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A review on co-cultivation of microalgae with filamentous fungi: Efficient harvesting, wastewater treatment and biofuel production

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ABSTRACT

As the third generation biofuel feedstock to confront with energy crisis, microalgae have great potential for the exploration of renewable energy fields, whereas the high cost related to biomass production and harvesting is the main bottleneck to hinder the applications on a large scale. To mitigate the environmental impacts in a sustainable mode, co-culturing filamentous fungi with targeted microalgae is a superior method to efficiently accumulate and harvest the total biomass. This paper serves as a base to review current advances in pelletization of microalgae with fungi for the co-cultivation process. The pellet formation is initially introduced, and then electrostatic interactions, hydrophobic interactions and specific components on cell walls as the main harvesting mechanisms are explored and generalized together with the inclusion of critical affecting parameters for efficiency promotion. Apart from the discussion about biomass harvesting, the latest studies of this co-cultivated technology on wastewater treatment in diverse types associated with corresponding removal mechanisms are analyzed as well. Subsequently, this article emphasizes the effects of fungal-algal cultivation on downstream processing for biofuel production, followed by the practical bioenergy conversion performances. Based on the policies support, the implications of this novel co-cultivation technology have shown the potential in further development. Meanwhile, the current challenges and future perspectives about harvesting on a large scale, removal of multiple pollutants and exploration of integrated biorefinery are pointed out systematically.

1. Introduction

Although human society develops rapidly in the 21st century, the excessive exploitation of fossil fuels has caused severe problems, such as energy crisis, environmental pollution and climate change. Therefore, finding a type of renewable energy with less ecological threats is urgent to meet the increasing demand. Biofuels, compared with commonly used fossil fuels, have tremendous advantages in lowering overall greenhouse gas emissions, advancing energy security, and saving energy export [1–3]. Traditional biofuels are mainly produced by economical crops, which usually need huge quantity of fresh water sources to obtain unbalanced oil yield. As the third generation biofuel feedstock, oleaginous microalgae have attracted great attention, due to their higher lipid contents, vast suitability and relatively short growth period [4]. In

addition, the bio-product acquisition can be combined with pollution control, because microalgae can survive in harsh conditions and utilize available nutrients in wastewater to accumulate biomass. Hence, the exploration of new energy fields through scaling up microalgae-based technologies is required. The key processes involved in practical applications are cultivation, harvesting, pretreatment and conversion of biomass into advanced biofuels [5]. However, the economic feasibility and sustainability of commercial production from microalgal biomass on a large scale are still limited. The undesirable properties, including growth in the diluted suspension, small size (<30 μm in diameter) and electrostatic repulsion between cells, make the separation of microalgae from media challengeable [6]. It is reported that harvesting even accounts for 20–30% of the overall cost in biomass production [7,8], becoming the major bottleneck to hinder microalgal commercialization.

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At present, microalgal biomass is recovered by physical, electrical, chemical and biological methods. Each harvesting technology has its typical pros and cons, and thus the selection of appropriate methods is crucial during the process [9]. Physical methods which consist of centrifugation, filtration and flotation can achieve high efficiencies, but the operational cost is far beyond the estimation. Though gravity sedimentation can greatly save energy during operation, the time consumption and species-specific feature limit its wide application. Negatively charged algae can also be concentrated by the electrical method, whereas the establishment of electric field requires massive capital expenditure. Chemical method that is involved with the utilization of organic and inorganic flocculants is able to aggregate microalgal cells in a short time, but the complicated reagents may result in biomass contamination and the decrease of biofuel values [10]. In order to ensure the high quality of biomass production, the current trend is to seek natural, chemical-free and efficient materials from biological origin to harvest microalgae. Culturing ubiquitous microorganisms, such as self-flocculating algae, fungi, bacteria and yeasts, to gather target algal strains through bioflocculation seems to be a comparatively optimal approach [11].

Filamentous fungi have ability to immobilize microalgae through mycelial interactions, so it is a novel way to co-culture algae with filamentous fungi for efficient harvesting [12]. Under the specific cultivation conditions, fungi and algae cells can form spherical morphology with many advantages, such as a large surface area, high mechanical stability and improved mass transfer rate to accumulate biomass. More importantly, the cell pellets can be well separated from the culture broth by sieve because of their relatively large size (>1 mm in average diameter), which also results in decreasing operational costs of microalgal harvesting process [13,14]. In general, there are two main co-cultivation modes for harvesting: fungal spore-assisted or pellet-assisted method. Fungal spore-assisted harvesting, as the name implies, is the process of co-culturing fungal spores with microalgae to form aggregations, while another mode is through adding pre-cultured fungal pellets directly [15]. In comparison with pellet-assisted method, inoculating fresh spores with algae can accumulate more biomass, but it usually consumes more time and organic carbon sources to finish the harvesting process. A comparative study on these two methods was carried out by Chen et al. [16], and the result showed that fungal spore-assisted method can harvest 99% algae cells within 28 h, while the highest flocculation efficiency (98.26%) was achieved in 2.5 h by fungal pellet-assisted method with less glucose input. In addition, co-cultivation requires nutrients, such as nitrogen, phosphorus and carbon dioxide, to satisfy the photosynthesis of the microalgae as well as heterotrophic metabolism of fungi [17,18]. Fungi secrete extracellular enzymes which can convert solid organic matters into soluble nutrients and carbon dioxide, making it easier for microalgae cells to be assimilated [19]. In turn, autotrophic microalgae release oxygen to promote fungal respiration. Therefore, the application of co-cultivation to simultaneously remove nutrients from the wastewater is proved to be a highly feasible technology, due to the establishment of synergistic metabolisms. The *A. fumigatus* and *Thraustochytrid* co-cultivation system could effectively assimilate $\text{NH}_4^+\text{-N}$ (86%) and $\text{PO}_4^{3-}\text{-P}$ (69%) after 48 h incubation in diluted swine wastewaters [20]. Compared with mono-system, fungi-assisted microalgae co-cultivation system could remove more nutrients from molasses wastewater with TN, TP and chemical oxygen demand (COD) removal efficiency of 67.09%, 88.39% and 70.67%, respectively [19]. Moreover, the utilization of wastewater as a co-culturing medium for biomass production with pollution control has a scale-up potential in a sustainable mode; meanwhile, the cost of wastewater treatment can partially offset the investment by breaking through technological barriers in exploring bioenergy fields.

The co-cultivation system of microalgae and filamentous fungi has been widely studied in biomass harvesting and wastewater treatment, as well the subsequent biofuel production. However, there is no critical review article to comprehensively and systematically discuss this novel

technology. Table 1 has summarized the advantages and current limitations about fungal-algal pelletization in practical applications. Meanwhile, as shown in Fig. 1, the sustainable development mode of co-cultivation of algae with fungi is presented for better visualization. With the aim of further improvement of the efficiency in biomass harvesting, the first concern is the mutual interactions between targeted algae and fungi. In consideration of fragmented information available, this review intends to reveal the electrostatic interactions, hydrophobic interactions and specific components on cell walls as the main harvesting mechanisms in the co-cultivation system, while the affecting factors such as strains, nutrient, inoculation ratio, pH and agitation are also analyzed in detail. Furthermore, successful application examples of co-cultivation of microalgae and fungi for wastewater treatment from diverse types as well as its removal efficiencies with corresponding mechanisms are discussed. In addition, the main aim of microalgae harvesting by a specific co-cultivation mode is to obtain the biomass for biofuel production, so the subsequent effects on downstream processing of biomass associated with the performances on bioenergy conversion are also critically discussed. Based on the policies support, the practical implications about fungal-algal cultivation are overviewed at the end of the paper. More importantly, the key challenges with future perspectives about further harvesting on a large scale, removal of multiple pollutants and exploration of integrated biorefinery are pointed out systematically in an effort to facilitate the sustainable development in a co-cultivation mode.

2. Co-cultivation mode for efficient harvesting

The primary aim of culturing microalgae with filamentous fungi is to harvest biomass more effectively, so the key point should concentrate on actual application process. In general, the co-cultivation for efficient harvesting depends on the establishment of symbiotic system between fungi and algae. Specifically, as exhibited in Fig. 2, the analysis of fungal-algal symbiosis for aggregation contains the mutually interactive mechanisms as well as the critical harvesting parameters with relevant efficiencies. Therefore, for further development of this novel method,

Table 1

Advantages and current limitations about co-pelletization of microalgae with filamentous fungi.

Advantages	Limitations
● Relatively low surface-to-volume ratio and accelerated mass transfer rate	● The species-specific properties of targeted microorganisms are complicated and suitable strains need to be identified in detail
● Establishment of symbiosis system to better exchange gas (CO_2 and O_2), utilize available nutrients and accumulate more biomass	● Adverse effects on total biomass accumulation may occur due to the competitive interaction between fungi and algae
● High mechanical stability with high cell loading	● Limited transport of nutrients into the interior of large pellets, causing differences in metabolic activities
● Improvement of harvesting efficiency, chemical-free flocculation and convenient separation biomass from culture medium	● The high retention time for co-pellet formation may affect the biochemical composition
● The co-cultivation performance on pollutant removal is better than treatment by algae or fungi	● The biomass quality is multi-factors dependent with uncertain results
● Partially promoting the quality of the total biomass for subsequent utilization (i.e., bioenergy, pharmaceuticals and cosmetics)	● The cost of maintaining cultivation conditions (i.e., mechanical agitation, pH and nutrient balance) limit further scaling up
● Combined fungal-algal biorefinery with multiple applications	● Undesired contamination in biomass with possible interference in downstream processing
● Pollution control, biofuel production and cost saving can be achieved through co-cultivation in wastewater environment	● Most of the co-pelletization applications are still at a laboratory level

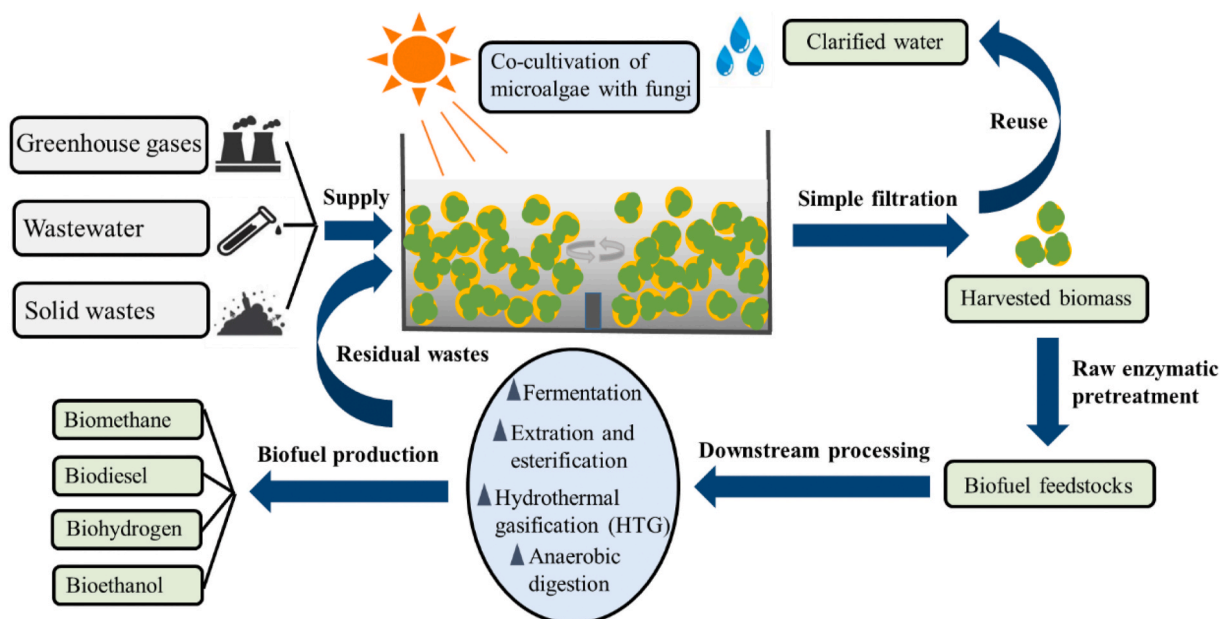


Fig. 1. The sustainable development mode on the co-cultivation of microalgae with filamentous fungi.

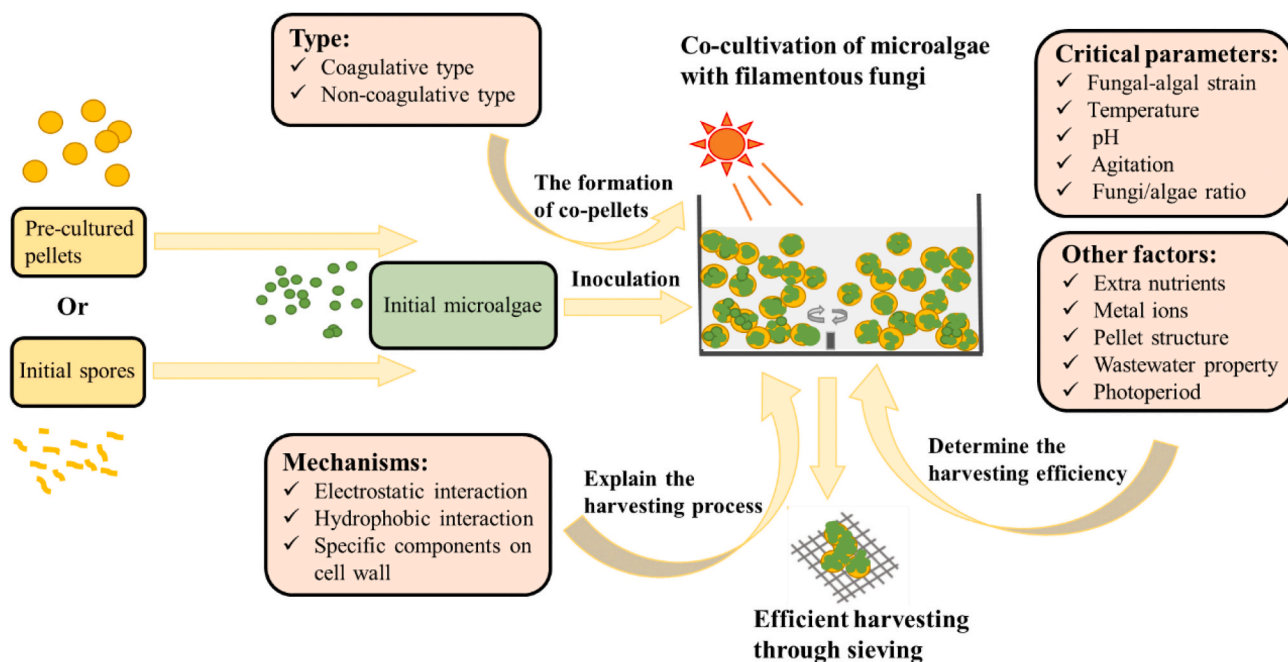


Fig. 2. Co-cultivation mode for efficient harvesting.

this part will comprehensively summarize the corresponding process in detail.

2.1. Fungal-algal pellet formation

Depending on various cultivation objectives, the morphological characteristics of specific filamentous fungi can vary from homogeneously dispersed mycelia to compact pellets of aggregated biomass [21]. Free filaments usually result in mycelial entanglement and medium viscosity increase, which in turn limit mixing and mass transfer. In contrast, the morphology of pellet has relatively lower surface to volume ratio, and thus the mass exchange rate of nutrients and oxygen are improved spontaneously [22]. Moreover, due to the high metabolic

property and cross-linking structure, pelletized form can self-immobilize well to efficiently entrap other microorganisms, forming a consistent dimension for subsequent utilization [23,24]. Based on the novel insights, many studies have combined filamentous fungi with microalgal cultivation to obtain fungal-algal pellets with the aim of efficient harvesting and valuable biomass recovery [13,25,26]. In general, there are two modes to achieve goals: Addition of pre-cultured fungal pellets into algal culture broth, or inoculation of fungal spores with algal cells to form pellets. No matter which co-pelletized method is taken, the practical results can be persuasive for further development, but the details may quite different. Therefore, in the present review, two modes are illustrated and analyzed.

