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A comprehensive review on hydrothermal liquefaction screening using microalgae in sewage water treatment and biofuel production

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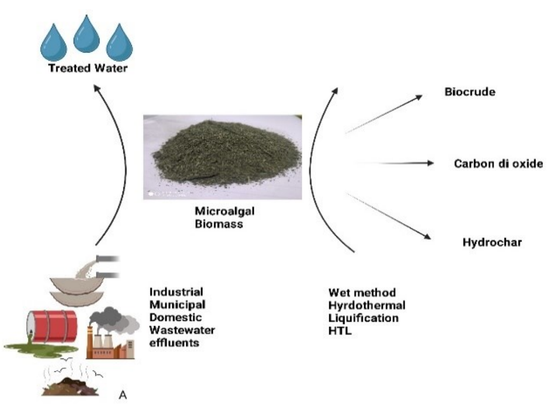
Graphical abstract



Abstract

Hydrothermal liquefaction (HTL) is involved in concurrently treating both sewage water and generating biofuels. It involves the analysis of various operating parameters, such as pressure, catalyst temperature, and reaction time, to determine the productivity and physiochemical properties of the resulting bio-oil. The critical assessment of over 100 algal strains, compared on the basis of their growth kinetics in different wastewater environments and their HTL-based yields, identified *Auxenochlorella pyrenoidosa* and *Microchaete* spirulina strains as the most suitable ones for optimised municipal wastewater treatment. Additionally, other strains like *Chlorella sorokiniana, Tetradesmus obliquus,* and *Desmodesmus* abundance are found to be effective for heavy metal remediation in municipal wastewater due to their high biosorption capacities. Furthermore, certain microalgal strains, namely *Auxenochlorella pyrenoidosa, Botryococcus braunii, Microchloropsis gaditana,* and *Microchaete spirulina*, were described as promising microalgae in the production of high crude oil yields through the HTL technique. The current review highlights the integrated approach of sustainable and economical HTL techniques using the best microalgal strains as a technologically and environmentally feasible solution selected through a systematic protocol for sewage water treatment and biofuel production.

Keywords: Biofuel; hydrothermal liquefaction; integration; microalgae; sewage water treatment

1. Introduction

The current circular economic principles demand not only to restrict pollution limits before wastewater release and reuse, but also focuses on the conventional wastewater treatment (WWTPs), enabling selected wastewater reuse, savings, and cost-effective processes implementation in wastewater recycling (Capodaglio, 2020; Mathews and Tan, 2016). Conventional wastewater treatment increases energy use, greenhouse gas emissions, recyclable resource waste, and solid landfilling. Alternatively, industrial, agricultural, and home-based effluents wastewater treatment systems that are comprised of low-carbon origin and high-resource recycling, encouraging biorefinery system and circular economy principles are in demand. The utilization of microalgal bioremediation in wastewater treatment has the potential to facilitate the growth of microalgae and effectively mitigate pollutants. According to a recent, study 1 kilogram of microalgal biomass necessitates the utilization of 1.83 kilograms of carbon dioxide with the potential to facilitate carbon dioxide sequestration and wastewater treatment (Shahid *et al*. 2020).

After the process of microalgal bioremediation of wastewater, it has been observed that the predominant component of the resulting sludge is microalgae. The activated sludge process typically necessitates an energy consumption of 1 kilowatt-hour per kilogram of biochemical oxygen demand (BOD) to provide oxygen. Cultivating microalgae in wastewater does not necessitate the presence of oxygen, as anaerobic digestion can generate 1 kilowatt-hour per kilogram of biochemical oxygen demand. Various energy recovery options for microalgal biomass encompass gasification, pyrolysis and biochar production, fermentation with hydrothermal liquefaction (HTL) as one of the technologies to generate bioethanol, bio-butanol, and biological crude (Shahid *et al*. 2020; Quintero-Dallos *et al*. 2019). The strains of microalgae grow swiftly and can be cultivated year-round and microalgae, particularly C. vulgaris strains, were reported to cultivate on vinasse medium and to have a higher oil yield and production of valuable metabolites. They are a potential source of bioenergy due to their higher growth rates than terrestrial biomass, their ability to reduce carbon dioxide (CO) while growing in solid and liquid media, and the presence of energy-rich compounds, such as lipids, as one of their cellular contents (Mishra *et al*. 2024; Huang *et al*. 2022). Cultivation processes of microalgae have also been advantageous because they can grow well on wastewater and can utilize NH_4^+, NO_3^- or PO_4^{3-} from the wastewater as their nutrient sources (Cavalcanti *et al*. 2024). However, if these nutrients are to be provided artificially, then the cost of nutrients is very high. The yield of microalgal bio-oil is around ten times greater than the yield and productivity of terrestrial biomass (Liu *et al*. 2024). Some drawbacks related to microalgal yield & productivity are its cultivation and harvesting. The cultivation and harvesting processes for microalgae biofuel production are too expensive and time-consuming. Generally, microalgae cultivation in a closed photobioreactor process is more expensive than an open raceway pond at a pilot scale. However, in open raceway ponds, cultivation limitations are contaminations with undesirable species; variations in light and temperature conditions; CO_2 loss; etc. (Fawcett *et al*. 2024). Harvesting or post-cultivation microalgae recovery from open raceway ponds is also one of the challenging tasks. 0.02–0.06% solids (dilute suspension), less than 25 mm microbial size, and negative charge microbial surface are the major factors that make harvesting difficult (Singh and Patidar =, 2018). Moreover, microbial concentrations in open raceway ponds after cultivation range from 0.1 to 2 gm/L (Pahl *et al*. 2013). Due to this, dewatering this diluted microbial suspension is a challenging task. There are various methods developed through which microalgae can be harvested to 10 to 45 gm/L concentration (Barros *et al*. 2015; Subhash *et al*. 2022). Microalgal growth in closed and open bioreactor is affected by various culture and operating parameters like pH, light intensity, salinity, temperature, nutrient concentration, CO_2 and dissolved oxygen (Sing *et al*. 2013).

