Flavour generation from microalgae in mixotrophic cultivation

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Abstract

Microalgae are known to produce several volatile organic compounds that can be obtained from the biomass or released extracellularly into the medium. The aim of this study was to evaluate the generation of volatile organic compounds with flavour potential from the microalga *Phormidium autumnale* in mixotrophic cultivation. The experiment was conducted in a New Brunswick Scientific BioFlo®310 bioreactor operating under a batch system, with a 1.5 L working volume. The experimental conditions were as follows: initial inoculum concentration 100 mg L^{-1}, temperature 25°C, pH adjusted to 7.6 and aeration of 1.0 volume air per culture volume per minute, supplemented with 5 $g.L^{-1}$ of sucrose and constant light intensity of 4 klux. The volatile compounds were isolated by solid phase micro-extraction applied in headspace of residence time (144 hours), separated by gas chromatography and identified by mass spectrometry (HS-SPME-GC/MS), co-injection of standards and Kovats index. The major products in the bioreactor were 2,4-decadienal (46.03%), 3-methyl-1-butanol (12.39%), hexanol (4.17%) and 2-ethyl-1-hexanol (3,51%). The descriptor flavour of the compounds detected in experiments was mainly classified as fried food, fruity, spice, and floral compounds. In conclusion, the results have shown that the mixotrophic cultivation of the *Phormidium autumnale* could be a potential biotechnological to produce natural flavours.

Introduction

Microalgae are a group of photosynthetic microorganisms typically unicellular and eukaryotic. Although cyanobacteria belong to the domain of bacteria, and are photosynthetic prokaryotes, they are often considered microalgae [1]. Microalgae and cyanobacteria are considered some of the most promising feedstocks for the supply of food and nonfood industries [2; 3]. Because they present a high content of macronutrients (proteins, carbohydrates, and lipids), microalgae have the potential to enhance the nutritional value of foods [4]. They may also be used as a feed source for many aquatic organisms and livestock [5]. Microalgae-based systems for chemicals production are an emergent area, representing a great promise for industrial application.

The growing interest in natural products guides the development of the technologies that employ microorganisms, including microalgae, which are able to synthesize specific volatile organic compounds. Therefore, the selection of a mode of cultivation of microalgae is of vital importance. Four major modes of microalgae cultivation can be adopted, namely photo-autotrophic, heterotrophic, photo-heterotrophic, and mixotrophic [6]. Mixotrophic microalgae use different energy and carbon sources so that they may use organic or inorganic sources and light in different combinations. Mixotrophy makes microalgae more flexible because it may gather both the carbon and energy demand from organic or inorganic sources and light simultaneously [7].

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The occurrence of volatile organic compounds in microalgae is a consequence of their versatile metabolism. The compounds produced may belong to different classes of compounds such as esters, alcohols, hydrocarbons, ketones, terpenes, carboxylic acids and sulphur compounds [8, 9]. Many of these volatiles present odour descriptors such as floral, fruity, spice, sweet, roasted, and can, therefore, be used as a flavouring agent in the food industry and others used in the pharmaceutical and fine chemicals industries.

Thus, the objective of this study was to evaluate the generation of volatile organic compounds with flavour potential from the microalga *Phormidium autumnale* in mixotrophic cultivation.

Experimental

Microorganism and culture conditions

Axenic cultures of *Phormidium autumnale* were originally isolated from the Cuatro Cienegas desert (26°59′ N, 102°03′, W. Mexico). Stock cultures were propagated and maintained in solidified agar-agar (20 g L^{-1}) containing BG11 medium [10]. The cultures were illuminated with 20 W fluorescent day light-type tubes (Osram Sylvania, Brazil), located in a photo period chamber at a photon flux density of 15 μmol photons $m^{-2}s^{-1}$ and a photoperiod of 12/12 h light/dark at 25°C. The photon flux density was adjusted and controlled by using a digital photometer (Spectronics, model XRP3000). To obtain the inoculum in liquid form, 1 mL of sterile medium was transferred to slants, and the colonies were scraped off and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

The experiment was conducted in a New Brunswick Scientific BioFlo®310 bioreactor operating under a batch system, with a 1.5 L working volume. The bioreactor including filtration units was sterilized by autoclaving at 121ºC for 20 min. The experimental conditions were as follows: initial concentration of inoculum of 100 mg L^{-1}, temperature of 26°C, pH adjusted to 7.6, aeration of 1.0 VVM (volume of air per volume of culture per minute per minute). The culture medium consisted of a BG11 synthetic medium supplemented with 5g L^{-1} of sucrose and a constant light intensity of 4 klux.

