ARTICLE IN PRESS

Algal Research xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Microzooplanktonic grazers – A potentially devastating threat to the commercial success of microalgal mass culture

John G. Day[a,*], Yingchun Gong[b,c], Qiang Hu[b,c,d,**]

[a] Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine Institute, Oban PA37 1QA, Scotland, UK
[b] Center for Microalgal Biotechnology and Biofuels, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[c] Key Laboratory for Algal Biology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China
[d] SDIC Microalgae Biotechnology Center, China Electronics Engineering Design Institute, Beijing 100142, China

ARTICLE INFO

Keywords:
Algal biofuel
Amoeba
Biological constraints
Ciliates
Grazers
Rotifers

ABSTRACT

Eukaryotic microalgae and prokaryotic cyanobacteria are globally the most important primary producers, forming the base of food web in aquatic ecosystems. As such, they are eaten by a huge diversity of protistan taxa (e.g., amoeba, flagellates and ciliates), as well as zooplanktonic and larger metazoan grazers. As in terrestrial agriculture, grazing has the potential to devastate the microalgal "crop" and this has obvious implications to the commercial success of the developing microalgal industry. Whilst in conventional agriculture thousands of years of exploitation of a relatively small number of crop plants, has resulted in tools, knowledge and strategies that can manage this issue, in the case of microalgal mass culture this is relatively undeveloped. This review explores our current understanding of the issue and where further research is needed, focusing on the diversity of grazers and how microalgae under various environmental regimes and culture conditions avoid being annihilated. In addition, the implications of algal mass culture, where the objective is to maintain a virtual monoculture, are discussed in the context of how infection could be prevented/minimised and if infection occurs, how this may be managed to prevent excessive losses in productivity or quality of the algal crop. The ultimate objective would be the development of robust methodologies for the early detection of "infection" of microalgal mass-cultures. This would allow the timely implementation of best management practices to prevent/reduce, damage caused by grazing. In reality, whilst there will be areas of commonality, as in terrestrial agricultural crops, methods will be need to be specifically tailored for each algal taxon, cultivation system and location.

1. Introduction

Microalgae are sunlight-driven cell factories that photosynthetically convert carbon dioxide, water and mineral nutrients to potential products such as: biofuels, human foods, animal feeds and high value compounds. The advantage of using microalgae rather than other higher plants have been well documented and include factors such as: many taxa have very high photosynthetic efficiencies and biomass productivities and can grow in conditions that are not favourable for terrestrial biomass production [1]. Thus, microalgae can provide an alternative to current unsustainable over-exploitation of natural resources, with possibility of providing a solution to the environmental dilemma of food versus energy production on high quality arable land. Whilst man has used microalgae, in particular *Spirulina/Arthrospira*, as a food for hundreds if not thousands of years [2,3], the origins of the current development of the microalgal biotech sector may be traced to the 1940s, where attempts to grow microalgae were focussed on finding alternative sources of chemicals for use in munition manufacturing during the Second World War, by examining the production of lipids by various micro-algae [4,5]. Later, during the oil-crisis of the 1970s, when the price of crude oil was high, microalgae were "revisited" for their potential in biofuels based on their ability to accumulate oil, usually in the form of triacylglycerol's [6]. Over the past ten years there has been an upsurge in interest in the commercial potential of microalgae stimulated by factors including: concerns associated with anthropogenic climate change, energy supply and security and increased interest in higher value metabolites for use in the food, pharma and wellness sectors. A variety of strategies have been proposed including coproduction of biofuels with high-value products [7]. To date, technologies have been developed for the production of a range of high and intermediate-value products at a commercial scale, such as health foods, aquaculture feeds and niche-market "healthy" oils and industrial

* Corresponding author.
** Correspondence to: Q. Hu, Center for Microalgal Biotechnology and Biofuels, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan 430072, China.
E-mail addresses: John.Day@sams.ac.uk (J.G. Day), huqiang@ihb.ac.cn (Q. Hu).

http://dx.doi.org/10.1016/j.algal.2017.08.024
Received 6 April 2017; Received in revised form 14 August 2017; Accepted 15 August 2017

Please cite this article as: Day, J.G., Algal Research (2017), http://dx.doi.org/10.1016/j.algal.2017.08.024

oils, as well as specific high-value products. The latter are mostly lipid-based nutraceuticals or cosmeceuticals such as carotenoids and omega-3 polyunsaturated fatty acids [8]. Some of the algae producing products, such as beta-carotene, are cultivated profitably at large scale in artificial, saline lagoons, and raceway pounds [9]. Other microalgae cultivated under less harsh environmental conditions, such as *Haematococcus pluvialis* for astaxanthin production, are more susceptible to being outcompeted by other microalgae and are most commonly grown in more enclosed photobioreactor systems [10,11].

Key to the commercial success is the cost of production at the commercial scale. However, there is relatively limited public domain knowledge about costs of microalgal cultivation and processing at the commercial scale. It has been suggested that model-based simulations, combined with pilot-plant production data, can fill this gap and a recent study by Ruiz et al. [12] performed a techno-economic evaluation of the whole process chain including cultivation, biorefinery and market exploitation for a 100 ha facility in six locations. Their projections indicated a current cost per unit of dry biomass of 3.4 Euro kg^{-1} for microalgae cultivation in Spain (excluding biorefining products), with an expected reduction to 0.5 Euro kg^{-1} in the next ten years. At the current production costs a range of high-value products (e.g., polyunsaturated fatty acids and pigments) would be currently profitable, but products aimed at the food and bulk chemical commodities markets require further production cost reductions to become economically viable. Efficient and sustainable microalgal cultivation is only likely to be commercially profitable if conducted in either photobioreactors, or open pond systems sited on land, within which growth conditions can be controlled and optimised. For most if not all envisaged products, a large number of factors require optimisation including choice of microalga(e), nutrients, pH etc., in addition to the engineering aspects of microalgal production and downstream processing. However, a key factor commonly overlooked is the issue of "crop" loss due to grazing. The implications of a reduction in productivity and quality of harvested biomass, due to removal of the microalgae by grazers, at best reduces profitability and where a catastrophic culture "crash" results from grazing pressure this could be an industry threatening issue.