More light intensity in the growth medium causes the death of microalgae due to degradation in chlorophyll-II and causes photo inhibition (Hossain and Mahlia, 2019). In addition, microalgae also excrete various inhibitors or metabolites like dissolved organic matter (DOM), which is the main reason for negative growth (Lu *et al*. 2019). Therefore, these culture parameters should be optimized to improve biomass productivity. The slow growth rate further requires to be optimized in terms of light, carbon fixation and high surface area during its growth phase (Patil *et al*. 2008; Chen *et al*. 2016). Biofuel from microalgae is referred to as 3rd generation biofuels produced from biomass, which is neither a food material nor cultivated in agricultural areas (Singh *et al*. 2011; Vlaskin *et al*. 2018). Microalgae are potential biomass to remediate various types of wastewaters (Subhash *et al*. 2022). Microalgae cultivation offers a prominent solution to treat secondary and tertiary stages of wastewater treatments due to its potential to utilize inorganic phosphorus and nitrogen for their growth and multiplication (Sawayama *et al*. 1998). Microalgae have also been found to be the potential biomass to remediate heavy metals and some toxic contaminants or organic compounds from wastewater (Ajiboye *et al*. 2021; Burakov *et al*. 2018). Moreover, along with a tertiary bio-treatment of wastewater, they produced various valuable bioproducts that can be further utilized in several applications (Yang *et al*. 2022). For sustainable production of 3rd generation biofuel, studies related to cultivation methods of microalgae and biofuel production from microalgae are focused. Both these processes need to be optimized to make the production of 3rd generation biofuel economic and sustainable. However, selecting microalgae strains appears to be the main task for increasing biomass productivity and lipid content (Hu *et al*. 2016; Neofotis *et al*. 2016; Taleb *et al*. 2016). The current study aims to screen suitable microalgae for large-scale integration of sewage water treatment and hydrothermal liquefaction process under optimized conditions.

2. The Necessity of pretreatment of wastewater

Wastewater may contain elevated concentrations of various compounds that can hinder the growth of microorganisms, such as microalgae (Yang *et al*. 2022). Similarly, the cloudiness and acidity level (pH) of wastewater can also impede the growth of microalgae (Hu *et al*. 2016). Treating wastewater with physicochemical processes can create more favorable conditions for microalgal growth and reduce the strength of the wastewater (Neofotis *et al*. 2016; Taleb *et al*. 2016). While ammonium is the preferred nitrogen source for microalgae, some strains can tolerate ammonium levels as high as 1000 μmol NH_4-N L^{-1} (Singh *et al*., 2011). To facilitate microalgal growth, pretreatment methods are necessary to lower the ammonium concentration in wastewater or dilute it to a level where the chosen strain can thrive efficiently (Chen *et al.*, 2016)

In various types of wastewaters, including those from metal, mining, paper, oil, and grease industries, electrocoagulation has been employed as a pretreatment step. This method effectively removes different chemical additives, cloudiness, and pathogens (Bharte and Desai, 2021). Cultivating microalgal biomass in pretreated wastewater can further reduce remaining nutrients and cloudiness in the treated water. Treating wastewater with a low nitrogen-to-organic carbon ratio (C/N) presents challenges. In such cases, organic supplements are often added to improve the efficiency of bacterial nutrient removal as an energy source. In contrast, microalgae can harness sunlight, soluble inorganic carbon dioxide, nitrogen, and other nutrients to increase their cell numbers while treating wastewater. The cellular nitrogen content of microalgae can vary from 3% to 10%, depending on the strain (Fasaei *et al*. 2018). Microalgal and cyanobacterial strains can assimilate various forms of nitrogen, both inorganic (such as ammonium, nitrate, nitrite, atmospheric nitrogen) and organic (like urea, glycine, etc.), although the efficiency may differ among strains and growth conditions.

From a cost perspective, using microalgae to remove phosphorus from wastewater may be a preferable option compared to chemical precipitation and engineered wetland-based phosphorus removal methods (Hoffman *et al*. 2017). Additionally, microalgae can selectively consume nitro and amino groups from different aromatic compounds (e.g., aminonaphthalenes and nitrobenzonates) as a nitrogen source, thereby reducing the toxicity of the original pollutants (Hoffman *et al*. 2017). High concentrations of heavy metals in wastewater can inhibit microalgal photosynthesis (Slegers *et al*. 2013). Nevertheless, microalgae can efficiently accumulate metal pollutants both internally and externally and can be utilized for the removal of metals from wastewater.

3. Microalgal harvesting system

Separation of microalgae from treated water in the open raceway pond is critical. Several methods are used for separating microalgae from the treated water. However, the process adopted for harvesting depends on the strain of microalgae, the application of algal biomass and the final desirable product (Bharte and Desai, 2021; Fasaei *et al*. 2018; Slegers *et al*. 2013). Various chemical, physical, and biological methods are used to collect microalgal biomass from treated water, including sedimentation, flocculation, filtration, flotation and centrifugation (Slegers *et al*. 2013). A combination of these two or more processes is used to increase the harvesting efficiency (Singh and Patidar, 2018). From the literature, it was found that a sedimentation time of 10 minutes was the optimum time to recover over 90% of *chlorella vulgaris* from the culture medium (Zhu *et al*. 2018). Microalgae strains are big enough for the sedimentation process, like *Scenedesmus* and *Chlorella* sp., to sediment easily from the growth medium without an agitator.

