



Review

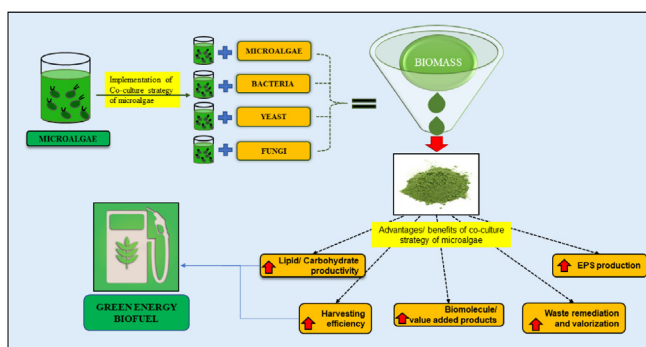
A review on co-culturing of microalgae: A greener strategy towards sustainable biofuels production

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HIGHLIGHTS

- Review describes a different co-cultivation strategy for algal biofuel production.
- Advantages and disadvantages of various co-cultivation methods were described.
- Different cultivation modes used for the co-cultivation strategy were discussed.
- Comprehensive review for biomass and lipid production using co-cultivation strategy
- Microalgal co-cultivation strategy supports eco-friendly and economical biorefinery.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:

Received 21 June 2021

Received in revised form 15 August 2021

Accepted 15 August 2021

Available online 20 August 2021

Editor: Qilin Wang

Keywords:

Microalgae-fungi

Microalgae-yeast

Microalgae-bacteria

Co-cultivation

Biomass

Lipid

ABSTRACT

There is a growing global recognition that microalgae-based biofuel are environment-friendly and economically feasible options because they incur several advantages over traditional fossil fuels. Also, the microalgae can be manipulated for extraction of value-added compounds such as lipids (triacylglycerols), carbohydrates, polyunsaturated fatty acids, proteins, pigments, antioxidants, various antimicrobial compounds, etc. Recently, there is an increasing focus on the co-cultivation practices of microalgae with other microorganisms to enhance biomass and lipid productivity. In a co-cultivation strategy, microalgae grow symbiotically with other heterotrophic microbes such as bacteria, yeast, fungi, and other algae/microalgae. They exchange nutrients and metabolites; this helps to increase the productivity, therefore facilitating the commercialization of microalgal-based fuel. Co-cultivation also facilitates biomass harvesting and waste valorization, thereby help to build an algal biorefinery platform for bioenergy production along with multivariate high value bioproducts and simultaneous waste bioremediation. This article comprehensively reviews various microalgae cultivation practices utilizing co-culture approaches with other algae, fungi, bacteria, and yeast. The review mainly focuses on the impact of several binary culture strategies on biomass and lipid yield. The advantages and challenges associated with the procedure along with their respective cultivation modes have also been presented and discussed in detail.

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1. Introduction

The present worldwide concern revolves around developing renewable and sustainable energy resources, replacing fossil fuels to meet the rising global energy demand (Nayak et al., 2013; Karpagam et al., 2021). Fossil fuel combustion is considered as one of the major causes of greenhouse gas (GHG) emissions, leading to hazardous climatic changes, hence a cleaner and greener energy is desirable (Nayak et al., 2016). Biofuel has emerged as a promising substitute for conventional fuels, which incurs the advantage of lesser GHG emissions and renewable in nature (Hwangbo and Chu, 2020; Nayak et al., 2020). Biofuels are classified into various generations based on the type of feedstock, where 1st generation and 2nd generation biofuels involve the use of food crops and lignocellulosic biomass as their potential feedstocks, respectively (Mat Aron et al., 2020). However, these biofuels come with various drawbacks, such as, using food crops as possible feedstock questions global food safety, and lignocellulosic biomass impedes the extraction of other intracellular components and requires pre-treatment escalating the overall production cost. In this regard, the area of 3rd generation biofuel has become a nationwide interest, using microalgae as the feedstock (Das et al., 2020; Oliveira et al., 2021; Banerjee et al., 2021; Mathimani et al., 2021; Zhang et al., 2021a), which comes with multiple advantages over the first two generations of biofuel, 1) no requirement of arable land for cultivation, 2) no seasonal breaks, 3) cultivable in any water, 4) high photosynthetic efficiency and CO₂ sequestration capacity (Ghosh, 2016; Nayak et al., 2019; Wang et al., 2018c). Microalgae is cultivable in wastewater and in presence of waste gas, thereby leading to wastewater and supplied waste gas or/flu gas remediation by heavy metal removal and mitigating CO₂ emission, apart from its contribution towards biofuel production. Moreover, microalgal biomass also acts as the raw material for value-added yield like, proteins, pigments, antioxidants, etc., which are marketable products in

pharmaceutical, cosmetic industries. The de-oiled microalgal biomass post lipid extraction is utilized for bioethanol, bio-oil, biogas, biochar, bio-fertilizer production (Nayak et al., 2019). However, algae-based biofuel bears multiple downsides, such as 1) dilute culture due to lower biomass concentration; hence biomass recovery is challenging, 2) hydrodynamic and environmental stress, 3) lower metabolite concentration, etc. Co-culturing approaches for microalgae have been followed to enhance microalgal biomass and biofuel. Co-culture methods have been in practice for decades, involving the exploitation of the various microbial interactions and using it to our advantage by culturing multiple strains of different species so that one strain would compensate for an enzymatic activity lacking in the other (Ji et al., 2013). Hence, they create a synthetic conglomerate based on a symbiotic relationship to attain the necessary requirements.

Monocultures of microalgae, even though they are mostly preferred for various bio-manufacturing, contribute to higher production costs and are more labor-intensive and more prone to getting contaminated. Therefore, to overcome axenic monocultures' tailbacks, co-cultivation strategies for microalgae have emerged and gained attention in recent times (Padmaperuma et al., 2018). Naturally, almost all the microorganisms exist in the form of mixed consortia, sharing multiple relationships among themselves; symbiotic, commensalism, mutualism, competition, etc. (Jiang et al., 2019a). The development of binary culture methods for microalgae in symbiotic association with microorganisms, such as bacteria, yeast, fungi, or other microalgal species, has proven to be a promising technique in enhancing biomass production for biofuel manufacturing (Das et al., 2021) (Fig. 1).

It was observed that a mixed consortium of microalgae followed a more stable and steady growth curve along with the fact that biomass yield and lipid productivity were also measured to be higher in mixed culture microalgal strains as opposed to microalgal monocultures (Johnson and Admassu, 2013). Primarily co-culturing mimics natural

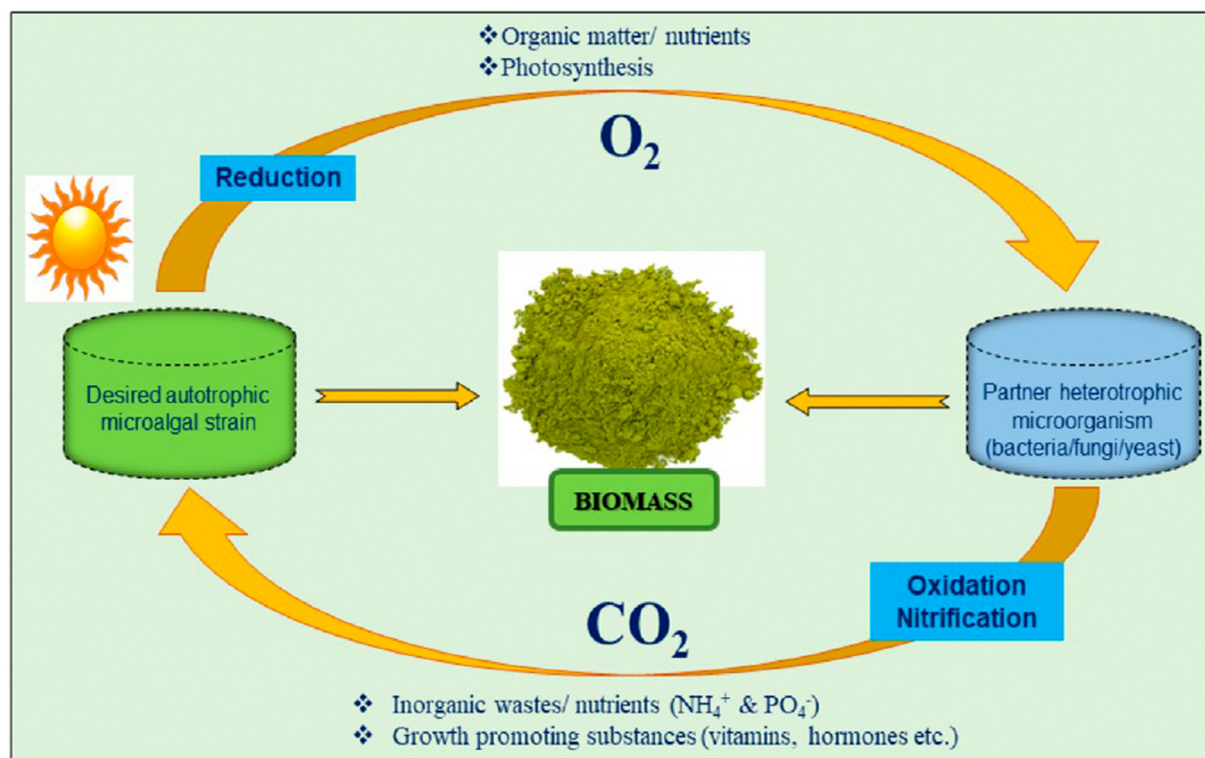


Fig. 1. Mechanism of microbial interaction via exchanging gases and metabolites in co-cultivation system.

environmental conditions more aptly, which is one of the major advantages of this strategy over monocultures. Furthermore, by developing an artificially mixed consortium, the co-culturing technique mainly focuses on broadening the feedstock potentiality and escalating the productivity of the target substrate or bioproducts value-added products (Jiang et al., 2019a). Biotechnologically, a well-constructed consortium would have additional advantages, such as robustness, scalability, self-reliance, and multifaceted in terms of feedstock potentiality or production purposes (Bernstein and Carlson, 2012; Gebreslassie et al., 2013; Markou and Nerantzis, 2013). However, few critical considerations have to be made while co-culturing microalgal strains, for example, recording population growth dynamics in order to eliminate any toxicity effects, competition, contamination, or any under/over-yielding effects, etc., wherein, if the partner culture exceeds the growth of the microalgae may act as an obstruction in the way of light reaching the microalgal cell population, affecting its biomass yield (Padmaperuma et al., 2018; Jayakumar et al., 2021).

Biomass yield in microalgae also depends on the mode of cultivation of the microalgal strain. Microalgae can be cultured under four different conditions; autotrophic, heterotrophic, mixotrophic or photoheterotrophic, among which autotrophic microalgae has shown a comparatively higher biomass yield ($73 \text{ t biomass ha}^{-1} \text{ y}^{-1}$) along with higher oil productivity (20%–40%) when cultivated in open ponds (Posten and Schaub, 2009; Borowitzka, 2013). The autotrophic mode of cultivation includes atmospheric carbon dioxide and/or industrial flue gases as a carbon source with light supply condition, whereas, in heterotrophic conditions, an external organic carbon source is provided to the media and cultivated without any light supply. Mixotrophic mode involves the utilization of an additional organic carbon source along with light supply condition, utilized as the energy source (Fig. 2).

Oleaginous microalgae have a lipid content of $\geq 20\%$ of their total dry weight biomass and improvement in the biomass yield of such microalgal strain using co-culture strategies would contribute towards higher lipid recovery leading to more efficient biofuel production (Brennan and Regan, 2020). Hence, this paper represents an overview of the diverse co-cultivation strategies practiced for oleaginous

microalgae to enhance algal biomass yield and the different pros and cons of this technique. The mode of cultivation for microalgae also plays a major role, and its effects on the co-culturing method have been discussed in detail. Overall, this paper reviews the contribution of co-culturing techniques of microalgae towards biomass and lipid production for biofuel prospective.

