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Research review paper

Flow cytometry for the development of biotechnological processes with microalgae

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ABSTRACT

The current interest in microalgae as a sustainable source of next generation biofuels and other valuable substances is driving exploration of their use as unique biotechnological production systems. To design and optimise appropriate production strategies, the behaviour of particular microalgal species should be well characterised under different culture conditions. Thus, flow cytometric (FCM) methods, which are already well established in environmental and toxicological studies of microalgae, are also useful for analysing the physiological state of microalgae, and have the potential to contribute to the rapid development of feasible bioprocesses. These methods are commonly based on the examination of intrinsic features of individual cells within a population (such as autofluorescence or size). Cells possessing the desired physiological or morphological features, which are detectable with or without fluorescent staining, are counted or isolated (sorted) using an FCM device. The options for implementation of FCM in the development of biotechnological processes detailed in this review are (i) analysing the chemical composition of biomass, (ii) monitoring cellular enzyme activity and cell viability, and (iii) sorting cells to isolate those overproducing the target compound or for the preparation of axenic cultures.

Contents

1. Introduction

Microalgae cover a broad group of eukaryotic and typically photoautotrophic microorganisms of very diverse phylogenetic positions.

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Accordingly, the ecology, morphology, physiology and biochemistry of microalgae are extremely diverse. The considerable metabolic versatility and flexibility of microalgae, also termed plasticity (Trainor, 2009), is awaiting exploitation in biotechnological manufacturing. To design and optimise appropriate production strategies, the behaviour of particular microalgal species should be well characterised under different culture conditions. These characteristics can be advantageously analysed using flow cytometric (FCM) methods, which are already well established in ecophysiological studies of microalgae (Collier, 2000; Sosik and Olson, 2007; Yentsch and Yentsch, 2008).

Products from microalgae are of emerging interest in a number of areas including supplements for human and animal nutrition, aquaculture, pharmaceutical and cosmetic products (Chisti, 2007; Rosenberg et al., 2008). Some most recent publications (Acien et al., in press; Heilmann et al., 2011) give the size of the microalgal biomass market worldwide as being about 5000 t year^{-1} of dry matter (Pulz and Gross, 2004) and generating a turnover of ca. $1.25 \cdot 10^9$ U.S. $ year^{-1} (Raja et al., 2008). The main commercial products are (in alphabetic order): β-carotene (*Dunaliella* sp.; Betatene or Western Biotechnology or Aqua-Carotene, Australia; Inner Mongolia Biological Engineering, China), astaxanthin (*Haematococcus* sp.; Cyanotech or Mera Pharmaceutcal, Hawaii, USA; Fuji Health Science, Japan), lipids and polyunsaturated fatty acids such as DHA (*Crypthecodinium* sp.; Martek or Omegatec, USA) or EPA (*Odontella* sp.; Innovalg, France), phycocyanine (*Spirulina platensis*), polysaccharides (*Chlorella* sp.; Ocean Nutrition, Canada; Solazyme, USA) and vitamins such as vitamin B_{12} (*Spirulina* sp.; Panmol or Madaus, Australia) (Pulz and Gross, 2004). Additionally, interest in microalgal biomass as a possible source for third generation biofuels has increased over the last decade (Montero et al., 2011). However, for economically sustainable production of biofuels from microalgal biomass, additional enhancement of the productivity and cell density of microalgal cultures would be necessary (Parmar et al., 2011).

Improving the productivity and reproducibility of cultivation processes is also of great importance in order to establish microalgae as a unique biotechnological production system for high-value substances (Bumbak et al., 2011; Guedes et al., 2011). Therefore, it is essential to gain more detailed information on microalgal metabolism in general and on cultivation and production processes in particular. Some basic information is available from environmental and toxicological studies of phytoplankton (e.g. Adler et al., 2007; Franqueira et al., 2000). These studies mostly incorporate the use of flow cytometry (FCM), which enables single cells with different features or physiological states to be counted, sorted and/or examined on the basis of quantification of scattered and fluorescent light signals. The special feature of microalgae is the presence of photosynthetic pigments that exhibit strong autofluorescence, requiring specific FCM approaches (e.g. selection of an appropriate fluorescent dye). The application of FCM methods (both combined and not combined with fluorescent staining) to determine morphological features of microalgal cells, to identify species, and to examine the physiological state of individual cells in marine and freshwater microalgal communities was described in detail in several review papers (Collier, 2000; Dubelaar and Jonker, 2000; Sosik and Olson, 2007; Veldhuis and Kraay, 2000; Yentsch and Yentsch, 2008).