Isolation of the volatile organic compounds

The volatile organic compounds were analysed at 144 h of the residence time using headspace solid-phase micro-extraction (HS-SPME) with a 50/30μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (Supelco, USA). Sample preparation was performed using 20 mL of culture medium, equally separated into two portions. Each of these portions was analysed by HS-SPME coupled with GC/MS for the quantitative determination of the volatile compounds. The aliquot was placed in a headspace septum vial containing 3 g of NaCl. The SPME fiber was inserted into the headspace of the vial containing the sample (previously kept at 40ºC for equilibration temperature) for 45 min at 40°C, with agitation provided by a magnetic stir bar. After this period, the fiber was removed from the vial and immediately desorbed into the injector of the GC. The analytical procedure was performed twice and in duplicate. Therefore, the data refer to the mean value of two repetitions.

GC/MS analysis

The volatile organic compounds were analysed in a GC system (Agilent 7890A) coupled to a mass spectrometer detector (Agilent 5975) using a DB-Wax fused silica

capillary column (60 m in length, 0.25 mm id and 0.25 μm film thickness). The initial oven temperature was held at 35°C for 5 min., followed by a linear increase at 5°C/min to 220°C, and held at this temperature for 5 min. For the identification of the compounds was based on GC-MS, electron-impact ionization voltage of 70 eV was applied, and helium was used as the carrier gas. The volatile compounds were identified by a comparison of their MS spectra with those provided by the computerized library (NIST MS Search). In addition, to assist with identification, each volatile linear retention index (LRI) was calculated using the retention times of a standard mixture of paraffin homologues prepared in hexane and compared with the LRI values published in the literature for columns with the same polarity (www.flavornet.net). Co-injection of the sample and the standard mixture provided experimental LRIs for the compounds, which were compared with those of standards analysed under similar conditions.

Results and discussion

The volatile organic compounds produced by *Phormidium autumnale* cultivated in mixotrophic conditions are presented in Table 1. A total of 16 compounds (aldehydes, alcohols, ketones, and hydrocarbons) with different odour descriptors were found. Among the chemical classes identified, 2,4-decadienal (46.03%), 3-methyl-1-butanol (12.39%) and 1-hexanol (4.17%) were the major compounds identified.

Table 1: Volatile organic compounds produced by *Phormidium autumnale* cultivated in a mixotrophic microalgal reactor. The odour description presented was extracted from the literature in comparison to the compound name, chromatographic column and Kovats index (www.flavornet.org).

Compound	*Kovats Index*	*Description of odour*	*Relative peak area (%)*
acetaldehyde	714	pungent, ether	2.37
hexanal	1084	grass, tallow, fat	1.96
2-methyl-1-propanol	1099	wine, solvent, bitter	0.73
3-methyl-1-butanol	1205	whiskey, malt, burned	12.39
1-pentanol	1255	balsamic	0.75
1-hexanol	1360	resin, flower, green	4.17
2-octenal (E)	1408	green	1.62
(E,E)-2,4-heptadienal	1463	nut, fat	3.02
2-ethyl-1-hexanol	1487	rose, green	3.51
benzaldehyde	1495	almond, burnt sugar	0.57
hexadecane	1600	alkane	3.28
2-octen-1-ol (E)	1608	soap, plastic	0.72
acetophenone	1645	must, flower, almond	1.43
2,4-decadienal (E,E)	1710	fried, wax, fat	46.03
trans-geranylacetone	1840	green	1.83
β-ionone	1912	seaweed, flower, raspberry	0.82
Other Compounds			14.80
Total			*100*

Mixotrophic cultivation occurs when the microalga uses photosynthesis and oxidation of organic compounds concomitantly: the oxygen produced in the photosynthesis is consumed in the heterotrophic route. At the same time, the carbonic gas generated in the oxidation of the organic compound is exploited in photosynthesis. This cultivation is already widely exploited in terms of biomass production [6, 7]. The volatile organic compounds biosynthesis mainly depends on the availability of carbon and

nitrogen as well as energy provided by primary metabolism. The formation of volatile organic compounds can occur during both primary and secondary metabolism of microorganisms as secondary products, thereby we can suggest that the presence of these compounds is due to the secondary metabolism of these microorganisms.

According to Santos [8], aldehydes proved to be the most prevalent volatile organic compounds and, due to their low odour threshold values, might be important headspace volatiles compounds contributing to desirable aromas as well as rancid odours and flavours. Saturated aldehydes have a green-like, hay-like, paper-like odour, whereas unsaturated aldehydes have a fatty, oily and frying odour. Whereas the shorter chain linear aldehydes are often derived from chemical lipid oxidation, branched and aromatic aldehydes are typically formed due to enzymatic lipid and protein oxidation.

Microalgae can produce a variety of industrially relevant volatile compounds that can represent an improvement in the supply of a large volume of inputs for different types of industry (odour, flavours, energy).

In conclusion, the results show that the mixotrophic cultivation of the *Phormidium autumnal* could be an alternative to obtain flavours by this biotechnological route. More knowledge about the biochemical routes should be taken into account, thereby increasing the production of compounds of interest and the use of all the products generated during the bioprocess.

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