1.1. Advantages of microalgae for lipid production

Microalgae incur various advantages compared to other feedstocks for biofuel production as it is renewable, they neither challenge global food security nor compete for arable lands or freshwater, they can be cultivated throughout the year. Ramírez-Verduzco et al. in 2012 reported that lipids extracted from such co-cultures have been observed to contain comparatively higher proportion of short-chain unsaturated fatty acids than saturated FAMES, which boost the fuel properties, such as, density, kinematic viscosity, iodine number, heating value, octane number etc. (Ramírez-Verduzco et al., 2012). Therefore, biofuel production from microalgae leads to a carbon-neutral process whereby no additional carbon is released into the atmosphere (Gambelli et al., 2017). Chisti (2007) stated that if compared per hectare of cultivation, algal bio-oil productivity is 23 times than that of palm oil and almost 300 times than that of soya bean oil yield (Chisti, 2007).

2. Different cultivation modes of microalgae

2.1. Photoautotrophic cultivation

Autotrophic or Photoautotrophic mode of cultivation is one of the earliest ways of cultivating microalgae, where the natural sunlight serves as the light source and microalgae depends on atmospheric CO_2 for carbon-source, however, external industrial exhaust gas or flue gas can also be supplied additionally (Fig. 2). This mode of cultivation can be categorized into (1) open pond system, (2) closed photobioreactor (PBR) system, the two major cultivating methods (Zhan et al., 2017). Open pond system may provide the ease of operation, also accessibility

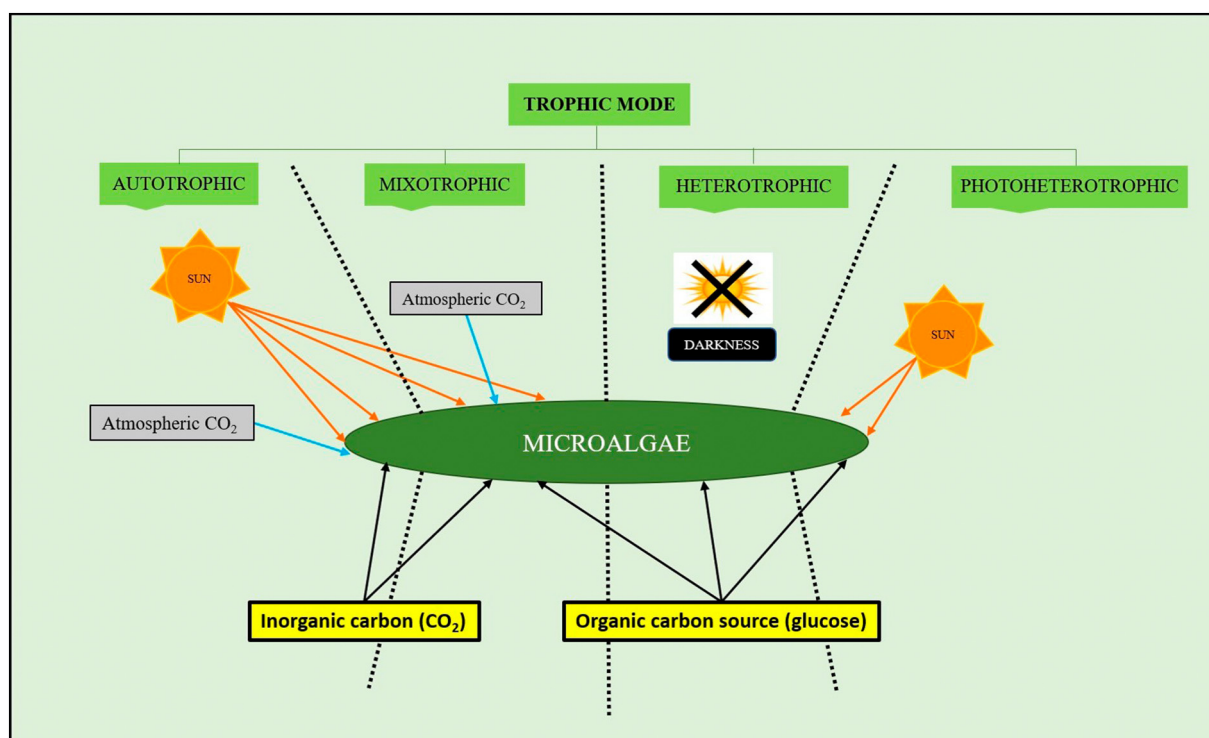


Fig. 2. An illustration of the different trophic modes of microalgal cultivation system.

of the microalgae to atmospheric CO_2 , and introducing submerged aerators to improve CO_2 absorption represents a potential option for microalgae cultivation (Zhan et al., 2017). The advantage behind using open pond system for cultivation could be explained by the fact that it facilitates a larger contact surface area for the accessibility to light and atmospheric CO_2 to the microalgae, additionally submerged aerators enhance the dissolved inorganic carbon (DIC) concentration in the medium and supply optimum carbon for microalgal growth. Furthermore, the ease of operation is also because it does not include a complex working design and is not labor-intensive which leads to an overall lower cost of operation than that of a closed photobioreactor. Moreover, open ponds provide a larger volume for cultivation, thus high amount of biomass can be harvested at once. However, there are few drawbacks, such as tending to get contaminated frequently, suffering a huge temperature difference between day and night hours, etc., making it tedious to maintain the culture conditions (Pulz and Scheibenbogen, 1998; Chojnacka et al., 2004; Chisti, 2007). A closed PBR system was developed to cope with the limitations of the open pond system. Photobioreactors are available in different forms, where the three major classifications are, Plate photobioreactor, tubular photobioreactor, and vertical column photobioreactor, however, it was suggested that tubular PBR was comparatively more efficient for algal cultivation (Sánchez Mirón et al., 1999) due to higher surface to volume ratio, even though, it was established that operation and production costs of closed cultivation systems are evidently higher than open pond cultivation systems. Nevertheless, autotrophic mode requires the dependence on natural weather conditions for various culture conditions (such as temperature, light), which is challenging to maintain at a steady state; moreover, high production costs make it not the most appropriate approach for industrial-scale microalgal cultivation (Zhan et al., 2017).

2.2. Heterotrophic cultivation

The heterotrophic mode of cultivation is independent of any light energy; hence microalgae are grown in complete darkness with a supply of organic carbon(C)-source (Fig. 2). Here the organic carbon

substrate is utilized as the sole sources for carbon and energy, since absence of light restricts microalgae from fixing inorganic (atmospheric) CO_2 by photosynthesis (Perez-Garcia et al., 2011). The absence of dependency on light energy directly reduces the necessity of the surface: volume ratio for the bioreactors (Lodi et al., 2005). Various organic compounds have been utilized as C-source for cultivation, such as glucose, cellulose hydrolysis products, glycerol, acetate, etc., moreover, glycerol provides additional advantages acting as an osmolyte to check cell osmotic balance and, even if present in higher concentration does not express any toxic effects on microalgal cells (Richmond, 2017; Behrens, 2005). It should be remembered that microalgal growth and biomass yield greatly varies on culture conditions, therefore the outcome will fluctuate with the supply of different organic C-source such as Zhang et al. (2013) observed that heterotrophically grown *Chlorella vulgaris* resulted in higher biomass and lipid productivity under the limited concentrations of glycerol and glucose as compared to acetate (Zhang et al., 2013). Nonetheless, the use of external carbon-source increases the risk of contamination as compared to autotrophic mode, however, the advantages of heterotrophy outweigh the shortcomings, since studies have concluded that heterotrophy offers a higher algal growth rate and enhanced biomass and lipid yield than autotrophy. Furthermore, such strategy can also be coupled with wastewater treatment methods reducing the operational cost (Zhan et al., 2017).

2.3. Mixotrophic cultivation

The mixotrophic mode of cultivation bridges between autotrophic and heterotrophic conditions and is the most advantageous and effective as it combines all the advantages and overcomes the shortcomings of both autotrophy and heterotrophy (Zhan et al., 2017). Therefore, the mixotrophic condition necessitates light energy as well as the supply of organic carbon sources (glucose, glycerol, acetate, etc.) accompanied by access to atmospheric CO_2 (Fig. 2), where the light is utilized as the energy source for atmospheric CO_2 fixation by photosynthesis along with the use of organic carbon substrate as the carbon source. Therefore, along with the intensification of biomass and lipid

productivity by utilizing organic carbon as opposed to autotrophic mode (no organic C-source present), it also photosynthesizes by inorganic carbon (CO₂) consumption, thereby reducing CO₂ emission in comparison to the heterotrophic mode, which is carried out in the dark, nevertheless, the energy conversion efficiency is decreased in mixotrophy due to photosynthetic losses (Yang et al., 2000; Abe et al., 2002; Zhan et al., 2017). It was reported by Endo et al. (1977) that for the microalgal strain *Chlorella regularis*, the cell growth rate under mixotrophic mode (acetate as the C-source) was almost the total of that produced in autotrophic and heterotrophic mode (Endo et al., 1977).

2.4. Photoheterotrophic cultivation

Photoheterotrophic mode of cultivation for microalgae is similar to mixotrophic mode with respect to the necessity of light source energy source for its growth, however, in contrary to mixotrophy, in photoheterotrophy the light supply is required for the microalgae to break and utilize the externally provided organic carbon substrate in the media as their sole carbon source (Chen et al., 2011). The utilization of inorganic carbon dioxide as the carbon source is restricted in this mode of cultivation as photoheterotrophism occurs in normal faint light conditions, not sufficient to support photoautotrophic growth and additionally triggered by using various photosynthetic inhibitors, such as, DCMU (3-(3,4-dichlorophenyl)-1,1-dimethylurea) (Orús et al., 1991; Chojnacka and Marquez-Rocha, 2004).

3. Co-culture strategy for enhanced biomass and lipid production

3.1. Microalgae-microalgae co-culture strategy

Microalgae binary culture, where one pure culture of microalgae was grown with another microalgal strain, has been observed to incur positive effects on both the biomass and lipid yield (Table 1). A study revealed that co-culturing *Chlorella* sp. U4341 with *Monoraphidium* sp. FXY-10 provided with variable lipid productivity yield based on the mode of cultivation. The initial cell counts taken for co-cultivation were, 3.41×10^6 cells mL⁻¹ for *Chlorella* sp. U4341 and 4.26×10^6 cells mL⁻¹ for *Monoraphidium* sp. FXY-10. It was observed that co-culturing under heterotrophic mode led to a lipid yield of $93.4\text{--}223.42$ g m⁻³ d⁻¹, whereas mixotrophic mode resulted in a drop in the lipid yield to 21.23 g m⁻³ d⁻¹. However, photoautotrophic conditions led to higher lipid productivity than the mixotrophic mode at 29.52 g m⁻³ d⁻¹ (Zhao et al., 2014a). The synergism explained this increase in lipid production by this binary culture strategy between the two microalgal strains, which is directly associated to the improved lipid yield under co-culturing conditions and was observed by previous reported studies (Gonçalves et al., 2016; Soydemir et al., 2016; Gautam et al., 2019; Qu et al., 2019; Rashid et al., 2019; Zhao et al., 2019a; Zhu et al., 2019; Cheng et al., 2020a; Zhang et al., 2021b).

