Review

Volatile organic compounds in aquatic ecosystems – Detection, origin, significance and applications

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HIGHLIGHTS

- Triggering of VOCs production by aquatic organisms and the possible ecological implications are analysed
- Current sampling and analysis techniques for VOCs are described
- A summary of the main VOCs detected in aquatic environments is provided
- Technological applications for monitoring controlled/non-controlled biological systems and future prospective are revised

GRAPHICAL ABSTRACT
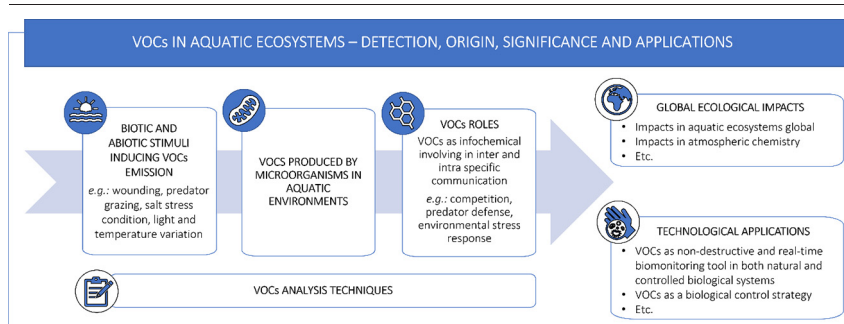
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ABSTRACT

Volatile organic compounds (VOCs) include a broad range of compounds. Their production influences a large number of processes, having direct and secondary effects on different fields, such as climate change, economy and ecology. Although our planet is primarily covered with water (~70% of the globe surface), the information on aquatic VOCs, compared to the data available for the terrestrial environments, is still limited. Regardless of the difficulty in collecting and analysing data, because of their extreme complexity, diversification and important spatial-temporal emission variation, it was demonstrated that aquatic organisms are able to produce a variety of bioactive compounds. This production happens in response to abiotic and biotic stresses, evidencing the fundamental role of these metabolites, both in terms of composition and amount, in providing important ecological information and possible non-invasive tools to monitor different biological systems. The study of these compounds is an important and productive task with possible and interesting impacts in future practical applications in different fields. This review aims to summarize the knowledge on the aquatic VOCs, the recent advances in understanding their diverse roles and ecological impacts, the generally used methodology for their sampling and analysis, and their enormous potential as non-invasive, non-destructive and financeable affordable real-time biomonitoring tool, both in natural habitats and in controlled industrial situations. Finally, the possible future technical applications, highlighting their economic and social potential, such as the possibility to use VOCs as valuable alternative source of chemicals and as biocontrol and bioregulation agents, are emphasized.

Contents

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Abbreviations

VOCs	Volatile Organic Compounds
OVOCs	Oxygenated Volatile Organic Compounds
GC	Gas Chromatography
MS	Mass Spectrometry
GC–MS	Gas Chromatography Mass Spectrometry
PTR-MS	Proton Transfer Reaction Mass Spectrometry
SPI-MS	Single Photon Ionization Mass Spectrometry
DAI	Direct Aqueous Injection
LLE	Liquid–Liquid Extraction
SD	Steam Distillation
SDE	Simultaneous Distillation Extraction
SFE	Supercritical Fluid Extraction
ME	Membrane Extraction
SHE	Static Headspace Extraction
SPME	Solid Phase Microextraction
SPDE	Solid-Phase Dynamic Extraction
P&T	Purge and Trap
WCOT	Wall Coated Open Tubular
PLOT	Porous Layer Open Tubular
MEMS	Microelectromechanical Systems
TCD	Thermal Conductivity Detector
FPD	Flame Photometric Detector
ECD	Electron Capture Detector
PID	Photoionization Detector
FID	Flame Ionization Detector
PDHID	Pulsed Discharge Helium Ionization Detector
MIMS	Membrane Inlet Mass Spectrometry
VUV	Vacuum Ultraviolet

