further, it involves DNA isolation, PCR amplification and sequencing, that enhance the accurate identification of *Chlorella* strains through genetic markers. A study by Zulkarnain et al [158] highlighted the growth analysis, astaxanthin production and molecular identification of *C. sorokiniana* using ITS and 18S rDNA. Further, next-generation sequencing technologies have significantly enhanced the study of microalgae through various methods like detection and quantification [159]. The term NGS is also known as a high-throughput sequencing technology that aids in the rapid and precise identification of microalgal cells [160].

Moreover, Gene expression analysis, such as RNA-seq, monitors gene expression profiles and identifies different growth phases of microalgae [161]. This enables valuable insight into microalgal composition, metabolic pathways, and gene expression patterns, facilitating the monitoring and optimization of microalgal culture [162]. Using metagenomic analysis, Zhou and his team [163] conducted a study to evaluate the stability of the endogenous *Chlorella* genes 18S, GTP, IDH, CYP, IDH, UBC, GAPDH, and α-TUB. As a consequence of this experimental analysis, GAPDH and α-TUB were identified as reference genes that exhibited instability in terms of expression fluctuation and inconsistency throughout the treatment procedure. Metagenomics, a closely related NGS technology, provides a genetic analysis of a microalgal sample that is directly recovered from the environment [164]. This technique is used to identify and characterize the microbial communities and to detect and quantify the potential contaminants. The tool also predominantly investigates the functional group of microbial communities within microalgal culture. This technique is invaluable, for assessing the diversity as well as the abundance of contaminants in the culture. This also plays a vital role in the early detection, identification and monitoring of contaminants.

Further, Microarrays or DNA chips are another powerful tool for studying multiple expressions of microalgal genes simultaneously [165]. This method mainly involves binding known microalgal DNA sequences on the solid surfaces, followed by microalgal culture DNA hybridization. Only limited experiments are conducted on microarray technologies for detecting and identifying algal species. Some examples include *Alexandrium* sp [166]*.*, *Dinophysis* sp [167]*, Heterocapsa* sp [168]*., Karenia* sp.[169]*, Micromonas* sp.[170]*, Prochlorococcus* [171]*, Protocentrum* sp [172]*.*, and *Synechococcus* [173][174][175].

The study of molecular diagnosis has become an essential tool for monitoring microalgae growth, detecting and identifying species, and analyzing their inhibitory activities in culture systems. Methods such as PCR, qPCR, NGS, FISH, and microarrays are widely employed to examine microalgal cultures and their activities in various environments. Additionally, advancements in metagenomic analysis enable the timely detection, quantification, and identification of cultured microalgal cells

5. Techno-economic challenge and successful demonstration of *Chlorella* treatment system in wastewater

Among various microalgal genera, *Chlorella* holds a unique ability to withstand organic and inorganic pollutants from wastewater. However, the wide adoption of the *Chlorella* system is hindered by certain drawbacks, particularly in terms of techno-economic challenges in both industrial and commercial applications. The term “Techno-economic challenge” (TEC) confers the difficulties and obstacles faced in the aspects of technological and economic processes or systems. In the context of wastewater treatment using the microalgal genus *Chlorella*, numerous hurdles exist, not only in the technological aspects but also in the economic feasibility and sustainability. Some techno-economic challenges in the wastewater treatment process are as follows

- Process design and optimization in wastewater treatment is crucial for ensuring effective mixing, gas exchange, and light penetration in the removal of both organic and inorganic pollutants. Both photobioreactors and open pond cultivation systems face unique challenges related to cost, scalability, and maintenance. For example, while photobioreactors provide precise control over growth conditions, they present significant techno-economic challenges due to their high capital costs and energy requirements. On the other hand, open pond systems are highly cost-effective and scalable, but they struggle with maintaining optimal growth conditions. These systems are also vulnerable to contamination and fluctuations in biomass productivity caused by environmental stress. Consequently, considerations of scalability, maintenance, and cost are essential for designing efficient and economically viable Chlorella-based wastewater treatment systems.

