Desalination and Water Treatment
www.deswater.com

doi: 10.5004/dwt.2021.27458

232 (2021) 16–25
August

The role of microalgae-based systems in the dynamics of odorous compounds in the meat processing industry. Part II – olfactometry and sensory relevance

Karem Rodrigues Vieira[a], Mariana Manzoni Maroneze[a], Bruna Klein[a], Roger Wagner[a], Maria Isabel Queiroz[b], Eduardo Jacob-Lopes[a], Leila Queiroz Zepka[a,*]

[a]*Bioprocess Intensification Group, Federal University of Santa Maria, UFSM, Roraima Avenue 1000, Santa Maria, RS 97105-900, Brazil, emails: lqz@pq.cnpq.br (L.Q. Zepka), merakvieira@gmail.com (K.R. Vieira), mariana_maroneze@hotmail.com (M.M. Maroneze), brunaklein06@yahoo.com.br (B. Klein), rogerwag@gmail.com (R. Wagner), ejacoblopes@gmail.com (E. Jacob-Lopes)*
[b]*School of Chemistry and Food, Federal University of Rio Grande (FURG), Rio Grande, RS, Brazil, email: queirozmariaisabel@gmail.com*

Received 1 February 2021; Accepted 23 May 2021

A B S T R A C T

This research evaluated the role of microalgae-based systems in deodorizing the meat processing industry by analyzing gas chromatography-olfactometry (GC-O). The olfactometric odorant profile of raw wastewater, the deodorization process along the residence time, and the high-value volatile organic compounds generated by heterotrophic cultures of *Phormidium autumnale* were assessed. The results presented thirty-seven compounds identified by GC-O in the raw wastewater. Indole and skatole were considered the main odor markers with the modified frequency of 91% and 75%, respectively. These compounds did not present sensory perception after 72 h of residence time, suggesting that were completely removed. At the same time, a total of 11 compounds were formed in the microalgae-based process. These compounds were classified as fruity, citrus, green, and resinous by the judges and can be used as a flavoring agent. Finally, the microalgal heterotrophic bioreactor was able to mitigate the most unpleasant odors of the meat processing wastewater, and, in addition, compounds of commercial interest were generated, suggesting the possibility of exploring them for application in the fine chemical or food industry.

Keywords: Microalgae/cyanobacteria; Agro-industrial wastes; Olfactometric analysis; Deodorization; Bioproducts

1. Introduction

Unpleasant odors emissions from wastewater treatment plants (WWTPs) represent a prominent threat to society by causing degradation of environmental quality, interference with business activities. In addition, the odor can cause effects on human health, ranging from mild discomfort (skin and eye irritation, headaches, dizziness, and nausea) to more severe symptoms (coughing, wheezing, and even breathing problems), depending on its intensity and time of exposure. If the odor lasts for a long time, it can affect a human's mood, anxiety, and stress level [1–3]. With the global trend of urbanization, the increasing population, and the shortage of land resources, the distance between residential areas and WWTPs has decreased, leading to a rise in public grievances against the occurrence of odorous compounds in areas adjacent to these facilities [5,6].

Odor can be defined as a sensation resulting from the interaction of volatile chemical species with relatively low molecular weight (30–200 g mol^{-1}) and pungent smell inhaled through the nose [7]. Among these molecules are volatile organic compounds (VOCs), which are the main pollutants in the atmospheric environment [8]. Some of these compounds have very low odor threshold values in

* Corresponding author.

terms of ppbv or pptv, where even at low concentrations, they can cause negative psychosomatic symptoms [9].

One of the main sources of environmental odors of anthropogenic origin is the food industry, especially meat processing plants. Although emissions of bad odors have always been associated with the animal protein production chain, only in recent decades has this attracted greater attention. This is related to the intensification of animal production in many countries since the global population growth has increased the demand for animal food sources. Representative VOCs emitted from meat processing facilities are mainly terpenes, alcohols, aldehydes, sulfuric compounds, amines, phenolic compounds, esters, and ketones [9].

To alleviate the issues related to odor emissions, strict environmental regulations are continually being developed and strengthened by the administrative authorities worldwide [10]. In this regard, a variety of odor treatment technologies have been proposed, which can be classified into physical/chemical (e.g., adsorption and chemical scrubbers) and biological (e.g., biofilters, biotrickling filters, bioscrubbers) techniques. Each available technology has advantages and disadvantages, cost, and specific application ranges since the wastewater from WWTPs is a complex mixture of compounds with different molecular weights, volatilities, and chemical functionalities [6,10]. Still, biological technologies are preferable in practical applications based on their efficiency and sustainability [11]. An innovative technology that has emerged is the application of microalgae-based systems for odor removal and the potential bioconversion of value-added products [12].

Microalgae-based systems applied to wastewater treatment have been used for almost 60 y [11,12]. However, the application of these microorganisms for deodorization of the volatile organic compounds of the wastewater treatment plant was first proposed by Vieira et al. [12], in Part I of this sequential research. In this study, the microalgae *Phormidium autumnale* was used to deodorize volatile organic compounds from wastewater, which regardless of polarity range and molecular weight, were removed with 99.6% of efficiency. In addition, was possible to observe the concomitant formation of compounds industrially interesting.

To characterize the olfactory impact of odorants, techniques that combine analytical and sensory measurements, such as olfactometry, have been key tools in odor control processes. Gas chromatography coupled with olfactometry (GC-O) allows to characterize compounds using odor descriptors, evaluate the potential sensorially relevant VOCs, thought the odor intensity and, so allow better estimation of odor impact [13,14]. As far as we know, there have been no reports on the olfactometric evaluation of wastewater deodorization processes.

Thus, the objective of this study was to evaluate the sensorial relevance of volatile organic compounds emitted by a deodorization process based on microalgae of meat processing wastewater. The study focused on the (i) characterization of the olfactometric odorant profile of raw wastewater, (ii) sensory evaluation of the deodorization process, and (iii) evaluation of high-value volatile organic compounds generated by *Phormidium autumnale*.

2. Material and methods

2.1. Microalgae and culture media

Axenic cultures of *Phormidium autumnale* were used in the experiments. Stock cultures were propagated and maintained in solidified agar-agar (20 g L^{-1}) containing synthetic BG11 medium [15]. The incubation conditions were 25°C, the photon flux density was 15 μmol m^{-2} s^{-1} and the photoperiod was 12 h. To obtain the inoculums in liquid form, 1 mL of sterile synthetic medium was transferred to slants; the colonies were scraped and then homogenized with the aid of mixer tubes. The entire procedure was performed aseptically.