A major advantage of the microalgae-microalgae co-culture strategy is that it results in a dense culture with a comparatively higher velocity growth than monocultures. Hence, in the case of binary culture techniques, more interactions between the microorganism lead to a rise in EPS production as a metabolic strategy to adapt and acclimatize in unfavorable conditions, such as nutrient starvation (Tribelli and López, 2011; Ramanan et al., 2016). However, there are certain concerns to be taken care of while performing such co-culture experiments. Excessive EPS accumulation may inhibit mass transfer obstructing nutrient uptake and inaccessibility of dissolved CO₂ to the microorganisms. Therefore, the choice of microalgal-microalgal consortia should be made carefully, and it might be limited for large-scale applications. In addition to enhanced biomass/lipid production, this co-culture strategy helps in bioremediation of different waste and facilitate bio-flocculation for efficient biomass harvesting (Gonçalves et al., 2016; Qu et al., 2019; Zhu et al., 2019; Cheng et al., 2020b; Ray et al., 2021) (Table 2). Rashid

et al. observed when *Chlorella* sp. is cocultured with *Ettlia* sp. results in the highest biomass yield of 740 ± 0.06 mg L⁻¹ d⁻¹ and lipid yield of 180.8 ± 14 mg g⁻¹ (Rashid et al., 2019). Therefore, it could be concluded based upon the various studies conducted on the effects of the microalgae-microalgae binary cultivation on biomass and lipid productivity that, even though it improves the overall biomass and lipid yield, yet more detailed study regarding the exact mechanism behind this symbiosis is needed in order to further enhance the productivity. Consequently, it can also be stated that the co-culture approach not only contributes towards bioenergy production, but simultaneously is efficient towards wastewater treatment, provided careful selection of the consortium is required for positive outcome (Das et al., 2021).

3.2. Microalgae-fungi co-culture strategy

The symbiotic relationship between microalgae and fungi has been utilized in this co-culture system for biomass and lipid enhancement (Fig. 1). Fungal partner consumes carbon produced by the microalgae in the medium via photosynthesis, whereas microalgae are provided with protection by the fungi through its water-retaining property and serve as a niche for mineral nutrients (Zoller and Lutzone, 2003). Lichen has been considered one of the most potent and efficient symbiotic associations between microalgae and fungi (Wrede et al., 2014). Xie et al. (2013) studied microalgal-fungal mixed consortium for lipid enhancement and simultaneous bio-flocculation via fungal pelletization. Therefore, this study provided a comparatively better understanding of the usage of the cell wall carbohydrates as well as exudation of cell-wall degrading cellulases (Xie et al., 2013).

The fungal-microalgal symbiotic association has been observed to provide an effective enhancement in biomass and lipid yield (Table 3). Liu et al. (2014) reported that co-culturing coprophilous fungi, such as *Byssoschlamys* sp. F52 with *Cladosporium* sp. F1 resulted in enhanced reducing sugar production, which can be further directed towards lipid conversion under controlled and specific conditions (Liu et al., 2014) and may be channelized to co-culturing with oleaginous microalgal strains to obtain enhanced lipid yield. Another study by Dash and Banerjee (2017) investigated symbiotic associations, *Chlorella minutissima* MCC 27 and *C. minutissima* UTEX 2219 separately with an oleaginous fungal strain *Aspergillus awamori*. In both the consortium, the mode of cultivation was mixotrophic. Both the system used pure glycerol as the organic carbon source instead of glucose, which primarily lowers the production cost. Compared to microalgal monocultures, the *Chlorella minutissima* MCC 27 co-culture system showed a 2.6-fold increase in biomass yield and a 3.4-fold increment in lipid accumulation. In contrast, the *C. minutissima* UTEX 2219 co-culture system provided a higher biomass escalation of around 3.9-fold and a lipid yield rise by 5.1-fold, where, in both the systems, C16:0 (31.26–35.02%) and C18:1 (21.14–24.21%) fatty acids were the major compounds (Dash and Banerjee, 2017). Previous reported studies have also observed substantial enhancement of biomass and lipid with fungal-microalgal co-culture strategy (Rajendran et al., 2017; Dash and Banerjee, 2017; Du et al., 2018; Srinuanpan et al., 2018a; Jiang et al., 2019b; Yang et al., 2019; Zorn et al., 2020).

Lichen, being regarded as one of the most effective algae-fungi symbiotic associations, provides the added advantage of lignin and cellulose degradation (Beckett et al., 2013; Wrede et al., 2014). Furthermore, white-rot fungi showed significant delignification patterns owing to their higher enzymatic activities. Coupling lignin degradation with lipid fermentation via co-culture strategy addresses the issue of lipid yield enhancement and causes a considerable reduction in the production cost for lipid extraction since pre-treatment steps are decreased (Liu et al., 2014). VanHeerden et al. (2008) also observed cellulose degradation property in the co-culture system for *Aspergillus flavipes* and *Pycnoporus sanguineus* on *Eucalyptus grandis* (VanHeerden et al., 2008). Another similar study revealed co-culture of the three rumen fungi, *Neocallimastix frontalis*, *Piromyces communis*, and *Caecomyces*

Table 1
Impact of microalgal-microbial co-culture system on biomass and lipid productivity as biodiesel feedstock.

Co-culture	Microalgae	Ratio	Media (volume)	Trophic mode	Reactor type	Cultivation conditions			Cultivation time	Biomass productivity	Lipid productivity		Reference
						Temperature	pH	Light source/intensity (photoperiod)			P_L , mg L ⁻¹ d ⁻¹	C _L , %	
Chlorella sp.	Scenedesmus sp., Chlorococcale sp.	-	Domestic wastewater (20 L)	Autotrophic	-	24 ± 2 °C	6.5–7.0	Day-light fluorescent lamps	-	-	-	26.2 ± 0.6	Soydemir et al., 2016
Synechocystis salina	Chlorella vulgaris, Pseudokirchneriella subcapitata, Microcystis aeruginosa	-	Synthetic medium (500 mL)	-	-	24.0 ± 1.0 °C	-	Fluorescent light 120 µE m ⁻² s ⁻¹	12	640 ± 0.004–970 ± 0.008	7.76 ± 0.38–11.10 ± 0.64	-	Goncalves et al., 2016
Desmodesmus sp. ZFY	Monoraphidium sp. QLY-1	-	300 mL	Mixotrophic	-	25 ± 1 °C	-	-	-	-	93.99	-	Zhao et al., 2019a
Chlorella vulgaris	Scenedesmus dimorphus	-	Modified Bristol medium (700 mL)	Mixotrophic	Conical flask	23 °C	6.8	80 µmol m ⁻² s ⁻¹	10	300–350	70–84	21–24	Zhu et al., 2019
Chlorella sp. HS2	Ettlia sp.	1:8	BG11 medium (1000 mL)	Autotrophic and mixotrophic	Bubble column bioreactor	25 ± 2 °C	-	LEDs	8	700 ± 0.02 (auto) 740 ± 0.06 (mixo)	109.5 ± 0.5 mg g ⁻¹ (auto) 180.8 ± 14 mg g ⁻¹ (mixo)	11	Rashid et al., 2019
Botryococcus braunii, Scenedesmus obliquus, Chlorella vulgaris	Nostoc muscorum, Anabaena variabilis, Nostoc muscorum	1:1	Nitrogen deficient medium	Autotrophic	-	28 °C	-	-	15/24	19.48	Not mentioned	12–25	Gautam et al., 2019
Scenedesmus obliquus strain FACHB 416	Wild algal strains (from lakes/ponds)	1:1	BG11 medium (150 mL)	Autotrophic	Conical flask	-	-	-	20	12.85–13.9	0.9–1.2	13	Qu et al., 2019
Chlorella zofingensis	Tribonema sp.	1:1	Swine wastewater diluted with fishery wastewater (5×) (600 mL)	-	-	-	-	-	8	0.195	-	44.12	Cheng et al., 2020a
Chlorella regularis	Scenedesmus obliquus	-	Modified BG11 medium + 5 g L ⁻¹ glucose (150 mL)	Heterotrophic	-	25 ± 1 °C	-	-	6	-	120	-	Zhang et al., 2021b

Table 2
Various applications of the different types microalgal binary culture systems.

Co-culture		Applications	Reference
Microalgae <i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> strain FACHB 416, <i>Synechocystis salina</i> , etc.	Microalgae <i>Scenedesmus dimorphus</i> , wild algal strains, <i>Chlorella vulgaris</i> , <i>Pseudokirchneriella subcapitata</i> , <i>Microcystis aeruginosa</i> , etc.	Nutrient removal (N, P, C)	Zhu et al., 2019, Qu et al., 2019, Gonçalves et al., 2016
<i>Ankistrodesmus falcatus</i> , <i>Tetraselmis suecica</i> , cell surface glycoproteins of <i>Ettlia texensis</i> , <i>Tribonema</i> sp. etc.	<i>Neochloris oleoabundans</i> , <i>Chlorella vulgaris</i> , <i>Chlamydomonas reinhardtii</i> , <i>Synechocystis</i> sp. etc.	Swine wastewater nutrient removal, bioflocculation for harvesting microalgal biomass for further biofuel production	Cheng et al., 2020a, 2020b, Ray et al., 2021
Microalgae <i>Chlorella vulgaris</i> 2714, <i>Chlorella variabilis</i> NC64A, <i>Chlorella</i> sp.	Fungi <i>Mucor circinelloides</i> UMN-B34, <i>Ganoderma lucidum</i> , <i>Aspergillus</i> sp.	Nutrient removal	Rajendran et al., 2017, Jiang et al., 2019b, Yang et al., 2019
<i>Chlorella vulgaris</i>	<i>Aspergillus niger</i>	Removal of cadmium by forming biopellets as well as harvesting microalgal biomass	Bodin et al., 2017
<i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> , <i>Scenedesmus capricornutum</i> , <i>C. vulgaris</i> (FACHB-31)	<i>Pleurotus geesteranus</i> , <i>Ganoderma lucidum</i> and <i>Pleurotus ostreatus</i>	Biogas slurry purification, biogas upgrading as well as nutrient, pharmaceutical removal and CO ₂ sequestration	Cao et al., 2017, Zhou et al., 2018, Zhao et al., 2019b
Multiple species (<i>Chlorella</i> sp., <i>Scenedesmus</i> sp. etc.)	Multiple species	Harvesting of microalgal biomass via flocculation	Bhattacharya et al., 2017, Srinuanpan et al., 2018a, Srinuanpan et al., 2018b, Hultberg and Bodin, 2018, Du et al., 2018, Ray et al., 2021
Microalgae <i>Chlorella sorokiniana</i> , <i>C. vulgaris</i> , <i>S. obliquus</i> , <i>Selenastrum capricornutum</i> etc.	Bacteria Proteobacteria, <i>Pseudomonas</i> sp., <i>Acinetobacter</i> sp., <i>R. brasiliensis</i> , <i>Rhodococcus</i> sp., <i>Mycobacterium</i> sp., <i>Burkholderia cepacia</i> , etc.	Organic chemical pollutant removal, greenhouse gas mitigation	Guieysse et al., 2002, Munoz et al., 2005, Munoz and Guieysse, 2006, Warshawsky et al., 2007, Perez-Garcia et al., 2011, Subashchandrabose et al., 2011, Magdouli et al., 2016
<i>Spirulina platensis</i> , <i>Chlorella</i> sp., <i>S. obliquus</i> , <i>C. sorokiniana</i> etc.	Sulfate-reducing bacterial species, <i>Rhodococcus</i> sp., <i>R. brasiliensis</i> , bacteria from wastewater	Heavy metal removal from wastewater, greenhouse gas mitigation	Safonova et al., 2004, Munoz and Guieysse, 2006, Perez-Garcia et al., 2011, Subashchandrabose et al., 2011, Magdouli et al., 2016
<i>C. vulgaris</i> , <i>C. sorokiniana</i> , <i>Spirulina platensis</i> , etc.	<i>Alcaligenes</i> sp., <i>Azospirillum brasiliense</i> , activated sludge bacteria etc.	Nutrient removal from wastewater (tannery effluent, pretreated sewage, swine/piggery/dairy wastewater etc.), greenhouse gas mitigation	de-Bashan et al., 2002, Gutzeit et al., 2005, Perez-Garcia et al., 2011, Subashchandrabose et al., 2011, Magdouli et al., 2016, Bohutskyi et al., 2019
<i>C. vulgaris</i>	Endophytic bacteria	Efficient nutrient removal for biogas purification	Xu et al., 2020
<i>Scenedesmus obliquus</i> UTEX B2630, <i>Chlorella vulgaris</i> UTEX 259, and <i>Chlorella sorokiniana</i> UTEX 1230	Activated sludge bacteria	Treatment of sodium hypochlorite pre-treated meat processing wastewater by co-immobilization, pollutant removal	Hu et al., 2020, Zhang et al., 2020
<i>Chlorella vulgaris</i> NIES-227	Indigenous bacteria (CV), activated sludge	Treatment of dairy-derived liquid digestate	Feng et al., 2020
<i>Chlorella</i> sp.	Activated sludge	Phycoremediation of sewage contaminated lake water, wastewater treatment (removal of nitrate, phosphate, COD)	Verma et al., 2020, Nguyen et al., 2020
<i>Scenedesmus obtusiusculus</i> (GM)	Alkaliphilic methanotrophic bacteria consortium (AMB), such as, <i>Methylocystis</i> sp., <i>Methylobacterium</i> sp., <i>Methylophaga</i> sp., and <i>Hyphomicrobium</i> sp.	Simultaneous sequestration of both CH ₄ and CO ₂ was achieved, where more than 70% of the initial methane was transformed into biomass and inorganic carbon	Ruiz-Ruiz et al., 2020
<i>Chlorella vulgaris</i>	Activated sludge	Total nitrogen removal efficiencies above 97% for both municipal and synthetic wastewater.	Leong et al., 2020
<i>Chlorella</i> sp. and <i>Scenedesmus</i> sp.	Multiple species	Bioflocculation of microalgal biomass for harvesting	Ray et al., 2021
Microalgae <i>Scenedesmus</i> sp.	Yeast Native wastewater yeast	100% nitrate, 100% TAN and 92.6% orthophosphate removal from wastewater (wastewater treatment)	Walls et al., 2019
<i>Chlorella vulgaris</i>	<i>Yarrowia lipolytica</i>	NH ₃ -N and SO ₄ ²⁻ removal rates from yeast industry liquid digestate were superior	Qin et al., 2019b
<i>Chlorella vulgaris</i>	<i>Saccharomyces cerevisiae</i>	In situ CO ₂ mitigation along with reduction in aeration costs of biotransformation processes.	La et al., 2019
Mixed microalgae from aerated activated sludge	Yeast present in tequila vinasse	Adding value to the vinasse which is produced during tequila production from agave	Barcia et al., 2020
<i>Chlorella</i> sp. and <i>Scenedesmus</i> sp.	<i>Rhodotorula</i> sp. And <i>Saccharomyces</i> sp.	Bioflocculation of harvesting microalgal biomass	Ray et al., 2021