As for the precultured pellet mode, the preliminary step is about how

to possess spherical morphology from filamentous fungi. Traditionally, the formation of fungal pellet is divided into coagulative and non-coagulative type [27,28]. In the first type, spores aggregate and germinate to grow hyphae, finally forming pellets [29]. *Aspergillus oryzae* and *Aspergillus niger* from genus *Aspergillus* and *Phanerochaete chrysosporium* are the typical fungi, and the pellet formation belongs to the first mechanism [30]. But for the second type, spores germinate firstly to form spherical pellets. This pellet formation mechanism has been reported for fungi belonging to *Penicillium* sp., *Rhizopus* sp. And *Mucor* sp [14,31]. Then, the pre-cultured pellets with uniform diameter are added into algal broth under the optimal conditions and agitated in an orbital shaker, finally obtaining harvested biomass from fungal-algal aggregations.

As for spore inoculation mode, fresh spores with certain initial concentration are inoculated to culture broth with microalgae under controlled conditions, and thus actually the pelletization and harvesting is a simultaneous and continuous process. Interestingly, the explanation of fungal-algal pellet formation can also be classified as the aforementioned types. In the coagulative pelletization, germinating spores may cluster together with suspended microalgal cells, partially because of specific spore-to-algae interaction [32]. The non-coagulative type can be explained by the fact that microalgal cells react with an individual germinating spore firstly, and then further hyphae might grow with each other to form grains. The co-pelletization can be considered as a way of co-cultivation, where fungi and algae use mutual metabolites to accumulate biomass in a symbiotic system [14]. However, the mechanisms about how fungal spores or pellets contribute to the harvesting of algal cells are still lack of a definitive conclusion.

2.2. Harvesting mechanisms

In this part of investigation, specific flocculation mechanisms have been explored to provide a unique understanding for the promotion of developing in prospective application. With the addition of fungal spores or pellets into the algae suspension under the control of conditions, algae cells are embedded in the fungal hyphae, finally intertwining with the mycelium to accomplish immobilization. Electrostatic interactions, hydrophobic interactions and interactions related to specific components on cell walls are the main mechanisms to explain the formation of fungal-algal pellets.

2.2.1. Electrostatic interaction

According to the previous studies, the surface of algal cells presented electron-donor properties favor the existence of negatively charged functional groups, including carboxylic (-COOH), phosphoryl (-POH) and amino (-NH₂) groups from lipoproteins, phospholipids and lipopolysaccharides, and SO₃ groups from sulfur clusters [33,34]. Depending on the pH value in the culture system, these ionizable groups can be protonated and deprotonated to create charges and potentials on the surfaces of algal cells. Mostly, the alkaline pH of microalgal culturing condition makes surface groups deprotonate (e.g., -COO⁻ and -PO⁻) to be negatively charged [35], and then the net negative charges on cell walls induce electrostatic repulsion between algal cells, keeping them suspended in water rather than aggregation. Due to the convenience about estimating the mobility of charged particles in an electric field, the zeta potential is an efficient indicator to measure the degree of electric force. Zheng et al. [36] measured the Zeta potential of *Chlorella vulgaris* and obtained a range from -10 mV to -35 mV. When the Zeta potential is below -20 mV, electrostatic repulsion between algal cells is relatively strong and the system is quite stable [35]. However, during the growth and metabolism period, filamentous fungi are known to secrete diverse organic acids (e.g., citric acid, gluconic acid and acetic acid) into the culture medium [37,38]. Once the fungal spores are inoculated to algal suspension for harvesting, the fluctuation about ambient pH may alter the charged properties of cell walls for a while to induce electrostatic interaction. As to the pellet-assisted mode, since pH value in fungal

medium is acidic, the surface functional (carboxylic and amine) groups of mycelium remain protonated, leading to the net positive charges of the fungal hyphae [39]. Therefore, when positively charged fungi contact with negatively charged algal cells sufficiently, charge neutralization can fully display advantages to eliminate the Zeta potential. Miranda et al. [17] reported that *Aspergillus fumigatus* pellets could almost harvest 100% *Synechocystis* cells with strong negative charges (-33.1 mV), and the final Zeta potential of fungal-algal pellet was only -2.5 mV. Once the systematic Zeta potential is near to zero, the repulsive cells can approach with each other by the attraction of charge, hydrogen bonding and van der Waals forces [40], subsequently occurring co-pelletization. But if surface charges are unevenly neutralized under the optimal conditions, electrostatic patch will be involved with the process, which means the opposite charged segments bind to microalgal cells and connect with each other by the attraction of charged patches [41]. Furthermore, the increase in the concentration of ambient electrolytes in culture broth can reduce the electric potential of cell walls, probably assisting fungi to harvest algal cells [42]. In other words, the electrostatic interactions are complicated, and more works should be focused on specific interactions between algae and fungus.

2.2.2. Hydrophobic interaction

As one of the determinants during pellet formation, hydrophobic interactions also play a vital role in adhering the surface of microalgal cells [43]. Through adding 1% of cetyltrimethyl ammonium bromide (CTAB, v/v) and four *Aspergillus niger* hsn26 pellets with the identical size into the 10 mL of culture medium (4.8×10^{10} cells/mL), Li et al. [44] investigated the effect of hydrophobicity on microalgae *Chlorella vulgaris* harvesting. After treatment, the results suggested that harvesting efficiency was 3.69 times higher than the control group with the only addition of equivalent pellets in 6 h. When CTAB exposes positively charged polar heads to connect with negatively charged algal cells, the hydrophobic tails in CTAB can strengthen the hydrophobic interactions with fungal mycelium to enhance the flocculation activity. Moreover, in a study of exploring the mechanism of microalgae *Nannochloropsis oceanica* aggregation, hydrophobic beads are proposed as the potential flocculant to replace bacteria for algal harvesting process [45]. Actually, the hydrophobicity of filamentous fungi is generated by surface active proteins, which are known as hydrophobins. The hydrophobins are too small to contain 100-150 amino acids with low molecular weight (≈ 10 kDa) and only found in fungal species, so the reactions are confined to the range of fungal secretion [46]. Considering the main functions in adhesion, these active proteins are capable of promoting surface coating, assembling hydrophilic-hydrophobic interfaces and mediating the interactions with external environment [47,48]. According to the previous study, amphipathic property is the most important feature of hydrophobic proteins, which means that the hydrophobic and hydrophilic parts of the proteins can form firmly amphipathic film to help fungus adhere to other microbial surface [49]. Specifically, the self-assembled amphipathic film has ability to make hydrophobic surface of solid materials wet, while the hydrophilic surface can be changed into the hydrophobic one as well [50,51]. Based on this amphipathic property, the hydrophobins from filamentous fungi can be utilized to immobilize suspended algae cells on surfaces via adhesive force. Moreover, through the modified adherence-to-hydrocarbon test, Garg et al. [52] also quantified the hydrophobicity of microalgae, such as *Chlorella* sp. And *Tetraselmis* sp. The hydrophobic parts of microalgae can contact with filamentous fungi to initiate hydrophobic interactions; meanwhile, the amphipathic film from fungal hydrophobins may regulate the surface property of algae cells, subsequently making it easier to form co-pellets. However, compared with marine *Chlorella* sp., Zhang et al. [53] found that the freshwater *Chlorella* sp. Preferred to adhere the hydrophilic surface rather than the hydrophobic site. Perhaps the species-specific property is one of the main reasons, and thus more studies are needed to reveal the hydrophobic interactions between fungi and algae in a more specific manner.

2.2.3. Specific components on cell wall

Developed by Derjagin et al. the DLVO theory was applied as quantitative and qualitative models to explain microbial adhesion by considering the function of cell wall macromolecules, which has the ability to bridge the cell to another surface [54]. According to this classical theory, the interactions between the surfaces of cells are responsible for biofloculation behaviour. In general, the mycelium is uniquely composed of glucans, lipids, chitin, polysaccharides and proteins [55]. Together, these components on cell walls contribute to the interactions with external environment and adhesions to other microbial cells. Talukder et al. [56] found the positive relationship between the content of chitin and microalgal immobilization efficiency, because the strong charged chitin could function as a cationic flocculant to neutralize the negative charges on the surface of algal cells. Performing proteinase K digestion assay, Li et al. [44] investigated the critical function of proteins on fungal mycelium during the flocculation process. When proteinase K was added into microalgal suspension with fungal pellets, the structure of proteins on cell walls was destroyed and the flocculation efficiency was only 0.11 times in comparison with the control without any addition. Similarly, the amorphous polysaccharides on cell walls, usually bonded with proteins as glycoproteins, also play an important role in cementing other microbial cells by delivering positive charges and viscous substances [57]. In addition, when certain divalent ions exist in the solution, the components on fungal cell walls can be linked with microalgal cells to form aggregations through ions bridging. Li et al. [58] measured the calcium binding ability of *Streptomyces* sp. Pellets, and the final results suggested that the addition of 5 mM CaCl₂ displayed the highest flocculation efficiency, while it was almost impossible to harvest *Chlorella vulgaris* biomass without calcium addition. Besides, fungal cell walls can secrete extracellular polymeric substances (EPS) with strong adsorption capability, due to the existence of multiple biomolecules (glycoproteins, humic-like substances, lipids and nucleic acids) [59]. These above-mentioned complex mixtures might react with other EPS secreted by microalgal cells to induce flocculation process. However, few studies have evaluated the function of concrete secretions.

To summarize, these mechanisms suggest that the interactions between fungi and algae are multi-factors dependent and species-specific, and thus the following section summarizes the critical parameters involved in the process in order to further improve the efficiency of biomass harvesting.

2.3. Critical parameters & harvesting efficiency

The previous studies on two different harvesting modes are accordingly presented in Table 2 and Table 3, including species, experimental conditions and corresponding efficiencies. In spite of the discrepancy between fungal spores and pre-cultured pellets, we should first focus on common factors (fungal-algal strain, nutrient, fungi:algae ratio, temperature, pH and agitation), which might have diverse impacts on two modes, and then make a comparison between them. Afterwards, other factors (such as metal ions, dissolved oxygen, pellet structure, etc.), which also have special influences on two modes, are characterized as well.

2.3.1. Fungal-algal strain

The fungal-algal strain is the primary factor in this co-cultivation technology, since it determines the harvesting efficiency directly through the combination of different and species-specific microorganisms. Bhattacharya et al. [60] verified the significance in algal strain by using *Aspergillus fumigatus* AML01 to harvest different biomass, and the results showed that more than 90% of *Chlorella pyrenoidosa* cells were obtained within 1.5 h, whereas it took nearly 3.5 h to harvest 90% of *Chroococcus* sp. Cells under the same conditions. The explanation on the differences in practical efficiency is that some specific characteristics of microalgal cells are involved in the adhesion, such as shape, size, hydrophobicity and the biochemical composition of cell walls [61,62]. Moreover, since the targeted algae species have diverse applications, the select criteria for cultivation must include adaptation, growth rate, lipid content and biomass composition. In other words, the specific properties of algal strains cause the diversity in co-cultivation performances, resulting in different harvesting efficiencies.

More importantly, the strain-dependent feature about filamentous fungi has multiple influences on the co-pelletization process. From the beginning, the initial inoculation for pelletization, which further influences the concentration of spores, germination, growth and morphology development, is species-specific [21]. Based on the diverse applications of co-pellets, before fungal spores are added into algal culture broth for efficient harvesting, it is necessary to confirm the properties of targeted strains. Moreover, as the pre-cultured pellets, the surface activity, intracellular structure and a variety of secretions, which are involved in interactions with algal cells, are also strain-dependent. For the purpose of screening the optimal strain to harvest marine

Table 2
Efficiency of pellet-assisted harvesting in the literature.

Algal strain	Fungal strain	Pellet size (mm)	Experimental conditions	Time (h)	Efficiency (%)	References
<i>Chlorella vulgaris</i> UTEX 259	<i>Cunninghamella echinulata</i>	1–2	Optimum fungi:algae ratio of 1:2 at 25 °C with 120 rpm	48	99	[80]
<i>Chlorella</i> sp.	<i>Aspergillus niger</i>	ND	Optimum dosage at 30 mg L ⁻¹ (dry weight) with 125 rpm	72	99	[96]
<i>Scenedesmus quadricauda</i>	<i>Aspergillus fumigatus</i>	2–5	25 °C, 150 rpm	48	>95	[20]
<i>Nannochloropsis</i> sp.	<i>Aspergillus nomius</i>	ND	Optimum fungi:algae ratio of 4:1 at 23 °C, pH 6.0, air through a sparger at the flow rate of 1 L/min	3	97.2	[56]
<i>Chlorella protothecoides</i>	<i>Aspergillus fumigatus</i>	ND	25 °C, 150 rpm	24	80	[63]
<i>Chlorella</i> sp.	<i>Penicillium</i> sp.	3–5	34 °C, 160 rpm, pH 4.0 with the fungi:algae ratio of 1:2	2.5	98.26	[16]
<i>Chlorella</i> sp.	<i>Aspergillus niger</i>	6–7	Optimum dosage at 30 g/L (wet weight), 125 rpm, pH 5.0–6.0	72	>95	[85]
<i>Chlorella</i> sp. NCU C01	<i>Pleurotus ostreatus</i>	ND	28 °C, 100 rpm, pH 3.0–4.0	2.5	64.86	[97]
<i>Synechocystis</i> sp. PCC 6803	<i>Aspergillus oryzae</i>	4–5	30 °C, 130 rpm, pH 7.4 with the fungi:algae ratio of 1:4.26	48	90	[98]
<i>Scenedesmus</i> sp.	<i>Trichoderma reesei</i>	3–5	30 ± 2 °C, 100 rpm with the fungi:algae ratio of 1:2	0.5	>99	[99]
<i>Synechococcus subsalsus</i>	<i>Aspergillus niger</i>	2.5	28 °C, 100 rpm, pH 6.0 with fungi:algae ratio of 1:5	48	98	[71]
<i>Synechocystis</i> PCC 6803	<i>Aspergillus fumigatus</i>	ND	25 °C, 150 rpm	48	97	[17]
<i>Chlorella pyrenoidosa</i>	<i>Aspergillus fumigatus</i>	ND	38 °C, 100 rpm with fungi:algae ratio of 1:5	3	99	[39]
<i>Chlorella vulgaris</i>	<i>Aspergillus niger</i> hsn26	10	120 rpm, pH 8.0–9.0 with addition of 5 mM CaCl ₂ at room temperature	24	>90	[44]
<i>Chroococcus</i> sp.	<i>Aspergillus lentulus</i>	ND	30 °C, fungi:algae ratio of 1:3	6	>99	[100]

Note: “ND” means not determined size.

Fungi:algae ratio refers the dry weight of biomass ratio.

Table 3
Efficiency of spore-assisted harvesting in the literature.

Algal strain	Fungal strain	Pellet size (mm)	Nutrient	Experimental conditions	Time (h)	Efficiency (%)	References
<i>Chlorella vulgaris</i> UMN235	<i>Aspergillus oryzae</i>	2–4	BG-11 medium with 10 g/L glucose	150 rpm, pH 4.0–5.0 with inoculums 1.1×10^4 spores/mL	72	93	[64]
<i>Chroococcus</i> sp.	<i>Aspergillus lentulus</i> FJ172995	ND	BG-11 medium with 10 g/L glucose	25 °C, 150 rpm with inoculums 2×10^4 spores/mL in 1.58 g/L initial algal concentration	24	≈100	[32]
<i>Chlorella</i> sp.	<i>Penicillium</i> sp.	3–5	BG-11 medium with 5 g/L glucose	40 °C, 160 rpm with inoculums 1.1×10^4 spores/mL	28	99	[16]
<i>Scenedesmus obliquus</i> SIT06	<i>Cunninghamella echinulata</i> TPU 4652	1–3.5	BG-11 medium with 20 g/L glucose	30 °C, 120 rpm, pH 5.5 with inoculums 1×10^6 spores/mL	24	92.65	[101]
<i>Chlorella vulgaris</i>	<i>Aspergillus</i> sp.	ND	pretreated molasses wastewater	35 °C, 80 rpm with inoculation ratio of spores:algae is 1:100 (cell number)	4	>97	[19]
<i>Chlorella vulgaris</i>	<i>Aspergillus niger</i>	3–6	Chu-10 medium with 15 g/L glucose	150 rpm, pH 5.0–7.0 with inoculums 1×10^4 spores/mL	72	>95	[65]
<i>Chlorella vulgaris</i> UMN235	<i>Aspergillus</i> sp. UMN F01	2–5	BG-11 medium with 20 g/L glucose	30 ± 2 °C, 100 rpm, pH 5.4 with 1.2×10^5 spores/mL	60	≈100	[102]
<i>Botryococcus braunii</i>	<i>Aspergillus fumigatus</i>	ND	BG-11 medium	25 °C, 100 rpm with inoculation ratio of fungal:algal broth is 1:40 (volume)	12	98	[74]
<i>Chlorella vulgaris</i> UTEX 2714	<i>Aspergillus niger</i> Ted S-OSU	3–4	Culture broth with 2 g/L glucose	27 ± 2 °C, 150 rpm with inoculation ratio of spores:algae is 1:300 (cell number)	72	>90	[68]
<i>Chlorella sorokiniana</i>	<i>Isaria fumosorosea</i>	1–2	Modified Bold's basal medium	125 rpm, pH 7.0–8.0 with inoculation ratio of spores:algae is 1:10 (biomass concentration)	72	94–97	[79]

Note: “ND” means not determined size.

microalgae *Tetraselmis suecica*, Muradov et al. [63] had isolated 33 fungal strains, but only *Aspergillus fumigatus* pellets could attain the efficiency of 90% after 24 h. However, both freshwater microalgae *Chlorella vulgaris* and marine microalgae *Nannochloropsis* sp. Were almost completely harvested (94–97%) by fungi *Aspergillus nomius* CCK-PDA 7#6 [56]. Therefore, the selection of fungal-algal strain is a crucial step and more detailed work is needed to combine with co-cultivation conditions for each targeted strain.