2.2. Meat processing wastewater

Meat processing wastewater (MPWW) samples were collected from industry in Santa Catarina, Brazil (27°14'02″S, 52°01'40″W). Samples were collected from the discharge point of an equalization tank over a period of 1 y. The collected MPWW samples were transferred to the analytical laboratory and stored at 4°C according to the standard methods for the examination of water and wastewater [16]. The characteristics of MPWW included chemical oxygen demand (COD), total Kjeldahl nitrogen (N-TKN), total phosphorus (P-PO_4^{-3}), total solids (TS), volatile solids (VS), fixed solids (FS), suspended solids (SS), and pH was determined according to APHA. The average composition of the wastewater was COD 4,100 ± 874 mg L^{-1}, N-TKN 128.5 ± 12.1 mg L^{-1}, P-PO_4^{3-} 2.84 ± 0.2 mg L^{-1}, TS 3.8 ± 2.7 mg L^{-1}, VS 2.9 ± 1.4 mg L^{-1}, FS 0.9 ± 0.3 mg L^{-1}, SS 1.9 ± 0.8 mg L^{-1}, and pH 5.9 ± 0.05.

2.3. Experimental condition

Cultivations were performed in a bubble column bioreactor, operating under a batch regime and fed on 2.0 L of wastewater [17]. The experimental conditions were determined as follows: initial concentration of inoculum 100 mg L^{-1}, temperature 25°C, pH adjusted to 7.6, and aeration of 1.0 VVM (volume of air per volume of culture per minute), absence of light, and residence time of 72 h. The experiments were performed twice and in duplicate. Therefore, data refer to the mean value of four repetitions.

2.4. Analytical methods

2.4.1. Isolation of the volatile organic compounds

The volatile compounds were isolated from the sample using a headspace solid-phase microextraction (HS-SPME) technique, employing a divinylbenzene/carboxen/polydimethylsiloxane (DVB/Car/PDMS) fiber (50/30 μm film thickness × 20 mm; Supelco®, Bellefonte, PA). Sample aliquots of 20 mL were collected each 24 h (0, 24, 48, 72) and equally separated into two portions. The same procedure was repeated for the wastewater and microalgae. Each portion was placed in a 20 mL amber glass vial containing 3 g of NaCl and 10 μL of a 3-octanol internal standard solution with a known concentration (0.082 μg mL^{-1}). The SPME fiber was exposed in the sample headspace for 45 min at 40°C, under constant stirring (400 rpm) with a magnetic stir bar. After this period, the fiber was removed from the vial and submitted to chromatographic analysis. The analytical procedure was performed twice and in duplicate. Therefore, data refer to the mean value of four repetitions.

2.4.2. GC-O and GC–FID analyses

The volatile compounds were quantified and sniffed by a Varian Star 3400 CX (CA, USA) gas chromatograph equipped with a flame ionization detector (GC–FID) and a sniffing port both interconnected by a flow splitter to the column exit. Eluting compounds were split at the end of the column at a 1:1 ratio between the FID detector and the olfactometric port. The fiber was thermally desorbed into the injection port at a temperature of 250°C for 10 min, in a splitless mode for 1.0 min. Hydrogen was used as carrier gas at constant pressure (15 psi) and flow rate (1.2 mL min^{-1}). The compounds were separated in a polar fused silica capillary column DB-WAX (CHROMPACK, USA; 30 m × 0.25 mm × 0.25 µm of film thickness). The initial column temperature was set at 35°C for 5 min, followed by a linear increase of 5°C min^{-1} to 250°C, and this temperature was held for 5 min. The temperature in the detector was kept at 250°C. Purified compressed air (flow rate 3.5 L min^{-1}) was used to carry the analytes from the heated GC transfer line until the sniffing port. The air was pre-heated and reach the judge's nose at 28°C.

The protocol of the study was approved by the Ethics Committee of the Federal University of Santa Maria (CAAE 98758718.8.0000.5346). A modified frequency technique was used for the evaluation of odors and their relative influence on the aroma of the sample. Sniffings were carried out by a panel composed of six experienced judges belonging to the laboratory staff. Sniffing time was approximately 47 min, and each judge evaluated a half part in one chromatographic run, and they participated one time per day. The panelists were asked to score the intensity of each volatile stimulus using a categorical 4-point scale: 0 = no odor; 1 = weakly recognizable odor; 2 = clear but not intense odor, and 3 = very intense odor. The olfactometric strategy carried out in this study combined measurements of intensity and frequency of detection, as has been reported in previous papers [18,19]. The signal obtained was the modified frequency (MF, %), a parameter which was calculated by Eq. (1) proposed by Dravnieks [20]:

$$\text{MF}(\%) = \sqrt{F(\%) \times I(\%)} \quad (1)$$

where F (%) is the detection frequency of an aromatic attribute expressed as a percentage of the total number of judges and I (%) is the average intensity expressed as a percentage of the maximum intensity.

The linear retention index (LRI) was calculated for each volatile compound using the retention times of a standard mixture of homologous series of n-alkanes (C6-C24) to aid identification [21]. This parameter was used to calculate the LRI of odoriferous stimuli.

2.4.3. GC/MS analysis

The volatile compounds were separated and identified in a Shimadzu QP2010 Plus gas chromatography coupled to a mass spectrometer (Shimadzu, Kyoto, Japan). The fiber was thermally desorbed for 10 min in a split/splitless injector, operating on the splitless mode (1.0 min splitter off) at 250°C. Helium was used as a carrier gas at a constant flow rate of 1.6 mL min^{-1}. Analytes were separated as described for a GC-O-FID. The MS detector was operated on electron impact ionization mode +70 eV and mass spectra were obtained by scan range from m/z 35 to 350.

The volatile compounds were identified by a comparison of experimental, mass spectra, and LRI with those provided by the computerized library (NIST MS Search) considering over 80% of similarity. Additionally, volatile olfactory descriptions were taken into account to identification when compounds possess odoriferous stimuli. The sample and the standard mixture were injected both separately and together to obtain the experimental LRI and mass spectra values for the purpose of compound identification by directed comparison.

3. Results and discussion

3.1. Compounds identified

Towards control odor at WWTPs, the first step is identifying the sensorially relevant VOC emitted, which should be monitored and managed [2]. Table 1 provides a complete list of VOCs identified in this study, along with their corresponding identifications, where the components are listed in order of their LRI on the DB-WAX column.

