Biotechnology Advances 33 (2015) 1228–1245

Contents lists available at ScienceDirect

Biotechnology Advances

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

Research review paper

Continuous cultivation of photosynthetic microorganisms: Approaches, applications and future trends

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ARTICLE INFO

Available online 14 March 2015

Keywords:
Continuous cultivation
Photobioreactor
Microalgae
Cyanobacteria
Technical–economic assessment

ABSTRACT

The possibility of using photosynthetic microorganisms, such as cyanobacteria and microalgae, for converting light and carbon dioxide into valuable biochemical products has raised the need for new cost-efficient processes ensuring a constant product quality. Food, feed, biofuels, cosmetics and pharmaceutics are among the sectors that can profit from the application of photosynthetic microorganisms.

Biomass growth in a photobioreactor is a complex process influenced by multiple parameters, such as photosynthetic light capture and attenuation, nutrient uptake, photobioreactor hydrodynamics and gas–liquid mass transfer.

In order to optimize productivity while keeping a standard product quality, a permanent control of the main cultivation parameters is necessary, where the continuous cultivation has shown to be the best option. However it is of utmost importance to recognize the singularity of continuous cultivation of cyanobacteria and microalgae due to their dependence on light availability and intensity.

In this sense, this review provides comprehensive information on recent breakthroughs and possible future trends regarding technological and process improvements in continuous cultivation systems of microalgae and cyanobacteria, that will directly affect cost-effectiveness and product quality standardization. An overview of the various applications, techniques and equipment (with special emphasis on photobioreactors) in continuous cultivation of microalgae and cyanobacteria are presented. Additionally, mathematical modeling, feasibility, economics as well as the applicability of continuous cultivation into large-scale operation, are discussed.

Contents

Abbreviations: PBR, Photobioreactor; EPA, Eicosapentaenoic acid; MEC, Major equipment costs

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http://dx.doi.org/10.1016/j.biotechadv.2015.03.004
0734-9750/

1. Introduction

Photosynthetic microorganisms, especially prokaryotic cyanobacteria and eukaryotic microalgae, are considered promising candidates for many potential applications ranging from direct use of biomass (e.g., aquaculture feed and food supplements), production of high-value compounds (e.g., vitamins, pigments and polyunsaturated fatty acids) and environmental applications (e.g., biofuel production, CO_2 mitigation and waste water treatment) (Fernandes et al., 2013, 2014; Klok et al., 2013a; Markou and Nerantzis, 2013; Mata et al., 2010; Wijffels et al., 2013). The commercial exploitation of these photosynthetic microorganisms raises the need for reliable, efficient and cost-efficient processes with a constant product quality (Kwon et al., 2012).

Currently, mass production of photosynthetic microorganisms is generally based on batch cultivation systems (Camacho et al., 1990; Marchetti et al., 2012; Rusch and Christensen, 2003). However these systems' performance is still seriously hindered by factors such as low productivity, high harvesting costs due to low cell concentrations, uncertain reliability and a variable product quality (Ganuza and Izquierdo, 2007; Guedes et al., 2014; Rusch and Christensen, 2003; Wang et al., 2013). This means that batch cultivation may not be the best choice for mass production of microalgae and cyanobacteria biomass (Camacho et al., 1990).

As the need for microalgae and cyanobacteria increases, continuous production systems are attracting interest once, according to many authors, these systems are the most suitable way to achieve successful large scale production of those photosynthetic microorganisms mainly due to higher volumetric productivities, constant product quality, reduction of space requirement, decrease of labor costs, lower investment and operational costs and decrease of "unprofitable" periods (Cuaresma et al., 2009; González-López et al., 2012; Gutierrez-Wing et al., 2012; Rusch and Christensen, 2003; Sforza et al., 2014; Tang et al., 2012; Zijffers et al., 2010).

This review presents the basic principles, the main advantages and challenges, the equipment, maintenance, monitoring, control and downstream processes associated to continuous cultivation of these photosynthetic microorganisms. The feasibility and economic aspects, the main applications and the future perspectives of these systems are also addressed.

1.1. Principles of continuous cultivation

Despite the first references to a continuous cultivation technique report to the 1920s (Cooney, 1979) and the first continuous cultivation of photosynthetic microorganisms to the 1940s (Myers and Clark, 1944), it were the studies developed by Monod (1950) and Novick and Szilard (1950) that marked the formal initiation and application of continuous culture. However the heyday of continuous cultivations was during the 1960s, where this technique was used very often as a tool to investigate biochemical, ecological, genetic and physiological aspects of different microorganisms (Hoskisson and Hobbs, 2005). The theory presented by these authors is based in the observation that, during microorganisms' growth in batch systems, substrates are depleted and products accumulate, which at a given point makes growth to cease due to limiting substrate depletion or growth-inhibiting products accumulation. So, in order to maintain cells' proliferation, the substrate needs to be replaced and the inhibitory products to be removed in a continuous way, which is basically what happens in continuous cultivations (Lee and Shen, 2004).

Generally, continuous culture can be defined as an open system in which fresh culture medium is continuously added to the bioreactor and the culture broth (including cells and metabolites) is also continuously removed (Brethauer and Wyman, 2010; Guedes et al., 2014; Paulová et al., 2013). Usually the volume of culture broth is kept constant inside the bioreactor and the microorganisms are in a nearly unchanged environment. This forces the microorganisms to adjust their physiology and composition to the environmental conditions provided and after some generations a steady-state is usually attained (Guedes et al., 2014). In this sense, the continuous cultivation theory makes use of the relationship between the availability of the limiting substrate and microbial growth. The mass balance in a bioreactor operating in continuous mode is usually defined by:

$$V \cdot dC_X/dt = \mu C_X \cdot V + F_{in} \cdot C_{Xin} - F_{out} \cdot C_{Xout} \quad (1)$$

Where V—working volume (L); dC_X/dt—biomass accumulation inside the bioreactor (g/(L h)); μ—specific growth rate (h^{-1}) C_X—biomass concentration (g/L) F_{in}, F_{out}·—volumetric inflow and outflow (L/h).

Assuming: (i) constant flow ($F = F_{in} = F_{out}$); (ii) constant volume; (iii) the steady state conditions ($dC_X/dt = 0$) (Lee et al., 2013) and taking in consideration the dilution rate definition ($D = F/V$), the Eq. (1) can be simplified to:

$$\mu = D \quad (2)$$

D is the reciprocal of the residence time, which is defined as the average time that a fluid element spends inside the bioreactor. Considering a constant working volume, the dilution rate can be manipulated and the continuous mode can operate at a defined (and constant) or variable dilution rate (Ferreira and Teixeira, 2003). Eq. (2) presumes that the specific death rate is negligible comparing to μ, which is not always true and must be taken in account (Wood et al., 2005).