2.3.2. Nutrient

As for the fungal spores-assisted harvesting method, it is worthy to note that co-culturing spores with algae in an autotrophic mode often results in a low efficiency [64]. Unlike microalgae, filamentous fungi are mainly subject to heterotrophic cultivation mode due to the lack of photosynthetic abilities, so the addition of organic carbon source is an essential step to ensure the metabolic activity. Although fungal species (e.g., *Aspergillus* sp.) can utilize polysaccharides from algal cell walls through secreting hydrolytic enzymes, the insufficient amount of organic nutrients will still limit spores germination and subsequent growth, resulting in the low efficiency in forming co-pellets [65,66]. When inoculated *Aspergillus oryzae* spores into microalgae *Chlorella vulgaris* suspension, no pellet formation was observed under the autotrophic mode; in contrast, 93% of algal cells could be quickly pelletized with fungi after the addition of carbon sources (10 g/L glucose) [64]. Moreover, microalgae in heterotrophic cultivation accumulate more biomass and lipids than those in autotrophy, mainly due to the promotion effects from organic substrates [67]. Considering that both microalgae and fungi under the heterotrophic mode need to assimilate organic carbon as energy sources, the selected nutrients must simultaneously meet the growth requirements of co-pellets. More importantly, the selection of various carbon sources such as glucose, glycerol and acetate, which have different effects on the metabolic capability of spores to germinate and form fungal-algal pellets, is strain-dependent [68,69]. In general, glucose is the most suitable organic carbon source for fungi and algae to be utilized, and the concentration ranges from 2 to 20 g/L in diverse conditions. Meanwhile, as fungi and algae can remove nutrients, wastewater (e.g., potato processing water) rich in compatible nutrients is an alternative medium for the co-cultivation of algae with fungal spores [70].

Compared with the spores-assisted method, fungal pellets are pre-cultured before the addition into the suspended algal medium, so the

effects of extra nutrients on harvesting are relatively slighter. Indeed, the supplementation of organic nutrients (e.g., sucrose) during the flocculation period can accelerate the metabolism of fungal pellet to aggregate microalgae more efficiently [71], but pre-made pellets are usually grow on the potato dextrose broth (PDB), which has already increased the total costs. However, it is worthy to note that available wastes such as starchy hydrolysate, raw glycerol and lignocellulosic hydrolyzates can be collected as feedstock to support fungal growth and pelletization [70], so the pre-cultured method may harvest algal cells with less costly nutrient input. For instance, 1% acid treated wheat straw was selected as the alternative nutrients for *Aspergillus fumigatus* pelletization to harvest freshwater algae (*Chlorella vulgaris* and *Scenedesmus quadricauda*), while the half maximal flocculation efficiency had no significant difference than that treated by glucose [20]. Therefore, the combination of waste recycling and biomass production is a promising solution, and more nutritional sources for co-pelletization need to be explored in further research.

2.3.3. Fungi:algae ratio

As one of the parameters influencing the harvesting efficiency, the ratio of fungi:algae has multiple effects during the process. Since the fungal spores are inoculated into the suspension, the initial spore/algae ratio (on cell number basis) can directly determine the growth of fungi and algae. In addition to symbiosis associations as lichen for synergetic mass transfer [72], fungal spores and algae during the growth period may compete limited resources in medium due to the inappropriate inoculation ratio, which results in the excessive predominance of one species. High concentration of spores means that more available nutrients in medium need to be assimilated by fungi to support metabolism, forming larger size of pellet to capture algae into the mycelium and further inhibiting the growth and biomass productivity of algal cells [13]. On one hand, previous studies proved that co-pelletization was similar to fungal pelletization, where high spore inoculation would limit the pelletization period, as well the size and number of pellets [65,73]. On the other hand, relatively low spore/algae (S/A) ratio can better create the suitable growth condition for algal cells, since the secreted organic acids from excessive spores will drastically reduce the pH value in medium. However, the ratio of *Aspergillus fumigatus*:*Botryococcus braunii* Kossou-4 with the range from 1:20 to 1:50 has decreased the harvesting efficiency from 97 to 35%, indicating that the inadequate inoculums of spores are also hard to form co-pellets [74]. Therefore,

only optimal S/A ratio, where the systematic symbiosis relationship between spores and algal cells is constituted, is beneficial for biomass yield and efficient harvesting simultaneously.

The pre-cultured fungal pellets mainly function as biofloculants to harvest microalgae, and thus adsorption capacity and time consumption also depend on the fungi:algae ratio [75]. The addition of a relatively high dosage of fungal pellets is a convenient method to aggregate algae in a short time, but it needs more energy input and might influence the composition of the total biomass. The low pellet/algae (P/A) ratio (on dry weight basis) means less pellets with the identical initial algae concentrations, and thus less surface on mycelium can be provided for the absorption of algal cells, leading to earlier saturation. *Aspergillus niger* could almost harvest all *Synechococcus subsalsus* cells with P/A ratio of 1:1, whereas the efficiency dropped to 59% under the ratio of 1:5 under the same conditions [71]. However, using *Aspergillus fumigatus* AML01 to harvest *Chlorella pyrenoidosa*, the data indicated that 99% of recovery efficiency was obtained with P/A ratio \geq 1:5, and 90% was obtained with 1:7 ratio within 4 h [60]. Interestingly, other microalgae species in co-pelletization experiment, named *Chroococcus* CC1 and algal consortia from an outdoor lake, could also be harvested efficiently under the similar conditions. The appropriate explanation on the difference in fungal adsorption capacity is mainly due to the species-specific property, so the future direction should focus on selecting fungal strains with the wide range of applicability to harvest diverse targeted microalgae. For the purpose of saving the total costs, the desirable mode is to use fewer amounts of filamentous fungi to harvest algal cells as much as possible, and more work needs to improve efficiency through the exploration of these strain-dependent details.

2.3.4. Temperature

In general, the increase of temperature in medium means the provision of sufficient kinetic energy for filamentous fungi to participate in the whole reaction and accelerate the flocculation process [76]. Likewise, the elevated temperature can facilitate spore germination and subsequent pelletization process. On one hand, the growth period has been shortened indeed, whereas the cost of temperature maintenance increases as well. On the other hand, fungal spores (e.g., *Penicillium* sp.) obtain higher metabolic activity when temperature is above 33 °C, but the proper cultivation temperature for most oleaginous microalgae is between 20 and 30 °C [77]. Although 99.3% of *Chlorella* sp. Cells are harvested under 50 °C, the scanning electron microscope (SEM) analysis about surface morphology shows some changes like cell wall deformation, breakage of structure and cell disruption to some extent [78]. Actually, the maximum temperature tolerance about most target algae is 40 °C for few hours, so it is necessary to consider the quality of harvested biomass when co-culturing spores and algae to form pellets. Inoculating *Aspergillus awamori* spores with *Chlorella minutissima* MCC 27, Dash and Banerjee [69] concluded that 25 °C was the suitable temperature for both biomass production and algal harvesting. The similar range of 25–30 °C is also appropriate for *Isaria fumosorosea* spores to recovery 94–97% of *Chlorella sorokiniana* biomass, while 32 °C is lethal [79].

In terms of pre-cultured fungal pellets function as flocculants to assist harvesting, optimum temperature must simultaneously ensure pellets and algal viability. *Cunninghamella echinulata* pellets have nearly the same applied temperature with *Chlorella* sp., so 99% of the algal cells are removed from the medium under 25 °C, with fungi:algae ratio of 1:2 in two days of co-pelletization [80,81]. Moreover, an increase in algal cell number during the process indicates the potential to achieve a continuous cultivation-flocculation mode under a constant temperature. Interestingly, it is also worthy to note that relatively high temperature may function as abiotic stress to increase the microalgae production of carbohydrates or lipids [82]. Therefore, through the regulation of the temperature during the co-cultivation period, the quality of the total biomass composition can be improved together with efficient harvesting.

2.3.5. pH

The pH of medium plays an important role in the whole process, from initial spore inoculation to fungal-algal pellet formation. Since fresh spores are added into algal suspension, the ambient pH can determine the biomass productivity and subsequent harvesting efficiency through the simultaneous regulation of the metabolic activity of fungi and algae. In general, a lower pH value promotes the metabolic activity of spores, but the percentage of microalgal biomass in co-cultured pellets is decreased [42]. This might attribute to the reason that acidic conditions better favor the growth of fungal spores than algae, and the co-pelletization period is shortened simultaneously. In other words, the adjustment of pH can influence the content of biomass, due to the differences in growth rate between spores and algae under the specific conditions. Indeed, the adjustment of pH has a potential in improving harvesting efficiency, whereas the variation in biomass composition of fungal-algal pellets may occur as the increase of initial cost.

The suspension pH also affects the surface properties of cells, such as charge distribution and EPS activity, directly impacting the flocculation efficiency [83]. The proton-active functional groups on the surface of algal cell wall, such as carboxylic, hydroxyl and amine groups, are sensitive to the pH fluctuations in medium [84]. Under the alkaline condition, the surface groups are deprotonated to generate negative potential, and then electrostatic repulsion would keep algal cell suspending rather than settling. Meanwhile, a decrease in pH might cause positive charges of fungal pellets, which helps to reduce the repulsion in negatively charged algal cells. Moreover, since the relatively acidic environment can better support the metabolism of filamentous fungi, the secretion of protein and polysaccharide compounds may also function as biofloculant to facilitate the harvesting process [40]. The optimal pH value for *Aspergillus niger* pellets to harvest *Synechococcus subsalsus* cells is 6, while the control group (initial pH = 8.1 without adjustment) shows the lowest efficiency of merely about 17% [71]. In contrast, the *Chlorella* sp. Harvesting experiments with pH range of 3.0–9.0 all have shown high efficiencies (88–98%), partially because of the wide pH tolerance about *Aspergillus niger* secretion [85]. In conclusion, the question on whether the procedure needs to adjust pH value for efficient harvesting is species-specific, and more details should be exploited to optimize cultivation conditions in future research.

2.3.6. Agitation

The agitation speed is another critical factor, since it affects the spore germination and subsequent morphology of filamentous fungi, which in turn facilitates the adsorption performance in the suspension systems. According to the previous studies, the hydrodynamic condition directly determines the efficiency in bioreactors, since it affects the mass transfer rate and biomass productivity [86,87]. After initial inoculation, the adoption of a suitable agitation speed is helpful to minimize the available nutrient concentration gradients and disperse nutritive particles in medium, so fungal conidia and algae can effectively utilize fluid nutrients for the rapid growth of co-pellets. More importantly, once fresh spores are cultivated in medium without mechanical agitation to provide hydrodynamics, the filamentous fungi even will not form the uniform morphology of pellet. In terms of co-culturing spores with algae, the stirring rate not only determines the formation of pellets, but also can adjust the spherical sizes [13]. When agitation speed ranges from 50 to 150 rpm, the average diameters of fungal-algal pellets are reduced by nearly 5 mm [64]. Small and relatively smooth pellets are obtained under a higher speed, and it is positive correlation with removal efficiency in a reasonable range. The improved metabolic activity and particle collision frequency are the key reasons, mainly because the sufficient mixing promotes the mutual interactions in fungal-algal system.

Compared with the spore-assisted method, pre-cultured pellets have a certain morphological shape during the flocculation process. The agitation provides fungal pellets with the sufficient contact opportunities, and thus hydraulic movements drive algae cells to get over

repulsion and finally aggregate together. However, the excessive agitation intensity for pellets might cause two consequences: disintegration of mycelia components on the surface area or the possibility of the whole pellet rupture [88]. The experiment on different velocity gradients for the flocculation of *Aspergillus fumigatus* pellets showed that the *Chlorella pyrenoidosa* cells removal efficiency could reach 99% at 100 rpm, while only 6% at 150 rpm [39]. Failure of the flocculation process under an over high speed suggests that shearing force overcomes the van der Waal's forces of adhesion, and even can cause algal cells lysis. Furthermore, relatively slow agitation promotes fungal pellet to produce extracellular polysaccharides, and thus microalgae might get entrapped with these sticky secretions. Overall, the optimal agitation speed is extremely needed to form co-pellets effectively with less energy costs.

2.3.7. Other factors

In addition to supplementing extra nutrients to accelerate metabolism for co-pelletization, the addition of metal ions can also influence the process. Xia et al. [89] found that Ca^{2+} could assist *Mucor circinelloides* spores to form compact pellets with smooth surfaces. Under the hydrodynamic condition, the Ca^{2+} might serve as a cross-linking material to facilitate the aggregation of fungal spores. Moreover, the growth rate and percentage of *Chlorella vulgaris* in co-pellets increased, as the increase of the concentration of Mg^{2+} , which suggest that Mg^{2+} could aid the entrapment of algal cells to fungal hyphae [42]. Therefore, wastewater that is abundant in metal ions should combine with co-cultivation technology for the simultaneous process of bioremediation and biomass harvesting. While considering the utilization of wastewater as culture medium, the corresponding impacts on biomass should be confirmed in detail. In the practical wastewater treatment process, the functional groups on the surface of biomass like carbonyls, hydroxyls and amides which contribute to the biosorption might be destroyed by multiple pollutants [90]. As the algal surface properties have changed, the bioflocculation efficiency of selected fungus will get fluctuations. For instance, heavy metals in wastewater environment, such as cadmium, copper and zinc, have ability to cause cell damage in culture system. Meanwhile, a study about bioaccumulation of cadmium and copper demonstrated that lipid productivity in microalgae *Chlorella minutissima* was increased by 21.07% and 93.90%, with the maximum adsorption of 35.36 mg/g and 3.28 mg/g, respectively [91]. Indeed, ambient stress within the acceptable range can stimulate microbial cells to synthesize intracellular substances, so wastewater is probably a better choice than artificial medium to co-cultivate microalgae with filamentous fungi.

When it comes to pellet formation, besides these factors such as strain, nutrients and inoculation ratio, the effect of dissolved oxygen is significant as well. Different from photosynthetic microalgae, the heterotrophic metabolism of filamentous fungi requires sufficient oxygen, and then the available substrates in medium can be assimilated to support biomass production. Through experimental determination, the results indicated that over 0.356 mmol- O_2 /L/h of oxygen transfer rate was needed for fungi *Neurospora intermedia* growth, whereas insufficient aeration would inhibit the pelletization directly [92]. To maintain suitable conditions, photosynthesis from algal cells, mechanical aeration and agitation are the main sources to provide oxygen, but more work is needed to optimize corresponding parameter. Moreover, with regard to fungal pellets, the harvesting efficiency also depends on the structural characteristics and relevant physiology. Li et al. [58] stated that relatively sparse mycelial structure could adsorb more algal cells onto the surface and even inside the pellets, thus achieving high flocculation activity. The harvesting efficiency of *Aspergillus fumigatus* pellets grown for 24 h was 95%, while pellets grown for 72 h experienced an efficiency reduction to less than 6%, partially because the dense structure hindered internal layers of hyphae through obtaining nutrients to metabolize [39, 87]. Likewise, as loosely packed mycelium has more interior space to uptake oxygen and transfer carbon dioxide, it would require less aeration input to support growth than densely packed fungi pellets [93]. Due

to the limited mass transfer in the core of compact pellet, both interior fungi and algae cells may even experience lysis, leading to the reduction in total biomass. Therefore, the relatively sparse structure in pellet is more preferable during the co-cultivation process for microalgae harvesting.