The compounds presented molecular weights ranged from 44.0 to 156.2 g mol^{-1} and included four sulfur compounds (compounds 1, 2, 10, and 28), eight aldehydes (compounds 3, 5, 6, 7, 8, 11, 35 and 43), one furan (compounds 4), two hydrocarbons (compounds 9 and 34), twelve alcohols (compounds 12, 19, 20, 24, 27, 31, 32, 33, 39, 42, 47, and 53), seven ketones (compounds 13, 14, 16, 23, 26, 29, and 44), eleven terpenes (compounds 15, 17, 18, 21, 22, 36, 37, 38, 40, 45, and 46), three amines (compounds 25, 54, and 55), 1 ester (compound 30), 1 carboxylic acid (compound 41), 4 phenolic compounds (compounds 48, 50, 51, and 52), and 1 nitrogen heterocyclic compounds (compound 49). Among them, sulfides, indoles, and phenols are generally listed as the most impacting odor classes in meat processing wastewater [22,23].

Among all the fifty-five odor compounds detected in this study, following the criteria of other authors [18,24], we considered odor-active compounds that were detected in at least half of the total sniffing analyses and reached a modified frequency value (MF) higher than 30%. Therefore, a total of 48 odor-active compounds were considered in this study.

3.2. Evaluation of odor characteristics along deodorization process with microalgae

Table 2 shows the volatile composition of the raw wastewater and the impact of the metabolic transformation as a function of time on the composition of volatile compounds in the microalgal heterotrophic bioreactor.

Thirty-seven compounds were identified by CG-O in the raw wastewater, and among them, indole had the highest MF value (91%). This compound is considered one of the main odor markers from animal production facilities

Table 1
List of VOCs identified by GC-O in this study

Compound number	LRI DB-WAX[a]	Identity	Chemical formula	Molecular weight (g mol^{-1})	Odor description[b]
1	<1000	Carbon disulfide	CS_2	76.1	Disagreeable, sweet
2	<1000	Dimethyl sulfide	C_2H_6S	62.1	Decayed cabbage, sulfurous
3	<1000	2-Propenal	C_3H_4O	56.1	Burnt, sweet
4	<1000	2-Methylfuran	C_5H_6O	82.1	Roasted meat, chocolate
5	<1000	Acetaldehyde	$C_6H_{14}O_2$	44.0	Pungent, fresh, green
6	<1000	Butanal	C_4H_8O	72.1	Sweet
7	<1000	2-Methylbutanal	$C_5H_{10}O$	86.1	Cocoa, almond
8	<1000	3-Methylbutanal	$C_5H_{10}O$	86.1	Malt, smell of oil
9	1053	Toluene	C_7H_8	92.1	Rubbery, tarry, mothballs
10	1089	Dimethyl disulfide	$C_2H_6S_2$	94.2	Rotten cabbage, putrefaction
11	1102	Hexanal	$C_6H_{12}O$	100.1	Grass, tallow, fat
12	1103	2-Methylpentanol	$C_6H_{14}O$	102.1	Pungent
13	1120	2-Methyl-3-hexanone	$C_7H_{14}O$	114.1	Fruity
14	1128	Acetyl valeryl	$C_7H_{12}O_2$	128.1	Butter, cheese, oily
15	1129	1,4-Cineole	$C_{10}H_{18}O$	154.3	Spice
16	1145	2-Heptanone	$C_7H_{14}O$	114.1	Fruity, spicy, sweet, herbal
17	1156	Limonene	$C_{10}H_{16}$	136.2	Lemon
18	1159	1,8-Cineole	$C_{10}H_{18}O$	154.3	Spice
19	1162	1-Pentanol	$C_5H_{12}O$	88.1	Balsamic, fruity
20	1166	3-Methylbutanol	$C_5H_{12}O$	88.1	Oil, alcoholic, fruity, banana
21	1169	α-Terpinene	$C_{10}H_{16}$	136.2	Lemon
22	1182	ρ-Cymene	$C_{10}H_{14}$	134.2	Lemon, fruity, fuel like
23	1184	Cyclohexanone	$C_6H_{10}O$	98.1	Pepper, acetone
24	1185	2-Heptanol	$C_7H_{16}O$	116.2	Herb
25	1184	Pyrrolidine-2,4-dione	$C_4H_5NO_2$	99.1	na[c]
26	1210	6-Methyl-5-hepten-2-one	$C_8H_{14}O$	126.1	Citrus, green, musty
27	1251	Hexanol	$C_6H_{14}O$	102.2	Flower, green
28	1247	Dimethyl trisulfide	$C_2H_6S_3$	126.3	Rotten, vegetables
29	1230	2-Nonanone	$C_9H_{18}O$	142.2	Fruity, sweet, cheese, green
30	1322	Methyl 3-methyl 2-hydroxy-butanoate	$C_6H_{12}O_3$	132.1	Apple
31	1341	Cyclohexanol	$C_6H_{12}O$	100.1	Camphor
32	1356	5-Ethyl-2-nonanol	$C_{11}H_{24}O$	172.3	na
33	1415	1-Heptanol	$C_7H_{16}O$	116.2	Chemical, green
34	1427	3-Propylcyclopentene	C_8H_{14}	110.2	na
35	1502	Benzaldehyde	C_7H_6O	106.1	Burnt, sweet
36	1511	Linalool	$C_{10}H_{18}O$	154.2	Flower, lavender
37	1522	Fenchol	$C_{10}H_{18}O$	154.2	Camphor
38	1528	4-Terpineol	$C_{10}H_{18}O$	154.2	Turpentine, nutmeg, must
39	1526	2-Octen-1-ol	$C_8H_{16}O$	128.2	Soap, plastic
40	1534	Menthol	$C_{10}H_{20}O$	156.2	Peppermint
41	1586	3-Methylpentanoic acid	$C_6H_{12}O_2$	116.1	Acidic, cheese, green
42	1591	1-Nonanol	$C_9H_{20}O$	144.3	Fat, green
43	1669	Phenylacetaldehyde	C_8H_8O	120.1	Honey, sweet
44	1685	Acetophenone	C_8H_8O	120.1	Must, flower, almond
45	1699	Linomen-4-ol	$C_{10}H_{16}O$	152.2	Fresh, mint
46	1741	α-Terpineol	$C_{10}H_{18}O$	154.2	Oil, anise, mint
47	1780	Benzyl alcohol	C_7H_8O	108.1	Sweet, flower
48	1819	2-Phenylethanol	$C_8H_{10}O$	122.1	Rosy
49	1832	Benzothiazole	C_7H_5NS	135.1	Gasoline, rubber
50	1829	o-Cresol	C_7H_8O	108.1	Medicinal, phenolic
51	1877	Phenol	C_6H_6O	94.1	Medicinal, phenolic plastic rubber
52	1876	ρ-Cresol	C_7H_8O	108.1	Fecal, horse stable-like
53	2015	1-Penten-3-ol	$C_5H_{10}O$	86.3	Pungent, green, vegetable
54	2264	Indole	C_8H_7N	117.1	Manure, fecal, nauseating
55	2500	Skatole	C_9H_9N	131.2	Fecal, nauseating

[a]Linear retention índices in the DB-WAX column;
[b]According to: Vieira et al. [12]; Acree and Arn [21];
[c]na: not available in the literature.