Table 3
Impact of microalgal-fungal co-culture system on biomass and lipid productivity as biodiesel feedstock.

Co-culture	Fungi	Ratio	Media (volume)	Trophic mode	Reactor type	Cultivation conditions		Cultivation time	Biomass productivity	Lipid productivity	Lipid content	Reference
						Temperature	pH					
							Light source/intensity (photoperiod)	T _c (d)	P _b , mg L ⁻¹ d ⁻¹	P _L , mg L ⁻¹ d ⁻¹	C _L , %	
<i>Chlorella vulgaris</i> 2714	<i>Mucor circinelloides</i> UMN-B34	-	Specific algal medium with glucose (microalgae) and Potato dextrose agar plates for fungi (100 mL)	Mixotrophic	Erlenmeyer flasks	26 °C	6.8	12	780	-	30.61	Rajendran et al., 2017
<i>Chlorella minutissima</i> (MCC 27 & UTEX 2219)	<i>Aspergillus awamori</i>	300:1	N11 medium furnished with different carbon and nitrogen sources	Mixotrophic	-	25–30 °C	5–8	9	289–322	50–55.5	17.1–18	Dash and Banerjee, 2017
<i>Nannochloropsis oceanica</i>	<i>Mortierella elongata</i>	-	Standard f/2 medium for algae; potato dextrose broth for fungi	Mixotrophic	Shaker flasks	23 °C	-	6	-	-	25	Du et al., 2018
<i>Scenedesmus obliquus</i> SIT06	<i>Cunninghamella echinulata</i> TPU 4652	-	BG11 medium	Photoautotrophic	-	30 °C	5.5	1	4450 ± 0.06	1210 ± 0.08	-	Srinuanpan et al., 2018a
<i>Chlorella variabilis</i> NC64A	<i>Ganoderma lucidum</i>	1:3	Simulated wastewater (500 mL)	Fungal (heterotrophically) microalgae (autotrophically, heterotrophically, and mixotrophically)	Conical flask	25 ± 1 °C	-	10	89	-	-	Jiang et al., 2019b
<i>Chlorella</i> sp.	<i>Aspergillus</i> sp.	-	Molasses wastewater	Autotrophic	-	35 °C	7.4	5	843	-	35.2	Yang et al., 2019
<i>Chlorella vulgaris</i> BMAK D07	<i>Mucor circinelloides</i> f. <i>griseo-cyanus</i> URM 4182	Varied	PDA (fungi), synthetic media + glucose (2 g L ⁻¹) (microalgae) (100 mL)	Mixotrophic	Erlenmeyer flasks	26 °C	-	16	72 ± 6	-	30.5 ± 0.5	Zorn et al., 2020

communis, promoted alfalfa hay (a complex assembly of cellulose, xylan, lignin) conversion (Bootten et al., 2011).

There are few filamentous fungi with self-pelletization ability, which is an algae-fungal co-culture system that has shown to cause bioflocculation of the microalgae (Nazari et al., 2020), apart from increasing the overall lipid productivity without the need of addition of any external chemical flocculants, thus potentially lowering the energy input, as studied with *Chlorella vulgaris* (Zhang and Hu, 2012; Gultom and Hu, 2013; Xie et al., 2013; Wrede et al., 2014). The self-pelletization ability has been studied and explained by coagulative and non-coagulative mechanisms (Luo et al., 2013; Xia et al., 2014). Al-Hothaly et al. (2015) also studied this flocculating activity with *Aspergillus fumigatus* at the pilot scale (500 L) and observed a significant harvesting efficiency of up to 98% for the species *Botryococcus braunii*, (Al-Hothaly et al., 2015). This assisted flocculating advantage provided by the fungal partner in the co-culture system also addresses one of the major bottlenecks behind the commercialization of algal biofuel; the inability to harvest microalgal biomass at a large scale due to the dilute algal culture. Wrede et al. (2014) also stated that this assisted bioflocculation tends to escalate the lipid and biomass yield (Wrede et al., 2014). Apart from biofuel production, the ability of the fungi to flocculate microalgal species and waste remediation also have been observed in earlier reported studies (Rajendran et al., 2017; Bhattacharya et al., 2017; Bodin et al., 2017; Cao et al., 2017; Du et al., 2018; Hultberg and Bodin, 2018; Srinuanpan et al., 2018a; Srinuanpan et al., 2018b; Zhou et al., 2018; Jiang et al., 2019b; Yang et al., 2019; Zhao et al., 2019b; Ray et al., 2021) (Table 2). Overall, it can be concluded that microalgae-fungi co-cultivation approach is one of the most potential strategies to enhance biomass and lipid productivity for biofuel production as well as bioremediation. The highest yield observed were 4450 mg L⁻¹ d⁻¹ (biomass) and 1210 mg L⁻¹ d⁻¹ (lipid) based on previous reports (Srinuanpan et al., 2018a) which is comparatively greater than that observed from other binary approaches. However, molecular level of study is required to understand the finer details of the interactions between the microorganisms so that the whole process, initiating from microalgal cultivation to biofuel production can be commercialized (Das et al., 2021). In addition, microalgae-fungal binary culture strategy benefits by minimizing the biomass harvesting cost as filamentous fungi help in flocculating microalgae with their mycelium.

3.3. Microalgae-bacteria co-culture strategy

Culturing microalgae-bacterial mixed consortia have gained attention with respect to bioremediation among researchers (Khoo et al., 2021) since various bacterial species are endogenic to most mixed microalgal cultures. It was studied that, factors such as biotin (vitamin B7), thiamin (vitamin B1), and particularly cobalamin (vitamin B12) principally acting as a cofactor for vitamin B12-dependent methionine biosynthesis (Croft et al., 2005), are major factors necessary for microalgal metabolism and growth. Auxotrophism for these factors has been observed in microalgae throughout evolution, and therefore, they depend on external vitamin sources (Croft et al., 2005). Bacteria act as the exogenous source for these compounds, explaining this tight-knit association between algae and bacteria. Such a vitamin-based symbiotic association was observed between green alga *Lobomonas rostrata* and bacterium *Mesorhizobium loti* in a binary culture system and between bacterium *Sinorhizobium meliloti* 1021 and *Chlamydomonas reinhardtii* (Kazamia et al., 2012; Grant et al., 2014). The biosynthetic pathways for these compounds are present in a vast array of bacterial species (Croft et al., 2005; Grant et al., 2014; Helliwell et al., 2014) and are also readily available for consumption. As an external source for these compounds and other bacterial properties (such as emulsification) could be steered towards escalating lipid productivity. This could be explained by the fact that bacteria incur the property of surfactant production, leading to an alteration in the cell surface hydrophobicity, facilitating a modification in the cell's direct

substrate uptake and, in return, lipid accumulation (Magdouli et al., 2016). Moreover, in a study by de-Bashan et al. (2008), it was observed that indole-3-acetic acid produced by bacteria could promote growth and lipid productivity and boosted the interaction between bacteria and microalgae (de-Bashan et al., 2008).

Various studies have established an intensification in the biomass and lipid productivity from this microalgae-bacteria co-culture system (Table 4), for instance, in a report by Do Nascimento et al. (2013), the co-culture of *Ankistrodesmus* sp. with *Rhizobium* strain 10II showed almost a ~30% rise in overall biomass and lipid yield with the lipid productivity touched 112 mg L⁻¹ d⁻¹ after parameter optimization for the oleaginous microalgal strain (Do Nascimento et al., 2013). Wu et al. (2012) performed binary culture cultivation for *Chlamydomonas reinhardtii* strain CC849 with *Bradyrhizobium japonicum* and observed an increased microalgal growth at around 3.9×10^7 cm⁻³, which directed a rise of 26% in the microalgal biomass yield along with 14-fold higher hydrogen production as opposed to algal monoculture (Wu et al., 2012). This enhanced growth and lipid yield were explained by the increased respiration rate of the co-culture system (7.71 mol kg⁻¹ h⁻¹), which may have caused higher O₂ consumption leading to the gradual formation of an anaerobic atmosphere in the system. This anaerobic atmosphere triggered higher Fe-hydrogenase activity, therefore a rise in H₂ production (Wu et al., 2012). A generally mixed consortium for microalgae and bacteria in landfill leachate was studied and characterized by Zhao et al. (2014b) and reported a maximum biomass yield of 1.58 g L⁻¹ and lipid productivity of 24.1 mg L⁻¹ d⁻¹, along with a simultaneous 90% total nitrogen (N) removal efficiency (Zhao et al., 2014b). The symbiotic association between *Rhizobium* sp. KB10 and *Botryococcus braunii* for co-culturing were developed and patented by Oh et al. (2014), which established a 9-fold increase in the microalgal biomass yield with a simultaneous escalation in the C18 (i.e., oleate) (Oh et al., 2014). To overcome the drawback of contamination, a collaborative approach for developing a mixed consortium with non-axenic bacterial culture (activated sludge) and microalgal strains such as, *Ettlia* sp. YC001 and *Chlorella protothecoides* (UTEX-1806), was investigated. The co-culture showed a significant rise to 28.7-fold in lipid production under photoheterotrophic mode as opposed to 17.3-fold under photoautotrophic conditions (Ryu et al., 2014). Previous reported studies also observed similar effect on biomass and lipid enhancement under microalgae-bacterial co-culture strategy (Do Nascimento et al., 2013; Higgins and VanderGheynst, 2014; Feng et al., 2020; Leong et al., 2020; Makut et al., 2020; Nguyen et al., 2020; Subasankari et al., 2020; Verma et al., 2020; Wei et al., 2020; Zhou et al., 2020; Wang et al., 2021) (Table 4).