Harvesting of *Chlorella vulgaris* was compared using natural flocculant (chitosan) and traditional flocculant (aluminium sulfate). Both chitosan and aluminium sulfate recovered more than 90% microalgal biomass ((Zhu *et al*. 2018). Five different strains of microalgae (*Chlorella vulgaris*, *Choricystis minor*, *Neochloris* sp., *Cylindrotheca fusiformis* and Nannochloropsis salina) were harvested by flocculation by using ferric chloride and aluminium sulfate as flocculants (Chatsungnoen and Chisti, 2016). Aluminium sulphate and Polyaluminium chloride were used to harvest Microchloropsis gaditana with a settling time of 15 minutes (Şirin *et al*. 2013). Ecotan and Tanfloc, as the natural flocculants, were used to harvest microalgae from wastewater treatment ponds and found a settling time of 6- 10 minutes (Gutiérrez *et al*. 2015).

Filtration of microalgae is a physical process which achieves 100% microalgal cell recovery (Drexler and Yeh, 2014). Filtration requires a semipermeable membrane that can retain microalgal cells and allow the water to pass through them (Hattab, 2015). However, filtration creates fouling and clogging, requiring a change in the filter or semipermeable membrane, increasing its processing cost (Milledge and Heaven, 2013). Polyacrylonitrile-based filter membrane was designed with different properties and applied for eight microalgae species. This membrane can harvest microalgae based on properties like shape, size, presence of cell wall, etc (Marbelia *et al*. 2016).

Table 1. Comparison of microalgae harvesting processes

Process	Advantages	Disadvantages	References
Sedimentation	The process is easy and Low cost	Slow process; Less Recovery; Applicable for large-size cells	(Pittman *et al*. 2011)
Flocculation	Low cost; No Equipment Demand	Require particular flocculant; Recovery of flocculant is difficult	(Chatsungnoen and Chisti, 2016)
Filtration	High Recovery; Low Energy Demand	Discontinuous; Clogging and fouling;	(Drexler and Yeh, 2014; bilad *et al*. 2012)
Flotation	Quick harvesting;	High capital cost; Require flotation tank	(Wiley *et al*. 2009)
Centrifugation	Continuous Process; 100 % Recovery; Efficient for the large scale	Applicable only for small-scale harvesting; Require centrifuge chamber; High capital cost	(Barros *et al*. 2015)

Flotation attaches microalgae cells with tiny bubbles to promote the floating of microalgae on the top of the culture media in the floatation tank for easy and quick harvesting (Ndikubwimana *et al*. 2016; Laamanen *et al*. 2016). However, generating micro-size bubbles (10–100μm) is energy-intensive (Wiley *et al*. 2009; Rubio *et al*. 2002). Centrifugation harvests microalgae from the water based on density, particle size and shape using centrifugal force (Sim *et al*. 1988; Rawat *et al*. 2013). This process is time-consuming and requires a particular centrifuge chamber to harvest microalgal cells, which is also energy intensive (Dassey and Theegala, 2013; Soomro *et al*. 2016). A Comparison of these microalgae harvesting processes is given in Table 1. From the comparison mentioned earlier, shown in Table 4 for microalgae harvesting, the flocculation process is the only process that does not require any equipment, and the energy demand is also less compared to other harvesting processes. Moreover, the flocculant can be reused further for flocculation after recovery from treated water. Therefore, flocculation is the most effective process for microalgae separation due to its quick harvesting with low cost, reusable and without equipment demand.

4. Microalgae as potential biomass for energy production

Microalgae have long been considered a potential biomass applicable to produce various value-added energy products like biodiesel/bio-oil, bioethanol, and biogas (Campbell *et al*. 2011; Borowitzka, 2013; Shimizu, 2003; Posten and Schaub, 2009). Apart from on production of value-added products from microalgae and its high growth rates, it can also grow in wastewaters (industrial, municipal and agriculture waste waters), and it can efficiently remove their primary nutrients like phosphorus, carbon, nitrogen, micro-pollutants & heavy metals (Boelee *et al*. 2011; Cong Nguyen *et al*. 2020; Kesaano and Sims, 2014). The production of renewable products can be integrated with wastewater treatment, CO_2 mitigation, medicinal value products, bio-fertilizer, bioplastics, and animal food (Tong *et al*. 2014; Gallezot, 2012; Muller-Feuga, 2000; Demirbaş, 2001). From these renewable products, biodiesel is one of the most common products produced by the transesterification process of algal lipids (Zhu and Ketola, 2012; Gonçalves *et al*. 2013; Davis *et al*. 2011). Gouveia and Oliveira selected six different strains of microalgae which include *C. vulgaris, Nannochloropsis sp., Spirulina maxima, S. obliquus, Neochloris oleoabundans* and *Dunaliella tertiolecta* for biodiesel production. Among these strains, Nannochloropsis sp. (marine microalgae) and N. oleoabundans (freshwater microalgae) were found to be more suitable biomass for the production of biofuel with a high oil content of 29.0% and 28.7% (Gouveia L. and Oliveira, 2009). However, biodiesel production through algal lipids needs microalgae drying, which is cost-ineffective and time-consuming (McKendry, 2002). Other strains as *Nannochloropsis spp*., were reported to produce the enzymes for commercial preparations to improve lipid recovery, influencing the efficiency of the treatment mentioned above. So, the alternative to biodiesel is bio-oil which can be produced by hydrothermal liquefaction of w*et al*gal biomass (Elliott, 2016; Brennan and Owende, 2010). A key benefit of hydrothermal liquefaction over conventional methods of dry biomass is that hydrothermal liquefaction can process wet biomass of 5-20% solids without involving energy consuming drying step. (Zuorro *et al*. 2015; Vardon *et al*. 2012; Peterson *et al*. 2008). Minowa *et al*. investigated bio-oil production using *D. tertiolecta* by thermochemical liquefaction. They concluded that liquefaction could also contribute to the bioenergy production system and its potential to mitigate global warming (Minowa *et al*. 1995). Bio-oil production through hydrothermal liquefaction of different microalgae strains is given in Table 1.