APCI-MS	Atmospheric Pressure Chemical Ionization Mass Spectrometry
SAW	Surface Acoustic Wave
µPE	Micro-Purge Extractor
µGC	Micro-Scale GC
HCA	Hierarchical Cluster Analysis
PCA	Principal Component Analysis
DFA	Discriminant Factorial Analysis
PLS	Partial Least Squart
ANN	Artificial Neural Network
NIST	National Institute of Standards and Technology
SWMD	Seaweed Metabolite Database
SOD	Superoxide Dismutase
POD	Peroxidase
CAT	Catalase
ROS	Reactive Oxygen Species
PDC	Programmed Cell Death
PAN	Product Peroxyacetyl Nitrate
DMSP	Dimethylsulfoniopropionate
DMS	Dimethyl Sulfide
DMDS	Dimethyl Disulfide
DMTS	Trisulfide
DTS	Dimethyl Trisulfide
PUAs	Polyunsaturated Aldehydes
PUFAs	Polyunsaturated Fatty Acids
GPP	Geranyl Diphosphate
GLVs	C6 Green Leaf Volatiles
2-MIB	2-methylisoborneol
OH	Hydroxyl Radical
HO_x	Reactive Hydrogen Radicals

1. Introduction

Volatiles, and in particular volatile organic compounds (VOCs), can be found in a wide range of environments (Herrmann, 2010; Rinnan et al., 2014; Zuo, 2019). Natural biotic VOCs are emitted by plants, fungi, bacteria and protists, such as microalgae. So far, a unique definition of VOCs is not available, instead there are several definitions according to the different international and national organizations. The World Health Organization (WHO) defines VOCs considering the boiling point ranges that have to fall within 50 °C–100 °C up to 240–260 °C (Jantunen et al., 1997). By comparison, the definition of the European Environmental Agency (EEA) is "*Organic chemical compounds that under normal conditions are gaseous or can vaporise and enter the atmosphere*" (EEA, 2006). Another example can be provided by the United States Environmental Protection Agency (EPA), that claims that VOCs are "*Any compound of carbon, excluding carbon monoxide, carbon dioxide, carbonic acid, metallic carbides or carbonates and ammonium carbonate, which participates in atmospheric photochemical reactions*" (EPA, 2020). Generally, the term VOCs comprises a broad range of organic compounds, able to diffuse relatively far from their source passing not only through atmosphere, but also through water. They are mainly characterized by low molecular mass (in the range of 50–200 Da), high vapor pressure under ambient temperature conditions (20 °C and 101.3 kPa) (Olsen and Nielsen, 2001) and from low to moderate hydrophilicity.

Volatiles arise by a variety of biosynthetic routes, and include several chemical classes such as terpenoids, sulphur compounds, alkanes, alkenes, hydrocarbons, aromatics, alcohols, aldehydes, acids, esters, amines, furans, ketones, carboxylic acids and thiols. These numerous types of VOCs can be both of biogenic and anthropogenic origin (Yu and Li, 2021; Santos et al., 2016; Zuo, 2019; Rowan, 2011; Achyuthan et al., 2017).

VOCs and their detection have an immense importance as they have direct and indirect effects on different fields, such as climate change, ecology, economy, and human health. For the field related to climate change and ecology, volatile detection as response to abiotic and biotic stresses provides an important non-invasive tool that allows researcher to monitor environmental interactions in habitats such as forests, microbial or marine communities, that are considered spatially complex or difficult to access (Rowan, 2011). VOCs also find a broad range of applications in different industries such as food and food processing, pharmaceutical, and cosmetics. For example, analysis of VOCs in food is important in order to monitor changes after processing, cooking and those occurring during shelf life, and to identify the components causing desirable or undesirable changes (Steinke et al., 2018b; Achyuthan et al., 2017; Srivastava and Mazumdar, 2011; Herrmann, 2010; Santos et al., 2016).

In terrestrial ecosystems, more than 30,000 VOCs have been identified as released from higher plants through secondary metabolism pathways. These compounds have a great ecological impact on the environment, acting as inhibitor/stimulator of seed germination or growth, as protector against herbivores and pathogens, and as a signal to internal and external communication (Zuo, 2019; Rowan, 2011). An interesting example of VOCs signalling is the interaction between insectivorous birds, the mountain birch *Betula pubescens* ssp. *Czerepanovii* and the herbivore *Epirrita autumnata*. As a consequence of herbivore-damage, plants emit significant amounts of β-ocimene, linalool and (*E*)-DMNT [(*E*)-4,8-dimethyl-1,3,7-nonatriene]. This VOCs emission, positively correlated with predation rate, seemed to act as a potential signal for predators of the herbivores, as well as an attractant for insect parasitoids and predatory mites (Mäntylä et al., 2008). Flower volatiles, together with flower colour, are also an important cue for pollinators. In bumblebee pollination of *Antirrhinum majus* flowers, for example, methyl benzoate was shown to be one of the major attractants to flower visits (Ruiz-Hernández et al., 2018).