Microalgae-bacterial symbiosis provides various advantages over algal monocultures, such as primarily algae provide with oxygen via photosynthesis, different organic exudates promoting bacterial growth, and secreted various toxic metabolites inhibiting undesired bacterial growth hence preventing competition among bacterial partner in the co-culture system (Riquelme and Avendaño-Herrera, 2003) (Fig. 1), whereas, the ecological niche being already occupied by the prevailing bacterial species also prevented other undesirable bacteria from invading the system. This phenomenon could be explained by the competitive exclusion principle of community ecology (Kazamia et al., 2012). Therefore, this approach could be beneficial at a large scale and additionally in places where maintaining a sterile environment is challenging.

Apart from its contribution towards biofuel commercialization, this co-culture strategy was investigated by coupling it with wastewater treatment to reduce the operating cost by utilizing the bacterial property to flocculate the microalgal partner (de-Bashan et al., 2002; Guiesse et al., 2002; Safonova et al., 2004; Gutzeit et al., 2005; Munoz et al., 2005; Munoz and Guiesse, 2006; Warshawsky et al., 2007; Perez-Garcia et al., 2011; Subashchandrabose et al., 2011; Magdouli et al., 2016; Bohutskyi et al., 2019; Feng et al., 2020; Hu et al., 2020; Leong et al., 2020; Nguyen et al., 2020; Ruiz-Ruiz et al., 2020; Verma

Table 4
Impact of microalgal-bacterial co-culture system on biomass and lipid productivity as biodiesel feedstock.

Co-culture	Microalgae	Bacteria	Ratio	Media (volume)	Trophic mode	Reactor type	Cultivation conditions			Cultivation time	Biomass productivity	Lipid productivity	Lipid content	Reference
							Temperature	pH	Light source/intensity (photoperiod)					
<i>C. minutissima</i>		<i>E. coli</i>	-	Acetate (10 g L ⁻¹)	Mixotrophic	-	28 °C	7.2	10,000 lx (16:8 h L:D).	5	428.9	72.5	16.9	Higgins and VanderGheynst, 2014
			-	Glucose (10 g L ⁻¹)							419.8	72.2	17.2	
<i>Ankistrodesmus</i> sp.		<i>Rhizobium</i> strain 1011	1:10	AEX (artificial medium for bacteria)	-	Photobioreactors	29 ± 1 °C	-	35–180 µmol photons m ⁻² s ⁻¹ (M)	8	250	112	47	Do Nascimento et al., 2013
				BG11-NO ₃ (microalgae)										
<i>C. pyrenoidosa</i>		<i>Kluyvera</i> sp. (heterotrophic ammonia-oxidizing bacterial strain FNS)	-	Municipal wastewater (600 mL)	Mixotrophic	Cylindrical reactor	25 ± 2.0 °C	7.1	Fluorescent lamps	5	70	28	39	Zhou et al., 2020
<i>Nannochloropsis oceanica</i>		<i>Halomonas aquamarina</i>	100:1	(Media not specified) 250 mL	Autotrophic	-	25 ± 1 °C	-	70 µmol photons m ⁻² s ⁻¹ (12:12 h L:D)	2/3	0.14	-	53.16 ± 1.08	Subasankari et al., 2020
<i>Chlorella vulgaris</i> NIES-227		Indigenous bacteria (CV), activated sludge	1:1	4 times diluted liquid digestate as wastewater	Mixotrophic	Photobioreactors	Room temperature	-	200 µmol photons m ⁻² s ⁻¹	8	270	-	-	Feng et al., 2020
<i>Chlorella sorokiniana</i> strain DBWC2 & <i>Chlorella</i> sp. strain DBWC7		<i>Klebsiella pneumoniae</i> strain ORWB1 & <i>Actinobacter calcoaceticus</i> strain ORWB3	1:1	Open raceway pond (350 L)	Autotrophic	Open raceway pond	Natural outdoor temperature	8.6	Sunlight (natural outdoor conditions)	19	213	-	Bio-crude oil yield of 21.7% (w/w)	Makut et al., 2020
<i>Chlorella</i> sp.		Activated sludge	2:1	Sewage-contaminated Lake water (350 mL)	Mixed	Erlenmeyer flask	26 ± 1 °C	-	35 W tubes (4000 lx light intensity) (12:12 h L:D)	10	780	-	-	Verma et al., 2020
<i>Chlorella</i> sp.		Activated sludge	3:1	Synthetic wastewater (14 L)	Autotrophic	Stirred photobioreactor		7.6	100 µmol photons m ⁻² s ⁻¹ (12:12 h L:D)	12	93.3	-	-	Nguyen et al., 2020
<i>Chlorella vulgaris</i>		<i>Mesorhizobium sangaii</i>	40:1	BG11-N (N-starvation condition)	Autotrophic	-	25 °C	-	130 µmol photons m ⁻² s ⁻¹ (14:10 h L:D)	10	0.189	96.77	51.2	Wei et al., 2020
<i>Chlorella vulgaris</i>		Activated sludge	0.75:1	Municipal wastewater (900 mL)	Autotrophic	Erlenmeyer flask	27 ± 1 °C	7.1 ± 0.1	LED light (1200 lx)	14	66.42 ± 2.14	16.4 ± 1.42	-	Leong et al., 2020
				Synthetic wastewater (900 mL)							48.57 ± 1.42	9.28 ± 0.71	-	
<i>Chlorella vulgaris</i>		Activated sludge	-	Pre-treated anaerobically digested swine manure (100 mL)	-	Shake flask	25 ± 0.5 °C	-	-	7	347.14	Not studied	-	Wang et al., 2021

et al., 2020; Xu et al., 2020; Zhang et al., 2020; Ray et al., 2021) (Table 2). This combination has been observed to be effective towards chemical oxygen demand (COD) removal. It was also observed that binary culture of microalgae with bacteria producing bioflocculant presented with comparatively higher advantage than bioflocculant non-producing bacterial partner as the former enhanced COD removal with simultaneous degradation of complex compounds. The approach mentioned above also ensured recycling of the residual nutrients and biomass from downstream processing which could be utilized for further algal growth, henceforth it benefitted by minimizing the energy costs (Magdouli et al., 2016). However, despite all the advantages described above of the co-culture approach, Perez-Garcia et al. (2011) stated that bacteria might exceed the microalgal growth rate and probably repress its growth may negatively affect the microalgal biomass yield and lipid content (Perez-Garcia et al., 2011). Also, the overgrowth of bacterial cells may cause inaccessibility to light and nutrients required for microalgal growth. Consequently, in order to carry out a successful microalgae-bacteria binary culture experiment, optimization of the parameters is a prerequisite for its construction. Therefore, such approaches could prove to be beneficial to meet the demands regarding improved biomass and lipid yield. According to the previous studies on this strategy, the biomass yield reached its peak at $429 \text{ mg L}^{-1} \text{ d}^{-1}$ and $112 \text{ mg L}^{-1} \text{ d}^{-1}$ for biomass and lipid productivity respectively (Higgins and VanderGheynst, 2014; Do Nascimento et al., 2013; Das et al., 2021). Further productivity can be enhanced by optimizing different carbon and nitrogen supply to the microalgal-bacterial co-culture.

3.4. Microalgae-yeast co-culture strategy

Naturally, microalgae are sunlight-propelled cell factories and prospective candidates for phosphorus as well as nitrogen removal and carbon utilization and generation of oxygen through photosynthesis. Simultaneously, microalgae providing with high oxygen levels and organic exudates in the culture promotes heterotrophic aerobic yeast growth, and in return, yeasts produce CO_2 through fermentation of organic compounds, triggering growth in microalgae along with lipid production (Ji et al., 2013; Magdouli et al., 2016) (Fig. 1). Previously reported studies observed substantial increase of biomass and lipid production with microalgae-yeast co-culture strategy compared to pure culture of microalgae as shown in (Table 5). Zhang et al. (2014a) concluded that the symbiotic association and the synergistic influences on the cell growth in such a co-culture were greatly dependent on factors, such as O_2 - CO_2 equilibrium, dissolved oxygen (DO), pH balance and substrate exchange in the mixed culture system (Zhang et al., 2014a).

Xue et al. (2010) studied the co-culture system for *Spirulina platensis* and *R. glutinis* for biomass and lipid escalation and achieved a significant rise in total lipid and total biomass accumulation as compared to monocultures, with a simultaneous reduction in chemical oxygen demand (COD) (73%) and nitrogen (35%) (Xue et al., 2010). Another study reported, mixed consortia of *Chlorella* sp. KKU S2 and oleaginous yeast, *Torulaspora globosa* YU5/2 or *Torulaspora maleeae* Y30 presented with an increase of almost 96% in the lipid productivity along with a significant rise in biomass yield when cultivated mixotrophically with sugarcane juice as an organic carbon source (Papone et al., 2012). A considerable number of studies have been performed revealing high biomass and lipid productivity via a co-culture approach, such as, in a report by Cai et al. (2007), the co-culture of yeast *Ambrosiozyma cicatricose* and microalga *Isochrysis galbana* 8701, led to a biomass yield of around 20.71 g m^{-3} (Cai et al., 2007). Autotrophically *C. protothecoides* presented with an improved lipid yield and cell growth when aerated with CO_2 -supplemented air from its heterotrophically grown culture to more than 55% for both biomass and lipid yield when equated with the bioreactor supplied only with air (Santos et al., 2011). On the other hand, for heterotrophically grown *C. protothecoides* when supplied with O_2 -rich air from its autotrophic

culture, resulting in a biomass and lipid throughput of almost $0.052 \text{ g L}^{-1} \text{ h}^{-1}$ and $28.6 \text{ mg L}^{-1} \text{ h}^{-1}$, respectively as compared with aeration using ambient air (Santos et al., 2011). Another interesting study by Santos et al. (2013) revealed that *C. protothecoides* cultivated under autotrophic conditions intensified lipid productivity ($2.2 \text{ g m}^{-3} \text{ h}^{-1}$) and biomass productivity ($15 \text{ g m}^{-3} \text{ h}^{-1}$) when the culture was supplied with off-gas from *Rhodospiridium toruloides*, as opposed to the same culture aerated fully with air, without gaseous exchange (Santos et al., 2013). *Chlorella* sp. has been studied vigorously in various combinations for co-culture development, such as Puangbut and Leasing (2012) carried out cultivation of *Chlorella* sp. KKU-S2 with the supply of CO_2 from *T. maleeae* Y30 culture, resulting in an enhanced lipid ($0.223 \text{ g L}^{-1} \text{ h}^{-1}$) and biomass productivity ($0.48 \text{ g L}^{-1} \text{ h}^{-1}$) (Puangbut and Leasing, 2012). Likewise, among various combinations studied, co-culturing *C. vulgaris* var. *vulgaris* TISTR 8261 with *T. spathulate*, established the highest biomass yield of 12.2 g L^{-1} with 47% mass fraction lipid content of the dried cells. (Kitcha and Cheirsilp, 2014). In a study by Zhang et al. (2014a), the co-culture system for oleaginous yeast *Rhodotorula glutinis* and *Chlorella vulgaris* showed a significant increment in the overall lipid yield. An upsurge of 17.3% in biomass yield and 70.9% in lipid productivity yield was established as compared to monocultures (Zhang et al., 2014a). Binary culture of yeast and *C. vulgaris* showed a biomass yield of 4.63 g L^{-1} and lipid productivity of 2.88 g L^{-1} when cultivated in the run-off from a seafood processing plant and molasses collected from a sugar cane plant (Cheirsilp et al., 2011). Yen et al. (2015) showed an escalation of 40–50% in biomass production and 60–70% in lipid yield from the mixed consortia of *T. obliquus* and *R. glutinis* as compared with single batch cultures (Yen et al., 2015). In addition to biofuel application, the co-culture strategy also facilitates waste remediation, bioflocculation etc. (La et al., 2019; Qin et al., 2019b; Walls et al., 2019; Barcia et al., 2020; Ray et al., 2021) (Table 2). Therefore, based on various reports, it could be concluded that microalgae-yeast co-cultivation strategy has also proved to be one of the most potential approaches for increasing biomass and lipid yield along with microalgae-fungi binary cultures. Highest productivity reached $2670 \text{ mg L}^{-1} \text{ d}^{-1}$ (biomass) and $1539 \text{ mg L}^{-1} \text{ d}^{-1}$ (lipid) based on previous reports which is comparatively higher than that observed from other binary approaches but almost similar to microalgae-fungi co-culture system. However, parameter optimization as well as detailed study on molecular level to have a better knowledge on the interrelationship between the microorganisms are also required for the successful implementation of the experiment (Liu et al., 2018b; Papone et al., 2012; Das et al., 2021)