Table 2
Odorants found in the microalgal heterotrophic bioreactor: gas chromatographic retention data, identify, and modified frequency percentage (MF, %)

			Modified frequency (%)				
Group	Peak	Identify	Waste	0 h	24 h	48 h	72 h
Sulfur compounds	1	Carbon disulfide	47	41	–	–	–
	2	Dimethyl sulfide	–	–	–	–	–
	10	Dimethyl disulfide	51	51	33	33	–
	28	Dimethyl trisulfide	71	58	41	–	–
Aldehydes	3	Acrolein	58	30	–	–	–
	5	Acetaldehyde	–	–	–	–	–
	6	Butanal	30	–	–	–	–
	7	2-Methylbutanal	58	51	–	–	–
	8	3-Methylbutanal	51	–	–	–	–
	11	Hexanal	–	–	–	–	–
	35	Benzaldehyde	68	68	–	–	–
	43	Phenylacetaldehyde	–	–	–	–	–
Furans	4	2-Methylfuran	47	37	–	–	–
Hydrocarbons	9	Toluene	31	30	–	–	–
	34	3-Propylcyclopentene	61	54	30	–	–
Alcohols	12	2-Methylpentanol	–	–	–	–	–
	19	1-Pentanol	88	78	–	–	–
	20	3-Methylbutanol	–	–	37	–	–
	24	2-Heptanol	71	74	–	–	–
	27	Hexanol	54	44	–	–	–
	31	Cyclohexanol	–	–	–	–	–
	32	5-Ethyl-2-nonanol	–	–	37	–	–
	33	1-Heptanol	51	58	–	–	–
	39	2-Octen-1-ol	51	30	–	–	–
	42	1-Nonanol	54	30	–	–	–
	47	Benzyl alcohol	68	–	–	–	–
	53	1-Penten-3-ol	–	51	43	51	41
Ketones	13	2-Methyl-3-hexanone	–	–	58	–	–
	14	Acetyl valeryl	–	–	–	–	–
	16	2-Heptanone	–	–	44	37	30
	23	Cyclohexanone	54	40	–	–	–
	26	6-Methyl-5-hepten-2-one	–	54	41	68	–
	29	2-Nonanone	–	–	41	–	–
	44	Acetophenone	30	30	–	–	–
Terpenes	15	1,4-Cineole	41	–	–	–	–
	17	Limonene	85	54	44	–	–
	18	1,8-Cineole	58	44	30	–	–
	21	α-Terpinene	41	51	–	–	–
	22	ρ-Cymene	71	97	–	–	–
	36	Linalool	41	47	–	–	–
	37	Fenchol	85	54	–	–	–
	38	4-Terpineol	58	54	41	–	–
	40	Menthol	–	–	44	53	58
	45	Linomen-4-ol	61	68	61	–	–
	46	α-terpineol	41	85	51	–	–
Amines	25	Pyrrolidine-2,4-dione	54	68	–	–	–
	54	Indole	91	82	71	50	–
	55	Skatole	75	44	–	–	–
Ester	30	Methyl 3-methyl 2-hydroxybutanoate	–	–	68	–	–
Carboxylic acid	41	3-Methylpentanoic acid	–	–	41	–	–
Phenolic compounds	48	2-Phenylethanol	58	–	–	–	–
	50	o-Cresol	44	33	30	–	–
	51	Phenol	61	44	41	–	–
	52	ρ-Cresol	65	54	54	–	–
Nitrogen heterocyclic compounds	49	Benzothiazole	–	41	54	47	33

by several authors [25–27]. Indole, as well as skatole, which had an MF of 75% in wastewater, are produced in the large intestine of animals and in manure by microbial deamination and decarboxylation of tryptophan. Both are detected low threshold concentration and contribute to the unpleasant and nauseating feces odors [28,29]. The other major compounds in the raw wastewater included 1-pentanol (88%), limonene (85%), skatole (75%), p-cymene (71%), 2-heptanol (71%), and dimethyl trisulfide (71%), whose main descriptors were balsamic/fruit, lemon, fecal/nauseating, lemon/fruit/fuel like, herb, and rotten, respectively.

Unsurprisingly, between the raw wastewater and the initial residence time (0 h), that is, shortly after inoculation, little change in the volatile profile was perceived. However, 3 compounds not identified in the wastewater, were detected at 0 h, 6-methyl-5-hepten-2-one, benzothiazole, and 1-penten-3-ol. These compounds are naturally found in the volatile fraction of microalgal cultures since they are derived from the carotenoids cleavage (6-methyl-5-hepten-2-one), fatty acids (1-penten-3-ol), and amino acids (benzothiazole) pathways [12,30,31].

A day after inoculation, important reductions in VOCs were noticed, as shown in Table 2. In this period 19 compounds were removed, mainly alcohols, terpenes, and aldehydes. Aldehydes are a group of great concern as air pollutants due to their reactivity and toxicity [32], so it is important to note that in 24 h all compounds in this class were removed. The term "removed" used in this article refers to changes in which it is unclear whether the compounds are biotransformed, metabolized, or removed from wastewater by any other mechanism. In addition, as a result of the microalgal heterotrophic metabolism, 8 new compounds were generated in the first 24 h of residence time, which are 3 ketones, 2 alcohols, 1 carboxylic acid, 1 terpene, and 1 ester, that will be discussed later.

Between 24 and 48 h, 9 compounds from the raw wastewater were removed, including compounds associated with malodors, such as dimethyl trisulfide, o-cresol, phenol, and ϱ-cresol. Moreover, 6 compounds formed by the microalgae disappeared. During this period no new compound was noticed.