Moreover, the separation of bio-oil from water is relatively easy, making hydrothermal liquefaction an attractive alternative for transforming energy from wet biomass, including microalgae (Pienkos and Darzins, 2009; Rahpeyma and Raheb, 2019). Presently, bioethanol is the most widely used biofuel, primarily produced from corn and sugarcane sugars. However, the technology is shifting towards using algal carbohydrates as potential raw materials for bioethanol production (Bothast and Schlicher, 2005; Basso *et al*. 2011; Goldemberg, 2007). Global bioethanol production has seen a significant surge, increasing from 1 billion to 39 billion liters in just a few years, and is projected to reach 100 billion liters in the near future (Licht, 2006). Microalgae are rich in various carbohydrates, including glycogen, starch, agar, and cellulose, which can be readily converted into fermentable sugars for bioethanol production (Ueda *et al*. 1996; Horn *et al*. 2000). Although bioethanol production from microalgae represents a promising step towards sustainable biofuels, there are still challenges related to scaling up production and commercialization of this clean biofuel. Key areas in the development of algal bioethanol technology that require optimization for commercialization include the selection of suitable algal biomass, pretreatment processes, and efficient fermentation methods. Increasing both the biomass and carbohydrate productivity of algal cells is essential for economically viable bioethanol production (Pulzand Gross, 2004; Usher *et al*. 2014). Certain carbohydrate-rich microalgae, such as *Chlamydomonas reinhardtii* and *Chlorella vulgaris*, are being considered for techno-economic analysis (TEA) in the context of bioethanol production (Mahlia *et al*. 2011). TEA assesses the feasibility of commercial bioethanol production from microalgae by evaluating factors like total investment, overall cost, and net profit (Arora *et al*. 2016; Yang *et al*. 2018).

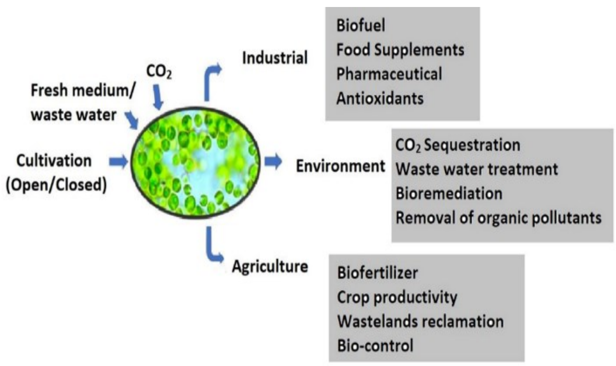


Figure 1. Applications of microalgae in industry, environment, and agriculture

The applications of microalgae are summarized in Figure 1.

5. An integrated approach

In recent times, microalgae have evolved beyond being utilized solely as single-cell proteins, and they are now envisioned as living-cell factories for the treatment of sewage, heavy metal removal and production of biofuels and a diverse range of beneficial biochemicals used in industries such as food, aquaculture, poultry, and pharmaceuticals.

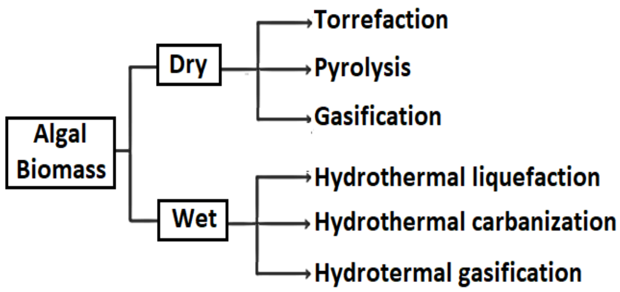


Figure 2. Block diagram on the two different routes for Biofuel production from algal biomass (Dry and Wet)

5.1. Bio-oil production from microalgae

Over the last few decades, work has been motivated by optimizing microalgae cultivation methods and processing microalgae for biofuels or energy production. Microalgae have rapid growth potential, and most microalgae strains have bio-oil content in the range of 20-60% dry weight of biomass (Sun *et al*. 2019). The growth phase of microalgae has a double rate of 3.5 h, and catalytic processes have shown their contribution through HTL and pyrolysis (Chisti, 2007; Zuorro *et al*. 2020; Spolaore *et al*. 2006). Biofuel from microalgae can be produced in wet or dry forms. The processes of biofuel production from microalgae in dry and wet form are represented in Figure 2.

For the sustainable production of biofuel, the balance between cost efficiency and drying efficiency should be made to optimize the net energy output of biofuels (Li *et al*. 2008). Biofuel production from dry algal biomass includes Torrefaction, pyrolysis and gasification. The pyrolysis of microalgae is a promising technique that converts dry algal biomass into bio-oil at a considerable temperature range of 400-700°C under inert conditions. Different chemicals are present in bio-oil extracted through the pyrolytic process, including hydrocarbons, alkanes, phenol derivatives, aromatics, ketones, esters, ethers, etc (Bridgwater, 2012). Moreover, the thermal breakdown of microalgae during the pyrolysis reaction degrades carbohydrates, lipids and proteins present in algal biomass. The characteristics of the pyrolysis reaction of microalgae in each component have been examined through TGA (thermogravimetric analysis) (Chen *et al*. 2018). During the pyrolysis reaction of microalgae, gas is produced due to the thermal degradation and decomposition of components in algal biomass. The composition of the pyrolysis gas was identified using FTIR (Fourier transform infrared spectroscopy), composed of CO_2, H_2, CO and CH_4 (Li, 2017). However, the extracted bio-oil exhibits disadvantages like low thermal and chemical stability, high water and oxygen concentration, and strong acidity that limits the bioenergy applications of the algal pyrolysis process.