4. Factors affecting the biomass and lipid productivity of the co-cultivation system

4.1. Strain selection

Strain selection is the primary and one of the major criteria to develop an efficient binary culture system. The pair selection for a binary culture system is mainly based on (a) screening from symbiotic associations existing in nature and/or (b) communication (metabolite/peptide) profiling, where selection depending upon communication profiling implies assessing the literature for secondary heterologous partner microorganisms that secrete compounds to trigger growth in the primary partner (Angelis et al., 2012; Padmaperuma et al., 2018). The primary partner (X) is represented by the desired microalgal species selected for biomass and lipid enhancement, whereas the secondary partner organism (Y) is the heterologous microorganism possessing few specific characteristics in order to provide a successful symbiotic association. Various studies investigated the parameters to be taken into consideration during the secondary partner selection, such as (1) absence of any inhibitory effects on the primary partner (Y) and non-toxic in nature, (2) ability to co-exist, (3) dynamics of its growth rate should be maintained and should match with the primary

Table 5
Impact of microalgal-yeast co-culture system on biomass and lipid productivity as biodiesel feedstock.

Co-culture	Yeast	Ratio	Media (volume)	Trophic mode	Reactor type	Cultivation conditions		Cultivation time	Biomass productivity	Lipid		Reference	
						Temperature	pH			Light source/intensity (photoperiod)	T _c (d)		P _B ,mg L ⁻¹ d ⁻¹
Microalgae		M:Y											
Chlorella sorokiniana	Cryptococcus curvatus	-	Food waste hydrolyzed broth	Mixotrophic	Conical flasks	25 °C	6	Fluorescent lamps	5	1500	428	28.6	Chi et al., 2011
	Rhodotomula glutinis	-	+ primary wastewater (1:4) (50 mL)							1040	204	19.6	
C. vulgaris	R. glutinis	1:1	Industrial effluents (50 mL)	-	Conical flasks	30 °C	-	3.0 klx with 16:8 h L:D	5	7.25	920	62.2	Cheirslip et al., 2011
Chlorella sp. KKU-S2	Tonilasporea maleeae 430	1:1	Sugarcane juice (500 mL)	Mixed/mixotrophic,	Erlenmeyer flasks	30 °C	-	Cool-white fluorescent lamps	7	1247	222.85	17.86	Papone et al., 2012
	Trichosporon globosae YUS/2	1:1								2670	345.72	30.21	
Chlorella sp.	Saccharomyces cerevisiae	1:2 2:1 1:1	Walne's nutrient medium (2500 mL)	Mixotrophic	Bubble column bioreactor	28 °C	-	1000-8000 lx	3	450 660 613	62.34 119.34 129.34	13.8 19.54 19.45	Shu et al., 2013
	C. protothecoides	No	Nitrogen limited + inorganic medium	Mixotrophic (M)	Photobioreactor (M), stirred vessel fermenter (Y)	30 °C (Y), 28 °C (M)	5.5 (Y)	White fluorescent light tubes (M)	15 h	0.22 g L ⁻¹ (M), 6.15 g L ⁻¹ (Y)	2.20 (M), 120 (Y)	26.90 (M), 27.69 (Y)	Santos et al., 2013
Chlorella vulgaris	R. glutinis	1:1 2:1	Winery wastewater (700 mL)	Photoautotrophic Heterotrophic	Conical flasks	26 °C	-	72 μmol photons m ⁻² s ⁻¹ (12:12 h L:D)	10	60 55	12 5	20 9.09	Zhang et al., 2017
	Trichosporonoides spathulata	1:1	Crude glycerol (2000 mL)	Mixed	Stirred tank bioreactor	room temperature	6	2000 lx light intensity with a 16:8 h L:D	5	2226	910	40.81	Kitcha and Cheirslip, 2014
Isochrysis galbana 8701	Yarrowia lipolytica	1:1	Liquid digestate/water (1:1) + glycerol (100 mL)	Mixotrophic	Conical flasks	28 ± 1 °C	-	45 ± 3 μmol photons m ⁻² s ⁻¹	6	270	51.7	18.98	Qin et al., 2018
	Ambrosiozyma ciliaricosa	1:1	Aged seawater + f/2 (2 g L ⁻¹ glucose) (2000 mL)	Mixotrophic	Erlenmeyer flasks	20 ± 1 °C	-	Fluorescent lamps	7	188.5	21.3	11.21	Cai et al., 2007
Chlorella vulgaris	R. glutinis	1:1 2:1	BC-11 + glucose (20 g L ⁻¹)	Mixotrophic	Shake flask or double system bubble column photo-bioreactor	30 °C	-	80 μmol m ⁻² s ⁻¹	4	2575 2175	222.85 262	20.8 15.1	Zhang et al., 2014a
	R. glutinis	2:1	Monosodium wastewater (50 mL)	Mixotrophic	Conical flasks		-	4000 lx	5	320	44	13.75	Xue et al., 2010
C. pyrenoidosa	R. toruloides	1:1	Distillery + local municipal wastewater (30 mL)	Mixotrophic	Conical flasks	30 °C	-	2000 lx light intensity with a 12:12 h L:D	5	1054	672	65.24	Ling et al., 2014
	R. glutinis	3:1	BBM + glucose (10 g L ⁻¹) (100 mL)	Mixotrophic	Erlenmeyer flasks	28 °C	6.1	100 μmol photons m ⁻² s ⁻¹	7	874	206	40.32	Liu et al., 2018a

<i>R. glutinis</i>	4:1	Cassava bagasse hydrolysate (100 mL)	Mixotrophic	Erlenmeyer flasks	28 °C	-	40 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$	12	2620	1539.2	41.27	Liu et al., 2018b
<i>Saccharomycopsis fibuligera</i>	-	BG-11 + starch (10 g L ⁻¹) (100 mL)	Heterotrophic	Erlenmeyer flasks	25 °C	-	-	5	724	79.16	26.2	Wang et al., 2018a
<i>S. cerevisiae</i>	-	BG-11 + sucrose (1%) (100 mL)	Heterotrophic	Erlenmeyer flasks	25 °C	-	-	4	520	145.6	28	Wang et al., 2018b
<i>Scenedesmus obliquus</i>	1:1	Glucose (30 g L ⁻¹) (5000 mL)	Mixed	Photobioreactor	24 °C	6	100 $\mu\text{mol m}^{-2} \text{ s}^{-1}$	4	2500	600	24	Yen et al., 2015
<i>S. elongatus</i>	1:1	BG-11 + yeast extract (250 mL)	Mixed	Conical flasks	28 °C	8.9	100 $\mu\text{mol m}^{-1} \text{ s}^{-1}$ with 16:8 h L:D	4	197.5	925	4.76	Li et al., 2017
<i>S. obliquus</i>	1:3	BG-11 (1000 mL)	Autotrophic	Glass columnar photobioreactors	26–28 °C	-	120 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$	6	566.66	86.5	31.3	Wang et al., 2016
<i>Chromochloris zofingiensis</i>	3:1	BBM + glucose + urea (100 mL)	Mixed	-	26 °C	6.5	100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$	12	566.66	91.2	31.8	
	3:1								600	97.3	31.6	
	3:1								385	120.12	31.2	Jiang et al., 2018
<i>Chlorella pyrenoidosa</i>	-	BG11 + YPD based mixed media (75 mL)	-	Conical flasks	28 $\pm$ 1 °C	<9.25	45 $\pm$ 3 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$	7	760	110 $\pm$ 0.11	14	Qin et al., 2019a
<i>Chlorella vulgaris</i>	-	Yeast industry liquid digestate (YILD) with 5 $\times$ and 10 $\times$ dilution (100 mL)	-	Conical flasks	28 $\pm$ 1 °C	6.7	45 $\pm$ 3 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$	10	594.38–650.13 (5 $\times$ dilution), 534.00–588.12 (10 $\times$ dilution)	7.3–15.4 (5 $\times$), 11.2–18.3 (10 $\times$)	6.66–10.07 (5 $\times$), 9.21–12.21 (10 $\times$)	Qin et al., 2019b
<i>C. pyrenoidosa</i>	40:1	Liquid BG-11 medium added 10 g L ⁻¹	Mixotrophic	Erlenmeyer flasks	25 °C	-	35 $\mu\text{mol m}^{-2} \text{ s}^{-1}$	6	488 $\pm$ 0.13	165.4 $\pm$ 5.3	28.2 $\pm$ 0.3	Tian et al., 2020
	30:1	Liquid BG-11 medium added 10 g L ⁻¹	Heterotrophic	Erlenmeyer flasks	26 °C	-	-	6	382 $\pm$ 0.17	124.3 $\pm$ 13.6	27.1 $\pm$ 1.8	
<i>Scenedesmus obliquus</i> , <i>Scenedesmus</i> sp.	-	sucrose Municipal wastewater (1000 mL)	-	Photobioreactor	24 $\pm$ 1 °C	-	White fluorescent tubes	12	140	242	27.77	Suastes-Rivas et al., 2020

partner, (4) secretion/production of growth-promoting factors or hormones and/or nutrients to promote growth in partner (X), (5) trigger multi-feedstock utilization in partner (Y), (6) utilization of the wastes produced by the primary partner as its feedstock for growth, (7) Also, the secondary partner should not undergo genetic shifts frequently, hence should be able to maintain genetic integrity over a longer period of time (Oren, 1993; Watanabe, 2001; Orphan, 2009; Schmidtke et al., 2010; Minty et al., 2013). Larkum et al. (2012) stated that microalgal biomass and lipid yield vary greatly based on culturing parameters, species, and strains; hence selecting indigenous microalgal strains are preferable if aiming commercialization of the production process since they are well-adapted and acclimatized to local climatic conditions, pathogens, and herbivores (Larkum et al., 2012; Chu, 2017). In the case of microalgal strain selection, Chu (2017) acknowledged that thermotolerance, along with a wide range of salinity tolerance, is an advantageous and desirable trait for biofuel production in the tropics (Chu, 2017; Ji et al., 2018).

4.2. Consortium selection

Many researchers have studied that nutrient-starvation provides synergistic effects on lipid and biomass enhancement in microalgae. A study by Mujtaba and Lee (2016) reported that the cultivation of *C. vulgaris* in a two-stage method, initially a nutrient-rich medium followed by a nitrogen-deficient medium, resulted in an almost 2-fold increase in lipid yield (Mujtaba and Lee, 2016). Also, the two-stage approach for *Ankistrodesmus falcatus* increased lipid productivity by 36.5–45.5% (Álvarez-Díaz et al., 2014). In another similar study by Mandal and Mallick (2009), the lipid accumulation by *T. obliquus* increased by about 10-fold when the microalgae were initially cultivated in glucose-supplemented medium followed by phosphate- and nitrate-limited conditions (Mandal and Mallick, 2009).