To date, FCM information has been successfully applied for the optimisation of growth conditions and for the production of a target compound only in yeast, bacterial and cell cultures (Davey, 2002; Davey and Kell, 1996; Weithoff, 2004; Yanpaisan et al., 1999). However, in principle, it can be also used with microalgae. In contrast to the routine methods used in bioprocess development and monitoring, FCM provides information on the intrinsic heterogeneity of a population in a bioreactor. The

Table 1
Applications of FCM analyses in bioprocesses with microalgae.

	Effect/product	FCM-method	Cultivation system	Microalga	Reference
Monitoring & optimisation of cultivation	Shear stress (>1 Pa) and rate (>10 s^{-1})	Viability (FDA)	Rotor-stator shear cylinders	*Chaetoceros muelleri*	Michels et al., 2010
	Shear stress (60 rpm)	Reactive oxygen species (DCFH, DHR 123)	Autotrophic, shaking flask	*Protoceratium reticulatum*	Rodriguez et al., 2009
	Ultrafiltration and cell density on growth	Cell cycle (PI)	Autotrophic, 1-L photobioreactor	*Chlorella vulgaris*	Javanmardian and Palsson, 1991
	Growth and DHA	Viability and membrane integrity (PI)	Heterotrophic, shaking flask	*Crypthecodinium cohnii*	Lopes da Silva and Reis, 2008
	Growth and oil	Viability and cell cycle (PI)	Heterotrophic, shaking flask	*Chlorella protothecoides*	Lopes da Silva et al., 2009b
	Cellulose and lipids, cell cycle phase and glucose concentration	Viability and cell cycle (PI), cellulose (CFW), neutral and polar lipids (NR)	Heterotrophic, shaking flask	*Crypthecodinium cohnii*	Kwok and Wong, 2005, 2010
	Growth and oil	Oil (NR)	Heterotrophic, shaking flask	*Chlorella protothecoides*	Lopes da Silva et al., 2009b
	Oil	Neutral and polar lipids (NR)	Autotrophic, outdoor photobioreactor	*Scenedesmus obliquus*, *Neochloris oleoabundans*	Lopes da Silva et al., 2009a
	Oil	Oil content (NR)	Autotrophic, 1-L air-lift reactor	*Neochloris oleabundans*	Gouveia et al., 2009
	Lipid and PUFA	Neutral and polar lipids, PUFAs (NR)	Autotrophic, shaking flask	*Tetraselmis suecica*	Guzman et al., 2010
	Lipid and DHA	Neutral and polar lipids (NR)	Heterotrophic, shaking flask	*Crypthecodinium cohnii*	de la Jara et al., 2003
	Astaxanthin	Autofluorescence	Autotrophic, shaking flask	*Haematococcus pluvialis*	Hu et al., 2008
Isolation	Neutral lipid overproducing strain	Cell sorting, neutral lipids (NR)	Autotrophic, air-lift photobioreactor	*Tetraselmis suecica*	Montero et al., 2011
	Lipid overproducing strain	Cell sorting, lipids (NR, autofluorescence)	Mixotrophic, shaking flask	*Nannochloropsis* sp.	Doan and Obbard, 2011a
	Carotenoid overproducing strain	Carotenoids (NR, autofluorescence), morphology (FSC, SSC)	Autotrophic, shaking flask	*Dunaliella salina*	Mendoza et al., 2008
	Carotenoid overproducing strain	Cell sorting (FSC, SSC, autofluorescence)	Autotrophic, outdoor reactor	*Dunaliella salina*	Nonomura and Coder, 1988
	Axenisation	Cell sorting (FSC, SSC autofluorescence)	Autotrophic, shaking flask	*Cyanophora paradoxa*, *Haematococcus* sp., *Monomastix* sp., *Scherffelia dubia*, *Spermatozopsis similis*	Sensen et al., 1993

All abbreviations of the designations of fluorescent dyes are explained in Table 2.