Construction of co-culture of microalgae with microorganisms for enhancing biomass production and wastewater treatment: a review

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Abstract. The development of microalgae cultivation in wastewater has been explored for years. Several wastewaters and nutrient sources related to biomass generation have been combined in recent years. On the other hand, several factors seem to reduce the possibility of industrialized this concept shortly. The growth rate and harvesting cost of the algae are often pointed as the leading cause of the gap for industrialization of this concept. In order to counteract these problems, constructing microalgae in the form of co-culture consortia with microorganisms, such as bacteria and yeast, have been reported to enhance the production of biomass under a short period of cultivation. This review highlights the strategies to combine microbial strains and microalgae for improving the process of biomass generation based on the comparison of the productivity of single and consortium of microalgae cultivation. Subsequently, mechanisms to enhance microalgae growth are scrutinized based on their interaction. Furthermore, critical factors regarding the construction of the consortia are discussed. Eventually, identifying gaps in this concept is displayed to describe the path of future focuses in this potential field.

1. Introduction

Microalgal biomass generation currently gains more attention since it has many possibilities to apply on an industrial scale and versatile applications [1]. Biomass products for secondary metabolites, lipid, and more fuel products are among the application of microalgae [2-4]. Low water footprint and higher productivity are mentioned as the advantages of microalgal biomass compared with terrestrial biomass from the crops and forests [5].

As microalgae cultivation faces nutrient source problems, coupling the cultivation with wastewater treatment rises as a promising method to grow the algae alongside the wastewater treatment process [6]. The high nutrient content of wastewater and the demand to remove the nutrient creates an opportunity to apply microalgae in the wastewater system to remove nutrients effectively [7]. At the end of this process, the outcomes are the biomass of algae and recovered water [8]. Production and harvesting processes are the most burdening factors for biofuel generation from microalgae [9]. The sequential process of flocculation and/or filter can only lead to the negative balance of the capital cost for such renewable energy [10]. Thus, many alternatives and solutions have addressed this barrier to reach biodiesel's feasibility generated from algae.
Among the solutions offered, a combination of algae and consortium of algae with other co-culture organisms are two main approaches to increasing this system's productivity in recent years [11]. Several strains of microalgae have been put together, and it resulted in great biomass yield [12]. Furthermore, the benefit of this interaction is also rendered in the harvesting process [13]. However, the combinations appear to have a more complicated system in which interactions among the organisms occur [14]. To obtain optimum biomass production enhancement from the interaction, it is vital to understand the various mechanisms of alga and other microbes. This review is aimed to (1) investigate microalgae and other microbial interaction in microalgal cultivation system, (2) identify and specify the effects of co-cultivation in biomass productivity, (3) summarize the application of co-cultivation in the harvesting process, and (4) draw the gap and future demand of knowledge regarding the practical application of microalga and other organism co-cultivation.

2. Interaction of algae-microbes in cultivation
Microalgae often interact with other organisms, either as mutualism or parasitism. Although there are other interactions between microalgae and other microbial such as commensalism, these two interactions are often referred to as an essential role in microalgal biomass generation. The mutualism interaction is shown by algae and bacteria, even though bacteria are often considered contaminants in algal cultures [15], while parasitism interactions are frequently observed in microalgae with pathogen microbes.

2.1. Mutualism
Mutualism interaction in wastewater is often regarded as an exchange product between microalgae and other microorganism (figure 1). The mutualism interaction between algae and bacteria are divided into four types, which are nitrogen-fixation, nutrient-exchange, signal transduction, and gene transfer [16]. In addition, bacteria also play a role as protector of algae from toxic compounds such as heavy metals [17] as some of these microorganisms were reported to be able to detoxify the heavy metals by transformation to less toxic forms, adsorption, or precipitation [18].
Moreover, the interaction between algae and bacteria occurs as nutrient-exchange [16], including macronutrients, vitamins, and other micronutrients [15]. Also, bacteria can mineralize many organic substrates, supplying the algae with CO₂, minerals, vitamins, and growth factors [19].

2.2. Parasitism
Besides the beneficial interaction between bacteria and algae, it also has an unfavorable condition. Bacteria can act as parasites through the interrupt of algal growth. The algal cell is lysed by the activity of certain enzymes such as cellulase, chitinase, glucosidase, and other enzymes [20]. Once the algal cell lysed, the bacteria can use algal intracellular compounds as nutrients sources. It is also noteworthy that healthy microalgal cells are able to control the bacteria colonization on their surfaces, and there may be
inhibiting the excessive growth of bacteria, in turn, reducing accessibility to nutrients and light. On the other hand, the biofilm of the bacteria could harm algae because the bacteria are able to penetrate algae tissue and cause disease [20].