Apart from these aforementioned parameters, other factors that include photoperiod, light wavelength and CO_2 concentration are also play important roles in the co-cultivation process for efficient harvesting [94]. More importantly, the retention time of fungal-algal pelletization is generally high, which has the potential in affecting the biochemical composition of harvested biomass and the economics of production [95]. However, few studies have emphasized this vital aspect in co-cultivation of filamentous fungi with targeted algae strains, so the biomass quality influenced by retention time should be paid more attention to during the harvesting process. Overall, the harvesting performance of filamentous fungi is efficient and multi-factors induced, and the process can be further optimized for the proposal of subsequent process.

3. Application in wastewater treatment

In addition to efficient harvesting, another major application area of co-cultivation is the coupling with wastewater treatment. Over the last few decades, microalgae have been successfully demonstrated as bio-resource for the removal of excessive nutrients in wastewater environment, such as nitrogen, phosphorus and carbon [103]. Moreover, current studies have focused on using microorganisms to remediate contaminated water, which contains multiple heavy metals, pesticides and pharmaceuticals. To improve treatment performances, co-cultivation of microalgae and fungi has been verified to be more efficient than the mono-system of microalgae [63]. Therefore, this part of investigation summarizes the synergistic mechanisms in pollutants removal, and the corresponding efficiency in removing pollutants by co-culturing microalgae with fungi in wastewater are reviewed as well.

3.1. Mechanisms of pollutants removal

The mechanisms of microalgal-fungal system which contributes to the excellent performance in the remediation of wastewater can be concluded as follows. First, the reason why applying co-cultivated mode to remove nutrients from wastewater performs more efficiently than traditionally pure cultivation is the establishment of symbiotic relationship. Fig. 3 depicts the involved mutual interactions between fungi and algae vividly. In general, the initial inoculation ratio of microalgae and fungi could explicitly determine final effects by influencing the growth of each other. Inappropriate ratios usually lead to unilateral growth of fungi or algae, and it might even cause adverse impacts on removal efficiency. Compared the efficiencies with the initial algae: fungi ratio ranging from 20 to 500 [19], the results showed that the most optimal inoculation ratio was 100, when the highest biomass yield (4.215 g/L) was obtained. Second, fungi are heterotrophic organisms that can convert organic matters into carbon dioxide through metabolism, while inorganic carbon sources are used as raw materials for autotrophic microalgae to accumulate biomass [104]. In this way, released oxygen from microalgal photosynthesis can fully supply fungi to respire and, in turn, delivering carbon dioxide back to algal cells. In addition to reducing the concentrations of nutrients, the transfer and utilization of diverse carbon sources in wastewater environment has also promoted biomass accumulation for value-added bioproducts, such as biodiesel, biogas and protein-rich feed [99,105,106]. Third, some nutrients, especially nitrogen and carbon, are embedded in suspended solids, which make them difficult for microalgae to be utilized directly. When coupled with co-cultivation mode, these macromolecular organic matters can be converted into soluble low-molecular-weight nutrients with the action of fungal extracellular enzymes. Thus, microalgae can efficiently remove more nutrients from wastewater through the

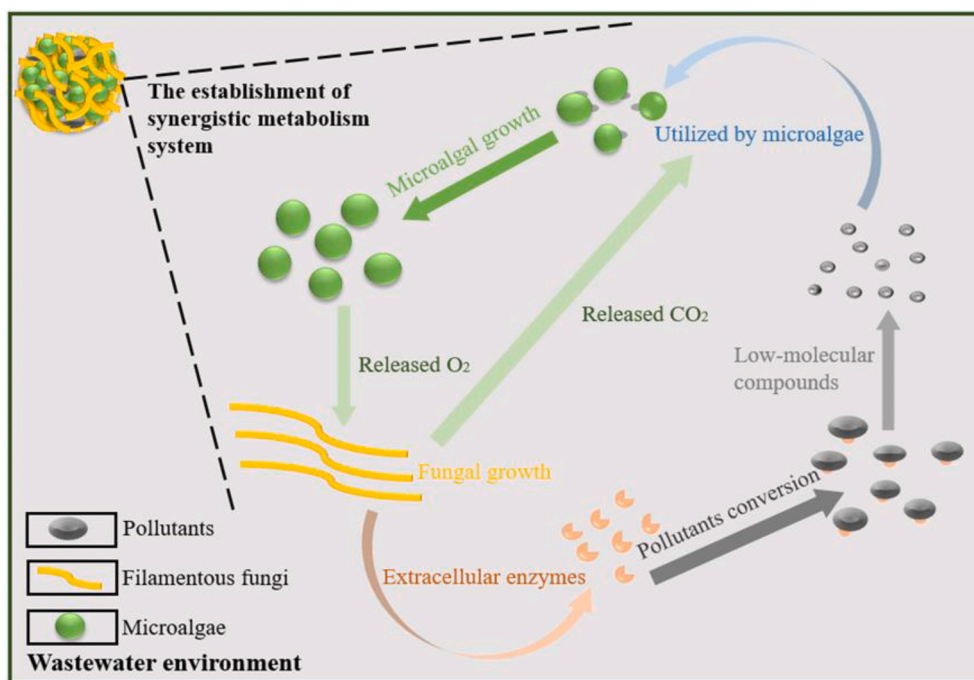


Fig. 3. The synergistic metabolism for pollutants removal in fungal-algal system.

assimilation of enzyme-treated soluble matters [107]. In other words, due to the mutually reinforcing mechanisms between microalgae and fungi, the co-cultivation system can be more efficient in the removal of nutrients (e.g. nitrogen, phosphorus and COD) than a mono-system.

Some heavy metals (e.g., zinc, copper, manganese and cobalt) are essential for the growth of microalgae and fungi as trace elements and involve in the enzymatic process and cell metabolism, while other heavy metals (e.g., arsenic, cadmium, lead, mercury and chromium) are toxic to organisms. Microalgae and fungi have been widely studied as bio-resources for removing heavy metals [108–111], while the co-cultivation of microalgae with fungi or fungal-algal pellets are also powerful bio-resources for the treatment of heavy metal contaminated wastewater. Bioremediation of heavy metal-containing wastewater by the co-cultivation of microalgae with fungi involves two stages. The first stage is characterized by its rapid extracellular passive adsorption (biosorption), which has nothing to do with cell metabolism [112]. Metal ions may attach to the cell surface through one or more of coordination, ion exchange, surface complexation, redox, micro-precipitation and physical adsorption [113]. Both microalgal and fungal cell walls are mainly composed of polysaccharides, proteins and lipids, which can provide abundant metal-binding functional groups (amino, hydroxyl, carboxyl, phosphoryl, etc.) [90]. In addition, the atoms of N, P, S and O in functional groups can provide heavy metal ions with a lone pair of electrons that are coordinate and complex, so that the heavy metals are tightly bound to the cell walls [114]. The accumulation of heavy metals inside the cell is the second stage, which is much slower than the initial stage, because the process is an energy-driven metabolism. After the adsorption on the surface of cells, heavy metals are actively transported into the cytoplasm through the cell membrane, followed by an intracellular positive diffusion and linking to the internal binding sites of proteins or peptides (glutathione, metallothionein and phytochelatins) [115]. Furthermore, once inside the cells, organelles such as chloroplasts, vacuoles and mitochondria can combine heavy metals with organic substances (sugar, protein and sulfide) to form complexes, and thus heavy metals are accumulated in cells in the form of sulfides or polyphosphates [111]. As for the larger molecular organic contaminants such as pharmaceuticals, pesticides, petro-alkane and detergents, the potential mechanisms for microalgae and fungi

bioremediation involves one of the following processes: (i) bio-adsorption, (ii) bio-uptake and (iii) biodegradation. The processes of bioadsorption and bio-uptake are similar, as to the bioremediation of heavy metals. However, the difference is that heavy metals cannot be degraded in cells, while organic pollutants can be degraded into small molecules through a series of biochemical reactions [116]. Therefore, based on the above mechanisms of microalgae and fungi for the removal of heavy metals and organic pollutants, the corresponding performances are discussed specifically in the following subsection as well.

3.2. Removal of organic pollutants

Nutrients, such as nitrogen, phosphorus as well as carbon dioxide or/and organic carbon, are necessary for the photosynthesis and growth of microalgae in the co-cultivation system [13,20]. Therefore, in order to simultaneously remove nutrients from wastewater, a highly feasible technology of fungal-microalgal pellets co-cultivation should be applied. The removal efficiency of nutrients by co-cultivating of fungi and microalgae is presented in detail in Table 4. Meanwhile, those types of wastewater (molasses wastewater, biogas slurry and swine wastewater) are rich in nitrogen, phosphorus as well as COD. In wastewater, nitrogen is mainly available in the forms of nitrites, nitrates and ammonia which play a very important role in the metabolic pathway through assimilation [117]. Microalgae are autotrophic microorganisms, so that they require nitrogen to synthesize proteins, phospholipids and nucleic acids [4]. In the recent study carried out by Yang et al. [19], *Aspergillus* sp. and *Chlorella vulgaris* were co-cultivated to treat molasses wastewater. The results showed that higher TN removal efficiency of 67.09% was reached through the co-cultivation system, whereas mono-system of fungi and microalgae only removed 44.39% and 18.20% of TN, respectively. Although the abilities of fungi to remove TN and $\text{NH}_3\text{-N}$ were not high (less than 20%), the introduction of fungi into wastewater treatment for the co-cultivation could remarkably improve the removal efficiency of TN, $\text{NH}_3\text{-N}$ and $\text{NO}_3\text{-N}$ [19,20]. In addition, phosphorous is another macronutrient which is also necessary for the synthesis of nucleic acids, lipids and adenosine tri phosphate (ATP) of the cells [118, 119]. Inorganic phosphates in form of H_2PO_4^- , HPO_4^{2-} and PO_4^{3-} are involved in the synthesis of organic compounds by phosphorylation,

Table 4

Removal efficiency of TN, TP, and COD in various types of wastewater by co-cultivation of microalgae with fungi.

Algal strain	Fungal strain	Type of wastewater	Initial concentration (mg/L)	Removal efficiency	Reference
<i>Chlorella vulgaris</i>	<i>Aspergillus</i> sp.	Molasses wastewater	TN = 364.4	TN (67.09%)	[19]
<i>Scenedesmus</i> sp.	<i>Trichoderma reesei</i>	Secondary effluent	TP = 28.6	TP (88.39%)	[99]
<i>Chlorella vulgaris</i>	<i>Ganoderma lucidum</i>	Biogas slurry	COD = 3894	COD (70.68%)	[94]
<i>Chlorella</i> sp.	<i>Aspergillus niger</i>	African catfish wastewater	TN = 144	TN (>93%)	[96]
<i>Chlorella vulgaris</i>	<i>Ganoderma lucidum</i>	Biogas slurry	TP = 18.6	TP (>44%)	(Zhao et al., 2019)
<i>Scenedesmus obliquus</i>	<i>Pleurotus ostreatus</i>	Biogas slurry	COD = 1239	COD (>74%)	[128]
<i>Pseudokirchneriella subcapitata</i>	<i>Ganoderma lucidum</i>	Biogas slurry	TN = 278	TN (75.57%)	[120]
<i>Chlorella protothecoides</i>	<i>Aspergillus fumigatus</i>	Swine wastewater	TP = 27.9	TP (78.26%)	[63]
<i>Tetraselmis suecica</i>	<i>Aspergillus fumigatus</i>	Swine wastewater	COD = 1495	COD (70.24%)	[63]
<i>Thraustochytrid</i> sp.	<i>Aspergillus fumigatus</i>	Swine lagoon wastewater	NH ₃ -N = 0.9	NH ₃ -N (98.6%)	[20]
<i>Tetraselmis chuii</i>	<i>Aspergillus fumigatus</i>	Swine lagoon wastewater	TP = 2.6	TP (75.1%)	[20]
			TN = 182.6	TN (89.83%)	
			TP = 17.96	TP (90.31%)	
			COD = 1061	COD (82.17%)	
			TN = 202.07	TN (61.98%)	
			TP = 20.92	TP (63.93%)	
			COD = 997.57	COD (59.17%)	
			TN = 51.84	TN (70.18%)	
			TP = 12.86	TP (82.41%)	
			COD = 289.98	COD (72.09%)	
			NH ₃ -N = 164.3	NH ₃ -N (73.7%)	
			TP = 38.7	TP (55.5%)	
			NH ₃ -N = 67.9	NH ₃ -N (94.3%)	
			TP = 18.7	TP (77.5%)	
			NH ₃ -N = 66.1	NH ₃ -N (95.6%)	
			TP = 16.1	TP (81.36%)	
			NH ₃ -N = 164.3	NH ₃ -N (77.5%)	
			TP = 38.7	TP (50.9%)	

leading to high nutrient removal efficiencies in wastewater [117]. In 25% diluted swine wastewater, 69.5% removal efficiencies of PO₄³⁻ was observed in the co-cultivation of *A. fumigatus* and *Thraustochytrid* sp. With the initial concentration of 38.7 mg L⁻¹, while *Thraustochytrid* sp and *A. fumigatus* respectively removed merely 45.7% and 49.3% [20]. Similar result was also observed in the study of Yang et al. [19]. After 5-day cultivation, the removal efficiency of TP in the co-cultivation system reached 88.39%, which was twice higher than fungi or microalgae in the mono-cultivation system. Moreover, the symbiotic system could be used to remove COD from wastewater environment, but it is worthy to note that the excessive concentration might inhibit the growth of microalgae and fungal cells [120]. Previous study proved that the fungi had much better performance in COD removal than microalgae [121]. On one hand, fungi are heterotrophic organisms which are prone to use the organic carbon as the only carbon source and major energy type, resulting in effective reduction of COD [99]. On the other hand, the small diameter of the channels helps transport materials through the microalgal plasma membrane, so relatively large size of organic matters cannot be absorbed by cells directly [122]. The results of fungi and algae co-cultivation indicated that the removal efficiency of COD reached 70.68%, but only 25.96% and 59.00% were accordingly removed by microalgae and fungi in the mono-cultivation system under the same initial concentration [19]. Based on these previous studies, it is obvious that the addition of fungi to induce mutualism with microalgae has more advantages in wastewater treatment than other pure-cultured modes, and the corresponding mechanisms are generalized in the coming section.

3.3. Removal of other pollutants

As mentioned before, co-cultivation of microalgae and fungi can effectively remove nitrogen, phosphorus and COD from several types of wastewater. Besides, this co-cultivation technique also enables effective removal of other pollutants. In the study of Bodin et al. [123], *Chlorella vulgaris*, *Aspergillus niger* and the bio-pellets composed of both microorganisms were applied in the removal of seven pharmaceuticals. Compared with the mono-system of microalgae and fungi, the results

indicated that co-cultivation of microalgae and fungi could significantly degrade the ranitidine. In comparison with the control, the co-cultivation of *Aspergillus niger* and *Chlorella vulgaris* was proved to remove the mixture of 38 pesticides effectively, and the result also suggested that fungi played a vital role in the degradation of organic contaminants in bio-pellets [124]. In addition, biological treatment of heavy metal pollutants is the main focus of current researches. The treatment performance of co-cultivation system via response surface methodology (RSM) was investigated for the removal of arsenic from water [125]. As a result, *Chlorella vulgaris* and *Aspergillus oryzae* pellets could absorb arsenic, followed by a reduction and extrusion into the surrounding environment, which exhibited a high arsenic tolerance. Cadmium is listed as a priority pollutant because of its serious toxicity in aquatic environments [126], but with the application of *Aspergillus niger-Chlorella vulgaris* pellets, cadmium with a relatively low concentration (1 µg/L) was successfully removed [127]. In conclusion, the ability of high accumulation and tolerance in fungal-algal pellets provides a new insight in the bioremediation of heavy metals-contaminated wastewater. Moreover, the application of fungi and microalgae co-cultivation not only has potential in removing pollutants from wastewater, but also facilitates the harvesting of microalgae. Therefore, the co-cultivation technique has dual functions in bioremediation of wastewater effluents and gathering of microalgae-based biomass products to form circular bioeconomy.

4. Application in subsequent biofuel production

Regardless of whether the co-cultivation occurs in wastewater or culture broth, it is necessary to evaluate the influences on total biomass for bioproduct processing after co-pelletization. Therefore, this part aims to illustrate the advantages of co-cultivating algae with fungi in biofuel production, and the corresponding effects with performances are demonstrated as well.

