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Extraction of carotenoids from *Chlorella vulgaris* using green solvents and syngas production from residual biomass

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ABSTRACT

A combined process for carotenoids extraction and efficient bioenergy recovery from the wet microalgae biomass is proposed. High added-value products could thus be extracted prior a hydrothermal gasification of the algal biomass into synthetic natural gas. The economic sustainability of biofuel production from algal biomass as well as the large energy demands of microalgae cultivation and harvesting is addressed in this paper. Two green solvents, ethanol and 2-methyltetrahydrofuran (MTHF), were used to achieve the maximum extractability of selected carotenoids. Pure MTHF was tested for the first time as an alternative renewable solvent for carotenoid extraction from wet biomass and promising results were obtained (30 min at 110 °C), with 45% of total carotenoids being extracted. The energy content of the residual biomass corresponds to a High Heating Value (HHV) of 18.1 MJ/kg. With a 1:1 mixture of both MTHF and ethanol, more carotenoids were extracted from wet biomass (66%) and the remaining HHV of the residual biomass was 15.7 MJ/kg. The perspectives of combined carotenoid extraction and energy recovery for a better microalgae valorization are discussed.

1. Introduction

There is a current worldwide interest in algal biomass and algae-derived compounds for their multiple applications ranging from biofuels and feed to health promoting products [1–3]. Microalgal biomass production is also regarded as a potential way to overcome the current reliance on fossil fuels; furthermore microalgae are of special interest as they have a higher photosynthetic efficiency and areal productivity compared to crop plants, and as they offer the possibility of using infertile land and are therefore not competing with the food and feed production [4,5]. However, for several reasons, the economics of biofuel production from microalgae does not look favorably at the present time. To cope with these limitations and to achieve economic sustainability in algal biofuel production, a promising option relies on a biorefinery concept where several products are obtained from the same raw material [6].

Microalgae are considered as rich sources of natural antioxidant molecules that exhibit unique properties. For instance, polyunsaturated fatty acids (PUFA) and carotenoids which are thought to play an important role in human health [7,8]. Epidemiological studies have shown an association between diets that are rich in carotenoids and reduced incidence of many forms of cancer [9]. Many studies have also proved the importance of carotenoids in the sequestration of free radicals released in the human body under stress conditions [9,10].

These lipophilic secondary pigments belong to the tetraterpenoids group, which can be classified according to the presence or the absence of oxygen atoms on the molecular structure to xanthophylls and carotenes, respectively. The latest consist only of carbon and hydrogen atoms such as beta-carotene, the most common carotene. Xanthophylls have one or more oxygen atoms; lutein is one of the most common xanthophylls [11].

The potential use of algal pigments as natural colorant in food or in

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Table 1
Hansen solubility parameters: solvent/solute interactions.
$MPa^{1/2}$ (equivalent to joules/cm^3; 2.0455 × $(cal/cm^3)^{1/2}$) at 25 °C. HSP is given by $\delta_{total}^2 = \delta_d^2 + \delta_p^2 + \delta_H^2$ which now consists of its three partitioned HSP in terms of dispersion force δ_d, polar (permanent dipole forces) δ_p, and hydrogen-bonding δ_H force, respectively [25].

Hansen solubility parameters ($MPa^{1/2}$)	Solvents				Solutes		
	MTHF	Ethanol	Water	Hexane	β-Carotene	Lutein	Zeaxanthin
δ_{total}	**17.69**	26.5	47.8	15	**17.5**	**18.4**	**18.5**
Dispersion δ_d	16.4	15.8	15.6	15	17.4	17.8	17.8
Polar δ_p	4.8	8.8	16.0	0	0.8	1.3	1.4
Hydrogen bonding δ_H	4.6	19.4	42.3	0	1.7	4.5	4.8

cosmetics offers an interesting perspective thanks to the extensive practical applicability and the relatively high market price of natural dyes, e.g. AlgalTechnologies (Israel) as well as Parry Pharmaceuticals (India) are two companies producing astaxanthin from *Haematococcus pluvialis* having a market value of 10,000 USD/kg [12]. However, such a product with a very high value typically have a low market size [12] and the production costs could be a major barrier. Therefore, establishing business models that look not only at the potential of algae for high value products but which are also considering the possibility of producing energy from the same raw material is of great interest. Moreover, new hydrothermal gasification technologies allow an energy efficient conversion of wet algal biomass (e.g. [13,14]), the elimination of problematic wastes, and recovery of the valuable nutrients for the algae production process [13].