Torrefaction is a thermochemical technique generally performed with dry biomass for solid fuel production. However, wet Torrefaction or hydrothermal Torrefaction is another technique which takes place at a temperature range of 180–260°C in an inert environment (Gan *et al*. 2018). Bach *et al*. performed wet Torrefaction of *Chlorella vulgaris* at different temperatures (160-180°C) and time duration (5-30 min), resulting in low solid yield (Bach *et al*. 2017). Moreover, cleaning the flue gases after Torrefaction and feedstock sensitivity are some challenges faced during Torrefaction.

The cost of the processes for drying microalgae is an important parameter that must be considered during the production of biofuels from algal biomass (McKendry, 2002). Algae having a more significant mass fraction of water (80-90%) (Patil *et al*. 2008), cannot economically undergo any thermochemical processes like gasification and pyrolysis (Amin, 2009; Guo *et al*. 2015. In such cases, more energy and time are required to dry the microalgal biomass, making the process unsustainable (Amin, 2009; Guo *et al*. 2015; Jena and Das, 2011).

Thermochemical conversion of wet microalgal biomass, such as hydrothermal liquefaction, hydrothermal gasification and hydrothermal carbonization, were the most suitable for biofuel production (Elliott, 2016; Vasudevan and Briggs, 2008). In these processes, whole microalgae in wet form are pyrolyzed and converted into bio-oil under hot compressed water (Han *et al*. 2019). The bio-oil is obtained as the final product along with aqueous (water-soluble hydrocarbon), gaseous and solid (biochar) as by-products (Jena and das, 2011; Vasudevan and Briggs, 2008; Han *et al*. 2019; Tian *et al*. 2014). Hydrothermal carbonization (HTC) of microalgae has received much attention over the past five years. HTC is a process in which mild temperature (about 200C), pressure (<2MPa) and time (<1h) conditions are employed to convert microalgae into a solid residue called hydrochar along with water-soluble products (López Barreiro *et al*. 2013). Experiments on several microalgae strains like *Chlamydomonas reinhardtii, Synechocystis sp., Aphanizomenon flosaquae, Spirulina sp., Chlorella sp.* and *Dunaliella salina* at different temperatures and reaction times (190-213°C and 0.5-3 h) were performed (Heilmann *et al*. 2010). The authors concluded that a mild temperature range of around 200ºC and a reaction time of around 30 minutes are substantial for appropriate carbonization of the algal biomass. However, the low yield of hydrochar and its high nitrogen content awaits research in the field of HTC. In addition, the aqueous phase products obtained as HTC by-products have high chemical oxygen demand (COD) and thus require further treatment (Rodriguez *et al*. 2021).

Hydrothermal liquefaction is a microalgae-to-bio-oil conversion process carried out in wet form at temperatures of 280-370℃ and pressures of 10-25MPa (Toor *et al*. 2011; Xu *et al*. 2020). Several publications were found related to Hydrothermal liquefaction of microalgae. The research findings of hydrothermal liquefaction report that it is challenging due to the different Strains of microalgae used, the reaction conditions, initial pressure, solvent, use of purging gas and catalysts used. A comparison of microalgae hydrothermal liquefaction experimental review, microalgae strain used, bio-oil yield reported, and experimental parameters are represented in Table 2.

Table 2. Review of comparison on microalgal strains and bio-oil productivity through hydrothermal liquefaction

Microalgae Strain	Temperature (°C)	Holding time (min)	Pressure (MPa)	Catalyst	Bio-Crude Oil Yield* (Wt. %)	References
Auxenochlorella pyrenoidosa	280	120	-	-	39.4	(Yu *et al*. 2011)
Auxenochlorella pyrenoidosa	280	120	-	-	39.4	(Yu *et al*. 2011)
Auxenochlorella pyrenoidosa	280	120	0.69	-	57.3	(Zhang *et al*. 2014)
Auxenochlorella pyrenoidosa	240	30	1.03	Raney-Ni; HZSM-5	72	(Zhang *et al*. 2014)
Auxenochlorella pyrenoidosa	240	30	1.03	-	70.8	(Zhang *et al*. 2014)
Botryococcus braunii	300	60	3	Na_2CO_3 (5%)	64	(Dote *et al*. 1994)
Botryococcus braunii	310	15	-	-	>60	(Ren *et al*. 2018)
Coelastrum	350	3	15	-	41.7	(Jazrawi *et al*. 2013)
Coelastrum	280	30	-	Porous Silica	32.5	(Xu *et al*. 2020)
Coelastrum sp	360	120	20	-	30	(Prapaiwatcharapan *et al*. 2015)
Dunaliella tertiolecta	300	60	3	-	42	(Minowa *et al*. 1995)
Dunaliella tertiolecta	360	50	-	Na_2CO_3	25.8	(Zou *et al*. 2009)
Microchaete spirulina	350	60	5	$Fe(CO)_5$-S	66.9	(Matsui *et al*. 1997)
Microchaete spirulina platensis	350	60	-	-	41	(Jena and Das, 2011)
Nannochloropsis	300	30	8	Ni/TiO_2	48.23	(Wang *et al*. 2018)
Microchloropsis gaditana	350	15	-	-	54,8	(Barreiro *et al*. 2015)
Nannochloropsis Sp.	350	60	-	-	43	(Brown *et al*. 2010)
Nannochloropsis Sp.	350	60	0.06	-	39	(Valdez *et al*. 2011)
Scenedesmus sp.	350	30	-	-	36	(Wądrzyk *et al*. 2018)

Hydrothermal conditions such as pressure, temperature, time and catalyst can significantly affect the yield and characteristics of crude oil. Table 2 summarizes the yields of crude oil reported in the literature at different conditions of the hydrothermal liquefaction process using different strains of microalgae. Temperature 240-280°C, reaction pressure 5 MPa, and 30-60 minutes were favourable conditions for hydrothermal liquefaction of microalgae (Zhang *et al*. 2021; Kumar and Strezov, 2021). Catalysts can improve the yield of crude oil and help in the reduction of oxygen and nitrogen contents in bio-oil and consequently increase its heating value (Sharma *et al*. 2019). Table 2 also summarizes different microalgae strains, which are considered potential sources for crude oil production through hydrothermal liquefaction. From Table 1, the most suitable temperature, time and operating pressure for the hydrothermal liquefaction process of microalgae were found to be 240°C, 30 minutes and 1 MPa. Whereas *Auxenochlorella pyrenoidosa, Botryococcus braunii, Microchloropsis gaditana* and *Microchaete spirulina* were identified as having high crude oil yield even without the involvement of catalyst as they have high lipid content.