4.1. Advantages of co-pelletization mode for biofuel production

In general, the investigation of fungal-algal pellet formation usually

falls into the scope of the effects on total biomass. Based on the symbiotic system, fungal cells consume oxygen and release carbon dioxide through heterotrophic metabolisms, while algal cells assimilate inorganic carbon and generate oxygen through autotrophic metabolisms [66]. Once the metabolic reactions are combined and complementary, this kind of co-pellet can accumulate more biomass for desired processing. In comparison with traditional mono-cultivation mode, the increase of total biomass means that more feedstock can be utilized for bioenergy field with less cultivation costs. More importantly, on a previous large-scale application, Al-Hothaly [129] analyzed the harvested *Aspergillus fumigatus-Botryococcus braunii* pellets by pyrolysis and concluded that this method had no adverse impacts on biofuel production. The similarity in biomass composition, which includes carbohydrates, proteins and lipids, is one of the major reasons. Likewise, the diversity in species-specific biochemicals and corresponding contents between fungi and algae can also enlarge the range of bioenergy production. Apart from this, microalgae-based downstream processing needs to disrupt the cell walls for the further extraction of intracellular substances, but traditional methods (e.g., ultrasound, pressing, microwave and high-pressure homogenization) usually follow with high energy consumption [130]. In contrast, the spontaneous pretreatment to maximize biofuel production appears in fungal-algal pellets. As the form of co-cultured pellets, fungi can convert components on algal cell wall into available carbon source through secreted hydrolytic enzymes, including hemicellulases, cellulases, pectinases and laccases. Hom-Diaz et al. [131] reported that enzymatic pretreatment from crude *Trametes versicolor* broth degraded algal cell wall to some extent and increased biomethane yield by 74%. In conclusion, due to the superiority in biomass accumulation, spontaneous pretreatment and various applications from a sustainable mode, co-cultivation of microalgae with filamentous fungi has great potential in exploring bioenergy field.

4.2. Performance on biofuel production

As a promising feedstock to confront with energy crisis, microalgae can grow at a rapid speed and accumulate lipids up to nearly 70% under optimum conditions [132]. Furthermore, the intracellular lipid content of some filamentous fungi which belong to *Mucor*, *Cunninghamella* and *Aspergillus* genus is over 20% of dry biomass [133]. When fungus and algae are harvested to apply in biodiesel industry, the first involved consideration should focus on the content of total lipids. Table 5 summarizes the biomass and lipid concentrations of separately cultured fungi and algae and co-cultured pellets. Compared with

mono-cultivation, the significant increase in biomass and lipid yields can be observed in co-cultured fungi-algae pellets, due to the establishment of symbiosis system. Maximum biomass and lipid concentrations of *Aspergillus fumigatus-Chlorella protothecoides* pellet were 8.96 ± 2.1 g/L and 2.042 ± 0.44 g/L respectively, while the corresponding contents in mono-cultured groups were less than half of the counterparts [63]. In addition, the lipid composition (measured by fatty acids), which relates to the biodiesel quality and fuel property, should also be concerned extensively. Zhou et al. analyzed lipid profile about *Aspergillus oryzae-Chlorella vulgaris* pellets and found that C16–C18 even accounted for 92.36% of total fatty acids [64]. In fact, C16–C18 with long carbon chains are the dominant fatty acids in harvested fungal-algal pellets (Table 5). Similarly, the main oil compositions in microalgal cells are palmitic (C16:0), almitoleic (C16:1), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acid [134]. Although fatty acids in fungi are quite different from microalgae, the co-cultured total lipid profile is still largely determined by oleaginous algal strains. Besides, pellet fatty acids that belong to fungal contribution might adjust the biofuel property. The *Aspergillus fumigatus* has improved the content of unsaturated fatty acid in fungi-algae system, since this strain contains nearly 60% of oleic (C18:1) and linoleic (C18:2) acid [17]. As a combination of fungal and algal lipids, the retaining of unsaturated fatty acids can ameliorate performances under low temperature and kinematic viscosity, while the saturated components aid in enhancing combustion properties and oxidative stability [130]. In other words, the change in saturated and unsaturated fatty acid content can also explain why the co-cultured pellet lipids are more advantageous than other mono-cultured methods.

Apart from biodiesel production, due to the abundance in lipids, carbohydrates, proteins and a variety of complex molecules, fungal-algal pellet could also be utilized for producing other biofuels. As the combination of fungal and algal biomass, this co-cultivation technology improves systematic suitability in biomethane generation through anaerobic digestion. In contrast with pure algal biomass, *Aspergillus lentulus-Chroococcus* sp. Pellet has enhanced >54% of digestibility and increased up to 50% in methane production [100]. The addition of fungal biomass could adjust the C/N ratios of harvested algae, so the quality of digested feedstock is relatively improved for biomethane production. Furthermore, fungal-algal pellets can also purify the raw biogas which contains high concentrations of CO₂ [106]. Despite initial biogas contains $36.17 \pm 1.97\%$ of CO₂, the final gas obtained nearly 90% of CH₄ by synergetic metabolisms from *Ganoderma lucidum-Chlorella vulgaris* pellets [13]. After treatment, both microbial biomass and calorific value in biogas are enhanced simultaneously. Moreover,

Table 5
Biomass and lipid yield of microalgae with fungi in mono-culturing and co-culturing mode.

Fungal/algal strain	Fungal/algal mono-cultivation			Fungal-algal co-cultivation			Reference
	Biomass (g/L)	Lipid (mg/L)	Fatty acid component* (%)	Biomass (g/L)	Lipid (mg/L)	Fatty acid component* (%)	
<i>Aspergillus fumigatus/Chlorella protothecoides</i>	2.21 ± 0.5/2.25 ± 0.4	240.20 ± 41.9/699.7 ± 120.4	C16:0 (18), C18:2 (28)/C16:0 (12), C18:2 (27)	8.96 ± 2.1	2041.9 ± 440.6	C16:0 (12), C18:2 (30)	[63]
<i>Aspergillus fumigatus/Tetraselmis suecica</i>	2.21 ± 0.5/1.77 ± 0.4	240.20 ± 41.9/215.55 ± 50.6	C16:0 (18), C18:2 (28)/C16:0 (18), C18:2 (21)	4.49 ± 0.9	578.29 ± 210.7	C16:0 (18), C18:2 (22)	[63]
<i>Aspergillus awamori/Chlorella minutissima</i>	ND/1.14 ± 0.1	ND/250 ± 19.0	C16:0 (8), C18:1 (37)/C16:0 (41), C18:1 (20)	2.80 ± 0.1	510.40	C16:0 (35), C18:1 (24)	[69]
<i>Aspergillus fumigatus/Chlorella vulgaris</i>	0.20 ± 0.03/0.20 ± 0.02	25.46 ± 4.1/45.74 ± 5.5	C16:0 (20), C18:2 (30)/C16:0 (20), C18:2 (28)	0.80 ± 0.1	146 ± 17.7	C16:0 (20), C18:2 (27)	[20]
<i>Trichoderma reesei/Scenedesmus</i> sp.	5.50 ± 0.5/1.00	1200 ± 500/ND	ND/ND	6.64 ± 0.7	1700 ± 90	C16:0 (32), C18:1 (25), C18:3 (14), C20:0 (11)	[99]
<i>Aspergillus fumigatus/Synechocystis</i> PCC 6803	1.50 ± 0.21/1.70 ± 0.24	60 ± 9/1.85	C18:1 (30), C18:2 (30)/C16:0 (49), C18:0 (18)	4.50 ± 0.6	250 ± 35.7	C16:0 (38), C18:1 (25)	[17]
<i>Cunninghamella</i> sp./ <i>Scenedesmus obliquus</i>	1.75 ± 0.1/1.99 ± 0.12	ND/810 ± 60	ND/C16:0 (62), C18:0 (30)	4.45 ± 0.06	1210 ± 80	C16:0 (52), C18:0 (35)	[101]
<i>Aspergillus niger/Chlorella vulgaris</i>	0.215 ± 0.02/0.142 ± 0.01	38.03 ± 10.05/53.11 ± 1.51	C16:0 (22), C18:1 (33)/C16:0 (27), C18:1 (33)	0.97 ± 0.129	275.88 ± 52.40	C16:0 (25), C16:1 (16), C18:1 (32)	[12]

Note: "ND" means the not determined content.

"*" represents the approximate value of fatty acid composition (%) in literature.

filamentous fungi are able to secrete hydrolytic enzymes to facilitate biomass fermentation process, which means the productivity of bioethanol could be improved apparently [135]. Besides, wet pellets have a potential as a sustainable feedstock for producing biohydrogen through hydrothermal gasification (HTG). The chemical compositions in *Isaria fumosorosea*-*Chlorella sorokiniana* pellets (measured as protein, carbohydrate and lipid content) are proven to be suitable for hydrothermal process, and the treated wastes can support fungal spores regrowth [79]. Due to the application of wet biomass in HTG, the energy costs in dewatering and drying process could be cut down, which improves the sustainability in microalgae-based bioenergy production. Overall, harvested fungi-algae pellets could be considered as value-added feedstock for the production of biofuels, including biodiesel, biomethane, bioethanol and biohydrogen, but further work is still required to scale-up this novel technique in bioenergy field.

5. The practical implications, key challenges and future perspectives

5.1. Policies support

Biomass energy is the fourth largest energy source behind fossil energy coal, oil and gas, accounting for 14% of the world's primary energy consumption. It has the characteristics of wide distribution, easy storage, cleanliness and renewability. Energy policies around the world are promoting the use and healthy development of renewable energy, which is also the main reason for the boom in first- and second-generation biofuels [136]. The variegated energy policies include financial subsidies, tax compromises, loan guarantees, biofuel infrastructure construction, etc. [137]. As a major supplier of agricultural products in the world, the United States is in a leading nation in the development of biofuels. The United States has authorized more than 10 legislations related to development of biofuels in recent years. In addition, in terms of taxation, the U.S. Senate Finance Committee has announced that it will provide a tax credit of \$1.01 per gallon for cellulosic biofuel production (including algae fuels) and a \$1 tax credit for biodiesel per gallon [138]. In European Union, the target of roadmap is set for low-carbon economy, which promotes the development of biofuels in a sustainable way. With the development of low-carbon technologies and the implementation of energy efficiency plans, greenhouse gas emissions will be reduced by 80% compared to 1990 by 2050 [138]. Compared with EU and U.S., China starts late in the development and utilization of biomass energy. The International Energy Agency (IEA) believes that by 2023 China may surpass EU to become the world's largest consumer of bioenergy. The main incentive policies adopted by China to promote the development of biomass energy include the immediate refund of value-added tax, the implementation of a fixed electricity price policy and electricity price subsidies. In recent years, microalgae are attracting more attention as the renewable feedstock coupled with high sustainability index. Considering the obstacles in scaling up, such as the high costs of cultivation, harvesting and subsequent multiple utilizations, co-cultivation of targeted microalgal strains with filamentous fungi is a promising way to achieve biomass accumulation and efficient separation through mutual symbiosis. Likewise, due to the variety in filamentous fungi biorefinery, fungal-algal pellet will have more applications than traditional mono-cultivation mode. Based on the policies support, if co-cultivation process exists with wastewater discharge and CO₂ emission industry, high cost of bioenergy production can be compromised and overcome one day [139]. Moreover, the wastes are considered as available nutrients to be utilized by both microalgae and fungi; meanwhile, the cost in waste treatment can partially offset the technological barriers in fungal-algal biomass utilization on a large scale. In conclusion, the integration of innovative policies support and financing projects are required to stimulate the market demands of co-cultivation for biofuel production in a progressive direction.

5.2. Further development for harvesting on large scale

As mentioned in section 2, co-cultivation of filamentous fungi with algae for biomass harvesting has been applied to many species on a laboratory scale, but several obvious obstacles need to be overcome for scaling up. High costs link the biomass production chain, as well as separation from medium and subsequent utilization, hindering microalgae-based technologies for further development. The growth optimization associated with nutrient balance is the main challenge in efficient harvesting, which shows that inappropriate co-cultivation conditions can lead to low biomass productivity and flocculation efficiency. Likewise, the co-culturing mode should also ensure the quality of biomass, whereas undesired results may occur because of the complexity in microorganisms. Therefore, several aspects regarding the potential in practical cultivation applications are presented.

First of all, the selection of fungal and algal strains is crucial, due to the species-specific properties. Competitive interactions between fungi and algae usually lead to poor performances, so the identification of suitable strains that have an ability to form mutual symbiosis is an essential step before co-cultivation process. The targeted microbial properties such as environmental compatibility, growth rate and lipid content, which are related to subsequent applications, should be taken into consideration thoroughly. For instance, if the primary objective of cultivating microalgae is to obtain lipids, co-cultivating oleaginous fungi with symbiotic compatibility could be beneficial for total biomass accumulation [140]. Meanwhile, recent strategies of metabolic and genetic engineering for the improvement of microalgal properties is also feasible, which will contribute to better performances in a co-cultivation process [141]. Second, heterotrophic co-cultivation can accumulate more biomass than autotrophic mode, but it is limited by the cost of extra nutrients. The prospective direction is to utilize nutrient-rich wastes to replace traditional culture broth. Therefore, from a long-term perspective of recycling waste resources, wastewater (such as biogas slurry, swine wastewater and landfill leachate) can serve as an alternative medium to co-cultivate microalgae with fungi, due to high concentrations of carbon, nitrogen, phosphorus and COD from wastewater. As a result, the nutrients are removed effectively with the achievement of valuable biomass recovery, which also fits the purpose of recycling resources. However, possible situations such as the concentration variation in wastewater, uncertainty of harvesting efficiency, undesired flocculation before co-cultivation termination and contamination of biomass should be considered before further scaling up. Besides, in order to maintain a homogenous distribution of fungi and algae, these two co-cultivation modes are usually performed in flasks shaking with a certain speed, so the quantities of machinery cost have limited its wide application. Future directions must also focus on the advancement of co-cultivation devices as well as the optimized conditions. On a large scale, a process modification with the low level of energy input can be practically feasible. Instead of operating orbital shaker, Al-Hothaly et al. [74] obtained fungal-algal pellets on a 500-L scale by supplying constant air flow. Expense could be further reduced through the application of simple facilities to optimize several parameters.

5.3. Removal of multiple pollutants

Due to the severe water pollution and the requirement of massive nutrients for biomass accumulation, the integration of fungal-algal cultivation with nutrient recycling in wastewater is a promising solution. Most of the relevant studies have investigated the solution only on a laboratory scale, so it is necessary to estimate the feasibility of removing multiple pollutants on a large scale. However, the key challenge associated with the utilization of wastewater as culture medium is the possible contamination because of wild types of microorganisms and pollutants existed. Moreover, the ambient concentration of necessary nutrients in diverse wastewater environment might experience fluctuation, so the variation in nutrient load is another challenge of cultivating

microalgae with filamentous fungi for pollutant removal. As mentioned in section 3, studies have proved that the pollutants (e.g., COD, TP, TN, NH₃-N and pharmaceuticals) in various wastewater (e.g., molasses, piggery and municipal wastewater) can be removed effectively by fungal-algal system on a lab scale. Considering the complexity of polluted water, the removal of diverse pollutants in aquatic environment should also be widely concerned during the application of co-cultivation technology. Even at relatively low concentrations, heavy metals are toxic, because of their different valences, metabolic profiles and lethal dosages. In a recent review [142], the removal efficiency of particular heavy metals as well as the microalgal detoxification mechanisms have been summarized in detail. Since the major mechanism is biosorption, it suggests that microalgal cells can accumulate excessive heavy metals after treatment. In order to avoid secondary pollution, co-cultivated algae should be harvested as soon as possible. However, under abiotic stress conditions which include non-lethal nitrate, salinity and heavy metals, microalgae also have a potential in accumulating intracellular lipids [91,143–145]. In other words, there is no definite conclusion on whether biomass is polluted or accumulated. Based on previous studies on co-cultivation of algae with fungi, it would be significant to explore the relationship between bioremediation efficiency and harvested biomass quality. In addition, microalgae are proved to have ability in removing organic micro-pollutants, such as pharmaceutical pollutants, antibiotics, steroidal hormones and other emerging contaminants [116, 146,147]. Perhaps, these emerging micro-pollutants might also contaminate biomass to some extent and cause secondary pollution. Few researches about co-cultivated mode have been involved in the removal of organic micro-pollutants, and thus further work should focus on the efficiency and mechanism regarding the bioremediation of various types of emerging pollutants in the fungal-algal system. Once the practical performances, efficiencies and mechanisms are explored in detail and the possible interferences can be removed effectively, the fungal-algal cultivation for multiple wastewater treatment will become more feasible on a large scale.