A wide spectrum of VOCs can also be found in aquatic ecosystems, where they are released from different organisms, such as algae and cyanobacteria, and which include terpenoids, furans, sulphur containing compounds, halogenated compounds, hydrocarbons, alcohols, aldehydes, ketones, esters, fatty acids, polyphenols and derivatives. These compounds are involved in different processes. They enhance the resistance to abiotic stresses, transfer information, play allelopathic roles, and protect against predation (Zuo, 2019; El Hattab, 2020). For example, in the case of *Caulerpa taxifolia* and *Caulerpa racemoset*, the most abundant cytotoxic sesquiterpene produced is the caulerpenyne, a compound that can be involved either in interspecific competition as antifeedant and/or antifouling activities or in the alga's chemical defence against herbivores (El Hattab, 2020).

Recently, an increasing interest in VOCs emissions from microalgae exists due to the high commercial value of these microorganisms in terms of high value compounds, biofuels, *etc.* (Steinke et al., 2018b; Achyuthan et al., 2017; Srivastava and Mazumdar, 2011; Herrmann, 2010; Santos et al., 2016).

Apart from biological VOC sources, aquatic VOCs may also be of anthropogenic origin. One example is the contamination of arctic environments with cyclic volatile methyl siloxanes. They derive from usage in industrial sectors such as cosmetic industry, surface treatment agents or construction materials. Contamination results from volatilization of these components to the atmosphere combined with a high potential for long-range transport (Warner et al., 2010). Another source of aquatic VOCs of anthropogenic origin is the discharge of wastewater into aquatic ecosystems, containing wastes from different origins such as industrial, domestic, urban or agricultural sources. The discharge into aquatic systems occurs from sources including surface runoff, septic systems, wastewater treatment facilities and runoff from storm drains. Inadequately treated wastewater effluent may lead to eutrophication caused by a nitrogen and phosphorus excess, resulting in algal clumps, algal blooms and odour production (Akpor and Muchie, 2013). An additional source of aquatic VOCs from anthropogenic origin may be exhaust gas produced *i.e.* during fuel combustion by international maritime transport. The produced compounds, including the semi volatile polycyclic hydrocarbons (PAHs), are emitted to the atmosphere and eventually deposited in the surface ocean (Endres et al., 2018).

The data collected by several studies indicate that aquatic VOCs are ubiquitous in the oceans, and that their emissions can have important ecological and atmospheric implications. Therefore, considering that about 70% of the earth's surface is covered with seas and oceans and that the aquatic VOCs emission can be influenced by multiple factors (Yu and Li, 2021; Achyuthan et al., 2017), the study of these compounds is an important and productive task, with a possible and interesting impact in future practical applications in different fields. This review aims to summarize on the recent advances in understanding the diverse roles and impacts of VOCs in aquatic environments focusing mainly on the emissions of VOCs *in vivo* or under natural conditions due to their high susceptibility to a wide variety of factors influencing their production, and their enormous potential as real-time biomonitoring tools.

2. Analysis of VOCs produced in aquatic environments

Our planet is predominantly covered with water. Hence, the aquatic VOCs, even if in a way still not completely clear, play a very important role in influencing the marine and freshwater ecology and the global atmospheric chemistry, such as the ozone formation in the troposphere and the carbon budget (Yu and Li, 2021; Achyuthan et al., 2017).

The emission of aquatic VOCs, both in terms of composition and amount, contain important and potentially useful information. Unfortunately, because of their extreme complexity, diversification and important spatial-temporal emission variation, they are yet largely unidentified (Zhang and Li, 2010; Yu and Li, 2021).

Due to their special features, VOCs' sampling and analysis is considered a technical challenge. Its analysis may include the identification of specific compounds, as well as the total or relative amount within samples. Quantification ideally is performed using calibration curves obtained from standards, a method often hampered by the fact that VOCs composition is often unpredictable. However, it was shown that other semi-quantitative methods such as the total integrated peak area per fresh weight, internal standard abundance, nearest *n*-alkane or percentage of emission are also reliable, valid methods for scent profiling (Ruiz-Hernández et al., 2018).