Part I of this sequential research [12] showed that dimethyl sulfide and indole were the most recalcitrant compounds, which were not completely removed, with efficiencies of 69% and 96%, respectively. In terms of sensory perception, these compounds were also the most persistent, being the last odors from wastewater to disappear. Both compounds play an important role in the negative effects on odor release from wastewater treatment plants. The odor impact of these compounds was assessed by the judges, and after 72 h of residence time, presented a modified frequency below 30%, concluding, therefore, that these compounds were completely removed (Fig. 1).

The VOCs identified by the panelists in the treated sample (72 h) were menthol (58%), 2-nonanone (57%), 1-penten-3-ol (41%), and benzothiazole (33%). Note that all of these compounds are the result of microalgae biotransformations since most of these structures were present in the inoculum and others, such as 2-nonanone and menthol were perceived during the process. Except for menthol, the compounds showed a reduction in their modified frequency in 72 h, characterizing the beginning of the senescence phase. According to the literature [33–35], the production rates of microalgal VOCs follow the same pattern as cell growth, which increases by several orders of magnitude during the exponential phase and decreases during senescence.

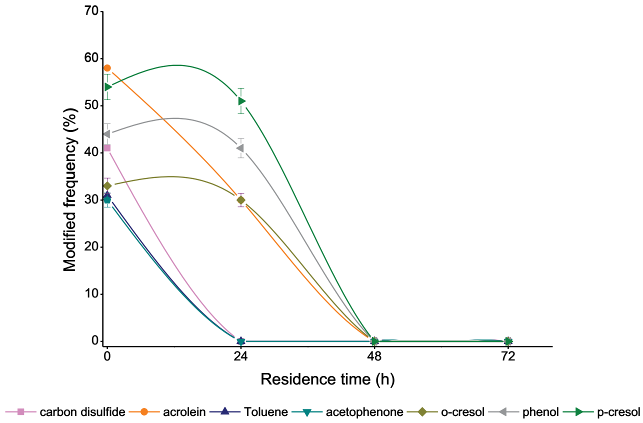


Fig. 1. Hazardous air pollutants biodegradation by *P. autumnale*.

Although some VOCs are considered pollutants due to their toxicity to many organisms, they have the potential to serve as sources of carbon for microalgae cultures, and consequently, as substrates for bioconversion into high-value products [36]. Six compounds found in the meat processing wastewater are listed as hazardous air pollutants (HAPs) by the United States Environmental Protection Agency [37]. The adverse effects on health from exposure to these toxic compounds can be as diverse as the substances themselves and therefore, their monitoring and controlling is imperative. The compounds classified as HAPs were carbon disulfide (1), acrolein (3), toluene (9), acetophenone (44), o-cresol (50), phenol (51), and ρ-cresol (52). Fig. 1 shows the HAPs biodegradation as a function of residence time.

In the raw wastewater, acrolein was found to be the most abundant species, followed by ρ-cresol, phenol, carbon disulfide, o-cresol, toluene, and acetophenone. The results obtained indicate that in one day of operation the heterotrophic bioreactor was able to reduce 43% of the compounds (carbon disulfide, acetophenone, and toluene) to levels undetectable by humans panelists in olfactometry. The phenolic compounds (ρ-cresol, phenol, and o-cresol) and acrolein were only eliminated in 48 h.

To help the study of the odor profile of each sample, the panel of six experienced judges generated a consensual list with twelve sensory descriptors: resinous, putrid, wood, hospital, fruity, sweet, mold, green, spice, floral, burnt, and fat, which are shown in Fig. 2.

The results presented in Fig. 2 corroborate what has already been discussed, where showed a clear change in the volatile profile of the wastewater along the residence time, were no longer detected. In 24 h of residence time, it can be observed that putrid odors were no longer detected. The changes were even more evident between 24 h and 48 h of process, where the descriptors wood, hospital, fruity, mold, spice, floral, and burnt disappeared. On the other hand, in all the samples analyzed, resinous was the descriptive term with the greatest impact.

The odors can be classified into pleasant, neutral, or unpleasant and the relative pleasantness of an odor can be measured by the hedonic tone. A comparative spider chart of the data from the raw wastewater and the end of the cultivation (72 h), considered the treated wastewater is shown in Fig. 3. Dravinieks et al. [38] developed a robust list of 150 odor descriptors and their respective hedonic tone. In this way, Fig. 3 also shows the hedonic tone values determined by these authors regarding the descriptors assigned in this study.

A total of eleven descriptors were generated in the raw wastewater, with resinous, putrid, and hospital being the most impact descriptors. The highest modified frequency occurred among the compounds, putrid (indole, 91%), resinous (1-pentanol, 88%), and fruity (limonene, 85%). At the end of the process, four compounds were perceived by the judges; a terpene, a ketone, alcohol, and a heterocyclic nitrogen compound, which have been described as green, spice, resinous and hospital.

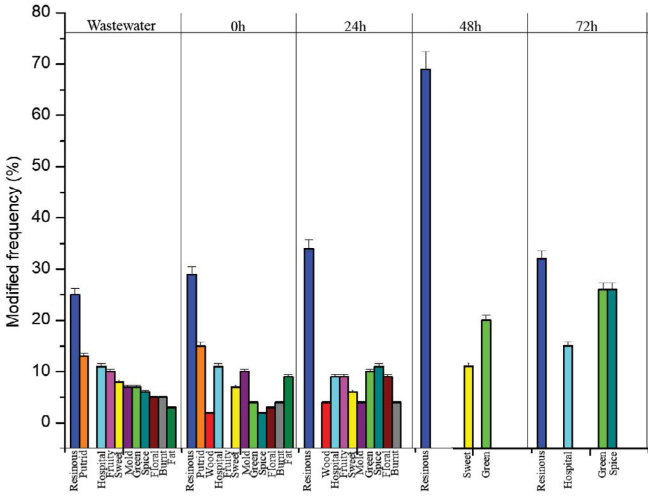


Fig. 2. Consensual list with twelve sensory descriptors the panel of six experienced judges generates.