5.4. Exploration of integrated biorefinery

Although microalgae and filamentous fungi has been used to separately produce various bioproducts, the integration of fungal-algal biorefinery in a sustainable mode, where the total biomass is converted into a wide range of materials, biochemicals, and bioenergy products with the emission of less wastes, remains to be explored. The economic feasibility is still the biggest barrier to limit the commercial application of microalgae-based technology on a large scale. The selection of highly efficient microbial strains, optimization of co-cultivation system with easy-controlled conditions and utilization of cheap nutrient sources from industrial leftovers have potential to make large-scale biofuel production from algae and fungi economically feasible. Moreover, the improvement of subsequent extraction and purification process as well as the multiple utilizations from diverse components in harvested biomass are the key concerns to achieve milestones in biorefinery directions. According to the study on an innovative roadmap for biorefinery [148], some of the ingredients in algal cells could be extracted to produce biofuels, while others could be converted into value-added byproducts, such as nutritious feed, cosmetics and pharmaceuticals. Likewise, in order to establish energy efficient and cost effective production mode, Troiano et al. [149] suggested to reduce, recycle and reuse waste components from algal bioenergy processing. From the sustainable perspective, the future direction in co-cultivation of microalgae with fungi also needs to focus on the development of integrated biorefinery. For instance, after the extraction of lipids from *Chlorella vulgaris* biomass, crude enzymes from fungi *Doratomyces nanus* can degrade the rest of algal biomass and achieve the saccharification efficiency of 76% without any pretreatment [150]. Through the combination of multiple utilizations, the leftovers after the value-added product processing can be converted into other co-products. More importantly,

filamentous fungi can produce a series of extracellular enzymes that can be used to break down biomass into available constituents. From technical and economic aspects, Troiano et al. [151] demonstrated the competitiveness of fungal biorefinery, which was also involved with the production of fungal enzymes and other biochemical products. Although the promising schemes of integrated biorefinery has presented, the current technologies of downstream processing such as drying and extraction are still hard to be applied on a large scale, which are associated with nearly 50–60% of the total costs. Meanwhile, key parameters need to be optimized separately for diverse biomass water contents, catalyst selection, co-solvent utilization, reaction time and waste recycling [152]. Considering the advantages of co-cultivation process, the exploration of fungal-algal interactions may better help to improve the feasibility in technological and commercial aspects, which can facilitate the refinement of multiple end products. In general, the disruption of the rigid cell wall of microalgae is an essential step to release the intracellular compounds such as lipids, carbohydrates and proteins for further processing [153]. Compared with commercial enzymes, raw enzymes from fungal secretions not only have a potential to cut down the cost of pellet processing, but also can function as co-cultured byproducts to be utilized effectively. However, few studies have systematically evaluated the functions of fungal enzymes in co-pellets, and future work on this co-cultivation technology should combine with multiple utilizations in a sustainable biorefinery mode. Overall, the key conclusions and suggestions in this part have provided the outlooks in co-cultivation of microalgae with filamentous fungi, and more specific details need to be confirmed for achieving efficient harvesting, wastewater treatment and biofuel production.

6. Conclusions

The review systematically summarizes current technologies on the co-cultivation of microalgae with filamentous fungi for biomass harvesting, wastewater treatment and subsequent biofuel production. Both fungal spores-assisted and pellet-assisted modes for microalgae harvesting can obtain high efficiency with the total biomass accumulation due to the mutual interactions. The potential mechanisms involved in the harvesting process as well as the affecting factors are critically discussed, which helps provide a unique understanding to promote the development in prospective applications. Meanwhile, wastewater serves as an abundant secondary source to displace culture medium, since it is rich in available nutrients. Therefore, the co-cultivation of microalgae with fungi has an ability to simultaneously achieve the dual purposes of pollutants removal and total biomass accumulation from recycled wastes. Most importantly, the advantages of co-cultivating algae with fungi for biofuel production are illustrated critically, whilst the corresponding effects with performances are discussed as well. Nonetheless, continuous research activities are indispensable to achieve the co-cultivation technology in large-scale harvesting, removal of multiple pollutants and development of integrated biorefineries. In addition, policy implementation is considered to be the basis for the large-scale launch of traditional biofuels, and similarly, systematic policy support is also an urgently needed agenda to ensure the sustainable development of microalgae-based biofuel.

CRedit author statement

Ruoyu Chu and Shuangxi Li contributed equally to this paper, writing the original draft and review. Liandong Zhu developed the concept and listed frames. Zhihong Yin and Dan Hu gave some important comments while Chenchen Liu and Fan Mo made some modifications to the tables.

Declaration of competing interest

The authors declare that they have no known competing financial

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References

- [1] Zhu L, Nugroho YK, Shakeel SR, Li Z, Martinkauppi B, Hiltunen E. Using microalgae to produce liquid transportation biodiesel: what is next? *Renew Sustain Energy Rev* 2017;78:391–400. <https://doi.org/10.1016/j.rser.2017.04.089>.
- [2] Ferreira GF, Ríos Pinto LF, Maciel Filho R, Fregolente LV. A review on lipid production from microalgae: association between cultivation using waste streams and fatty acid profiles. *Renew Sustain Energy Rev* 2019;109:448–66. <https://doi.org/10.1016/j.rser.2019.04.052>.
- [3] Jankowska E, Sahu AK, Oleskiewicz-Popiel P. Biogas from microalgae: review on microalgae's cultivation, harvesting and pretreatment for anaerobic digestion. *Renew Sustain Energy Rev* 2017;75:692–709. <https://doi.org/10.1016/j.rser.2016.11.045>.
- [4] Zhu L, Li S, Hu T, Nugroho YK, Yin Z, Hu D, et al. Effects of nitrogen source heterogeneity on nutrient removal and biodiesel production of mono- and mixed-cultured microalgae. *Energy Convers Manag* 2019;201:112144. <https://doi.org/10.1016/j.enconman.2019.112144>.
- [5] Mubarak M, Shaija A, Suchithra TV. Flocculation: an effective way to harvest microalgae for biodiesel production. *J Environ Chem Eng* 2019;7:103221. <https://doi.org/10.1016/j.jece.2019.103221>.
- [6] Singh G, Patidar SK. Microalgae harvesting techniques: a review. *J Environ Manag* 2018;217:499–508. <https://doi.org/10.1016/j.jenvman.2018.04.010>.
- [7] Zhu L, Hu T, Li S, Nugroho YK, Li B, Cao J, et al. Effects of operating parameters on algae *Chlorella vulgaris* biomass harvesting and lipid extraction using metal sulfates as flocculants. *Biomass Bioenergy* 2020;132:105433. <https://doi.org/10.1016/j.biombioe.2019.105433>.
- [8] Ge S, Champagne P. Nutrient removal, microalgal biomass growth, harvesting and lipid yield in response to centrate wastewater loadings. *Water Res* 2016;88:604–12. <https://doi.org/10.1016/j.watres.2015.10.054>.
- [9] Roy M, Mohanty K. Valorization of waste eggshell-derived bioflocculant for harvesting *T. obliquus*: process optimization, kinetic studies and recyclability of the spent medium for circular bioeconomy. Elsevier Ltd; 2020. <https://doi.org/10.1016/j.biortech.2020.123205>.
- [10] Rwehumbiza VM, Harrison R, Thomsen L. Alum-induced flocculation of pre-concentrated *Nannochloropsis salina*: residual aluminium in the biomass, FAMES and its effects on microalgae growth upon media recycling. *Chem Eng J* 2012;200–202:168–75. <https://doi.org/10.1016/j.cej.2012.06.008>.
- [11] Alam A, Vandamme D, Chun W, Zhao X, Foubert I, Wang Z, et al. Bioflocculation as an innovative harvesting strategy for microalgae. *Rev Environ Sci Biotechnol* 2016;15:573–83. <https://doi.org/10.1007/s11157-016-9408-8>.
- [12] Zhang J, Hu B. A novel method to harvest microalgae via co-culture of filamentous fungi to form cell pellets. *Bioresour Technol* 2012;114:529–35. <https://doi.org/10.1016/j.biortech.2012.03.054>.
- [13] Zhao Y, Guo G, Sun S, Hu C, Liu J. Co-pelletization of microalgae and fungi for efficient nutrient purification and biogas upgrading. *Bioresour Technol* 2019;289. <https://doi.org/10.1016/j.biortech.2019.121656>.
- [14] Gultom SO, Hu B. Review of microalgae harvesting via co-pelletization with filamentous fungus. *Energies* 2013;6:5921–39. <https://doi.org/10.3390/en6115921>.
- [15] Wan C, Alam MA, Zhao XQ, Zhang XY, Guo SL, Ho SH, et al. Current progress and future prospect of microalgal biomass harvest using various flocculation technologies. *Bioresour Technol* 2015;184:251–7. <https://doi.org/10.1016/j.biortech.2014.11.081>.
- [16] Chen J, Leng L, Ye C, Lu Q, Addy M, Wang J, et al. A comparative study between fungal pellet- and spore-assisted microalgae harvesting methods for algae bioflocculation. *Bioresour Technol* 2018;259:181–90. <https://doi.org/10.1016/j.biortech.2018.03.040>.
- [17] Miranda AF, Taha M, Wrede D, Morrison P, Ball AS, Stevenson T, et al. Lipid production in association of filamentous fungi with genetically modified cyanobacterial cells. *Biotechnol Biofuels* 2015;8:1–18. <https://doi.org/10.1186/s13068-015-0364-2>.
- [18] Zhu L, Wang Z, Shu Q, Takala J, Hiltunen E, Feng P, et al. Nutrient removal and biodiesel production by integration of freshwater algae cultivation with piggy wastewater treatment. *Water Res* 2013;47:4294–302. <https://doi.org/10.1016/j.watres.2013.05.004>.
- [19] Yang L, Li H, Wang Q. A novel one-step method for oil-rich biomass production and harvesting by co-cultivating microalgae with filamentous fungi in molasses wastewater. *Bioresour Technol* 2019;275:35–43. <https://doi.org/10.1016/j.biortech.2018.12.036>.
- [20] Wrede D, Taha M, Miranda AF, Kadali K, Stevenson T, Ball AS, et al. Co-cultivation of fungal and microalgal cells as an efficient system for harvesting microalgal cells, lipid production and wastewater treatment. *PloS One* 2014;9. <https://doi.org/10.1371/journal.pone.0113497>.
- [21] Krull R, Wucherpfennig T, Efsandabadi ME, Walisko R, Melzer G, Hempel DC, et al. Characterization and control of fungal morphology for improved production performance in biotechnology. *J Biotechnol* 2013;163:112–23. <https://doi.org/10.1016/j.jbiotec.2012.06.024>.
- [22] Espinosa-Ortiz EJ, Rene ER, Pakshirajan K, van Hullebusch ED, Lens PNL. Fungal pelleted reactors in wastewater treatment: applications and perspectives. *Chem Eng J* 2016;283:553–71. <https://doi.org/10.1016/j.cej.2015.07.068>.
- [23] Yu L, Chao Y, Wensel P, Chen S. Hydrodynamic and kinetic study of cellulase production by *Trichoderma reesei* with pellet morphology. *Biotechnol Bioeng* 2012;109:1755–68. <https://doi.org/10.1002/bit.24433>.
- [24] Yu T, Wang L, Ma F, Yang J, Bai S, You J. Self-immobilized biomixture with pellets of *Aspergillus Niger* Y3 and *Arthrobacter* sp ZXY-2 to remove atrazine in water: a bio-functions integration system. *Sci Total Environ* 2019;689:875–82. <https://doi.org/10.1016/j.scitotenv.2019.06.313>.
- [25] Wang X, Bao K, Cao W, Zhao Y, Hu CW. Screening of microalgae for integral biogas slurry nutrient removal and biogas upgrading by different microalgae cultivation technology. *Sci Rep* 2017;7:1–12. <https://doi.org/10.1038/s41598-017-05841-9>.
- [26] Hom-Díaz A, Jaén-Gil A, Bello-Laserna I, Rodríguez-Mozas S, Vicent T, Barceló D, et al. Performance of a microalgal photobioreactor treating toilet wastewater: pharmaceutically active compound removal and biomass harvesting. *Sci Total Environ* 2017;592:1–11. <https://doi.org/10.1016/j.scitotenv.2017.02.224>.
- [27] Pirt SJ. A theory of the mode of growth of fungi in the form of pellets in submerged culture. *Proc R Soc Lond Ser B Biol Sci* 1966;166:369–73. <https://doi.org/10.1098/rspb.1966.0105>.
- [28] Nielsen J, Johansen CL, Jacobsen M, Krabben P, Villadsen J. pellet formation and fragmentation in submerged cultures of *Penicillium chrysogenum* and its relation to penicillin production. *Biotechnol Prog* 1995;11:93–8. <https://doi.org/10.1021/bp00031a013>.
- [29] Zhang J, Zhang J. The filamentous fungal pellet and forces driving its formation. *Crit Rev Biotechnol* 2016;36:1066–77. <https://doi.org/10.3109/07388551.2015.1084262>.
- [30] Veiter L, Rajamanickam V, Herwig C. The filamentous fungal pellet—relationship between morphology and productivity. *Appl Microbiol Biotechnol* 2018;102:2997–3006. <https://doi.org/10.1007/s00253-018-8818-7>.
- [31] Ummalyma SB, Gnansounou E, Sukumaran RK, Sindhu R, Pandey A, Sahoo D. Bioflocculation: an alternative strategy for harvesting of microalgae - an overview. *Bioresour Technol* 2017;242:227–35. <https://doi.org/10.1016/j.biortech.2017.02.097>.
- [32] Prajapati SK, Kumar P, Malik A, Choudhary P. Exploring pellet forming filamentous fungi as tool for harvesting non-flocculating unicellular microalgae. *Bioenergy Res* 2014;7:1430–40. <https://doi.org/10.1007/s12155-014-9481-1>.
- [33] Yuan H, Zhang X, Jiang Z, Wang X, Chen X, Cao L, et al. Analyzing the effect of pH on microalgal adhesion by identifying the dominant interaction between cell and surface. *Colloids Surf B Biointerfaces* 2019;177:479–86. <https://doi.org/10.1016/j.colsurfb.2019.02.023>.
- [34] Ozkan A, Berberoglu H. Physico-chemical surface properties of microalgae. *Colloids Surf B Biointerfaces* 2013;112:287–93. <https://doi.org/10.1016/j.colsurfb.2013.08.001>.
- [35] Ndikubwimana T, Zeng X, He N, Xiao Z, Xie Y, Chang JS, et al. Microalgae biomass harvesting by bioflocculation-interpretation by classical DLVO theory. *Biochem Eng J* 2015;101:160–7. <https://doi.org/10.1016/j.bej.2015.05.010>.
- [36] Zheng Y, Huang Y, Xia A, Qian F, Wei C. A rapid inoculation method for microalgae biofilm cultivation based on microalgae-microalgae co-flocculation and zeta-potential adjustment. *Bioresour Technol* 2019;278:272–8. <https://doi.org/10.1016/j.biortech.2019.01.083>.
- [37] Liaud N, Giniés C, Navarro D, Fabre N, Crapart S, Gimbert IH, et al. Exploring fungal biodiversity: organic acid production by 66 strains of filamentous fungi. *Fungal Biol Biotechnol* 2014;1:1–10. <https://doi.org/10.1186/s40694-014-0001-z>.
- [38] Yang L, Lübeck M, Lübeck PS. *Aspergillus* as a versatile cell factory for organic acid production. *Fungal Biol Rev* 2017;31:33–49. <https://doi.org/10.1016/j.fbr.2016.11.001>.
- [39] Bhattacharya A, Mathur M, Kumar P, Prajapati SK, Malik A. A rapid method for fungal assisted algal flocculation: critical parameters & mechanism insights. *Algal Res* 2017;21:42–51. <https://doi.org/10.1016/j.algal.2016.10.022>.
- [40] Pugazhendhi A, Shobana S, Bakonyi P, Nemestóthy N, Xia A, Banu JR. A review on chemical mechanism of microalgae flocculation via polymers. *Biotechnol Reports* 2019;21:e00302. <https://doi.org/10.1016/j.btre.2018.e00302>.
- [41] Yin Z, Zhu L, Li S, Hu T, Chu R, Mo F, et al. A comprehensive review on cultivation and harvesting of microalgae for biodiesel production: environmental pollution control and future directions. *Bioresour Technol* 2020;301:122804. <https://doi.org/10.1016/J.BIORTECH.2020.122804>.
- [42] Zamalloa C, Gultom SO, Rajendran A, Hu B. Ionic effects on microalgae harvest via microalgae-fungi co-pelletization. *Biocatal Agric Biotechnol* 2017;9:145–55. <https://doi.org/10.1016/j.bcab.2016.12.007>.
- [43] Ogawa M, Bisson LF, García-Martínez T, Mauricio JC, Moreno-García J. New insights on yeast and filamentous fungus adhesion in a natural co-immobilization system: proposed advances and applications in wine industry. *Appl Microbiol Biotechnol* 2019;103:4723–31. <https://doi.org/10.1007/s00253-019-09870-4>.
- [44] Li Y, Xu Y, Liu L, Li P, Yan Y, Chen T, et al. Flocculation mechanism of *Aspergillus Niger* on harvesting of *Chlorella vulgaris* biomass. *Algal Res* 2017;25:402–12. <https://doi.org/10.1016/j.algal.2017.06.001>.
- [45] Powell RJ, Hill RT. Mechanism of algal aggregation by *Bacillus* sp. strain RP1137. *Appl Environ Microbiol* 2014;80:4042–50. <https://doi.org/10.1128/AEM.00887-14>.