3. Co-cultivation of microalgae for enhancing biomass generation

Application of microalgae consortium has been recognized for wastewater treatment as well as generating biomass for the algae culture. Many studies have been developed, and the consortia were also tested into many wastewater samples and compositions (table 1). The co-culture of the bacteria, fungi, and consortium from indigenous wastewater sources creates a wide opportunity to generate a high concentration of microalgal biomass using a low clean water footprint with remarkable removal.

Table 1. Consortium containing microalgae for biomass generation with wastewater as the main substrate.

| Microalgae                  | Co-culture                                             | Wastewater                                         | Biomass         | Nutrient Removal |
|-----------------------------|--------------------------------------------------------|----------------------------------------------------|-----------------|-----------------|
| *Chlorella sorokiniana*     | Aerobic sludge (activated sludge microbial communities) | The treated liquid fraction of pig manure          | 26.3 mg/L d⁻¹   | 86a             |
| *Chlorella vulgaris*        | photobioreactor treating swine slurry bacterial seed (bacteria consortium) | Swine slurries                                    | 0.057 g/L       | 90b >95c        |
| & *Scenedesmus obliquus*    |                                                        |                                                    |                 |                 |
| *Chlorella pyrenoidosa*     | Leachate indigenous bacteria                            | Municipal wastewater and landfill leachate         | 1.58 g/L        | 90c             |
| *C. vulgaris*               | *Ganoderma lucidum* (fungus)                           | Anaerobically digested swine wastewater           | 4.77 g/L        | 79b 76c 85d     |
| Unknown microalgae consortium | from microbial consortium *Bacillus* sp. (bacteria) Fracturing wastewater (synthetic) | Primary effluent municipal wastewater             | 0.018a          | 83b 100d        |
| *C. vulgaris*               | *Bacillus* sp.                                        | Palm oil mill effluent                            | 2.23 g/L        | 45b             |
| *Coelastrella* sp., *Chlamydomonas* sp. & *Scenedesmus* sp. | Native bacteria consortium                             |                                                    | 1.2-1.5 g/L     | 70b 40c         |

Table 1. Consortium containing microalgae for biomass generation with wastewater as the main substrate.

3.1. Co-culture with bacteria

Bacteria can help to increase the algal biomass during the microalgae cultivation processes. Vitamins and amino acids have been identified as the main mediator molecules that regulate the interaction between bacteria and microalgae during biomass production [14]. Two bacteria strains, *Muricausa* sp., and *Alteromonas* sp., were reported to be able to enhance the accumulation of *Dunaliella* sp. biomass [27]. A substantial increase was also recorded in nitrogen incorporation, which suggested that nitrogen availability for microalgae growth was affected by these bacteria activity [27]. The co-culture of
microalgae and other organisms has been documented widely as the emerging solution for increasing biodiesel's feasibility by nutrient exchange and additional production of hormones [28].

The co-cultivation of microalgae is not limited to the unique culture of bacteria nor yeast to obtain an optimum biomass productivity increase. Freshwater microalgae Chlorella sp. has been reported to be cultivated with activated sludge microbial communities from the sewage treatment plant [29]. With the ration 4:3 of activated sludge and microalga, 130 mg/L of microalgal biomass has been reached. Furthermore, Qi W, et al. (2018) studied the pure culture of microalgae Chlorella sorokiniana with the three most dominant bacteria from fermentation wastewater after treatment, and the treatment resulted in a one-fifth increase of the algal biomass compared with the pure culture cultivation [30]. Nevertheless, the same study also reported the co-cultivation only resulted in 42% of flocculation efficiency.

3.2. Co-culture with yeast
Yeast Rhodotorula glutinis and microalga S. obliquus showed a mutualistic interaction of gas exchange and trace elements for one another, resulting in higher biomass up to two-fold [31]. Frequent interaction of yeast and microalgae relates to the release-uptake of CO₂ and pH balance. Organic acid released from yeast and exudate from microalgae in day time tends to play a critical role in pH balance [32]. Moreover, Yen H W, et al. (2015) found that temperature and carbon source played an important role in determining the co-cultivation of algae biomass's total lipid production [31].