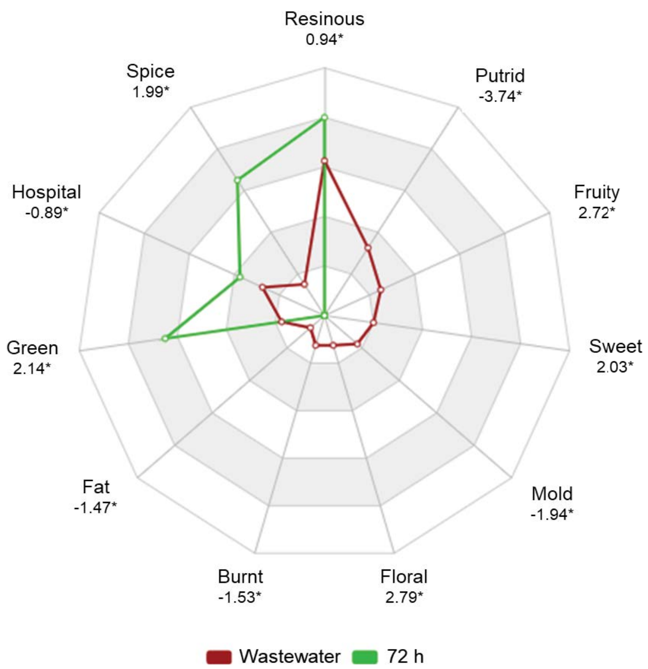


Fig. 3. Spider chart of the sensory profile of mean attribute values for the raw wastewater and the treated wastewater. *Hedonic tone determined by Dravnieks et al. [38].

Regarding the hedonic tone, the 11 descriptors associated with the raw wastewater presented values from –3.74 to 2.79, as showed in Fig. 3. The odor annoyance is subjective, and the perception of pleasantness or dislike depends on the individual's level of tolerance, the exposure time, the emotions of the moment, in addition to being influenced by intercultural differences. Typically, the hedonic tone, that is the level of odor pleasantness or unpleasantness, is measured in a numeric scale ranging from –4 to 4, where –4 is the most unpleasant odor, 0 is neutral, and 4 is the least unpleasant odor. Among the eleven descriptors, five were classified as unpleasant due to their negative value, namely putrid (–3.74), mold (–1.94), burnt (–1.53), fat (–1.47), and hospital (–0.89).

The diagram of comparison (Fig. 3) shows that seven odor characteristics disappeared after microalgae treatment, including the four with the lowest hedonic tone value (putrid, mold, burnt, and fat). At the same time, the descriptors resinous, green, spice, and hospital, with a hedonic tone of 0.94, 2.14, 1.99, and –0.89, increased the impact with the microalgae treatment. By analyzing the results presented above, it can be seen that the microalgal heterotrophic bioreactor was capable of mitigating the most unpleasant odors of the meat processing wastewater.

3.3. Biogeneration of volatile organic compounds

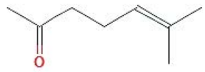
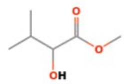
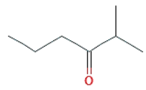
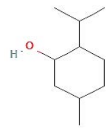
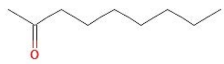
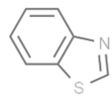
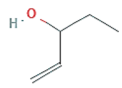
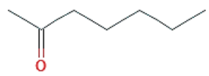
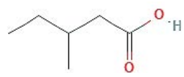
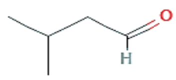
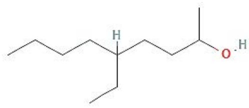
Volatile organic compounds represent an important part of the microalgae metabolome, with expressive possibilities for industrial applications. These structures could be used as a significant alternative source of aromas, fragrances, food additives, pharmaceutical products, and energy [35]. Still, VOCs have been neglected for a long time. However, scientific advances in recent years and the increasing consumers' preference for natural compounds have driven researchers and companies to explore the volatile fraction of microalgae-based processes [39,40].

In this sense, the volatile organic compounds produced by *Phormidium autumnale* cultivated in meat processing wastewater under heterotrophic conditions are presented in Table 3, as well as its potential industrial applications and chemical structure.

A total of 11 compounds produced in the microalgae-based process were identified, with 4 ketones, 3 alcohols, 1 terpene, 1 ester, 1 carboxylic acid, and 1 heterocyclic nitrogen compound. Among the chemical classes identified, 6-methyl-5-hepten-2-one (68%), methyl-3-methyl-2-hydroxybutanoate (68%), 2-methyl-3-hexanone (58%), menthol (58%), and 2-nonanone (57%) were the most impactful in terms of modified frequency.

Ketones, such as 6-methyl-5-hepten-2-one, 2-nonanone, and 2-heptanone are used mainly as flavors, and fragrance agents, due to their description as fruity, citrus, and green [35]. 2-Methyl-3-hexanone, another ketone produced by

Table 3
Ranking of the volatile profile by average modified frequency percentage of the compounds formed

Compound	MF %	Main applications	Structure
6-Methyl-5-hepten-2-one	68	Analytical standard, flavor, fragrance agents	
Methyl-3-methyl-2-hydroxybutanoate	68	Research chemical	
2-Methyl-3-hexanone	58	Analytical standard, research chemical	
Menthol	58	Medicines, ointments, flavor, fragrance agents	
2-Nonanone	57	Flavor, fragrance agents	
Benzothiazole	54	Analytical standard, fragrance agents, cosmetic, chemical industry	
1-Penten-3-ol	51	Analytical standard, flavor, fragrance agents	
2-Heptanone	44	Flavoring agent, adjuvant	
3-Methylpentanoic acid	41	Flavoring agent, adjuvant	
3-Methylbutanol	37	Flavoring agent, adjuvant	
5-Ethyl-2-nonanol	37	Research chemical, building blocks	

P. autumnale is applied as a research chemical and analytical standard and is not recommended for flavor use [41]. Except for 6-methyl-5-hepten-2-one, which was already present in the microalgal inoculum, the other ketones were noticed only in 24 h of culture.

In the alcohol class, 1-penten-3-ol was identified in all samples after inoculation, which might exert an important effect on the flavor of microalgae, which was defined as resinous by the judges and generally is used as a flavoring agent. This compound is typically found in microalgae since it is a product of the lipid oxidation of n-3 fatty acids [42,43]. 3-Methylbutanol and 5-ethyl-2-nonanol were only perceived in 24 h of cultivation, being described as wood and green, respectively. 3-Methylbutanol is allowed to be used in foods, as a flavoring and adjuvant agent, while 5-ethyl-2-nonanol is used for other purposes, such as research chemicals and building blocks [42].