- [46] Kulkarni S, Nene S, Joshi K. Production of hydrophobins from fungi. *Process Biochem* 2017;61:1–11. <https://doi.org/10.1016/j.procbio.2017.06.012>.
- [47] Vergara-Fernández A, Revah S, Moreno-Casas P, Scott F. Biofiltration of volatile organic compounds using fungi and its conceptual and mathematical modeling. *Biotechnol Adv* 2018;36:1079–93. <https://doi.org/10.1016/j.biotechadv.2018.03.008>.
- [48] Fernandes S, Simões LC, Lima N, Simões M. Adhesion of filamentous fungi isolated from drinking water under different process conditions. *Water Res* 2019;164. <https://doi.org/10.1016/j.watres.2019.114951>.
- [49] Wessels JGH. Developmental regulation of fungal cell wall formation. *Annu Rev Phytopathol* 1994;32:413–37. <https://doi.org/10.1146/annurev.py.32.090194.002213>.
- [50] Wösten HAB, Scholtmeijer K. Applications of hydrophobins: current state and perspectives. *Appl Microbiol Biotechnol* 2015;99:1587–97. <https://doi.org/10.1007/s00253-014-6319-x>.
- [51] Zampieri F, Wösten HAB, Scholtmeijer K. Creating surface properties using a palette of hydrophobins. *Materials* 2010;3:4607–25. <https://doi.org/10.3390/ma3094607>.
- [52] Garg S, Wang L, Schenk PM. Effective harvesting of low surface-hydrophobicity microalgae by froth flotation. *Bioresour Technol* 2014;159:437–41. <https://doi.org/10.1016/j.biortech.2014.03.030>.
- [53] Zhang X, Yuan H, Jiang Z, Lin D, Zhang X. Impact of surface tension of wastewater on biofilm formation of microalgae *Chlorella* sp. *Bioresour Technol* 2018;266:498–506. <https://doi.org/10.1016/j.biortech.2018.06.082>.
- [54] Chen Ming-Ju, Kreuter JY-TK. Nanoparticles and microparticles for drug and vaccine delivery. *J Anat* 1996;189(Pt 3):503–5. <https://doi.org/10.1002/bit.10002>.
- [55] Garcia-Rubio R, de Oliveira HC, Rivera J, Trevijano-Contador N. The fungal cell wall: *Candida*, *Cryptococcus*, and *Aspergillus* species. *Front Microbiol* 2020;10:1–13. <https://doi.org/10.3389/fmicb.2019.02993>.
- [56] Talukder MMR, Das P, Wu JC. Immobilization of microalgae on exogenous fungal mycelium: a promising separation method to harvest both marine and freshwater microalgae. *Biochem Eng J* 2014;91:53–7. <https://doi.org/10.1016/j.bej.2014.07.001>.
- [57] Binupriya AR, Sathishkumar M, Swaminathan K, Kuz CS, Yun SE. Comparative studies on removal of Congo red by native and modified mycelial pellets of *Trametes versicolor* in various reactor modes. *Bioresour Technol* 2008;99:1080–8. <https://doi.org/10.1016/j.biortech.2007.02.022>.
- [58] Li Y, Xu Y, Zheng T, Wang H. Flocculation mechanism of the actinomycete *Streptomyces* sp. hsn06 on *Chlorella vulgaris*. *Bioresour Technol* 2017;239:137–43. <https://doi.org/10.1016/j.biortech.2017.05.028>.
- [59] Li J, Jiang Z, Chen S, Wang T, Jiang L, Wang M, et al. Biochemical changes of polysaccharides and proteins within EPS under Pb(II) stress in *Rhodotorula mucilaginosa*. *Ecotoxicol Environ Saf* 2019;174:484–90. <https://doi.org/10.1016/j.ecoenv.2019.03.004>.
- [60] Bhattacharya A, Malik A, Malik HK. A mathematical model to describe the fungal assisted flocculation process. *Bioresour Technol* 2017;244:975–81. <https://doi.org/10.1016/j.biortech.2017.08.062>.
- [61] Vandamme D, Foubert I, Muylaert K. Flocculation as a low-cost method for harvesting microalgae for bulk biomass production. *Trends Biotechnol* 2013;31:233–9. <https://doi.org/10.1016/j.tibtech.2012.12.005>.
- [62] Wang JH, Zhuang LL, Xu XQ, Deantes-Espinosa VM, Wang XX, Hu HY. Microalgal attachment and attached systems for biomass production and wastewater treatment. *Renew Sustain Energy Rev* 2018;92:331–42. <https://doi.org/10.1016/j.rser.2018.04.081>.
- [63] Muradov N, Taha M, Miranda AF, Wrede D, Kadali K, Gujar A, et al. Fungal-assisted algal flocculation: application in wastewater treatment and biofuel production. *Biotechnol Biofuels* 2015;8. <https://doi.org/10.1186/s13068-015-0210-6>.
- [64] Zhou W, Min M, Hu B, Ma X, Liu Y, Wang Q, et al. Filamentous fungi assisted bio-flocculation: a novel alternative technique for harvesting heterotrophic and autotrophic microalgal cells. *Separ Purif Technol* 2013;107:158–65. <https://doi.org/10.1016/j.seppur.2013.01.030>.
- [65] Alrubaie G, Al-Shammari RHH. Microalgal *Chlorella vulgaris* harvesting via co-pelletization with filamentous fungus. *Baghdad Sci J* 2018;15:31–6. <https://doi.org/10.21123/bsj.2018.15.1.0031>.
- [66] Li T, Jiang L, Hu Y, Paul JT, Zuniga C, Zengler K, et al. Creating a synthetic lichen: mutualistic co-culture of fungi and extracellular polysaccharide-secreting cyanobacterium *Nostoc PCC 7413*. *Algal Res* 2020;45:101755. <https://doi.org/10.1016/j.algal.2019.101755>.
- [67] Leong WH, Lim JW, Lam MK, Uemura Y, Ho YC. Third generation biofuels: a nutritional perspective in enhancing microbial lipid production. *Renew Sustain Energy Rev* 2018;91:950–61. <https://doi.org/10.1016/j.rser.2018.04.066>.
- [68] Gultom SO, Zamalloa C, Hu B. Microalgae harvest through fungal pelletization - Co-culture of *Chlorella vulgaris* and *Aspergillus Niger*. *Energies* 2014;7:4417–29. <https://doi.org/10.3390/en7074417>.
- [69] Dash A, Banerjee R. Enhanced biodiesel production through phyco-mycocultivation of *Chlorella minutissima* and *Aspergillus awamori*: an integrated approach. *Bioresour Technol* 2017;238:502–9. <https://doi.org/10.1016/j.biortech.2017.04.039>.
- [70] Muniraj IK, Xiao L, Hu Z, Zhan X, Shi J. Microbial lipid production from potato processing wastewater using oleaginous filamentous fungi *Aspergillus oryzae*. *Water Res* 2013;47:3477–83. <https://doi.org/10.1016/j.watres.2013.03.046>.
- [71] Oliveira HR, Bassin ID, Cammarota MC. Bioflocculation of cyanobacteria with pellets of *Aspergillus Niger*: effects of carbon supplementation, pellet diameter, and other factors in biomass densification. *Bioresour Technol* 2019;294:122167. <https://doi.org/10.1016/j.biortech.2019.122167>.
- [72] Renuka N, Guldhe A, Prasanna R, Singh P, Bux F. Microalgae as multi-functional options in modern agriculture: current trends, prospects and challenges. *Biotechnol Adv* 2018;36:1255–73. <https://doi.org/10.1016/j.biotechadv.2018.04.004>.
- [73] Metz B, Kossen NWF. The growth of molds in the form of pellets—a literature review. *Biotechnol Bioeng* 1977;19:781–99. <https://doi.org/10.1002/bit.260190602>.
- [74] Al-Hothaly KA, Adetutu EM, Taha M, Fabbri D, Lorenzetti C, Conti R, et al. Bio-harvesting and pyrolysis of the microalgae *Botryococcus braunii*. *Bioresour Technol* 2015;191:117–23. <https://doi.org/10.1016/j.biortech.2015.04.113>.
- [75] Letelier-Gordo CO, Holdt SL, De Francisci D, Karakashev DB, Angelidaki I. Effective harvesting of the microalgal *Chlorella* proteocholeoides via bioflocculation with cationic starch. *Bioresour Technol* 2014;167:214–8. <https://doi.org/10.1016/j.biortech.2014.06.014>.
- [76] Supramaniam U, Lam MK, Uemura Y, Shuit SH, Lim JW, Show PL, et al. Flocculation of *Chlorella vulgaris* by shell waste-derived bioflocculants for biodiesel production: process optimization, characterization and kinetic studies. *Sci Total Environ* 2020;702:134995. <https://doi.org/10.1016/j.scitotenv.2019.134995>.
- [77] Suthar S, Verma R. Production of *Chlorella vulgaris* under varying nutrient and abiotic conditions: a potential microalga for bioenergy feedstock. *Process Saf Environ Protect* 2018;113:141–8. <https://doi.org/10.1016/j.psep.2017.09.018>.
- [78] Kothari R, Pathak VV, Pandey A, Ahmad S, Srivastava C, Tyagi VV. A novel method to harvest *Chlorella* sp. via low cost bioflocculant: influence of temperature with kinetic and thermodynamic functions. *Bioresour Technol* 2017;225:84–9. <https://doi.org/10.1016/j.biortech.2016.11.050>.
- [79] Mackay S, Gomes E, Holliger C, Bauer R, Schwitzgebel JP. Harvesting of *Chlorella sorokiniana* by co-culture with the filamentous fungus *Isaria fumosorosea*: a potential sustainable feedstock for hydrothermal gasification. *Bioresour Technol* 2015;185:353–61. <https://doi.org/10.1016/j.biortech.2015.03.026>.
- [80] Xie S, Sun S, Dai SY, Yuan J S. Efficient coagulation of microalgae in cultures with filamentous fungi. *Algal Res* 2013;2:28–33. <https://doi.org/10.1016/j.algal.2012.11.004>.
- [81] Li S, Yu H, Liu Y, Zhang X, Ma F. The lipid strategies in *Cunninghamella echinulata* for an allostatic response to temperature changes. *Process Biochem* 2019;76:85–94. <https://doi.org/10.1016/j.procbio.2018.11.005>.
- [82] Zhao Y, Wang HP, Han B, Yu X. Coupling of abiotic stresses and phytohormones for the production of lipids and high-value by-products by microalgae: a review. *Bioresour Technol* 2019;274:549–56. <https://doi.org/10.1016/j.biortech.2018.12.030>.
- [83] Ndikubwimana T, Zeng X, Liu Y, Chang JS, Lu Y. Harvesting of microalgae *Desmodesmus* sp. F51 by bioflocculation with bacterial bioflocculant. *Algal Res* 2014;6:186–93. <https://doi.org/10.1016/j.algal.2014.09.004>.
- [84] Rosa M, Ward JE, Holohan BA, Shumway SE, Wikfors GH. Physicochemical surface properties of microalgae and their combined effects on particle selection by suspension-feeding bivalve molluscs. *J Exp Mar Biol Ecol* 2017;486:59–68. <https://doi.org/10.1016/j.jembe.2016.09.007>.
- [85] Mohd Nasir N, Mohd Yunus FH, Wan Jusoh HH, Mohammad A, Lam SS, Jusoh A. Subtopic: advances in water and wastewater treatment harvesting of *Chlorella* sp. microalgae using *Aspergillus Niger* as bio-flocculant for aquaculture wastewater treatment. *J Environ Manag* 2019;249:109373. <https://doi.org/10.1016/j.jenvman.2019.109373>.
- [86] Olmos E, Mehmood N, Haj Husein L, Goergen JL, Fick M, Delaunay S. Effects of bioreactor hydrodynamics on the physiology of *Streptomyces*. *Bioproc Biosyst Eng* 2013;36:259–72. <https://doi.org/10.1007/s00449-012-0794-1>.
- [87] Papagianni M. Fungal morphology and metabolite production in submerged mycelial processes. *Biotechnol Adv* 2004;22:189–259. <https://doi.org/10.1016/j.biotechadv.2003.09.005>.
- [88] Cui YQ, Van Der Lans RGM, Luyben KCAM. Effect of agitation intensities on fungal morphology of submerged fermentation. *Biotechnol Bioeng* 1997;55:715–26. [https://doi.org/10.1002/\(SICI\)1097-0290\(19970905\)55:5<715::AID-BTT2>3.0.CO;2-E](https://doi.org/10.1002/(SICI)1097-0290(19970905)55:5<715::AID-BTT2>3.0.CO;2-E).
- [89] Xia C, Zhang J, Zhang W, Hu B. A new cultivation method for microbial oil production: cell pelletization and lipid accumulation by *Mucor circinelloides*. *Biotechnol Biofuels* 2011;4:1–10. <https://doi.org/10.1186/1754-6834-4-15>.
- [90] Pradhan D, Sukla LB, Mishra BB, Devi N. Biosorption for removal of hexavalent chromium using microalgae *Scenedesmus* sp. *J Clean Prod* 2019;209:617–29. <https://doi.org/10.1016/j.jclepro.2018.10.288>.
- [91] Yang J, Cao J, Xing G, Yuan H. Lipid production combined with biosorption and bioaccumulation of cadmium, copper, manganese and zinc by oleaginous microalgal *Chlorella minutissima* UTEX2341. *Bioresour Technol* 2015;175:537–44. <https://doi.org/10.1016/j.biortech.2014.10.124>.
- [92] Osadolor OA, Jabbari M, Nair RB, Lennartsson PR, Taherzadeh MJ. Effect of media rheology and bioreactor hydrodynamics on filamentous fungi fermentation of lignocellulosic and starch-based substrates under pseudoplastic flow conditions. *Bioresour Technol* 2018;263:250–7. <https://doi.org/10.1016/j.biortech.2018.04.093>.
- [93] Osadolor OA, Nair RB, Lennartsson PR, Taherzadeh MJ. Empirical and experimental determination of the kinetics of pellet growth in filamentous fungi: a case study using *Neurospora intermedia*. *Biochem Eng J* 2017;124:115–21. <https://doi.org/10.1016/j.bej.2017.05.012>.
- [94] Cao W, Wang X, Sun S, Hu C, Zhao Y. Simultaneously upgrading biogas and purifying biogas slurry using cocultivation of *Chlorella vulgaris* and three different fungi under various mixed light wavelength and photoperiods. *Bioresour Technol* 2017;241:701–9. <https://doi.org/10.1016/j.biortech.2017.05.194>.