- Scaling up the cultivation of Chlorella from the laboratory to a large-scale operation presents significant challenges in maintaining optimal growth conditions, maximizing biomass productivity, and ensuring long-term reliability and adaptability. For example, Algae Systems, a US-based algae wastewater treatment company, has encountered substantial difficulties in scaling up Chlorella cultivation within their treatment process. While pilot and laboratory scales showed promising results, transitioning to a large-scale system has revealed major obstacles related to reactor design, nutrient supplementation strategies, and growth parameters [176]

5.1 Successful innovation in commercial wastewater treatment

Several innovative technologies have already successfully demonstrated the commercial use of algal systems to treat wastewater. For instance, Gross-Wen Technologies, based in Slater, Lowa, developed a revolving algal biofilm (RAB) treatment system for treating municipal and industrial wastewater. This treatment system removed nutrients (such as N and P) from wastewater, resulting in value-added algal biomass. This system further generated revenue to balance the system's operational costs [177]. Similarly, EcoDuna an Austrian company developed an innovative solution for scaling up large-scale cultivation systems utilizing vertical photobioreactor. These photobioreactors were designed in a way to maximize the utilization of light and minimize land use, making them more viable for treating wastewater [178]. Further, LGem a Dutch company primarily specializing in the development and production of microalgal cultivation systems holds a wide range of patented photobioreactor systems. This stood as a highly productive and cost-effective system for the treatment of wastewater. Their photobioreactor system was designed in a way to address techno-economic challenges. LGem technology pioneered a modular solution in wastewater treatment by delving into stable microalgal production, with low operational cost and high biomass productivity [179].

5.2 Challenges and Possible Solutions in Scaling up Microalgal Cultures to Meet Industry Standards

Engineering *Chlorella* for wastewater treatment on an industrial scale is quite challenging. This could be addressed by selecting proper algae strains with optimized growth conditions, in both monoculture and polyculture systems. The potential of the chlorella to thrive in industrial wastewater under specific conditions should have to be optimized before testing in an industrial setup. Some industrial effluents prefer photobioreactor-based closed systems to open ponds due to environmental variability and hence adaptability of algae cultures in different wastewaters across different design systems should be studied before implementation on industrial systems. This ensures maximized biomass productivity and minimizes the cost of production of *Chlorella*. Also, real-time monitoring and automation are vital to monitor key parameters that control the growth of algal cultures and ensure that the system runs in optimized process conditions. Optimizing the whole process in the pilot plant system before expansion to full-scale implementation determines the economic and technical feasibility of implementing chlorella-based wastewater treatment in specific industries. Moreover, industries may establish collaborative initiatives and partnerships among government and non-government organizations, industry, and academia to address techno-economic challenges effectively to adopt a *Chlorella*-based wastewater treatment system

6. Future prospects of *Chlorella* in wastewater treatment

The microalgal genus *Chlorella* offers an innovative and sustainable solution to global challenges, primarily in wastewater treatment. Over the past five years, numerous studies based on *Chlorella* have demonstrated their remarkable efficiency in removing organic and inorganic pollutants from wastewater. These studies also highlight "*Chlorella*" as a valuable asset in the wastewater treatment processes due to its versatility and resilience [180].

Looking ahead, ongoing advancements in biotechnology and bioprocess engineering can lead to substantial development in enhancing the overall performance of *Chlorella* effectively by addressing environmental impacts in wastewater treatment. Emerging biotechnological techniques such as CRISPR-Cas 9, TALEN, ZFN, CRISPRi and CRISPRa play a crucial role in modifying undesirable traits or metabolic pathways through the knocking in and out method. These techniques can aid in improving *Chlorella's* biomass productivity, nutrient intake, and ability to withstand environmental stress. Additionally, synthetic biology is vital in designing, constructing, and redesigning biological systems, allowing for the engineering of metabolic pathways to enhance their effectiveness in large-scale wastewater bioremediation processes.

Technological development in upstream and downstream processing can improve the efficiency and scalability of *Chlorella*-based wastewater treatment systems. For instance, combining Chlorella cultivation with anaerobic digestion effectively removes organic and inorganic contaminants, improving degradation efficiency and resource utilization. This approach to sustainable wastewater management techniques is accredited by the world government and regulatory agencies, which provide incentives and support to encourage using microalgae-based treatment technologies at the industrial and commercial levels. Such initiatives help promote the implementation of environmentally friendly wastewater treatment solutions, contributing to the advancement and resource conservation of Chlorella.