4.3. Inoculum ratio of each strain of the consortium

The inoculum ratio for each strain in the consortium is another major concern since it will affect the final outcome and the general structure of the co-culture study. Different factors need to be considered, such as the growth rate of each strain since overgrowth or any negative competition between the partner strains is not desirable, the growth phase as well as the timing at which the inoculums are added into the binary culture set-up. This phenomenon has been referred as the priority effect by Fukami (2015) and Chase (2003) and regarded as one of the most integral factors in a bioreactor system (Chase, 2003; Fukami, 2015).

A study revealed that the co-culture of *C. vulgaris* and *R. glutinis*, accomplished significant escalation in both biomass and lipid yield, reaching 17.3% and 70.9%, respectively, when both the strains were inoculated in a ratio of 1:1 while both were in their exponential phase. Similar conditions were preferred for co-culturing *Thalassiosira pseudonana* (a diatom) and *Dinoroseobacter shibae* (a bacterium), where the diatom was required to be in its log phase before the bacterial inoculum is introduced (Paul et al., 2012).

4.4. Trophic mode of cultivation

The mixotrophic mode of cultivation has been one of the preferable trophic modes of cultivation for this microalgal co-culture strategy (Zhan et al., 2017). Andrade and Costa (2007) and Rym et al. (2010) revealed that in microalgal species, the photosynthetic features observed under the mixotrophic mode are rather different from that observed under autotrophy and heterotrophy (Andrade and Costa, 2007; Rym et al., 2010). A comparative study was performed by Kang et al. (2004) for *Synechococcus* sp. PCC 7002 in mixotrophic and autotrophic mode established that both the growth rate and the net photosynthetic rate ($263 \mu\text{mol O}_2 \text{ mg Chl}^{-1} \text{ h}^{-1}$) of mixotrophy were considerably

higher than that of autotrophy (Kang et al., 2004). Similarly, Yu et al. (2008) showed that the cultivation of *Nostoc flagelliforme* under mixotrophic mode resulted in a biomass yield of 4.98 times that in autotrophic mode and 2.28 times under heterotrophic mode during harvesting (Yu et al., 2008). Another comparative analysis was studied by Rym et al. (2010) for the various photosynthetic parameters for the two-stage cultivation of *Arthrospira platensis* under mixotrophic and autotrophic growth conditions. It was observed that the peak instantaneous growth rate, respiratory rate and net photosynthetic rate under mixotrophic mode resulted in about 1.5 times higher than that under autotrophic conditions at the initial stage (the first 3 days) (Rym et al., 2010). Giovanardi et al. (2013) also concluded from his study on *Neochloris oleoabundans* that cultivation under mixotrophic mode using carbon-rich manure as the C-source, derived from apple vinegar production, the algal cell density was found to be 150% higher than the algal density in autotrophic culture mode, therefore establishing mixotrophy being the desired trophic mode of cultivation to enhance microalgal biomass (Giovanardi et al., 2013). There is a considerable number of studies by different researchers (Barea and Cardenas, 1975; Gao et al., 2010; Heredia-Arroyo et al., 2010; Baldisserotto et al., 2016; Mondal et al., 2016; Wang et al., 2018c; Oliveira et al., 2021) who concluded and acknowledged that mixotrophy resulted in higher algal growth rate as opposed to autotrophy and/or heterotrophy; moreover, lipid content too has been observed to be comparatively higher in microalgae cultivated under mixotrophic mode.

4.5. Light intensity and photoperiod

Microalgae are sunlight-driven batteries producing O_2 and organic exudates as photosynthesis products, therefore the light energy source is an essential factor for its cultivation affecting its overall growth rate. Utilization of light energy with low intensity may lead to retard photosynthetic rates in microalgae, whereas high illumination may cause photoinhibition by damaging the photosynthetic pigments (Zuroff and Curtis, 2012). Moreover, Huo et al. (2011) reported that microalgal biochemical composition is manipulated by the type of illumination supplied. A high-intensity light may trigger cellular stress-causing intracellular accumulation of triacylglycerols (TAGs) (Huo et al., 2011). Therefore, optimizing light intensity is important to find a balance between maintaining the photosynthetic receptors and achieving high lipid accumulating phase. The significance of photoperiod in the co-culturing system was discussed by Juneja et al. (2013), where it was stated that light: dark periods determine the light-capturing efficiency of microalgae, as extended dark hours with high-intensity illumination facilitates complete utilization of the accepted photons by the photosynthetic machinery and convert it into chemical energy, hence higher photosynthetic yield is promoted by avoiding the effects of photoinhibition (Juneja et al., 2013). Furthermore, Das et al. (2011) reported that blue light triggers growth and lipid productivity in microalgae as opposed to red light (680 nm) (Das et al., 2011). Moreover, it should also be considered that screening of partner organisms for co-culturing should focus on selecting organisms matching the growth rate of the microalgal strains; otherwise, it may so happen that the heterologous partner may outcompete the algal population, with limited light supply due to competition and shading, being the determining factors for the final product yield (Subashchandrabose et al., 2011; Grant et al., 2014; Praveen and Loh, 2015; Padmaperuma et al., 2018).

4.6. Cultivation time

Cultivation time is optimized depending on the types of strains used, for instance, most of the yeast strains and the oleaginous microalgae show high lipid accumulation in the early stationary phase of their growth curve (Sitepu et al., 2013). The study also reported that these microorganisms have their lipid peroxidation pathway activated on

entering into the late stationary phase, consequently, TAG content gradually drops (Sitepu et al., 2013). Hence, the maximum lipid accumulation phase (LAP) for the co-culture system is determined by the culture time, and achieving maximum LAP is crucial to obtain the highest lipid yield during harvesting biomass (Arora et al., 2019).

4.7. Culture pH, temperature

Microalgal growth is highly specific and dependent on culture parameters (pH, temperature, etc.). Optimizing the culture conditions is a prerequisite for carrying out a successful co-culture system (Chu, 2017). Maintenance of the pH of the culture broth is necessary since it modulates and determines the CO₂ solubility and accessibility along with other essential nutrients (Juneja et al., 2013; Arora et al., 2019).

It has been observed that a rise in the pH of the growth medium results in limited CO₂ availability to the microalgae, thereby increasing the lipid accumulation ability in the microalgae as the growth is restricted (Huo et al., 2011; Juneja et al., 2013). Cheirsilp et al. (2011) reported that for a microalgae-yeast co-cultivation system, a pH of 5 was optimized for a balanced growth rate.

However, a challenge frequently faced while co-culturing microalgae with other heterologous partner microorganisms is that as the microalgae grows, the pH of the growth medium gradually rises with time because atmospheric CO₂ in water forms bicarbonate (HCO₃⁻) at neutral pH, which is further converted to CO₂ and hydroxide ions (OH⁻), during the phototrophic process (Nayak et al., 2018). As a result of this, when microalgae consume CO₂ from the medium for their growth, excess OH⁻ ions are left in the medium, resulting in an increased pH (Zhang et al., 2014b).

Optimizing the culture growth temperature is crucial to achieve maximized growth rate with significant nutrient uptake (Juneja et al., 2013). In microalgae, low temperatures lead to a reduced CO₂ fixation rate, hence a slower electron transport. Moreover, fluctuations in temperatures, above or below the optimum range, leads to problems, such as inhibition of photosystem II (PS II) (Juneja et al., 2013), alterations in the fatty acid profiles, mainly fatty acid unsaturation (Harwood, 1998) and this tendency to get converted to unsaturated forms could be explained by the fact that this form helps in maintaining cell membrane fluidity cultured at lower temperatures (Harwood, 1998; Chu, 2017). Furthermore, alterations in temperature (ranging from 10 to 40 °C) induce increment in protein/lipid ratio in both yeast and microalgae (Juneja et al., 2013; Vanhercke et al., 2013). Sharma et al. (2012) concluded that instability in lipid profiles in a microalga due to temperature changes might thereby affect the properties of algal biodiesel as a whole under different climatic conditions and seasons (Sharma et al., 2012).

However, co-culturing psychrophile (optimal temperature < 15 °C), halotolerant (salt tolerance < 2.5 M salt) or thermophile (temperature ranging from 41 to 122 °C) yeast and microalgae could be considered as an approach since they could be grown in a wide range of climatic conditions with no requirement for maintaining the temperature, hence decreasing the production cost (Arora et al., 2019).

4.8. Culture agitation strategy

Optimization of the agitation speed is another critical point since it modulates mass transfer rate, thereby controls the exchange of O₂-CO₂ in the broth (Arora et al., 2019). A study by Cheirsilp et al. (2011) observed a linear relationship between the growth rate and the agitation rate (~150 rpm), however, as the speed was increased in revolutions per minute beyond that, it showed no visible effect on the growth rate of the microalgae-yeast co-culture.

4.9. Sterile/nonsterile condition

Generally, for the binary culture approach, sterile conditions are maintained, nonetheless, supporting such sterile conditions while

scaling up the whole process is an energy-demanding as well as an expensive step, as a result of which the industrialization of such strategies are mostly limited. To study the effects of sterile/non-sterile conditions, Chi et al. (2011) carried out the second yeast cultivation step under non-sterile conditions and observed reduced lipid yield as opposed to that under sterile conditions; however, under non-sterile conditions, the organism showed more efficient COD removal than that under sterile conditions. In a similar study, Ling et al. (2014) reported for a co-culture of *R. toruloides* and *C. pyrenoidosa*, where a lipid content of 63.46% (w/w) and 4.60 g L⁻¹ of lipid productivity and 95.4% of soluble COD removal rate was observed after 5 days of mixed cultivation with distillery wastewater and urban wastewater mixture (1:1) under nonsterile conditions as the culturing medium (Ling et al., 2014; Iasimone et al., 2018).

5. Life cycle assessment, economic perspective and biorefinery approach

Life Cycle Assessment (LCA) is a methodological tool to analyze the various environmental impacts, health hazards linked with life, different life processes of a product and its activities. In this review, LCA evaluates the sustainability, feasibility as well as ecological impacts associated with the production process of microalgal biomass and lipid, utilizing the co-cultivation strategy. LCA works by considering the input (raw materials) and the output (emissions, by-products) related to the life cycle of the product and quantifying them along with a comparison to the various health and environmental impacts (Morales et al., 2015; Nezamhahalleh et al., 2018; Ubando et al., 2019). This co-cultivation strategy of microalgae facilitates flue gas/CO₂ sequestration, and wastewater treatment along with biomass production as bioenergy feedstock. The co-cultivation methods assist in the removal of heavy metals from wastewater (municipal/industrial) and minimizing air pollution. It can be stated that the overall life cycle analysis of the different high-value yields from the microalgae co-culture, such as biomass, lipids, carbohydrates, pigments, proteins, etc., is a necessary step to improve the sustainability of the whole procedure. In addition, optimization of the culturing parameters, proper co-product or by-product rescue, spent media recycling, etc., are a prerequisite. Proper implementation of the co-cultivation strategy of microalgae can lead to a better environmental profile of the final product (Chew et al., 2017; Banu et al., 2020; Karpagam et al., 2021).