5.2. Microalgae for sewage-water treatment

The Microalgae water treatment system is an environmentally friendly biotechnological process. Microalgae have a fast growth rate biological source that can grow well in wastewater containing nutrients by absorbing organic nutrients, carbonaceous phosphorus and nitrogenous material (Liu *et al*. 2013; Renuka *et al*. 2015; Slocombe *et al*. 2020). All kinds of wastewater can be used for the commercial production of microalgae, including municipal, agricultural, paper, refinery, and other industrial effluents, along with varying efficiencies in treatment performance and microalgae growth. The various microalgae strains possess immense potential in removing nitrogen, phosphorus, and heavy metals from industrial wastewater through biosorption and adsorption processes at the surface of algal cells using industrial wastewater as their growth medium (Chinnasamy *et al*. 2010; Cai *et al*. 2013; Ji *et al*. 2013; Han *et al*. 2019). Microalgae are not yet used on a large scale in wastewater treatment. However, notable examples of commercial systems exist. Microalgae shows better growth in municipal wastewater after settling of sludge because of the presence of higher nutrients in sewage water (Tam and Wong, 1989; Mohsenpour *et al*. 2021; Lakshmi *et al*. 2015). Municipal wastewater after the primary treatment unit (sedimentation tank) can be used to cultivate microalgae on a large scale to produce 3rd generation biofuels (Cho *et al*. 2011; Yang *et al*. 2011). The critical factors that need to be studied for the screening of a microalgae strain for wastewater treatment are microalgae growth rate, environmental conditions, nutrient source, the ease of harvesting from water, doubling time and level of nutrient reduction. The screening of microalgal strains for the biological removal of these materials from wastewater effluents has been investigated by several studies. Microalgae *Chlorella sp.* has proven the capability of reducing phosphorus, nitrogen and COD with different retention times ranging from 10 h to 42 days (Tam and Wong, 1989).

In the context of wastewater treatment using microalgae, studies primarily focus on removing nitrogen, phosphorous and organic contaminants. A symbiotic relationship between microalgae and bacteria occurs in wastewater treatment systems. Thus, co-culturing microalgae with heterotrophic microorganisms enhances wastewater treatment efficiency and increases biomass productivity. A mixed consortium of microalgae and bacteria was used in a study to treat municipal wastewater in photo-sequencing batch reactors (Foladori *et al*. 2018). The authors concluded that the hydraulic retention time (HRT) for pollutant removal was reduced considerably, achieving high removal efficiencies for COD and TKN (Total Kjeldahl Nitrogen). COD of sewage water is measured for testing water quality which is calculated not only to determine the concentration of biologically active compounds like bacteria but also to determine the biologically inactive organic substances in water. The treated municipal wastewater can be reused in waterbodies to mitigate the freshwater shortage. Boelee *et al*. investigated that microalgae-based biofilms are also used for simultaneously removing N and P from municipal wastewater (Boelee *et al*. 2011). Gai *et al*. studied that the presence of iron and magnesium ions in wastewater can enhance phosphorous removal efficiency by microalgae (Mao *et al*. 2021). In a recent study, *Chlorella vulgaris* was cultivated in a 50 L open tank containing sewage water for bioremediation as well as bioenergy generation (Pooja *et al*. 2022). The results showed that nitrates, COD, and BOD (Biochemical Oxygen Demand) were reduced to 93%, 95% and 92%, respectively. The treated sewage water was utilized as a biofertilizer to grow tomato plants. Wu *et al*. studied the importance of light given, exploring the removal effects using an algae-bacteria system grown in municipal wastewater (Cheng *et al*. 2022). The results revealed that illumination of 60 $\mu mol\ m^{-2}s^{-1}$ removed more than 90% of the insecticide Imidacloprid and about 82.3% of nitrogen. A similar study was conducted to check the influence of solar irradiance on municipal wastewater treatment by microalgae-bacteria agglomerates in an 80 L outdoor pond (Arcila and Buitron, 2017). The authors concluded that low irradiance levels, i.e., less than 3800Wh $m^{-2}d^{-1}$, promoted high removal efficiencies for total nitrogen (60 ± 5%), COD (89 ± 3%) and phosphates (28 ± 7%).

The removal performances of microalgae-based treatment systems are influenced by environmental (nutrient concentration, pH, temperature, N:P ratio, illumination, etc.) and operational factors (initial biomass concentration, HRT, mixing, type of reactor, etc.) (Wang *et al*. 2017). Thus, control over these technological parameters provides the most reliable results. Biosorption, biodegradation and bioaccumulation are the three mechanisms involved in environmental remediation using microalgae (Abdelfattah *et al*. 2023). Different forms of nitrogen, such as NH_4^+, NO_3^-, urea, etc., are directly synthesized to form amino acids and proteins. In the same way, phosphates are converted to ATP and phospholipids (Huang *et al*. 2023). The applicability of microalgae for bioremediation of nutrients from wastewater has been extensively analyzed in laboratories. *Chlorella* and *Scenedesmus* species members are widely studied due to their dominance in freshwater ecosystems (Whitton). However, cyanobacteria, diatoms and other Chlorophyceae species are not widely investigated for wastewater treatment application and await further research. Conventional algae-based wastewater systems must be integrated with more advanced technologies for commercial sustainability. Hence, the multifaceted role of microalgae provides green solutions to implement a sustainable economy. The comparison of different microalgal strains for the reduction of nitrogen, Phosphorus and COD based on literature is represented in Table 3. From the abovementioned comparison of different microalgae strains, *Chlorella* and *spirulina* were the most effective for municipal wastewater treatment as they caused maximum percentage reduction of nitrogen, phosphorus and COD, respectively.