Here, it is essential to mention that the application of this combination needs deep consideration regarding the organisms' behavior. Microalgae prefer a neutral pH environment to grow [33], while several oleaginous fungi prefer low pH [34]. Another notable constraint is that in the microalgae and yeast system, organic carbon shall be supplied into the system to support the high quality of lipid production [35]. Furthermore, the condition of microalgae in the reactor lacks stabilization since the pure culture is very susceptible to slight change and contamination.

3.3. Microalgal consortium
Strategies for growing more strains of microalgae are based on the different niche between strains. In order to obtain optimum removal in wastewater, and high lipid production from biomass, the occupation of each nutrient and source of carbon needs to be optimized. Since more than one microalgae are competing for similar resources, it is important to pay attention to the initial seed composition for each alga.

Here, more than one strain that grown together tends to have more competition to obtain high nitrogen, and thus nitrogen depletion can be easily triggered. Nitrogen starvation creates an increase of acetyl-CoA content, which is a precursor in the fatty acid pathway [36]. Mechanism of communication among strains is related to the production of chemicals extracellular interaction that can affect or increase lipids production [37]. However, as the application of removal focuses on both algal biomass generation and contaminant removal in wastewater, the application of microalgal consortium, especially the indigenous ones, is preferably here. The production of lipid from indigenous strains was lower than high lipid content strains in the cultivation using carpet mill wastewater, the nutrient removal of indigenous strain was much higher than that using the introduced strains [38].

4. Harvesting processes and importance of co-culture for enhancing the removal
As mentioned earlier, harvesting is also holding a vital factor that interferes with the industrialization of biofuel from microalgae [39]. Flocculation is the first step in the utilization of the algae. The process is started by adding chemicals, or flocculants, to neutralize or reduce the negative surface charge on algae cells [40]. Some organic and biological flocculants were employed to increase the flocculation efficiency, such as chitosan and tannin, or using biological agents as flocculants such as bacteria [41,42]. The bioflocculant group is preferable since it has sustainability and lower total cost compared with the former one [41].

It was reported by Li Y, et al. (2017) that the algae were flocculated by a novel Actinomycetes activity [43]. The flocculation was examined by creating mycelial pellets to attract and attach the microalgae
cell. CaCl₂ addition was also reported to initiate the process of flocculation. That the activity of flocculation from some biological agents related to the amino acid in the cell walls [44]. It was also not related to the viability of the cells. However, there is no specific study with this microbial on a much larger scale, which is essential as well.

5. Current gaps and future prospect
There is no specific condition where all organisms (microalgae, bacteria, and fungi) can grow simultaneously and equally contribute to lipid production [35]. However, optimum production of lipids is the main goal for producing biofuel from wastewater as the substrate, and thus mutualism relationship is the primary goal of this application. Here, co-cultivation is expected to efficiently convert the inorganic and organic contaminants in wastewater into biomass. Based on this concept, several factors are needed to be deeply deliberated for optimizing the concept.

Firstly, co-cultivation needs to have the ability to remove carbon, nitrogen, phosphorous, heavy metal, and other hazardous components in wastewater [45]. In this situation, co-cultivation has advantages to cope with these contaminants removal because the microorganisms have a wide range of niche, and interaction among organisms can create a broader range of possibilities to involve such a vast amount of compound in the removal process. Secondly, advantageous interaction between microalgae and co-cultivate can be detrimental to the wastewater containing strong microbiome stability [46]. Thirdly, more studies are needed to follow up on the co-cultivation process until the harvesting process. As mentioned before, some co-cultivation can improve the harvesting process by attachment to form floc or pellets [43]. Several co-cultivations were also found not to have such interaction or advantages [46]. Here, a comprehensive process is demanded to be conducted to improve the holistic system. Taking together, in the future, the study in this field may strongly related to (1) application of modeling concept on nutrient mass-balance of nutrients by the co-culture, (2) essential investigation of interaction in wastewater that contains dynamic microbial composition, and (3) holistic application for cultivation and harvesting process.

6. Conclusion
Application of microalgal consortium and co-cultivation of other microbes can create a great step forward of the industrialization of microalgae cultivation for wastewater treatment and algal biomass production. Mechanisms of interaction and the response for growing in the wastewater are essential to be developed in this method. To the best of our knowledge, many aspects still need to reconsider namely (1) removal efficiency and consistency after several cycles of application, (2) entire extracellular communication for a holistic picture of application and mitigating the change of wastewater constituents, and (3) holistic application for not only biomass production but also biomass harvesting process as the most expensive step.

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