Generally, analysing aquatic volatiles involves different steps: collect and prepare the liquid samples, extract the analytes, and finally operate the detection and measurement by specific instrumentations such as gas chromatography mass spectrometry (GC–MS), proton transfer reaction mass spectrometry (PTR-MS) and single photon ionization mass spectrometry (SPI-MS).

2.1. Sampling methods and sample preparation

Depending on the type of study and the experimental design of the research work, the sampling itself may require a specific design. In many studies it may be necessary to transport and eventually storage the samples. This type of needs requires the evaluation of several factors and parameters before selecting a specific method of transport and storage such as: conservation temperature, eventual transportation time, potential risk of contamination, sample storing and volume, stability of VOCs subjected on investigation, *etc.* For example, the samples can be stored in a gas-phase using thermal desorption tubes, SPME fibers, bags made from polymers, or canisters (Achyuthan et al., 2017; Cavalcante et al., 2010). In general, samples collected for analysis should be stored in an ice chest at 4 °C, unless they will be analysed within 1 h of collection. Storage of water samples for 24 h or more should include the addition of sodium thiosulphate (0.2 mL of a 3% solution per 200 mL sample is suggested) as a preservative, if necessary. The dichlorination helps to maintain the actual state of water quality at the moment of sample collection (Kamp et al., 2016). It is recommended to flush the vials about seven times (removing the air) before the final volume is retained in it. Filling the sample vials to the bottom of the neck using a very slow flow speed is recommended. Then, very slightly, each vial must be overfilled with additional sample water. If the vial is overfilled excessively, the preservatives will be diluted and may not be effective. Once filled, the vial should be closed and inverted at least 5 times to mix the sample and preservative. It is important to check for trapped air bubbles. If air bubbles are detected, the vial must be carefully opened (right side up), and more sample added. Storage should be done at 6 °C or less but above the freezing point of water until transported to the laboratory. Consistent identification of the samples is essential for analysis so, a data-coding strategy is highly recommended (Shelton, 1997).

Another important step is sampling preparation, that is fundamental because it may influence measurements sensitivity and accuracy (Cavalcante et al., 2010). Different kind of techniques were developed, including direct aqueous injection (DAI), liquid–liquid extraction (LLE), steam distillation (SD), simultaneous distillation extraction (SDE), supercritical fluid extraction (SFE), membrane extraction (ME), static headspace extraction (SHE), solid phase micro-extraction (SPME), solid-phase dynamic extraction (SPDE) and dynamic headspace extraction (P&T, purge and trap). The advantages and disadvantages of each technique are listed in Table 1. A brief description of each technique is given below.

2.1.1. DAI

It is a fast and easy technique which allows the passage of a liquid sample directly from the syringe into a chromatographic column previous vaporisation into a hot inlet and without the necessity of sample pre-concentration and pre-treatment steps. One main weakness lies in the possible presence of a matrix effect (Demeestere et al., 2007; Golfinopoulos et al., 2001), a term indicating the combined effect caused by all sample components excluding the analyte on the quantity measurement (Guilbault and Hjelm, 1989). This technique is applicable only for a small number of VOCs highly concentrated. Alternatively, selective highly sensitive detectors could be used (Demeestere et al., 2007).

2.1.2. LLE

It is one of the oldest pre-concentration methods in analytical chemistry (Demeestere et al., 2007). It involves the addition of organic solvents to the liquid sample and the subsequent injection of the extracts into a gas chromatograph (GC) for the VOCs detection (it is possible to also use liquid chromatography). In the GC, two types of columns with distinct polarities

are used to achieve a complete isolation and identification of each compound. Manual extraction by a separation funnel (through shaking) or automated extraction employing a continuous liquid–liquid extractor is all options. As a major disadvantage, the separation between the aqueous and organic phase is often intertwined by the emulsion formation (especially with manually extraction protocols). In the latter explained situation, continuous LLE or the use of extube extraction columns are commonly better options (Golfinopoulos et al., 2001).

