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Micelle mediated extraction of fatty acids from microalgae cultures: Implementation for outdoor cultivation

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ABSTRACT

In this work micelle-mediated extraction was investigated in order to develop an alternative process for the *in situ* extraction of hydrophobic substances from microalgae cultures. The main requirements for an *in situ* extraction process such as biocompatibility, phase separation behavior and partitioning of the hydrophobic target substance between micellar- and aqueous phases were studied for a number of surfactants. The cloud point temperatures (CPT) as well as the biocompatibility of seven nonionic surfactants with the microalgae *Scenedesmus obliquus* were determined as a function of time. For the most biocompatible surfactant, Triton X-114 with a biocompatibility of 98%, the kinetics of phase separation in the temperature range between 30 and 40 °C have been investigated. The fastest phase separation (12 min) took place at a Triton X-114 concentration of 3 wt% at 40 °C, in contrast to the slowest (55 min) at a Triton X-114 concentration of 5 wt% at 30 °C. The partitioning of representative hydrophobic substances between the both phases was predicted using the model COSMO-RS, the results were compared to experimental data with satisfying accordance ($Log P_{calc}$. palmitic acid: 0.82; $Log P_{exp}$. palmitic acid: 0.75). Based on these results the extraction of valuable compounds from the microalgae *S. obliquus* was realized on a pilot plant and compared to the lab scale experiments.

1. Introduction

The interest in microalgae products is constantly growing. Different species of microalgae are cultivated in order to produce valuable compounds like β-carotene (*Dunaliella salina*), astaxanthine (*Haematococcus pluvialis*) etc. [1,2]).

Special attention is given to the extraction of these valuable components from algae cultures. State of the art processing of microalgae currently includes energy consuming cell harvesting, dewatering, cell disruption and lipid extraction from the algae biomass using classical extraction methods [3]. Commonly applied extraction methods use supercritical fluids like carbon dioxide or organic solvents (mainly n-alkanes like hexane), as well as ultrasonic-assisted extraction procedures [4]. All these techniques have in common that the algae cells are disrupted, followed by a time and cost consuming re-growing step of the algae biomass. None of the described methods allows a simultaneous cultivation of algae cells and an extraction of the hydrophobic target substances (*in situ* extraction approach).

The *in situ* extraction is generally based on two-phase systems, where one phase is the culture medium containing microorganisms and the other phase an extractive agent (it can be an organic solvent or a surfactant containing solution), which is biocompatible with the cultivated organisms. With such a system the extracted substances can be obtained directly from the culture medium, or from the cells after permeabilization of their cell walls or cell lysis. In an extraction processes, all of these mechanisms are of interest [5]. The dodecane-based extractions from the microalgae *D. salina* in two-phase bioreactors have been extensively studied by Wijffels et al. in a series of publications. They investigated selective extraction of β-carotene directly from the microalgae culture [6], [7]. Thereby n-dodecane was found to be efficient for extraction but showed certain cell toxicity during longer contact times [8].

Extraction solvents with high molecular weight and low polarity show high biocompatibility with microorganisms. Thereby $log P_{o/w}$ (logarithm of the solvent partition coefficient in a standard octanol/water system) is often used for the characterization of such systems [9]. $Log P_{o/w}$ value equal or greater than 5 indicates possible biocompatibility; however it varies for different cultures [10]. Alternative extraction media for the *in situ* extraction of algae can be nonionic surfactant water mixtures. The amphiphilic molecules of nonionic surfactants form micellar aggregates in aqueous solutions, when their concentration reaches the surfactants critical

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micelle forming concentration (CMC). The hydrophobic head of the molecule is directed to the bulk water and the hydrophobic tails form the hydrophobic micelle core. These solutions often form temperature induced two-phase systems. After a temperature increase above the cloud point temperature (CPT), the homogeneous micellar system divides into two phases: a surfactant-rich phase and an aqueous phase [11]. A typical liquid–liquid-equilibrium of a nonionic surfactant (here Triton X-114) water mixture is shown on Fig. 1 [12] (see Fig. 2).

After phase separation of the cloud point system, hydrophobic solutes (solubilized in the micelles) accumulate in the surfactant rich phase [13]. Some nonionic surfactants, e.g. Triton X-100 and Triton X-114 or Tergitol 15-s-7, show relatively low cloud point temperatures (65, 23, and 37 °C), which allows the application of the corresponding cloud point system as a two phase extraction media for whole cell bioprocesses. Such a process is called cloud point extraction (CPE). It was demonstrated that *Monascus purpureus mold* can be cultivated in Triton X-100/water mixture without significant cell growth inhibition [14]. Furthermore, the extraction of the valuable pigments was implemented with permeabilization of the cell membrane. After carrying out the CPE, microbial cells predominantly accumulate in the aqueous phase [15–17]. Hydrophobic molecules like oleic acid, polycyclic hydrocarbons and other fatty acids were rapidly solubilized in micelles formed by nonionic surfactants [18–20]. So far, the nonionic surfactants Triton X-114 and Tergitol 15-s-7 were used for these processes. It was shown that the CPE process based on Triton X-114 is implementable in continuous counter current extraction columns which are alternatives to batch extraction systems [12].

