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Review

Co-culture for lipid production: Advances and challenges

S. Magdouli, S.K. Brar, J.F. Blais*

Institut national de la recherche scientifique (Centre Eau, Terre et Environnement), Université du Québec, 490 rue de la Couronne, Québec, QC G1K 9A9, Canada

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ABSTRACT

The exploration of microbial communities to efficiently produce biofuels has become a critical approach among biochemical processes. Co-cultures have been intensively studied to address the limitations in substrate utilization by individual strains for the production of other bioproducts. Accordingly, many concerns have arisen about the effects of this strategy on lipid productivity. Despite the extensive research on lipid production by oleaginous microorganisms, co-culture strategy has been only well-reviewed in algal species and most of the original research has been concentrated on the different nutritional growth modes (e.g. heterotrophic and mixotrophic). Moreover, current literature indicates scarce information on strategies for the improvement of lipid production with other species rather than microalgae. From a systematic perspective, this review will highlight co-culture systems existing for the improved biomass and lipid productivity, among other species. The review first discloses the current state of microalgal assemblies and their strategies for lipid production. Subsequently, it summarizes other assemblies aimed at lipid production. Finally, it discusses the relative advantages and disadvantages and the possibilities to overcome inherent challenges.

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Contents

1. Introduction

Intense utilization of fossil fuels for energy production has resulted in global environmental pollution as well as resource depletion, and climate change. This requires an urgent research for new attractive substrates with pre-determined patterns for energy production [1]. Hence, the necessity of developing an environmental friendly and sustainable energy source that can meet the global requirements and satisfy the existing quality standards has become a pressing challenge. An alternative way to produce biodiesel is the use of microbial oils through the cultivation of

* Corresponding author.
E-mail addresses: sara.magdouli@ete.inrs.ca (S. Magdouli), satinder.brar@ete.inrs.ca (S.K. Brar), jean-francois.blais@ete.inrs.ca (J.F. Blais).

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microbial communities. These microbes in turn can present many advantages, such as short-life cycle, higher production rate, less labor requirement and easy scale-up [2,3]. Oil-rich microbes, called single cell oils (SCO), are mainly produced by oleaginous microorganisms known for their high lipid productivity and oil content that exceeded 20% of biomass weight [4]. Most common studies on microbial oils have been carried out on single cell cultures. However, recent concerns in this field have been oriented towards co-culture system for biodiesel production. This system has been recognized as an efficient model due to the existing interactions between different cultures in most natural environments. Commonly, microbes; clusters of microbial cells, are found in close association, they grow and survive in the same biocoenosis as long as the nutrient sources are available, either in mutualistic relationship or as antagonists. Therefore, microbial interactions (e.g. physical and biological) existing among cultures could be exploited and reproduced at laboratory scale. This concept is not new and for decades, mixed cultures have played primary role in treating wastewaters [5,6] producing biomass and bioactive compounds [7] and degrading halogens and hydrocarbons [8,9]. Other applications are illustrated in Table 1. The use of co-culture system for value-added products (VAP) production is challenging, so that only few works have been reported for the production of polyhydroxyalkanoates (PHA) and biohydrogen using the co-culture of *Enterobacter aerogenes* and/or *Rhodobacter spheroides* and *Rhodopseudomonas* BHU01 [10,11].

The objective of the mixed culture was mainly based on the mixture of more than one species in such a way that one strain possesses an enzymatic activity that the other is lacking. Among successful trials, the mixture of amylolytic microorganism with a non-amylolytic producer strain to hydrolyze starch as carbon source was highly representative. *Saccharomycopsis* (*Endomycopsis*) *fibuliger* was used as the amylolytic microorganism either in combination with bacteria or yeasts to produce many metabolites, such as single cell protein (SCP) [12], lactic acid [13] and ethanol [14], extracellular polymeric substances (EPS) through the co-culture of microalgae/cyanobacteria and macromycetes [15].