To determine the economic feasibility of the whole process, techno-economic analysis (TEA) is implemented at the research and development stages (Karpagam et al., 2021). In this regard, the co-cultivation strategy of microalgae benefits by enhancing the biomass and lipid yield/productivity. It is estimated that with lower biomass concentration, an open pond microalgal cultivation system incurs 45.73% of the total cost (Banu et al., 2020) and hence considered as one of the bottlenecks for the commercialization of algal-based biofuel. This approach of co-culturing method helps to minimize the overall biomass harvesting cost which covers around 20–30% of the overall production revenue. Flocculation with the co-cultivation approach is feasible due to the inherent flocculating characteristics of bacteria, fungi, yeast, etc. (Nazari et al., 2020). Furthermore, using wastewater as the nutrient source and flue gas as the carbon source for microalgae binary cultivation systems minimize the overall production cost of biomass/biofuel. Coupling wastewater treatment with microalgal cultivation not only resulted in a reduced overall production cost but is observed to have contributed towards 12–27% biofertilizer recovery as well as 19–39% CO₂ mitigation (Judd et al., 2017). Gong and You (2014) had projected a model for capturing CO₂ from a coal power plant and its conversion to high-value bio-products by utilizing the sequestered CO₂ for cultivating microalgal biomass. Furthermore, it was observed that on proper execution of the biorefinery model, there was cost reduction from \$33.65/ton of CO₂ to \$9.52/ton of CO₂ for a 300–2400 MW power plant for optimum unit CO₂ sequestration and operational cost respectively. Moreover, substituting conventional methods like nitrification-denitrification for wastewater treatment with microalgal

co-cultivation, a savings of around \$172.41 t⁻¹ biomass is estimated (Kumar and Singh, 2019). In another study by Fasaie et al. (2018), it was stated that, the overall operational cost and energy usage fall in the range of 0.5–2 € kg⁻¹ algae and 0.2–5 kWh kg⁻¹ of algae, respectively in open pond cultivation systems, whereas, in closed cultivation systems, the cost and energy utilization are within 0.1–0.6 € kg⁻¹ algae and 0.1–0.7 kWh kg⁻¹ algae, respectively (Fasaie et al., 2018). Furthermore, fungi/yeast/bacteria/self-flocculating microalgae aided bio-flocculation of microalgae is another area that is being studied vigorously (Ray et al., 2021) and it has been reported that harvesting microalgal biomass by bio-flocculation would approximately cost about A\$0.13 m⁻³ of the medium (Lee et al., 2010), which significantly reduce the total harvesting cost.

The major aim of constituting an algae-based biorefinery is to minimize the use of resources with an assured maximized productivity and negligible waste contribution. The microalgal co-culture strategy provides a biorefinery platform by virtue of its ability to be incorporated in wastewater/industrial effluents remediation along with the production of high-value compounds eliminating the use of freshwater resources and ensuring water recycling. Makut et al. (2019) carried out co-cultivation with four different strains for consortia development; two microalgal strains, *Chlorella sorokiniana* DBWC2 and *Chlorella* sp. DBWC7 and two bacterial strains *Klebsiella pneumoniae* ORWB1 & *Acinetobacter calcoaceticus* ORWB3. This co-culturing method was coupled with wastewater treatment and effectively resulted in almost 85–95% nitrate removal as well as about 80–90% COD removal (Makut et al., 2019). Moreover, in co-cultivation methods using flue gases as carbon source ensures the whole bio-production procedure is a carbon-neutral process since CO₂ can be fixed by microalgae, and CO₂ released by respiration is utilized by the partner heterotrophic microorganisms in the binary culture as their carbon and energy source. This altogether enables the yield of a spectrum of bio-products which are renewable, sustainable, bio-degradable along with wastewater/waste gas bioremediation, thereby helping in developing a suitable biorefinery system (Rosero-Chasoy et al., 2020). Biomass obtained from the microalgal co-culture system can be utilized for manufacturing a number of novel bio-compounds, where researchers have already chemically identified about 15,000 of them (Rawat et al., 2013; Rosero-Chasoy et al., 2020). In this scenario, residual biomass from the co-cultivation system can be utilized to produce various high-value products like carbohydrates, lipids, pigments, proteins, antioxidants, etc. These multiple outcomes help to contribute not only to bioenergy production but also has implementations in the pharmaceutical, cosmetic industries (Chew et al., 2017). Proteins are beneficial as health supplements, moreover, complete rescue and re-utilization of the residual/de-oiled biomass after lipid extraction is another major step for a biorefinery approach. De-oiled biomass from the algal co-culture system can be reprocessed as a raw material for further production of bioethanol, bio-butanol, biogas, bio-oil, and biochar (Özdenkçi et al., 2017). De-oiled biomass can also be used as bio-fertilizer for enhancing crop production and maintaining soil fertility (Nayak et al., 2019). Therefore, several factors determine a robust algal biorefinery, such as 1) energy consumption, 2) ratio of raw material used to productivity, 3) quality of the bio-products, 4) environmental impacts, etc. Proper implementation of the co-cultivation method with careful consideration of the aforementioned factors, techno-economic analysis (TEA), and life cycle assessment (LCA) will further strengthen the biorefinery framework of the whole process and can contribute towards a greener environment (De Bhowmick et al., 2019; Banu et al., 2020).

6. Challenges, recommendations, and future outlook

6.1. Challenges of the co-cultivation strategy

Co-culturing strategy alongside benefitting us also incurs multiple disadvantages, such as bacterial-microalgal binary culture, extracellular

secretion of various algicidal toxic metabolites, cell-wall degrading enzymes, etc., may cause algal cell lysis and inhibit their growth. (Cole, 1982; Fukami et al., 1997; Fergola et al., 2007; Mujtaba and Lee, 2016). Moreover, both open (raceways ponds) and closed (conventional photobioreactors) systems at an industrial scale are prone to contamination frequently (Dias et al., 2019). Therefore, the industrialization of such co-culture methods involves sterilizing the scaled-up culture system and utilizing of low-cost substrates, where such a large-scale sterilization step increases the global production cost (Dias et al., 2019).

For a binary culture, suitable strain selection is one of the major challenges since in absence of symbiosis the growth of microalgae could be negatively hampered leading to undesirable outcomes. Furthermore, to commercialize co-culture method, the scaled-up system will be exposed to various environmental changes, such as fluctuating nutrient availability, temperature and pH fluctuations due to gradients generated caused by irregular and insufficient stirring of the culture broth leading to a more heterogeneous medium, making these factors major limitations for proper implementation of this co-cultivation strategy at industrial-scale. Hence, developing a suitable culture medium which would support the growth of both the strains in the binary culture is another major task. Another critical point while operating large scale bioreactors for such co-cultivation processes is the formation of some O₂, or CO₂ depleted areas while cultivating obligate aerobic microorganisms (mostly yeasts) or autotrophic microorganisms such as microalgae (da Silva and Reis, 2015) and such dead zones will trigger poor mass transfer, biomass settling thereby cellular stress. Fortunately, these patches of depleted areas are possible to mitigate through the synergetic O₂-CO₂ exchange between mixotrophs/autotrophs/heterotrophs in non-axenic cultures (da Silva and Reis, 2015). Therefore, it is essential to optimize the aeration and agitation rates for binary cultures, both at the lab and industrial scale; otherwise, high aeration and stirring rate may also negatively affect the cells, causing hydrodynamic stress and cell damage (Dias et al., 2019). Furthermore, designing a reactor with optimized culture parameters is a prerequisite for a successful co-cultivation process (Dias et al., 2019).

Another crucial aspect of this co-culture system that needs attention is the accessibility to the light source while performing co-cultivation of high cell density symbiotic (microalgae/yeasts) organisms. Such a dense population will obstruct the light pathway, inhibiting microalgal growth and lipid productivity (da Silva and Reis, 2015).

6.2. Recommendations of the co-cultivation strategy

Different researchers have suggested various approaches in order to combat the loopholes of this co-cultivation strategy. Firstly, as Olguín (2012) stated, extrapolating data from laboratory-scale studies should be avoided in terms of biomass and lipid or any value-added product yield and biofuel productivity (Olguín, 2012). Secondly, to alleviate the high operational cost that sterilization steps incur at industrial scale studies, utilization of detergent and phenol-based disinfection/sanitation to avert contamination of the culture broth. Apart from these approaches, many other cheaper methods could be implemented to control contamination (Iasimone et al., 2018). The study by Iasimone et al. (2018) also stated that due to photosynthetic activity by microalgae, the culture broth suffers an elevation in the pH and DO values which may inhibit other fungal and bacterial invasions, specifically by the end of the growth period (Iasimone et al., 2018).

It was suggested by Mata-Gómez et al. (2014) and Chagas et al. (2015) that valorization of different microbial biomasses may be a preferred option since a few oleaginous microbial strains, apart from producing triacylglycerols, also give efficient yield for value-added compounds such as carotenoids and poly-unsaturated fatty acids with commercial interest which may result in increased revenue of the overall process and enhance the economic viability of the bioprocess (Mata-Gómez et al., 2014; Chagas et al., 2015).

6.3. Future outlook of the co-cultivation strategy

There is limited information on the mechanism of symbiosis between microalgae and other partner organisms, such as the involvement of amino acids and vitamins-dependent interactions that have been identified between microalgae and bacteria, whereas detailed study at the molecular level is lacking. It is also stated that optimized control of such chemical interactions has been regarded as an effective method to escalate productivity in these cultures; hence research at the molecular level should be focused on. Future studies should be aimed at understanding the biochemistry of these symbiotic interactions focusing on reduced nutrient expenditure, intracellular cellular product recovery, harvesting algal biomass, and efficient biofuel production (Olguín, 2012).

Also, the recognition of the correct symbiotic partner organism for co-culturing microalgae requires further detailed study and will provide profound implications for future utilization and manipulation of microalgae in the field of bioenergy and other biotechnological applications (Yao et al., 2019). Another approach that might be focused on is the development of genetically modified strains by classifying the various stress-reactive genes which could be engineered into the strains to achieve a better symbiotic interaction to ensure an enhanced lipid productivity along with other value-added products, assuring an effective algal biorefinery. Moreover, approaches to couple this co-culture strategy primarily aimed at biomass and lipid enhancement with wastewater treatment by utilizing it as the culture medium instead of synthetic media could further reduce the overall cost of the process (Gupta et al., 2019). Therefore, future research should pay attention to associating microalgae, bacteria, fungi and co-cultivate them with specific enzymatic steps and major metabolic pathways to facilitate a higher and improved degree of operations control (Roccuzzo et al., 2020). With culturing microalgae by co-cultivation strategy can lead to a successful biorefinery for production of different value-added products with enhanced quality in addition to the biofuel production.

7. Conclusion

Microalgae have been widely acknowledged as one of the most potential, renewable energy feedstocks. Despite many advantages, microalgae have low biomass productivity and a high-cost harvesting process. Implementation of co-culturing techniques on microalgae with other microbes enhances the overall biomass production and facilitates biomass harvesting via bio-flocculation. Several studies have recently focused on such co-cultivation experiments of microalgae and have observed improved productivity of lipids as well as other value-added products along with biomass, hence have a wide range of applications: biofuel production, health care, cosmetic industries, etc. Successful industrialization of microalgal-based fuels and other byproducts largely depends on the optimization of growth conditions, pretreatment costs and methods, efficient solvent separation, harvesting, and conversion to biofuel. In this review, co-cultivation strategies were discussed in detail to critically assess the pros and cons of their implementation in technology advancement. Also, various trophic modes and their impact on various co-cultivation strategies were discussed. Systematic studies might bring a major advancement in microalgae co-cultivation in an economically and eco-friendly feasible way to scale up microalgal-based fuels. Besides, employing biodegradation of waste materials as the major nutrient source for microalgal co-cultivation along with parameter optimization to achieve multifaceted values would develop an effective algal biorefinery leading to greener and carbon-neutral energy.

Declaration of competing interest

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

Acknowledgments

AG acknowledges the support from Department of Science and Technology (Grant No. CRG/2020/002080), Department of Biotechnology (Grant No. BT/RLF/Re-entry/06/2013) and Scheme for Promotion of Academic and Research Collaboration (SPARC), MHRD, Govt. of India (Grant No. SPARC/2018-2019/P265/SL). MN acknowledges the support from Department of Biotechnology (DBT), Govt. of India, New Delhi-Ramalingaswami fellowship (No. BT/RLF/Re-entry/43/2018). The authors also gratefully acknowledge their institute, IIT Kharagpur and AUUP, for the infrastructural support.

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