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Ecology-based selective environments as solution to contamination in microalgal cultivation

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Large-scale production of energetic storage compounds by microalgae is hampered by competition and evolution. Both phenomena result in contamination and arise due to a mismatch between the desired productive microalgal strain and the constructed environment. The prevailing approach to solve this issue involves increasing the survival potential of the desired strain, for example by working in closed systems or at extreme conditions. We advocate adjusting the environment in such a way that lipid production, or any other desired characteristic, gives a competitive advantage. Competition and evolution become a value rather than a threat to processes in which the desired characteristic is ensured by a selective environment. Research and cultivation efforts will benefit from this approach as it harnesses the microalgal diversity in nature.

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Current Opinion in Biotechnology 2015, 33:46-51

This review comes from a themed issue on **Energy biotechnology** Edited by **Eleftherios Terry Papoutsakis** and **Jack T Pronk** For a complete overview see the <u>Issue</u> and the <u>Editorial</u> Available online 20th November 2014 http://dx.doi.org/10.1016/j.copbio.2014.11.001 0958-1669/© 2014 Elsevier Ltd. All rights reserved.

Introduction

Microalgae have the potential to supply a biobased society with essential photosynthetic building blocks like sugars and lipids. In nature, production of these storage compounds enables algae to endure dynamic growth conditions like the alternating presence and absence of light. From a biofuel production point of view, sugars can, for example, be fermented to ethanol [1] or digested to produce methane containing biogas [2] whereas lipids can be transesterified to yield biodiesel [3^{••}]. Biodiesel is the preferred microalgal fuel product since it can be implemented in existing infrastructure. As a consequence, microalgal research has been mainly focussed on lipid production. At this moment the large-scale production of low-value commodities such as lipids is economically not viable. A key parameter for the economics of lipid production by microalgae is the area-specific lipid productivity [4]. Different strategies including genetic modification [5–7], wildtype screening programmes [8] and optimization of cultivation conditions [9] are applied to increase lipid productivity. Even with increased lipid productivity, the large-scale cultivation of microalgae is challenging. The phototrophic nature of microalgal cultivation leads to light limitation at relatively low biomass concentrations. Scaling up of phototrophic processes will therefore occur on surface basis and not on volume basis as is standard practice in chemotrophic processes [10]. Large surface areas and diluted cultures result in challenges concerning gas-liquid mass transfer, down-stream processing and contamination. Contamination has a detrimental effect on production [11,12^{••}] and is therefore ranked as a major bottleneck in microalgal cultivation in open systems [12^{••},13,14[•]]. This paper will address two ways to deal with contamination: either strain driven or founded on ecology-based selective environments.

Contamination

Contamination comes in the form of herbivores (cladocerans, copepods, rotifers, etc.), pathogens (bacteria and viruses) and competing microalgae [12^{••},15]. Chemical and ecological methods can be applied to reduce the effect of herbivores and pathogens [11,12^{••},13,14[•],16[•]]. Competing microalgae seem to be the most difficult form of contamination to control, since the biological and physical properties of the contaminant are largely similar to those of the desired species. We, therefore, define contamination in this paper as the unwanted introduction of microalgal strains and functionalities in any microalgal cultivation system, open or closed.

Contamination by competing algal strains is a logical consequence of the operation of microalgal cultivation systems. Although cultivation strategies that combine biomass and lipid production are investigated [17[•]], the standard microalgal cultivation strategy consists of a period with excess nutrients to produce biomass followed by a nutrient depleted phase in which lipids are produced [18,19]. The first period creates an environment in which fast growth is rewarded. In the second phase lipids are produced, but this functionality is not rewarded. Consequently, the process faces two challenges. Competing microalgae, with a higher growth rate than the desired lipid producing species, can outcompete the desired species and threaten the process from the outside. Even

if the desired species is maintained by properly operating under axenic conditions, the desired characteristic can be lost through evolution [20]. In this case evolution may lead to an increased growth rate at the cost of a decreased lipid production capacity [21[•]]. This allows a mutated strain to outcompete the original high productive strain. Evolution can, therefore, be classified as a danger from the inside and is as such coined 'strain degeneration'.

With a mismatch between the desired lipid producing species and the constructed cultivation environment, two approaches emerge. One could either increase the

Figure 1

survival potential of the desired species or create an environment that favours lipid production. Figure 1 gives a schematic overview of these two lines of thought and points out where current research is focussed.

Increasing the survival potential of the desired strain

The majority of measures taken to avoid contamination aims at increasing the survival potential of the desired strain (left part of Figure 1). Axenic cultures are established in closed cultivation systems by imposing a physical barrier between the desired and competing



Two ways to achieve large-scale lipid production using microalgae. Left panel: Strain-based approach. The point of action of working with closed systems (closed systems), using a herbicide-resistant strain (herbicides), under extreme conditions (extreme conditions) or with regular inoculation (inocula) with the desired strain is highlighted. Right panel: Ecological approach. The point of action of the 'Survival of the Fattest' approach (SotF) is highlighted. Do not enter sign indicates: aims to block, checkmark indicates: benefits from.

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