Carotenoids extraction could thus contribute to make microalgal biofuel production economically feasible. However, conventional extraction techniques of these compounds are time consuming and generally require using dry algal biomass with < 10% of water content [14]. For instance, to perform supercritical carbon dioxide extraction it is necessary to remove water after harvesting biomass, which consumes large amounts of energy [15]. Currently, efforts are devoted to develop processes for extracting products directly from wet algal biomass. For instance, lipid-rich extraction methods have been successfully developed by the direct extraction from wet microalgae [16–19]; among them, the Pressurized Liquid Extraction (PLE) method has demonstrated to be a promising alternative in order to reduce energy consumption and increase the extraction yield of lipids and carotenoids [20]. Nevertheless, there are also issues related to the use of toxic organic solvents that are generally required in high amounts to carry out the extraction step. The uncommon environmentally friendly, efficient viable technologies for extraction of those valuable pigments are currently a matter of intense research [21]. Concerns about health effects have also led to a search for alternatives [21–22]. This is mainly due to the fact that some of the carotenoids may undergo a structural change leading to possible loss of functionality or nutritional deterioration during the extraction with the hydrocarbon based solvents (generally sourced from fossil resources). Therefore, new green solvents were proposed. The main advantage of these ecofriendly solvents including the ones that are produced from biomass feedstock and ecofriendly petrochemical-based solvents are non-toxic and/or biodegradable. For instance, terpenes such as p-cymene, terpinolene, α-pinene, β-pinene or D-limonene have been tested for carotenoids extraction from different raw materials [22–23]. For instance, D-limonene (bio-solvent) was used instead of dichloromethane for pigments extraction from tomato and promising results were obtained [24].

2-Methyltetrahydrofuran (MTHF) is a green solvent derived from renewable resources (lignocellulosic biomass) [25] and has the advantages to be biodegradable and easy recyclable. MTHF appears to be a potential alternative solvent to n-hexane for the extraction of carotenoids due to its unique properties [26].

The present research focused on using wet biomass and opted for more environmentally friendly extraction techniques that employ green solvents (MTHF alone or combined with ethanol) instead of petroleum-derived ones. The chemical profile of the extracts was determined by High-Performance Liquid Chromatography coupled with Diode Array Detector (HPLC-DAD). The effect of this pre-processing step on the residual microalgae biomass was evaluated for the potential energy recovery by calculating the high heating value. The perspective about integrating the carotenoids extraction step into an existing hydrothermal energy recovery process for better microalgae valorization is also discussed [27–28].

2. Material and methods

2.1. Hansen solubility parameters

To predict the compatibility of the tested solvent and solutes, Hansen solubility parameters (HSP) were studied (Table 1) to forecast miscibility and solvation and were compared with hexane, which is generally used for carotenoid extraction [25]. The Hansen method provides a convenient and efficient way to characterize solute/solvent interactions.

HSP is given by $\delta total^2 = \delta d^2 + \delta p^2 + \delta H^2$ which consists of its three partitioned HSP in terms of dispersion force δd, polar (permanent dipole forces) δp, and (hydrogen-bonding force) δ_H, respectively. In general, the more similar the two δtotal are, the greater the affinity between solutes and solvents. MTHF was chosen for carotenoid extraction since it has a δtotal which is very close to the one of carotenoids and since it is a green solvent.

2.2. Microalgae cultivation and harvesting

Chlorella vulgaris (strain CCAP 211/52) was cultivated in an open thin-layer photobioreactor situated in a greenhouse on the Grüental campus of the Zurich University of Applied Sciences in Wädenswil, Switzerland. The design of the reactor has been developed at the Institute of Microbiology, Academy of Sciences of the Czech Republic, at Trebon [29]. With *C. vulgaris*, cultivation reaches a photosynthetic efficiency of approximately 7% and cell concentrations of up to 50 g/L [29]. In this study concentrations as high as 30 g/L were reached.

The algal biomass was harvested using a conical plate centrifuge (Type SB7-47-076, Westfalia Separator AG), obtaining slurry with 20% dry weight content, and then stored at − 20 °C. Prior to extraction, the microalgae biomass was then freeze dried using a lyophilizer (CHRIST, LCG lyo Chamber Guard) at − 70 °C and at low pressure. As the experiment addressed extraction from both wet and dry biomass, the lyophilized biomass was either rehydrated to 50% moisture content, or used directly without hydration (moisture content < 5%).

2.3. Chemicals and reagents

Analytical and HPLC-grade solvents (acetonitrile, methanol, ethyl acetate and acetone) were obtained from Merck (Darmstadt, Germany). Trans carotenoids used as external and internal standards (astaxanthin, β-carotene, canthaxanthin β-apo-8′-carotenal, violaxanthin) were purchased from Sigma Aldrich, USA, whereas lycopene and lutein were