2.1.3. SD and SDE

Are classical sampling techniques, generally characterized by multiple steps, the need of large amounts of solvents and long extraction times. SD is a process where VOCs are carried out from the distillation system by water vapor followed by solvent extraction. Differently, SDE technique simplifies experimental protocols, reducing sample degradation during sample transfer and saving toxin organic solvents by combining vapor distillation and solvent extraction. The usage of high temperatures might lead to decomposition and deterioration of unstable volatiles such as alkene, ester, and some unsaturated VOCs may possibly be thermally decomposed and deteriorated (Zhang and Li, 2010).

2.1.4. SFE

It is a method that utilizes, as the extraction solvent, supercritical fluids such as carbon dioxide (CO_2) either in its pure form or in combination with other chemicals modifiers (Zhang and Li, 2010; Rowan, 2011).

2.1.5. ME

It is a technique that uses a membrane as a "selective barrier". The analytes are extracted by passing them through the "selective barrier" and, thanks to a carrier stream, they are carried to a trap, to be concentrated and inserted into a detection device (Yu and Li, 2021; Theis et al., 2001).

2.1.6. SHE

In this technique, volatiles of the sample, which are diffused in the vial and heated at a definite temperature for a precise time period, are first collected thanks to a withdrawal of a known volume of headspace air through the use of a gas-tight syringe, an operation performed when the equilibrium between the concentration of the analyte in the headspace and in the sample is reached, and then injected into a GC equipped with a capillary column, for the analysis (Yu and Li, 2021; Rowan, 2011; Soria et al., 2015; Demeestere et al., 2007). Critical parameters that affect the SHE sampling and sensitivity are the samples' temperature and volume in the vial, and the combined effect of the partition coefficient (K, the ratio of gas phase and analyte concentrations in the condensed sample) and the phase ratio (β, the ratio between sample phase and volumes of gas), respectively. This technique can be improved in terms of sensitivity thanks various strategies, such as the "salting-out effect" a method that involves the addition of salts to the analyte solution (*e.g.*, ionic liquids), so the solubility of the hydrophobic analytes in the water solution decreases, increasing in ionic strength, leading to a higher concentration of these analytes in the headspace. Another possibility, that can be used mainly with analytes with low solubility in the condensed phase, is using low volume ratios between headspace and sample (Soria et al., 2015).

2.1.7. Dynamic headspace extraction, P&T

It is a method which allows VOCs extraction in function of volatility. This technique involves the use of an inert purge gas (*e.g.*, nitrogen or helium), which passes through the samples, leading VOCs to be trapped thanks an adsorbent. Subsequently, VOCs may be desorbed and transferred into a GC for analysis by rapid heating. An alternative, even so less sensitive option, may be the elution with organic solvents. This method has achieved widespread acceptance. P&T method can be used to quantify a number of VOCs and for analysis of trace gases with very low concentrations (Zhang and Li, 2010; Yu and Li, 2021; Rowan, 2011; Soria et al., 2015; Golfinopoulos et al., 2001).

Table 1
Description and comparison of different type of VOCs sampling methods.

Sampling method	Advantages	Disadvantages	References
Direct aqueous injection (DAI)	• Rapidity and simplicity of execution • Minimizing of sample contamination • Reduction of analyte loss • No sample preconcentration and pre-treatment steps required	• Low sensitivity • Liquid sample's incompatibility with most capillary column stationary phases • Possible matrix effects	(Demeestere et al., 2007; Golfinopoulos et al., 2001)
Liquid–liquid extraction (LLE)	• Possibility to use manual or automatic extraction	• Long analysis times • Large consumption of solvents • Use of toxic solvents • Limitation of the injectable solvent and moderate detection limits	(Demeestere et al., 2007; Golfinopoulos et al., 2001)
Simultaneous distillation extraction (SDE)	• Simple experimental protocols • Reduction of the sample degradation and the use of toxic organic solvent	• Large consumption of solvents • Long extraction times • Possible thermally decomposition and deterioration of unsaturated VOCs	(Zhang and Li, 2010)
Supercritical fluid extraction (SFE)	• Using supercritical fluids as the extraction solvent	• High analytical cost • Limited dissolving power of non-polar CO_2	(Rowan, 2011; Zhang and Li, 2010)
Membrane extraction (ME)	• Possibility to be coupled to continuous online analysis instrumentations	• Short lifetime membranes	(Yu and Li, 2021)
Static headspace extraction (SHE)	• Direct sample placing into the headspace vial (no additional preparations required for analysis) • Simple equipment • Good repeatability • High sensitivity	• Detect limitation of analytes with fairly high volatilities	(Cavalcante et al., 2010; Golfinopoulos et al., 2001; Yu and Li, 2021)
Purge and trap (P&T)	• Possibility of quantification with low concentrations • Minimal sample manipulation and preparation • Good repeatability • High sensitivity	• Water vapor interference at dry purge phase • Possible cross contamination • Complex instrumentation • High investment costs	(Cavalcante et al., 2010; Golfinopoulos et al., 2001; Rowan, 2011; Soria et al., 2015; Yu and Li, 2021; Zhang and Li, 2010)
Solid-phase microextraction (SPME) Solid-phase dynamic extraction (SPDE)	• Portable instrumentation and possibility to reuse fibers • The different fiber coat materials allow to analyse many VOCs • Possibility of extraction with direct immersion or in the headspace (SPDE) • Improvement in the needle resistance (SPDE) • Short extraction times (SPDE) • Increase in the extraction phase volumes (SPDE)	• Requirement of a specific design for each class of compounds • Dependency of the extraction efficiency on the fiber coating type	(Demeestere et al., 2007; Yu and Li, 2021; Zhang and Li, 2010)

