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Optimal selection of organic solvents for biocompatible extraction of β-carotene from *Dunaliella salina*

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Abstract

In the aim of β-carotene biocompatible extraction, toxicity of various pure solvents belonging to different homologous series has been investigated for *Dunaliella salina*. The results showed that solvents having $\log P_{oct} > 5$ or having a molecular weight over 150 g/mol can be considered biocompatible for this microalga. The membrane critical solvent concentration for each series of solvents has been calculated applying Osborne's model, showing that the aliphatic chlorinated hydrocarbon is the most toxic family studied. Mixtures of a biocompatible solvent (decane) with a toxic solvent (CH_2Cl_2, MEK, MTBE) have been studied. The β-carotene extraction ability of CH_2Cl_2-decane mixture was found six times more efficient than with pure decane. It has been demonstrated that the extraction ability of solvent depends on its affinity with the product extracted and on its concentration incorporated in the cellular membrane.

Keywords: β-Carotene; *Dunaliella salina*; Biphasic systems extraction; Mixed solvents

1. Introduction

Microalgae are important sources of food and pharmaceutical additives such as colorants and antioxidants (León et al., 2001). Furthermore they are considered as potential producers of a large number of new natural products, as β-carotene. This molecule is used as food additive for its antioxidant and vitamin properties (Metting, 1996), and is added to numerous cosmetic and body-care products as a non-harmful colorant to improve the attractiveness of the product (Dufossé et al., 2005).

The β-carotene is a secondary pigment in photosynthetic organisms such as microalgae (León et al., 2003). Its main natural source is the extremophile green microalga *Dunaliella salina*, discovered in 1960. It can accumulate high concentrations of β-carotene (up to a concentration of 10% of dry weight) in oil globules in the cells (Ben-Amotz, 1982). Actually, more than 90% of commercialized β-carotene is chemically synthesized (León et al., 2003). Several industrial exploitations of *Dunaliella* are operational in Australia, Israel (Ben-Amotz, 1995), USA (Borowitzka, 1999) and China (Raja et al., 2007). Table 1 summarizes several methods for β-carotene extraction from algae, where the β-carotene is extracted in organic solvents or edible oils, directly or after breaking cells by osmotic or mechanical shock.

However, β-carotene production using microalgae is limited by the slow growth rate of photosynthetic microorganisms (compared to bacteria or yeasts). So, if a method can be applied in which the produced biomass is re-used for the production of metabolites, this can be a new approach to improve the productivity of biosynthetic processes while producing continuously the β-carotene. In this case, a biphasic aqueous/organic system offers a solution in which both production and extraction occur simultaneously (Hejazi et al., 2003; León et al., 2003). A biocompatible organic solvent is in contact with the aqueous phase where the cells are carrying out the bioconversion (Hejazi and Wijffels, 2003); the product is continuously extracted into the organic phase due to a permeability effect of the solvent on cell membrane (Hejazi et al., 2002; Xu et al., 2004). However, a direct contact between microorganisms and a solvent may result in significant losses in biomass (Choi et al., 2000; Osborne et al., 1990). The selection of the solvent is of critical relevance (León et al., 1998) where it must satisfy certain requirements such as

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biocompatibility, maximum solubility for β-carotene and important extraction ability (Daugulis, 1988). Main constraint is that it must perform the product extraction with preservation of the cell viability. A significant amount of work has been done and published, focusing on this topic (Laane et al., 1987; Santhanam and Shreve, 1994; Hejazi et al., 2002; León et al., 2003). León et al. (2003) have studied some organic solvents and shown that the ability of decane and dodecane for biocompatible extraction of β-carotene is respectively about 20% and 10% of the maximum.

Biocompatibility and extraction ability of the organic phase is not easy to achieve simultaneously; several solutions have been proposed to minimize the solvent toxicity and make biocompatibility a less restrictive criterion. Mixtures of toxic but good extractive solvents with poor extractive but biocompatible ones (Bruce and Daugulis, 1991) are one of the solutions proposed to protect cells from solvent toxicity.

A method to predict the solvent ability to meet these strict requirements would be of great practical use, since experimental data are seldom available for a specific system, and there are hundreds of solvents from which to choose. We report in this paper a new strategy of solvent selection by providing new experimental information for the selection of appropriate organic solvents suited for the biocompatible extraction of β-carotene from *Dunaliella salina*, leading to a significant decrease of the amount of experimental work required.

Table 1
Methods for β-carotene extraction from *Dunaliella salina*[a]

Breaking method	Extracting solvent
Osmotic shock	Ethanol, hexane, cyclohexane and benzene
Thermal treatment	Halogenated, aliphatic or aromatic hydrocarbon
Homogenization	Edible oil
No additional breaking	Super-critical CO_2
Osmotic shock mechanical methods	Hexane, cyclohexane and petroleum ether
Thermal treatment	Edible oil
Strong solvent (methanol)	Methylene chloride and ethanol
Strong solvent	Acetone, methanol and di-ethylether
Thermal treatment and organic solvents	Mixture of one acid ester and oil

[a] From Hejazi et al. (2002).

2. Materials and methods

2.1. Organism and culture conditions

Dunaliella salina (CCAP 19/18) was gathered from the culture collection of IFREMER (Nantes, France). *D. salina* was cultivated in artificial medium composed of: 1.5 M NaCl, 10 mM KNO_3, 0.4 mM KH_2PO_4, 5 mM $MgSO_4$, 185 μM H_3BO_3, 0.2 mM $CaCl_2$, 2 μM $FeCl_3$, 7 μM $MnCl_2$, 1 μM $ZnCl_2$, 1 μM $CoCl_2$, 1 μM $CuCl_2$, 1 μM $(NH_4)_6Mo_7O_{24}$, 5 μM Na_2EDTA

Table 2
Physical properties of solvents investigated[a]

Solvents	Max. solubility in water (mg l^{-1})	Molecular weight (g mol^{-1})	log P_{oct}
Aliphatic chlorinated hydrocarbons			
Chloroform	7.95×10^3	119.38	1.97
Dichloromethane	13×10^3	84.93	1.25
Trichloroethane	1.29×10^3	133.41	2.49
Aliphatic ketones			
Acetone	10^6	58.08	−0.24
Methyl ethyl ketone	2.23×10^5	72.11	0.29
Methyl isobutyl ketone	19	100.16	1.31
Aliphatic ethers			
Methyl *tert*-butyl ether	5.1×10^4	88.15	0.94
Diethyl ether	6.04×10^4	74.12	0.89
Di-isopropyl ether	8.8×10^3	102.18	1.52
Aliphatic alcohols			
Pentanol	22×10^3	88.15	1.51
Hexanol	5.9×10^3	102.18	2.03
Heptanol	3.27×10^3	116.2	2.31
Saturated aliphatic hydrocarbons			
Decane	52×10^{-3}	142.29	5.01
Dodecane	3.7×10^{-3}	170.34	6.1
Hexadecane	0.9×10^{-3}	226.45	8.25
Aromatic hydrocarbons			
Toluene	526	92.14	2.73
Dichlorobenzene	156	147	3.43
Oil			
Ethyl oleate	579×10^{-6}	310.52	8.51

[a] Data obtained from Handbook of Chemistry and Physics (Weast, 1972).