Table 3. Comparison of physical factors and their reduction in using different microalgal strains for bioremediation of municipal wastewater

Microalgae Species	% Reduction			References
	Nitrogen	Phosphorus	COD	
Auxenochlorella pyrenoidosa	93.9	80	-	(Tam and wong, 1989)
C. sorokiniana	71	72.8	46	(Chen *et al*. 2017)
C. sorokiniana	62	47	-	(Covarrubias *et al*. 2012)
Chlorella sp.	89.1	80.9	90.8	(Li *et al*. 2011)
Chlorella sp.	93	86	24.8	(Licht, 2006)
Chlorella sp., Scenedesmus sp.	92.94	82.85	85.44	(Kim *et al*. 2014)
Chlorella vulgaris	84.81	36.12	82.3	(Choi and Lee, 2012)
Chlorella vulgaris	-	-	97	(Shen *et al*. 2017)
Chlorella vulgaris	-	94.1	76.3	(Chaudhary *et al*. 2018)
Microchaete spirulina platensis	85.5	91	98.7	(Li *et al*. 2018)
Tetradesmus obliquus	-	91.3	75.9	(Chaudhary *et al*. 2018)

5.3. Heavy metal remediation

Due to anthropogenetic activities, the concentration of various heavy metals has significantly increased in the environment over the past years. This increase in the concentration has led to increased exposure to heavy metals which finally leads to an increase in heavy metal-associated disorders. The heavy metals which are most problematic include copper (Cu), chromium (Cr), Cadmium (Cd), magnesium (Mn), zinc (Zn), iron (Fe), lead (Pb) and mercury (Hg). These heavy metals are toxic in low or trace concentrations (Javanbakht *et al*. 2014). Wastewater produced from industries like agriculture, mining, battery manufacturing etc., is often polluted due to containing vast quantities of heavy metals. Various conventional adsorbents synthesized, including activated carbons, clays and zeolites, and various nanostructures were found to be effective for reducing these heavy metals from wastewater (Burakov *et al*. 2018). Moreover, various biosorbents like plant material, fungi and microalgae were also used to remove these heavy metals from industrial and municipal wastewater (Ajiboye *et al*. 2021).

Table 4. Microalagal strains in the removal of heavy metals from various waste wasters through HTL processing technology (Goswami *et al*. 2022)

Microalgae Strains	Heavy metals	Type of wastewater	% of removal after HTL
Nanochloropsis	Iron, Aluminium	Municipal and wastewater with high salt concentration	95
Nannochloropsis oculata	Copper	Mines wastewater	99.92 _ 0.04%
Pavlova lutheri	Iron, Aluminium	Municipal and wastewater with high salt concentration	95
Tetraselmis chuii	Iron, Aluminium	Municipal and wastewater with high salt concentration	95
Chaetoceros muelleri	Iron, Aluminium	Municipal and wastewater with high salt concentration	95
Scenedesmus incrassatulus	Chromium, Copper, Cadmium	Artificial wastewater	25–78%
Scenedesmus sp.	Zinc and Iron	Acid mine wastewater	Zinc: 84.14% Iron 65.76%
Chlorella vulgaris	Copper and molybdenum	Metal mine tailings wastewater	Cu: 64.7%, Mo: 99.9%
Scenedesmus spinosus	Copper and molybdenum		

The concentration of the heavy metals in wastewater of Bindal pul, Dehradun, Uttarakhand is Pb 0.88 mg/l, Cu 0.45 mg/l, Zn 0.83 mg/l, Ni 0.94 mg/l, Cd 0.13 mg/l and Cr 0.58 mg/l). They found the maximum bioaccumulation of heavy metals of Cu (36.75 ± 6.19 mg/kg), Pb (196.91 ± 8.13 mg/kg), Ni (125.48 ± 5.97 mg/kg), Zn (305.54 ± 14.30 mg/kg), Cr (93.06 ± 3.25 mg/kg) and Cd (29.58 ± 4.26 mg/kg), in agricultural wastewater by using *Beta vulgaris, Spinacea oleracea*, *Brassica oleracea* and *Phaseolus vulgaris* (Chopra and Pathak, 2015). Both microalgae and macroalgae have the potential of biosorbents for heavy metals from wastewater (Zhu and Hiltunen, 2018; Brinza *et al*. 2007; Li *et al*. 2019). Microalgae, *Parachlorella*, Kessler were inoculated as a biosorbent for heavy metals reduction from synthetic wastewater. The biosorption efficiency of *Parachlorella kessleri* for lead (II) was 99.54% in 9 days, and remediate of each heavy metal was in the order of Pb(II) > Co(II) > Cu(II) > Cd(II) > Cr(II) (Sultana *et al*. 2020). Microalgae, F. vesiculosus, was found to remediate 70.1 ± 1.9 nickel, 143.2 ± 7.5 cadmium and 516.3 ± 12.5 lead (mg/g), respectively (V.R.M *et al*. 2019). Similarly, Khajavian etal. observed the bioremediation ability of brown algaeCystoseria indicant by remediating 55.34mg/g cadmium and 18.17mg/g Nickel (Khajavian *et al*. 2019). Anbaena spharicawas also reported to remediate 121.95mg/g lead and 111.1mg/g Cadmium using fresh water aqueous medium (Abdel-aty *et al*. 2013).

