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Factors affecting algae biofilm growth and lipid production: A review

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ABSTRACT

Algae is recognized as a potentially valuable source of biofuels and biochemicals; however, a major limitation to commercialization is in the high cost of harvesting, de-watering, and downstream processing of dilute algae biomass when it is grown planktonically. Growing algae as a biofilm offers potential advantages for biomass processing because biofilms are immobilized and orders of magnitude more concentrated. For these reasons there has been an emerging interest in algae biofilm biofuel research over the past several years. Additionally, there has been a considerable amount of work on understanding algae biofilms in nature, and on using algae biofilms for tertiary wastewater treatment. This review paper draws from all of this literature to describe algae biofilm composition, and their growth responses to the key environmental factors affecting growth and internal lipid concentrations; the emphasis being on optimizing biomass and lipid productivity. Additionally, the paper summarizes key things known about planktonic algae growth and bacterial biofilm growth in order to make inferences about the potential growth of algae biofilms. The paper identifies many key knowledge gaps in the potential for producing biomass and lipids from algae biofilm growth systems.

Contents

1. Introduction

Algae growth systems show great potential for the production of biofuels and bioproducts. The main reasons for this are due to their high growth rates – doubling times as low as 7–8 h [1] – and

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high concentrations of valuable biocompounds. Coupled together, high biomass growth rates and biocompound concentrations results in high overall productivities of valuable biocompounds. For instance, Mata et al. [2] estimated that microalgae could generate between 58,700 and 136,900 L/ha yr of biodiesel – depending on biomass lipid contents of 30% or 70% – compared to 5366 L/ha yr for the best terrestrial biodiesel crop (Palm Oil). Other valuable biocompounds of interest within algae biomass are amino acids, fatty acids, pigments, carbohydrates, polysaccharides, vitamins, and antioxidants, which can be used to generate nutraceuticals, pharmaeucticals, functional foods and food additives, farm and aquaculture feed, biofuels, cosmetics and bioplastics [2–4]. In addition to high productivities, growing algae has advantages over conventional biofuel sources because it can be grown on non-arable lands, growth ponds and reactors can be scaled vertically, and algae can be grown on wastewater [5–7] and flue gas waste streams [8,9], thereby providing essential nutrients for growth to the algae while simultaneously mitigating pollution from these waste streams.

Researchers have identified several key limitations to commercialization of biofuels and bioproducts from algae growth systems, including optimizing growth rates and internal biochemical compositions through genetic modification of species, optimization of growth conditions and reactor design, and developing the biorefinery concept for algae [10]. Many researchers agree that one of the most significant economic limitations to commercialization, however, is the harvesting and dewatering of algae biomass grown planktonically – estimated to be up to 30% of the production costs [11–13]. By growing algae as a biofilm there is potential to significantly reduce these costs because the biomass is immobilized and much more concentrated – 0.4% (g/g) for planktonic systems [10], compared to 8–16% in biofilms [14,15]. The immobilized nature may also make downstream processing easier and more economical.

There has, however, been a limited amount of work conducted on the production of biofuels and biochemicals with algae biofilms compared to planktonic growth systems. Of the work that has been done on algae biofilms for biofuel production, much of it revolves around the study of the attachment, and subsequent growth, of algae biofilms to various growth materials [14,16–23]. There has also recently been a significant amount of algae biofilm research on novel reactor designs studying biomass and lipid production rates [14,17–18,20,21,24–29]. Other studies on algae biofilms have related not specifically to biofuel and bioproduct production, but rather, on using them to treat wastewaters of nitrogen and phosphorus using algal turf scrubbers and novel biofilm growth systems [5–6,30–38]. Additionally, there are many studies on algae biofilms from a fundamental perspective i.e. oxygen profiles under various conditions, species composition and succession, affects of shear rate, affects of temperature, etc., which is helpful in understanding how to optimize growth rates and lipid concentrations for biofuel and biochemical production. Lastly, there is a large breadth of knowledge on bacteria biofilms and planktonic algae growth systems that may provide insights into algal biofilm systems.

This review paper summarizes the current knowledge of algae biofilms, and how it relates to the production of biomass and biofuels. The overall objective of this paper is to provide an overview for what we currently know about algae biofilm development and composition, and growth parameters that affect growth rates and internal biomass lipid concentrations. The review will include studies done on algae biofilms for biofuel and biochemical production, on algae biofilms studied from a fundamental perspective, and on algae biofilms used to treat wastewater streams. It will also draw from the extensive literature on bacterial biofilms and planktonic algae growth systems to make inferences about algae biofilm growth systems. This review will not focus on reactor design, since reactor designs were covered extensively by Berner et al. [39]. A review such as this is important as algae biofilm biofuel and biochemical growth systems have emerged as a promising biotechnology over the past 5 years, evidenced by the growing number of groups working on such systems. To the best of our knowledge no such review has been written to date.

2. Composition and structure of an algal biofilm

Biofilms are communities of microorganisms attached to each other and a solid growth substratum. They form virtually anywhere water exists for extended periods of time. Photosynthetic biofilms in nature are often referred to as periphytic or algae biofilms, which are mostly composed of algae, cyanobacteria, and heterotrophic bacteria living in symbiosis. The composition and structure of photosynthetic biofilms will vary according to abiotic and biotic factors within the environment.

2.1. Extracellular polymeric substances and matrices

In biofilms, extracellular polymeric substances (EPS) are biomolecules and inert solids that bind cells to each other and to solid materials. Extracellular polymeric substances are located on the outside of cells, generated through active secretion, cell lysis, shedding of cell surface material, and adsorption from the environment [40,41]. The predominant EPS are polysaccharides and proteins; however, nucleic acids, lipids, and suspended solids can also make up the EPS matrix [42].

In well-developed biofilms the EPS forms a matrix that creates a microenvironment for the cells. This microenvironment protects cells from environmental stress such as dehydration, and fluctuations of pH, temperature, and nutrient concentrations [41,43,44]. Additionally, EPS matrices are known to act as a nutrient reservoir as enzymes within the matrix digest EPS and inert solids, [45], and the EPS acts as an ion exchange resin as it traps nutrients through sorption [40,46]. In these ways the EPS matrix helps accumulate and concentrate nutrients from the bulk medium. Although EPS is 99% water and collapses upon itself when dehydrated [47], it can compose up to 90% of the organic matter in some (bacterial) biofilms [46].

During biofilm formation and growth, microalgae will respond to environmental circumstances by increasing or decreasing the expression of specific promoters that affect EPS production. For instance, Becker [48] demonstrated that the diatom *Amphora coffaeformis* increases EPS production when it is in contact with materials that have good adhesion strength with EPS. A growth material effect on algae EPS production was also demonstrated by Shen et al. [28]. Additionally, Domozych [49] and Shen et al. [28] demonstrated that increasing nutrient concentrations, particularly nitrogen, would increase EPS production from diatom and green algae species. This is likely because a significant fraction of EPS is composed of proteins [28], allowing the cells to 'over-produce' amino acids while nitrogen is abundant and environmental conditions are favorable. There is some evidence that suggests algae cells increase their EPS production as their colonies age and mature [28,48]. This could be a result of mature colonies allocating less resources into reproduction i.e. reaching a stationary growth phase, and more into stabilizing their biofilm community. Lastly, there is evidence that temperature stress and mineral (calcium) accumulation stress adversely affect EPS production from algal cells [49]. Although it is clear that algae cells produce EPS according to environmental stimuli, compared to bacterial systems, the literature on EPS production and EPS matrices in axenic and mixed community algal biofilms is limited. This is an opportunity for fundamental algal biofilm knowledge development.