Terpenes are particularly important in the flavor market, especially menthol-flavored compounds, that are used extensively as additives in oral hygiene products and flavors in food and beverages. Menthol isomers are derived from limonene, and it is possible to see (Table 2) that the modified frequency of menthol increases as that of limonene decreases, which may give evidence of the biotransformation of these compounds [44]. Benzothiazole is another natural component of the VOCs of *P. autumnale*, where its biggest modified frequency was in 24 h (54%). Nitrogen heterocycles compounds, especially benzothiazole and its derivatives are of great interest to the fine chemical and pharmaceutical industries due to their wide range of biological activities, like anticancer, antifungal, antiviral, anticonvulsant, anti-inflammatory, and antidiabetic activities [45].

Finally, among the VOCs originating from *P. autumnale*, typical microalgal compounds that cause an unpleasant odor, such as 2-methylisoborneol and geosmin, were not detected, as already reported by Santos et al. [35]. Despite the possibility of broad industrial application of microalgal VOCs, the unit operations of isolation, fractionation, and purification operations are still substantial bottlenecks in the process that need to be solved.

4. Conclusions

GC-O analysis has demonstrated been a key tool in odor control processes and contributed to proving that there was a transformation in the volatile profile of compounds released in wastewater treatment plants by the microalgae-based system proposed. This research shows the potential of the microalgal heterotrophic bioreactor in odor emission abatement in meat processing wastewater and production concomitant of new compounds. Thus, the microalgae-based systems can become essential support in the consolidation of new technologies in the wastewater treatment industry, with simultaneous odor and wastewater treatment by microalgal heterotrophic bioreactor.

Acknowledgements

The authors are grateful to the National Academic Cooperation Program PROCAD/CAPES, National Counsel of Technological, Scientific Development (CNPq), and Brasil Foods, Inc for the financial support.

Symbols

COD	—	Chemical oxygen demand (mg L^{-1})
N-TKN	—	Total nitrogen (mg L^{-1})
$P\text{-}PO_4^{-3}$	—	Total phosphorus (mg L^{-1})
TS	—	Total solids (mg L^{-1})
SS	—	Suspended solids (mg L^{-1})
VS	—	Volatile solids (mg L^{-1})
FS	—	Fixed solids (mg L^{-1})
VVM	—	Volume of air per volume of wastewater per minute
HS-SPME	—	Headspace solid-phase microextraction
DVB/Car/PDMS	—	Divinylbenzene/carboxen/polydimethylsiloxane
GC/MS	—	Gas chromatography-mass spectrometry
LRI	—	Linear retention index
GC-FID	—	Gas chromatography equipped with a flame ionization detector
GC-O	—	Gas chromatography coupled with olfactometry
MF	—	Modified frequency
F (%)	—	Detection frequency of an aromatic attribute
I (%)	—	Intensity

References

[1] N. Xue, Q. Wang, J. Wang, J. Wang, X., Sun, Odorous composting gas abatement and microbial community diversity in a biotrickling filter, Int. Biodeterior. Biodegrad., 82 (2013) 73–80.

[2] I. Wysocka, J. Gębicki, J. Namieśnik, Technologies for deodorization of malodorous gases, Environ. Sci. Pollut. Res. Int., 26 (2019) 9409–9434.

[3] H. Bu, G. Carvalho, Z. Yuan, P. Bond, G. Jiang, Biotrickling filter for the removal of volatile sulfur compounds from sewers: a review, Chemosphere, 277 (2021) 130333, doi: 10.1016/j.chemosphere.2021.130333.

[4] L. Liang, P. Gong. Urban and air pollution: a multi-city study of long-term effects of urban landscape patterns on air quality trends, Sci. Rep., 10 (2020) 18618, doi: 10.1038/s41598-020-74524-9.

[5] J. Liu, X. Kang, X. Liu, P. Yue, J. Sun, C. Lu, Simultaneous removal of bioaerosols, odors and volatile organic compounds from a wastewater treatment plant by a full-scale integrated reactor, Process Saf. Environ. Prot., 144 (2020) 2–14.

[6] P. Karageorgos, M. Latos, C. Kotsifaki, M. Lazaridis, N. Kalogerakis, Treatment of unpleasant odors in municipal wastewater treatment plants, Water Sci. Technol., 61 (2010) 2635–2644.

[7] P. Márquez, A. Benítez, A. Caballero, J.A. Siles, M.A. Martín, Integral evaluation of granular activated carbon at four stages of a full-scale WWTP deodorization system, Sci. Total Environ., 754 (2021) 142237, doi: 10.1016/j.scitotenv.2020.142237.

[8] E. Nie, G. Zheng, C. Ma, Characterization of odorous pollution and health risk assessment of volatile organic compound emissions in swine facilities, Atmos. Environ., 223 (2020) 117233, doi: 10.1016/j.atmosenv.2019.117233.

[9] K. Barbusinski, K. Kalemba, D. Kasperczyk, K. Urbaniec, V. Kozik, Biological methods for odor treatment – a review, J. Cleaner Prod., 152 (2017) 223–241.

[10] R.H. Bogan, O.E. Albertson, J.C. Pluntz, Use of algae in removing phosphorus from sewage, J. Saint. Eng. Div., 86 (1960) 1–20.

[11] W.J. Oswald, C.G. Golueke, Eutrophication trends in the United States: a problem?, J. Water Pollut. Control Fed., 38 (1966) 964–975.

[12] K.R. Vieira, P.N. Pinheiro, A.B. Santos, A.J. Cichoski, C.R. Menezes, R. Wagner, L.Q. Zepka, E. Jacob-Lopes, The role of microalgae-based systems in the dynamics of odors compounds in the meat processing industry, Desal. Water Treat., 150 (2019) 282–292.

[13] R.M. Fisher, R.J. Barczak, I.H. Suffet, J.E. Hayes, R.M. Stuetz, Framework for the use of odour wheels to manage odours throughout wastewater biosolids processing, Sci. Total Environ., 634 (2018) 214–223.

[14] R. Ríos-Reina, M.P. Segura-Borrego, M.L. Morales, R.M. Callejón, Characterization of the aroma profile and key odorants of the Spanish PDO wine vinegars, Food Chem., 311 (2020) 126012, doi: 10.1016/j.foodchem.2019.126012.

[15] R. Rippka, J. Deruelles, J.B. Waterbury, M. Herdman, R.Y. Stanier, Generic assignments strain histories and properties of pure cultures of cyanobacteria, J. Gen. Microbiol., 111 (1979) 1–61.

[16] APHA, Water Environment, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, D.C., 2005.

[17] E.C. Francisco, T.T. Franco, R. Wagner, E.J. Lopes, Assessment of different carbohydrates as exogenous carbon source in cultivation of cyanobacteria, Bioprocess Biosyst. Eng., 37 (2014) 1497–1505.