- [95] Sui Y, Jiang Y, Moretti M, Vlaeminck SE. Harvesting time and biomass composition affect the economics of microalgae production. *J Clean Prod* 2020; 259:120782. <https://doi.org/10.1016/j.jclepro.2020.120782>.
- [96] Nasir NM, Bakar NSA, Lananan F, Abdul Hamid SH, Lam SS, Jusoh A. Treatment of African catfish, *Clarias gariepinus* wastewater utilizing phytoremediation of microalgae, *Chlorella* sp. with *Aspergillus Niger* bio-harvesting. *Bioresour Technol* 2015;190:492–8. <https://doi.org/10.1016/j.biortech.2015.03.023>.
- [97] Luo S, Wu X, Jiang H, Yu M, Liu Y, Min A, et al. Edible fungi-assisted harvesting system for efficient microalgae bio-flocculation. *Bioresour Technol* 2019;282: 325–30. <https://doi.org/10.1016/j.biortech.2019.03.033>.
- [98] Choi YN, Cho HU, Utomo JC, Shin DY, Kim HK, Park JM. Efficient harvesting of *Synechocystis* sp. PCC 6803 with filamentous fungal pellets. *J Appl Phycol* 2016; 28:2225–31. <https://doi.org/10.1007/s10811-015-0787-y>.
- [99] Srinuanpan S, Chawpraknoi A, Chantarit S, Cheirsilp B, Prasertsan P. A rapid method for harvesting and immobilization of oleaginous microalgae using pellet-forming filamentous fungi and the application in phytoremediation of secondary effluent. *Int J Phytoremediation* 2018;20:1017–24. <https://doi.org/10.1080/15226514.2018.1452187>.
- [100] Prajapati SK, Bhattacharya A, Kumar P, Malik A, Vijay VK. A method for simultaneous bioflocculation and pretreatment of algal biomass targeting improved methane production. *Green Chem* 2016;18:5230–8. <https://doi.org/10.1039/c6gc01483f>.
- [101] Srinuanpan S, Cheirsilp B, Prasertsan P, Kato Y, Asano Y. Photoautotrophic cultivation of oleaginous microalgae and co-pelletization with filamentous fungi for cost-effective harvesting process and improved lipid yield. *Aquacult Int* 2018; 26:1493–509. <https://doi.org/10.1007/s10499-018-0300-0>.
- [102] Zhou W, Cheng Y, Li Y, Wan Y, Liu Y, Lin X, et al. Novel fungal pelletization-assisted technology for algae harvesting and wastewater treatment. *Appl Biochem Biotechnol* 2012;167:214–28. <https://doi.org/10.1007/s12010-012-9667-y>.
- [103] Sutherland DL, Howard-Williams C, Turnbull MH, Broady PA, Craggs RJ. Enhancing microalgal photosynthesis and productivity in wastewater treatment high rate algal ponds for biofuel production. *Bioresour Technol* 2015;184:222–9. <https://doi.org/10.1016/j.biortech.2014.10.074>.
- [104] Abinandan S, Subashchandrabose SR, Venkateswarlu K, Megharaj M. Microalgae-bacteria biofilms: a sustainable synergistic approach in remediation of acid mine drainage. *Appl Microbiol Biotechnol* 2018;102:1131–44. <https://doi.org/10.1007/s00253-017-8693-7>.
- [105] Sutherland DL, Heubeck S, Park J, Turnbull MH, Craggs RJ. Seasonal performance of a full-scale wastewater treatment enhanced pond system. *Water Res* 2018;136: 150–9. <https://doi.org/10.1016/j.watres.2018.02.046>.
- [106] Guo P, Zhang Y, Zhao Y. Biocapture of CO₂ by different microalgal-based technologies for biogas upgrading and simultaneous biogas slurry purification under various light intensities and photoperiods. *Int J Environ Res Publ Health* 2018;15. <https://doi.org/10.3390/ijerph15030528>.
- [107] Zhao C, Xie S, Pu Y, Zhang R, Huang F, Ragauskas AJ, et al. Synergistic enzymatic and microbial lignin conversion. *Green Chem* 2016;18:1306–12. <https://doi.org/10.1039/C5GC01955A>.
- [108] Santos FM, Mazur LP, Mayer DA, Vilar VJP, Pires JCM. Inhibition effect of zinc, cadmium, and nickel ions in microalgal growth and nutrient uptake from water: an experimental approach. *Chem Eng J* 2019;366:358–67. <https://doi.org/10.1016/j.cej.2019.02.080>.
- [109] Zhang C, Tao Y, Li S, Tian J, Ke T, Wei S, et al. Simultaneous degradation of trichlorfon and removal of Cd(II) by *Aspergillus sydowii* strain PA F-2. *Environ Sci Pollut Res* 2019;26:26844–54. <https://doi.org/10.1007/s11356-019-05811-w>.
- [110] Li F, Wang W, Li C, Zhu R, Ge F, Zheng Y, et al. Self-mediated pH changes in culture medium affecting bioadsorption and biomineralization of Cd²⁺ by *Bacillus cereus* Cd01. *J Hazard Mater* 2018;358:178–86. <https://doi.org/10.1016/j.jhazmat.2018.06.066>.
- [111] Leong YK, Chang JS. Bioremediation of heavy metals using microalgae: recent advances and mechanisms. *Bioresour Technol* 2020;303:122886. <https://doi.org/10.1016/j.biortech.2020.122886>.
- [112] Urrutia C, Yañez-Mansilla E, Jeison D. Bioremoval of heavy metals from metal mine tailings water using microalgae biomass. *Algal Res* 2019;43:101659. <https://doi.org/10.1016/j.algal.2019.101659>.
- [113] Shen Y, Li H, Zhu W, Ho SH, Yuan W, Chen J, et al. Microalgal-biochar immobilized complex: a novel efficient biosorbent for cadmium removal from aqueous solution. *Bioresour Technol* 2017;244:1031–8. <https://doi.org/10.1016/j.biortech.2017.08.085>.
- [114] Shen L, Wang J, Li Z, Fan L, Chen R, Wu X, et al. A high-efficiency Fe₂O₃@Microalgae composite for heavy metal removal from aqueous solution. *J Water Process Eng* 2020;33:101026. <https://doi.org/10.1016/j.jwpe.2019.101026>.
- [115] Ibuot A, Dean AP, McIntosh OA, Pittman JK. Metal bioremediation by CrMTP4 over-expressing *Chlamydomonas reinhardtii* in comparison to natural wastewater-tolerant microalgae strains. *Algal Res* 2017;24:89–96. <https://doi.org/10.1016/j.algal.2017.03.002>.
- [116] Sutherland DL, Ralph PJ. Microalgal bioremediation of emerging contaminants - opportunities and challenges. *Water Res* 2019;164:114921. <https://doi.org/10.1016/j.watres.2019.114921>.
- [117] Cai T, Park SY, Li Y. Nutrient recovery from wastewater streams by microalgae: status and prospects. *Renew Sustain Energy Rev* 2013;19:360–9. <https://doi.org/10.1016/j.rser.2012.11.030>.
- [118] Abinandan S, Shanthakumar S. Challenges and opportunities in application of microalgae (Chlorophyta) for wastewater treatment: a review. *Renew Sustain Energy Rev* 2015;52:123–32. <https://doi.org/10.1016/j.rser.2015.07.086>.
- [119] Peccia J, Haznedaroglu B, Gutierrez J, Zimmerman JB. Nitrogen supply is an important driver of sustainable microalgae biofuel production. *Trends Biotechnol* 2013;31:134–8. <https://doi.org/10.1016/j.tibtech.2013.01.010>.
- [120] Guo G, Cao W, Sun S, Zhao Y, Hu C. Nutrient removal and biogas upgrading by integrating fungal-microalgal cultivation with anaerobically digested swine wastewater treatment. *J Appl Phycol* 2017;29:2857–66. <https://doi.org/10.1007/s10811-017-1207-2>.
- [121] Li S, Wang P, Zhang C, Zhou X, Yin Z, Hu T, et al. Influence of polystyrene microplastics on the growth, photosynthetic efficiency and aggregation of freshwater microalgae *Chlamydomonas reinhardtii*. *Sci Total Environ* 2020;714: 136767. <https://doi.org/10.1016/j.scitotenv.2020.136767>.
- [122] Lu Q, Zhou W, Min M, Ma X, Chandra C, Doan YTT, et al. Growing *Chlorella* sp. on meat processing wastewater for nutrient removal and biomass production. *Bioresour Technol* 2015;198:189–97. <https://doi.org/10.1016/j.biortech.2015.08.133>.
- [123] Bodin H, Daneshvar A, Gros M, Hultberg M. Effects of biopellets composed of microalgae and fungi on pharmaceuticals present at environmentally relevant levels in water. *Ecol Eng* 2016;91:169–72. <https://doi.org/10.1016/j.ecoeng.2016.02.007>.
- [124] Hultberg M, Bodin H. Effects of fungal-assisted algal harvesting through biopellet formation on pesticides in water. *Biodegradation* 2018;29:557–65. <https://doi.org/10.1007/s10532-018-9852-y>.
- [125] Li B, Zhang T, Yang Z. Immobilizing unicellular microalgae on pellet-forming filamentous fungus: can this provide new insights into the remediation of arsenic from contaminated water? *Bioresour Technol* 2019;284:231–9. <https://doi.org/10.1016/j.biortech.2019.03.128>.
- [126] Zhang C, Liu S, Li S, Tao Y, Wang P, Ma X, et al. Enhanced biosorption of Cu(II) by magnetic chitosan microspheres immobilized *Aspergillus sydowii* (MCMAs) from aqueous solution. *Colloids Surfaces A Physicochem Eng Asp* 2019;581:123813. <https://doi.org/10.1016/j.colsurfa.2019.123813>.
- [127] Bodin H, Asp H, Hultberg M. Effects of biopellets composed of microalgae and fungi on cadmium present at environmentally relevant levels in water. *Int J Phytoremediation* 2017;19:500–4. <https://doi.org/10.1080/15226514.2016.1244170>.
- [128] Zhou K, Zhang Y, Jia X. Co-cultivation of fungal-microalgal strains in biogas slurry and biogas purification under different initial CO₂ concentrations. *Sci Rep* 2018;8:1–12. <https://doi.org/10.1038/s41598-018-26141-w>.
- [129] Al-Hothaly KA. An optimized method for the bio-harvesting of microalgae, *Botryococcus braunii*, using *Aspergillus* sp. in large-scale studies. *Methods* 2018; 5:788–94. <https://doi.org/10.1016/j.mex.2018.07.010>.
- [130] Menegazzo ML, Fonseca GG. Biomass recovery and lipid extraction processes for microalgae biofuels production: a review. *Renew Sustain Energy Rev* 2019;107: 87–107. <https://doi.org/10.1016/j.rser.2019.01.064>.
- [131] Hom-Diaz A, Passos F, Ferrer I, Vicent T, Blázquez P. Enzymatic pretreatment of microalgae using fungal broth from *Trametes versicolor* and commercial laccase for improved biogas production. *Algal Res* 2016;19:184–8. <https://doi.org/10.1016/j.algal.2016.08.006>.
- [132] Ma Y, Gao Z, Wang Q, Liu Y. Biodiesels from microbial oils: opportunity and challenges. *Bioresour Technol* 2018;263:631–41. <https://doi.org/10.1016/j.biortech.2018.05.028>.
- [133] Carota E, Crognale S, D'Annibale A, Petruccioli M. Bioconversion of agro-industrial waste into microbial oils by filamentous fungi. *Process Saf Environ Protect* 2018;117:143–51. <https://doi.org/10.1016/j.psep.2018.04.022>.
- [134] Sajjadi B, Chen WY, Raman AAA, Ibrahim S. Microalgae lipid and biomass for biofuel production: a comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. *Renew Sustain Energy Rev* 2018;97: 200–32. <https://doi.org/10.1016/j.rser.2018.07.050>.
- [135] Prasad RK, Chatterjee S, Mazumder PB, Gupta SK, Sharma S, Vairale MG, et al. Bioethanol production from waste lignocelluloses: a review on microbial degradation potential. *Chemosphere* 2019;231:588–606. <https://doi.org/10.1016/j.chemosphere.2019.05.142>.
- [136] Suparmaniam U, Lam MK, Uemura Y, Lim JW, Lee KT, Shuit SH. Insights into the microalgae cultivation technology and harvesting process for biofuel production: a review. *Renew Sustain Energy Rev* 2019;115:109361. <https://doi.org/10.1016/j.rser.2019.109361>.
- [137] Li S, Hu T, Xu Y, Wang J, Chu R, Yin Z, et al. A review on flocculation as an efficient method to harvest energy microalgae: mechanisms, performances, influencing factors and perspectives. *Renew Sustain Energy Rev* 2020;131: 110005. <https://doi.org/10.1016/j.rser.2020.110005>.
- [138] Su Y, Zhang P, Su Y. An overview of biofuels policies and industrialization in the major biofuel producing countries. *Renew Sustain Energy Rev* 2015;50: 991–1003. <https://doi.org/10.1016/j.rser.2015.04.032>.
- [139] Patel A, Gami B, Patel P, Patel B. Microalgae: antiquity to era of integrated technology. *Renew Sustain Energy Rev* 2017;71:535–47. <https://doi.org/10.1016/j.rser.2016.12.081>.
- [140] Spagnuolo M, Yaguchi A, Blenner M. Oleaginous yeast for biofuel and oleochemical production. *Curr Opin Biotechnol* 2019;57:73–81. <https://doi.org/10.1016/j.copbio.2019.02.011>.
- [141] Park S, Nguyen THT, Jin ES. Improving lipid production by strain development in microalgae: strategies, challenges and perspectives. *Bioresour Technol* 2019;292: 121953. <https://doi.org/10.1016/j.biortech.2019.121953>.
- [142] Nagarajan D, Lee DJ, Chen CY, Chang JS. Resource recovery from wastewaters using microalgae-based approaches: a circular bioeconomy perspective. *Bioresour Technol* 2020;302:122817. <https://doi.org/10.1016/j.biortech.2020.122817>.
- [143] Ranadheer P, Kona R, Sreeharsha RV, Venkata Mohan S. Non-lethal nitrate supplementation enhances photosystem II efficiency in mixotrophic microalgae

- towards the synthesis of proteins and lipids. *Bioresour Technol* 2019;283:373–7. <https://doi.org/10.1016/j.biortech.2019.03.089>.
- [144] Abedini Najafabadi H, Malekzadeh M, Jalilian F, Vossoughi M, Pazuki G. Effect of various carbon sources on biomass and lipid production of *Chlorella vulgaris* during nutrient sufficient and nitrogen starvation conditions. *Bioresour Technol* 2015;180:311–7. <https://doi.org/10.1016/j.biortech.2014.12.076>.
- [145] Guo X, Wang P, Li Y, Zhong H, Li P, Zhang C, et al. Effect of copper on the removal of tetracycline from water by *Myriophyllum aquaticum*: performance and mechanisms. *Bioresour Technol* 2019;291:121916. <https://doi.org/10.1016/j.biortech.2019.121916>.
- [146] Tiwari B, Sellamuthu B, Ouarda Y, Drogui P, Tyagi RD, Buelna G. Review on fate and mechanism of removal of pharmaceutical pollutants from wastewater using biological approach. *Bioresour Technol* 2017;224:1–12. <https://doi.org/10.1016/j.biortech.2016.11.042>.
- [147] Xiong JQ, Kurade MB, Jeon BH. Can microalgae remove pharmaceutical contaminants from water? *Trends Biotechnol* 2018;36:30–44. <https://doi.org/10.1016/j.tibtech.2017.09.003>.
- [148] Zhu L. Biorefinery as a promising approach to promote microalgae industry: an innovative framework. *Renew Sustain Energy Rev* 2015;41:1376–84. <https://doi.org/10.1016/j.rser.2014.09.040>.
- [149] Mishra S, Roy M, Mohanty K. Microalgal bioenergy production under zero-waste biorefinery approach: recent advances and future perspectives. *Bioresour Technol* 2019;292:122008. <https://doi.org/10.1016/j.biortech.2019.122008>.
- [150] Monjed MK, Robson GD, Pittman JK. Isolation of fungal strains for biodegradation and saccharification of microalgal biomass. *Biomass Bioenergy* 2020;137:105547. <https://doi.org/10.1016/j.biombioe.2020.105547>.
- [151] Troiano D, Orsat V, Dumont MJ. Status of filamentous fungi in integrated biorefineries. *Renew Sustain Energy Rev* 2020;117:109472. <https://doi.org/10.1016/j.rser.2019.109472>.
- [152] Sitepu EK, Heimann K, Raston CL, Zhang W. Critical evaluation of process parameters for direct biodiesel production from diverse feedstock. *Renew Sustain Energy Rev* 2020;123:109762. <https://doi.org/10.1016/j.rser.2020.109762>.
- [153] Khoo KS, Chew KW, Yew GY, Leong WH, Chai YH, Show PL, et al. Recent advances in downstream processing of microalgae lipid recovery for biofuel production. *Bioresour Technol* 2020;122996. <https://doi.org/10.1016/j.biortech.2020.122996>.