In developing countries like India and China, water pollution is a major concern due to the huge industrial revolution and overpopulation. Considering these factors, the governments of India and China have initiated various efforts to promote sustainable wastewater management using microalgae-based treatment solutions [181]. They initiated different pilot-scale projects to treat both industrial and domestic wastewater using microalgae [182]. To fully realize the benefits of microalgal wastewater treatment, governments need to implement additional initiatives. These measures will promote the commercial use of Chlorella in both upstream and downstream processes. Furthermore, offering incentives to start-up companies can encourage them to engage in large-scale wastewater treatment, thereby facilitating the widespread adoption of microalgal-based solutions. The Australian government's Commonwealth Scientific and Industrial Research Organisation (CSIRO) and the Australian Renewable Energy Agency (ARENA) are some good examples of statutory authorities that promote algal environmental research. These initiatives firmly focused on improving wastewater treatment processes to address water quality issues and mitigating the environmental impacts, particularly in the region where the conventional treatment system is inadequate or non-existent. These enterprises led by the government contributed to improving scientific research in algal water treatment technology and solving the greatest challenges in achieving a cleaner and healthier environment. Technologies such as IoT sensors, remote sensing, and machine learning algorithms also play a vital role in monitoring and identifying *Chlorella* growth and wastewater treatment for a sustainable future. In addition, these real-time monitoring strategies help to improve system reliability and prolong the lifespan of algal treatment.

More studies on *Chlorella* consortia utilizing bacteria, fungi, and microalgae have been reported in wastewater treatment. Nevertheless, most research articles fall short of addressing the *Chlorella*-yeast co-culture system for the bioremediation of wastewater. Indeed, it is found that some yeasts like *Saccharomyces cerevisiae, candida* sp*, Schizosaccharomyces pombe, Pichia pastoris* etc, help degrade organic and inorganic pollutants effectively when co-cultured with *Chlorella*. As a result, understanding the critical influence of *Chlorella-y*east cultures in the remediation of industrial and municipal effluents is significant.

In summary, consortium systems of *Chlorella* – algae, *Chlorella*- bacteria, *Chlorella*- fungi in wastewater treatment systems have pioneered a promising future in the circular economy by offering scalability, resource recovery, and environmental sustainability. It is reported that the global *Chlorella* market is expected to reach $506.99 million by 2030, with a CAGR of 8.5%, and contributes significantly to the transition of resource-efficient economy [183].

7. Conclusion

This review predominantly highlights the microalgal genus *Chlorella* as a versatile and efficient candidate for removing pollutants from industrial and domestic effluents, particularly through single and co-culture systems. With strong photosynthetic ability and wide diversity, live *Chlorella* cells demonstrate exceptional competence in removing a wide range of pollutants. The studies discussed in this review illustrate the remarkable effectiveness of *Chlorella* in achieving significant reductions in the level of COD, TN, TP, NH_3-N, and other contaminants from different industrial and domestic wastewater sources. Incorporating Chlorella into the wastewater treatment process highlights the removal of pollutants while also contributing to ecological balance and sustainable solutions.

Studies on the Chlorella-bacteria and Chlorella-fungi consortium systems reveal new possibilities for treatment processes by utilizing the symbiotic interactions between Chlorella and other microorganisms, such as nitrogen-fixing bacteria or heterotrophic fungi. Additionally, this consortium system offers benefits in terms of pollutant degradation efficiency and biomass productivity. The selection of compatible species and the optimization of co-culture conditions are crucial for maximizing treatment efficiency. The microalgal genus Chlorella and its associated consortium systems demonstrate significant mechanisms, including biodegradation, bioaccumulation, and biosorption, which effectively degrade contaminants from industrial, municipal, and agricultural effluents. These mechanisms involve the metabolic and enzymatic breakdown of pollutants, active accumulation within the cells, and the binding of contaminants to the cell surface through functional groups and extracellular polymeric substances.

This review highlights the vital role of molecular diagnostic tools in monitoring the growth, detection, identification, and inhibitory activities of microalgae in culture systems. Techniques such as PCR, qPCR, FISH, NGS, and microarrays offer high sensitivity and rapid analysis of microalgal cultures and their interactions in various wastewaters. These methods provide significant advantages over traditional techniques like manual cell counting, dry cell weight measurements, optical density assessments, and flow cytometry. Therefore, future research should also focus on developing various molecular probes to monitor different microalgal species effectively.

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Figure 3. Bioaccumulation activity of BDE 47 and BDE 209 in *Chlorella* sp.,.Reproduced with permission from (Lv et al., 2020).

Figure 4. Removal Mechanism of Emerging Contaminants (ECs) using microalgae. Reproduced with permission from (Gondi et al., 2022).

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