Currently, co-cultivation system is aimed to overcome the contradiction existing between biomass productivity and lipid content to obtain significantly higher lipid productivity. In fact, high lipid content is often offset by lower growth rates and the increase in lipid content does not result in increased lipid productivity, however, it leads to lower biomass and lipid productivity. So far, many studies have focused on increasing the lipid productivity through promotion of the accumulation of total biomass and lipid yield via co-immobilization technique using various bacterial species. Generally, research regarding this approach has been reported for algal species. For instance, the assembly between *Chlorella vulgaris* and *C. sorokiniana* co -immobilized with *Azospirillum brasilense* has resulted in an increase in the lipid content of the cells more than 350 (μg/g dw). Not only did the lipid content increased, but also, a remarkable variety of fatty acids increased from five to eight different fatty acids in microalgae co–immobilized [16]. Additionally, coupling algal growth with other microbial species, either, algae, yeast or fungi has been reported for many other species, such as *Monoraphidium* sp FXY10 [17,18], *Rhodotorula glutinis* and *Ambrosiozyma cicatricose* [18–21]. Thus, co-culture system between different microorganisms either algae, yeast or bacteria is challenging to address for lipid production.

This review, thus, aims to highlight the different approaches of co-culture systems designed for lipid production and to identify the key parameters to overcome some of the technical challenges associated with such systems. Furthermore, the relative advantages and disadvantages will be also summarized in the review. Finally, the processes or technologies currently available on lipid accumulation in co-culture systems to overcome the challenges inherent to this field of work will also be discussed.

Table 1
Biotechnological potential of mixed cultures.

Purposes	References
Harvesting bioflocculants	[9,10]
Wastewater Treatment	[11,12]
Production of EPS	[13]
Single cell protein	[14–16]
Flocculation process	[18,20]
Electricity generation	[22]
PHA production	[7,8,23]
Organic acids production	[24,25]
Heavy metals removal	[26]
Biohydrogen production	[7,8,28]
Ethanol production	[27,29–31]
Growth promotion and Lipid production	[11,32,34,35]

2. Microalgae for lipid production

Microalgae have been well-reviewed as a potential factory for lipid production for biofuels [22]. It is presumed that achieving high yields and titers for industrial production might require improvement of algal strains through genetic engineering or recombinant DNA technologies [23,24]. Previous studies on lipid accumulation via multispecies microbial consortia were limited. Accordingly, relations existing between algal species, beneficial or antagonistic, mutualistic or symbiotic are being studied and further research is currently underway. Besides, microbial consortia can perform more complex tasks as compared to mono-cultures and can carry out difficult functions impossible for individual strains or species [25]. Numerous studies have focused on Chlorophyta, which have higher oil contents, and can be easily cultivated, particularly, *Chlorella* species [23,26]. In nature, several assemblages between microbial communities of microalgae and other species have been cited. The present section reports the existing interactions studied to lipid production.

2.1. Microalgae-microalgae interactions for lipid production

To reduce the cost of raw materials for biodiesel production, the co-culture system presented a proficient and safer alternative. Recently, *Chlorella* sp. *U4341* and *Monoraphidium* sp. *FXY-10*, potential feedstock for biodiesel production [27–29] were tested in co-culture system for lipid production under photoautotrophic conditions. This combination was advantageous and permits *Monoraphidium* sp. to increase their lipid productivity 20-folds. Although the mixture fermentation was feasible, the difficulty was in the step of harvesting and separation. The challenge was to release lipids in a way so that there was low energy cost and with possible recovery of high-value products after lipid extraction. Harvesting accounts for up to 50% of the total cost of biodiesel production [30] and having an extract without contamination by cellular components such as chlorophyll was a key requirement. Recent trends have focused on screening of valuable approaches, based principally on selective decomposition of cell wall with low cost. Due to small micro algal cells (2–20 μm) and their colloidal stability in suspension [31], sedimentation was reported to be not efficient; this fact restricts their potential use. Further, the increased energy requirements and the addition of chemicals delimit this process. Novel approaches were reviewed in literature, such as centrifugation, filtration, flocculation, and flotation [32–35]. Despite higher efficiency (e.g. 90% recovery), the higher energy input cost of centrifugation, especially with a low value

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