Chlorella sorokiniana was found to be a strong microalga which can tolerate high concentrations of heavy metals from wastewater even in different ranges of temperature, pH and other environmental conditions (Izadpanahb *et al*. 2018; AKHTAR, 2004; Yoshida *et al*. 2006). Similarly, *Chlorella sorokiniana* successfully tolerated upto 250µM Cadmium from the wastewater, the most carcinogenic and mutagenic heavy metal in municipal wastewater (León-Vaz *et al*. 2021; Tchounwou *et al*. 2012).

Microalgae, *Scenedesmus* and *Chlorella*species were reported to be the most used microalgae for the reduction of heavy metals. These species were found to have high biosorption capabilities (Chugh *et al*. 2022). The microalgae genus*Chlorella* is a single-celled and spherical microalgae with a diameter of 2-10µm. Moreover, *Chlorella* is also currently the most cultivated microalgae strain worldwide due to its fast growth rate and high photosynthetic efficiency with substantial nutritional value (Masojídek and Torzillo, 2008). Chlorella cells contain 70% protein (dry biomass), which is also very valuable biomass in the food industry (Liu and Hu, 2013). *Chlorella vulgaris* is the most reported strain of *Chlorella* species with high heavy metal reduction.

Microalgae genus *Scenedesmus* was also found to be the most commonly freshwater-based microalgae which are commonly used for various applications such as wastewater treatment and oil production (Pignolet *et al*. 2013). A very dense cell wall in the *Scenedesmus* species makes it more digestion resistant.

Microalgae consortia with other bacteria like cyanobacterial species*, Clostridium needles* and *Chlamydomonassalina* were prepared for the reduction of arsenic heavy metal. This was adsorbed by the cell wall of microalgae consortia with bacteria through various functional groups like carbonyl, hydroxyl and thiol present on the surface of their cell wall (Hussain *et al*. 2021). The maximum remediation rate of arsenic was reported by Gao *et al*., where consortia of *Aspergillusoryzaeand Chlorella vulgaris* biomass at pH 7, 140 rpm with five g/L concentration of glucose (Gao *et al*. 2020). Bodin *et al*. also reported the remediation of Cadmium through the bio pellets synthesis from microalgae *Chlorella vulgaris* and fungi*Aspergillus niger*and found to be more in consortia from 40% to 56% when it is compared to*Chlorella vulgaris* alone (Bodin *et al*. 2017).

Microalgae, *Desmodesmus abundans* in living and nonliving form act as a biosorbent for the remediation of Cadmium and copper from water (Terry and Stone, 2002). Moreover, the other microalgal species like *Chlamydomonas reinhardtii, Microchaete spirulina platensis, Auxenochlorella pyrenoidosa, Planothidium lanceolatum, Pleurococcus miniatus,* efficiently remediate the heavy metals from wastewater (Macfile and Welbourn, 2000; Sandau *et al*. 1996; Yan and Pan, 2002; Sbini, 2012; Lau *et al*. 1999, Malik *et al*. 2023).

6. Conclusion

Integrating a 3rd generation biofuel production and sewage water treatment system using microalgae must be optimized to make it technically and economically more feasible (Sanchez-Galvis *et al*. 2020; rangel_Basto *et al*. 2018; Malik *et al*. 2023). A detailed study is required to select appropriate strains from the abundant varieties of algal species and their wastewater treatment efficiency in a large-scale open system, along with their potential to produce biofuel. More than 100 microalgae strains were identified and compared for integrating sewage wastewater and HTL. Microalgae, *Auxenochlorella pyrenoidosa*, *Botryococcus braunii*, *Microchloropsis gaditana* and *Microchaete spirulina* can produce high crude oil yield even without a catalyst. From these strains, *Auxenochlorella pyrenoidosa* and *Microchaete spirulina* sp. were also identified and used for municipal wastewater. Other microalgal strains like *Chlorella sorokiniana, Tetradesmus obliquus* and *Desmodesmus abundans* were identified and used for heavy metals remediation from municipal wastewater. These microalgae can also be the source for bio-oil production through hydrothermal liquefaction, as only a little literature work has been identified related to these strains. Harvesting processes by self-flocculation and sedimentation were most suitable for economically separating microalgal biomass from water. Natural fiber-Chitosan and Chemical flocculant- Aluminum Sulfate are identified as suitable reagents for feasible harvesting of microalgal biomass.

7. Future perspectives

Microalgae can remove various pollutants from effluent and several novel technologies has been implemented in harvesting of microalgal cells. They can efficiently assimilate and metabolize organic compounds, nitrogen, phosphorus, and other nutrients present in wastewater. They also could purify heavy metal-contaminated water. Toxic contaminants, including Cadmium, lead, mercury, and chromium, threaten human and environmental health. In addition, because of their high lipid content, they are used to produce bio-oil that can be used in neat or blended form as an alternative to conventional fuels. Moreover, the aqueous phase hydrocarbon obtained as a by-product of HTL can be the source of microalgae cultivation. So, significant contributions are made by microalgae to wastewater remediation, heavy metal removal, and bio-oil production. Their adaptability, nutrient-removal capabilities, heavy metal-binding properties, and lipid-rich composition make them a promising tool for addressing environmental issues and investigating sustainable energy alternatives.

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Data Availability Statement

Data will be made available on request.

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Abbreviations

ATP- Adenosine Triphosphate

BOD-Biological Oxygen Demand

COD- Chemical Oxygen Demand

HTC- Hydrothermal carbonization

HTL - Hydrothermal Liquefaction

N- Nitrogen

P-Phosphorus

Conflicts of Interest

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

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