2.1.8. SPME

It is a method that uses a fused silica fiber coated with a thin film of sorbent that is housed within a syringe needle. Analytes are first exposed to the adsorbent, then extracted and concentrated on the fiber, and subsequently desorbed off the fiber thanks to a thermal desorption into the heated injection GC port (Yu and Li, 2021; Rowan, 2011).

2.1.9. SPDE

It is a technique based on the same SPME's principle, utilising a 2.5 mL headspace syringe connected to a stainless-steel needle characterized by an immobilized extraction phase coat on its inner side. The extraction can be made both with direct immersion of the needle into the aqueous sample or in the headspace. This technique includes numerous extraction cycles (aspirating and dispensing), followed by a thermal desertion from the internal coating into a GC.

2.2. VOCs identification and quantification

Once the analytes have been collected and separated from the samples, the next stage involves the identification and quantification of the VOCs. Several tools can be used to complete this step (Achyuthan et al., 2017).

Regarding the use of GC, a number of parameters and their interactions must be adjusted to optimise the separation. This process is influenced by both physical and parametric column variables such as: length, flow velocity, internal diameter, stationary phase and temperature.

Habitually, capillary columns are used to separate VOCs. Their main advantages are the efficient separation, the high coating efficiency, the stationary phase high stability, and the low catalytic and absorption property towards a solute (Mametov et al., 2019).

Capillary chromatographic columns are characterized by a stationary phase made by a thin film of liquid phase coating the inner wall, a low resistance to gas flow and a general internal diameter of 250–320 μm,

considered as an optimal compromise between sample capacity, speed, resolution, and ease of procedure (Rahman et al., 2015; Mametov et al., 2019). In most applications, separation of VOCs is based mainly on the interaction with the stationary phase, since interactions with mobile phases, such as He, N_2 or H_2, are negligible (Dewulf et al., 2002).

A classic capillary column material is polyethylene glycol (PEG), a non-silicone stationary phase with a high polarity (Mametov et al., 2019). This kind of columns present different disadvantages such as the susceptibility to be affected by oxygen and water, the low thermal stability and the restricted ability to modify the phase, thus the selectivity of the column (Rahman et al., 2015; Mametov et al., 2019).

The silicone-type wall coated open tubular (WCOT) column is one of the most used for VOCs separation (Mametov et al., 2019). In WCOT columns, the wall is directly coated with the stationary-phase layer at a film thickness of 0.05–3 μm. Another type of column is the Porous Layer Open Tubular (PLOT) column containing a porous layer of a solid adsorbent such as alumina, carbon sieve materials or Porapak (Mametov et al., 2019; Dewulf et al., 2002; Rahman et al., 2015).

Commonly, the columns mostly used in GC are the general-purpose columns, which length can vary from 25 to 60 m and are characterized by a high thermal resistance, a low polarity and the use of 5%-phenyl-methylpolysiloxane as a typical stationary phase (Mametov et al., 2019).