2. Grazers

Algae are crucial to the "health" of the planet, contributing approximately 50% of the total global photosynthetic activity [13] and forming the basis of the food chain for over 70% of the world's biomass [14]. In almost all natural aquatic environments top down control, i.e., grazing by ciliates, amoeba, rotifers and other zooplankton form a key aspect of the food web and as such have significant influence on ecosystems and are critical to the effective functioning of the microbial loop [15]. Although there is much variability from site to site worldwide, on average meiofauna, i.e., organisms with a body size of < 1 mm, alone graze at a rate of 0.01 h^{-1}, or 1% of the standing stock of both heterotrophs and autotrophs per hour [16]. There is a considerable body of literature on microzooplanktonic grazers, which includes reviews on freshwater and marine ecosystems [17,18], mesocosm studies [19] and the roles of grazers in manmade environments such as wastewater treatment plants [20]. Clearly, their capacity to ingest microalgae has major implications to the development of algal mass culture systems, with respect to productivity, sustainability and commercial viability. The key factors that require consideration are the impact that grazers have on algal productivity and, in mixed populations, competition between different algal taxa. This largely depends on the mode of grazing and the selectivity of the grazer(s). It is known that an individual grazer taxon may exhibit preference for certain food items and selection has been shown to be influenced by prey size [21,22], motility [23], as well as the chemical characteristics of the food particle/alga [24]. Furthermore, some species are able to discriminate against inert particles [25]. However, the morphological variety of both grazers and their potential food (Fig. 1) is such that in reality most potentially commercially exploitable algae are at risk of being eaten by grazers.

Industry threatening grazers range from macroscopic insects to microflagellates, barely larger than the alga(e) they ingest. Aquatic insect larvae have been reported to graze in *Spirulina* ponds [26,27] and are effectively unavoidable in open freshwater pond systems. Larger zooplankton such as the Brine shrimps *Artemia* and *Paraartemia* may be a significant problem in marine, or even hypersaline pond systems where the salt levels drop below 15% (w/v) NaCl [6]. In freshwater, brackish and marine media-based production systems rotifers and cladocerans may be the major grazing zooplankton having the potential to reduce algal concentrations and production to low levels within just a few days or weeks [28,29]. For example, rotifers and cladocerans at high densities ($>10^5 L^{-1}$) have been reported to reduce algal cell density by 90% within 2 days [30] and Cauchie et al. [31] measured a 99% reduction in algal chlorophyll-a due to *Daphnia* grazing over several days in an open pond system. Debatably protozoa, because of their size, diversity and speed of reproduction, pose the largest threat to commercial exploitation of algae. The authors have observed a 90% reduction in the cyanobacterium *Oscillatoria* within 5 to 6 days, with a corresponding 100-fold increase in the grazing ciliate *Nassula* [32]. In a recent study by the authors, scale-up of *Chlorella* production in an open pond system was severely constrained by repeated contamination and grazing by one species of chrysophyte, which was identified as *Poterioochromonas malhamensis* [33]. Additionally, we have observed on cultivation of *Scenedesmus* that the microalga usually grew well, however, culture quality and productivity deteriorated when it was invaded by vampyrellids [34]. This effect is not restricted to freshwater taxa, grazing ciliates have been observed to clarify dense outdoor mass cultures of *Dunaliella salina* within 2 days [35]. Furthermore, in *Dunaliella* ponds when the salinity drops below 20% (w/v) NaCl, amoeba and ciliates can rapidly decimate the algal culture [36]. In the context of ensuring consistency of productivity, grazing is a widespread problem and there is a growing literature on the topic (Table 1). It is worth noting that many of the microzooplanktonic grazers listed are capable of forming resistant resting stages, cysts spores etc. that may remain viable for many years. These may remain dormant in sediments or biofilms within production facilities, or be spread by wind currents [37] and as such form a major threat to open pond production systems in particular. Defences of algae against predation and the prevention of infection of production facilities are discussed in the following sections.

3. Natural defences

3.1. Morphological adaptation

Planktonic microalgae, especially nanoplanktonic species with dimensions of 2–30 μm are highly susceptible to zooplankton grazing [69,70]. However, algae with larger cell sizes, 20–30 μm or more in longest dimension, are generally less susceptible to being ingested by microzooplanktonic grazers, simply because of their size [69]. This morphological "solution" of becoming too large to be consumed conflicts with the selection pressure of resource acquisition, which generally favours algae with smaller cell sizes and short doubling times [71]. The capacity for rapid resource acquisition and growth is a major factor in the selection of biotechnologically exploitable algal strains [8] and to some extent explains the focus on organisms with small cell size including *Chlorella* and *Nannochloropsis*.

Many algal taxa have relatively plastic phenotypes, with different cell sizes and morphological features being observed at different points in their life cycle, or under different environmental conditions. The prokaryotic cyanophyte *Microcystis aeruginosa*, a common bloom-forming organism, on cultivation in the lab invariably grows as a unicellular suspension. In nature and under experimental conditions, including when a culture of the flagellate grazer *Ochromonas* sp. was placed in dialysis tubing in a culture of the cyanobacterium, *M. aeruginosa* colony formation was induced [72]. This