[18] E. Campo, V. Ferreira, A. Escudero, J. Cacho, Prediction of the wine sensory properties related to grape variety from dynamic-headspace gas chromatography-olfactometry data, J. Agric. Food. Chem., 53 (2005) 5682–5690.

[19] V.C. Resconi, M.M. Campo, F. Montossi, V. Ferreira, C. Sañudo, A. Escudero, Relationship between odour-active compounds and flavour perception in meat from lambs fed different diets, Meat Sci., 85 (2010) 700–706.

[20] A. Dravnieks, ed., Atlas of Odor Character Profiles, ASTM, Philadelphia, 1985.
[21] T. Acree, H. Arn, Flavornet and Human Odor Space, Cornell University, College of Agriculture and Life Sciences, New York State Agricultural Experiment Station, USA, 2017. http://www.flavornet.org/f_kovats.html/ (last accessed: 9 December 2019).
[22] H.X. Huang, G.Y. Miller, M. Ellis, T. Funk, Y.H. Zhang, G. Hollis, A.J. Heber, Odor management in swine finishing operations: cost effectiveness, J. Food Agric. Environ., 2 (2004) 131–136.
[23] K. Kaikiti, M. Stylianou, A. Agapiou, Use of biochar for the sorption of volatile organic compounds (VOCs) emitted from cattle manure, Environ. Sci. Pollut. Res., (2020) 1–9, doi: 10.1007/s11356-020-09545-y.
[24] L. Culleré, B.F. de Simón, E. Cadahía, V. Ferreira, P.H. Orte, J. Cacho, Characterization by gas chromatography-olfactometry of the most odor-active compounds in extracts prepared from acacia, chestnut, cherry, ash and oak woods, LWT Food Sci. Technol., 53 (2013) 240–248.
[25] J. Schaefer, Sampling, characterization and analysis of malodours, Agric. Environ., 3 (1977) 121–127.
[26] S.E. Curtis, Environmental Management in Animal Agriculture, Iowa State University Press, Ames, 1993.
[27] P.J. Hobbs, T.H. Misselbrook, B.F. Pain, Emission rates of odorous compounds from pig slurries, J. Sci. Food Agric., 77 (1998) 341–348.
[28] Y. Nagata, N. Takeuchi, Measurement of odor threshold by triangle odor bag method, Odor Measur. Rev., 118 (1990) 118–127.
[29] P.D. Le, A.J. Aarnink, N.W. Ogink, P.M. Becker, M.W. Verstegen, Odour from animal production facilities: its relationship to diet, Nutr. Res. Rev., 18 (2005) 3–30.
[30] R.G. Berger, Biotechnology as a source of natural volatile flavours, Curr. Opin. Food Sci., 1 (2015) 38–43.
[31] M.I. Hosoglu, Aroma characterization of five microalgae species using solid-phase microextraction and gas chromatography–mass spectrometry/olfactometry, Food Chem., 240 (2018) 1210–1218.
[32] S.S. Schiffman, J.L. Bennett, J.H. Raymer, Quantification of odors and odorants from swine operations in North Carolina, Agric. For. Meteorol., 108 (2001) 213–240.
[33] J. Nuccio, P.J. Seaton, R.J. Kieber, Biological production of formaldehyde in the marine environmental, Limnol. Oceanogr., 40(1995) 521–527.
[34] E.J. Lopes, C.H.G. Scoparo, M.I. Queiroz, T.T. Franco, Biotransformations of carbon dioxide in photobioreactors, Energy Convers. Manage., 51 (2010) 894–900.
[35] A.B. Santos, A.F. Fernandes, R. Wagner, E.J. Lopes, L.Q. Zepka, Biogeneration of volatile organic compounds produced by *Phormidium autumnale* in heterotrophic bioreactor, J. Appl. Phycol., 28 (2016) 1561–1570.
[36] A.V. Lindner, D. Pleissner, Utilization of phenolic compounds by microalgae, Algal Res., 42 (2019) 101602, doi: 10.1016/j.algal.2019.101602.
[37] US EPA, Initial List of Hazardous Air Pollutants with Modifications, U.S. Environmental Protection Agency, Washington, D.C., 2008.
[38] A. Dravnieks, T. Masurat, R.A. Lamm, Hedonics of odors and odor descriptors, J. Air Pollut. Control Assoc., 34 (1984) 752–755.
[39] K.R. Vieira, P.N. Pinheiro, L.Q. Zepka, Volatile Organic Compounds from Microalgae, E. Jacob-Lopes, M.M. Maroneze, M.I. Queiroz, L.Q. Zepka, Eds., Handbook of Microalgae-Based Processes and Products, Elsevier, 2020.
[40] E. Jacob-Lopes, A.B. Santos, I.A. Severo, M.C. Deprá, M.M. Maroneze, L.Q. Zepka, Dual production of bioenergy in heterotrophic cultures of cyanobacteria: process performance, carbon balance, biofuel quality and sustainability metrics, Biomass Bioenergy, 142 (2020) 105756, doi: 10.1016/j.biombioe.2020.105756.
[41] I.A. Severo, P.N. Pinheiro, K.R. Vieira, L.Q. Zepka, E.J. Lopes, Biological conversion of carbon dioxide into volatile organic compounds, Inamuddin, A.M Asiri, E. Lichtfouse, Eds., Conversion of Carbon Dioxide into Hydrocarbons Vol. 2 Technology, Springer, 2020.
[42] J.V. Durme, K. Goiris, A. Winne, L. Cooman, K. Muylaert, Evaluation of the volatile composition and sensory properties of five species of microalgae, J. Agric. Food. Chem., 61 (2013) 10881–10890.
[43] L. Zhou, J. Chen, J. Xu, Y. Li, C. Zhou, X. Yan, Change of volatile components in six microalgae with different growth phases, J. Sci. Food Agric., 97 (2017) 761–769.
[44] H.S. Toogood, A.N. Cheallaigh, S. Tait, D.J. Mansell, A. Jervis, A. Lygidakis, N.S. Scrutton, Enzymatic menthol production: one-pot approach using engineered *Escherichia coli*, ACS Synth. Biol., 4 (2015) 1112–1123.
[45] R.S. Keri, M.R. Patil, S.A Patil, S. Budagumpi, A comprehensive review in current developments of benzothiazole-based molecules in medicinal chemistry, Eur. J. Med. Chem., 89 (2015) 207–251.