In our previous work we have shown that it is generally possible to use nonionic surfactant systems to extract hydrophobic substances from microalgae. Therefore, the biocompatibilities of different nonionic surfactants, as well as the phase separation behavior were firstly studied [21]. It was shown that a mixture of the microalgae culture with the nonionic surfactant Triton X-114 forms stable phases after 20 min at 37 °C, whereby the microalgae cells predominantly accumulate in the aqueous top-phase.

The aim of this work is a broad surfactant screening for a potential surfactant based *in situ* extraction process of microalgae cultures, as well as the implementation of the *in situ* surfactant based extraction in a pilot plant for outdoor cultivation of microalgae. Therefore, the main process requirements, namely biocompatibility of the surfactant with microalgae, phase separation kinetics of the resulting two phase system and the partitioning of the target substance between the micellar and the aqueous phase, were studied.

2. Materials and methods

2.1. Microalgae

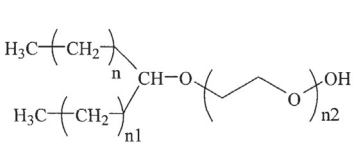
The used microalgae *Scenedesmus obliquus* (isolate from surface waters in Hamburg) was cultured as described by Leupold et al. [22]. *S. obliquus* is a commonly used microalgae strain in mass culture, due to its ability to accumulate lipids [23]. After analyzing the biomass of *S. obliquus*, it contains predominantly the saturated palmitic (16:0) and stearic (18:0) fatty acids. Myristic acid (14:0) is also detected, but in less significant amount [24].

2.2. Surfactants screening

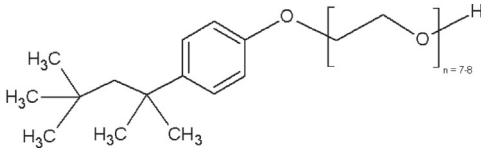
Triton X-114 (AppliChem, Germany), Tergitol 15-S-7 (Sigma Aldrich, USA) and Ecosurf SA-7, Ecosurf EH-6, Triton DF-12, Triton DF-16, Triton CF-32 (DOW, Germany) in laboratory grade were studied. Triton X-114, Tergitol 15-S-7, Triton DF-12, Triton DF-16, and Triton CF-32 are alcohol ethers of ethylene oxide. Ecosurf SA-7 and Ecosurf EH-6 are manufactured by reaction of an alcohol with ethylene oxide and propylenoxyde. An aromatic ring is present in the alcohol chain of all Triton surfactants (manufacturer data). These were chosen due to their cloud point temperature below or equal to 40 °C, which is the upper limit for the cultivation of *S. obliquus* (Table 1).

The solvent screening procedure included tests of the toxicity and of the clouding behavior in aqueous solution.

Following the available chemical structures of two surfactants under study were shown. Unfortunately there are no concrete Structures of the remaining Surfactants due to the corporate secret of the manufacturers.



Tergitol 15-S-7, n+nl = 12, n2 = 6
[Patent No.: PCT/US2006/061347]



Triton X-114 chemical structure [Sigma Aldrich datasheet]

2.3. Surfactant biocompatibility experiments

Changes in the photosynthetic activity (PA) were used to determine toxic effects of the surfactants and thus their biocompatibility. PA was measured using an MAXI – Imaging-PAM Chlorophyll Fluorometer (Heinz Walz GmbH) as described in our previous work [21]. All analyses were carried out in triplicates, whereas the surfactant concentration was varied from 1 to 5 wt% in steps of 1 wt%. During all experiments, PA was measured after 5 min of dark adaptation after 10, 30, 50, 70, 90, 120, 150 and 220 min.

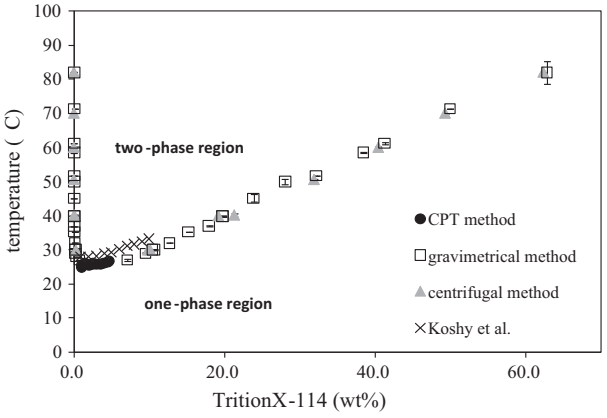


Fig. 1. Liquid–liquid-equilibrium for the Triton X-114/water system [12].