Besides that, the necessity of an on-site and portable system, led to the attempt of a size reduction of GC columns (one of the most voluminous components in GC equipment's) using the microelectromechanical systems (MEMS) technology. For example, in microchip GC, short columns are used, which are mainly made of silicon, and are made-up with various types of cross section over plane-shaped structures (Soria et al., 2015; Mametov et al., 2019).

Additionally, there are the multichannel chromatographic columns, characterized by multiple capillaries of few micrometres in diameter. This kind of columns works in parallel, improving the sample capacity and the general performance. Nevertheless, compared with the unique

capillary column, preparing the stationary phase coating is a more complex process.

The embedding microcolumns are another type of columns characterized by an increased inner specific surface area (thanks to their height that is the same of the capillary depth within the channels) and a great efficiency in separation and sample capacity, thanks to the columns fabrication on a 1.8 cm^2 plate and in double-spiral layout (Soria et al., 2015; Mametov et al., 2019). Besides that, due to the improvements and introduction of columns with a broader variety of applications, also packed columns resumed popularity (Mametov et al., 2019).

New advancements led to introduce the use of modern materials for columns stationary phases such as the ionic liquids (ILs) and the metal organic frame structures (MOFs). The former can be described as low-melting-point salts able to retain polar and non-polar solutes at the same time. The latter is formed by inorganic subunits associated to multidirectional organic linkers with chelating groups characterized by strong ion covalent or dative bonds. ILs based columns have a high thermal stability, the possibility to change and adjust the solvent and its selectivity characteristics and a retentive performance for precise functional groups. MOFs are characterized by a complex structure with significant impact in adsorption properties. This material allows particular separation performance for analytes with different polarities thanks to the influence of the positive poles located in metal sites (Soria et al., 2015; Mametov et al., 2019; Rahman et al., 2015).

Other materials were integrated into chromatography equipment to enhance analyte identification and separation. For example, different nanomaterials (*e.g.*, graphene quantum dots and sheets or graphene in combination with polydopamine) were used as stationary phases for GC columns. Moreover, as mobile phase, supercritical fluids (SFCs) may also be used (Mametov et al., 2019).

GC–MS is one of the commonly employed instrumentation for VOC analysis (Achyuthan et al., 2017; Rowan, 2011). It provides different advantages such as: high chromatographic resolution and sensitivity, the ability to quantify the detected compounds, distinguish unknown volatiles and identifying specific VOCs (Yu and Li, 2021; Rowan, 2011).

The use of MS as detector, combined with the current improvement in data management strategies (mainly thanks metabolomics), allows to obtain reliable spectra that can be considered a representative "fingerprint" of the analysed sample, and these spectra are therefore also useful for classification and profiling aims (Soria et al., 2015).

Even though MS is still the most popular GC detector in the case of complex samples, there are other alternative detectors that can be coupled with GC such as: thermal conductivity (TCD), flame photometric (FPD), electron capture (ECD), photoionization (PID), flame ionization (FID) and pulsed discharge helium ionization (PDHID) as the most relevant. Sulphur chemiluminescence detector (SCD) and nitrogen chemiluminescence detector (NCD), where SO and NO radicals, respectively, collide with ozone generating light emission that is detected by a photomultiplier, are especially sensitive to these compounds. SCD and NCD are highly selective and are typically used to detect trace amounts of S and N in complex sample matrixes with little or no interference. Multidetector system can provide all the information needed for the confirmation purposes (Achyuthan et al., 2017; Dewulf et al., 2002). In order to obtain as much information as possible, detectors can be connected in parallel or in series at the GC column's outlet, considering their ability in providing structural information, selectivity and sensitivity (Dewulf et al., 2002).

Moreover, it is also possible to use alternative analytical instrumentation to GC, like membrane inlet mass spectrometry (MIMS), a technique for direct liquid sample VOCs analysis using a semipermeable membrane to capture the analytes and evaporating it into a MS; PTR-MS, a real-time measurement technique characterized by a high sensitivity and fast response time; SPI-MS, where analytes with a lower ionization energy than the vacuum ultraviolet (VUV) photon energy are ionized once exposed to the VUV radiation; and atmospheric pressure chemical ionization mass spectrometry (APCI-MS), where the produced fragments of ions of the analytes are examined allowing the classification of unknown species (Yu and Li, 2021).

Another type of instrumentation is the electronic nose, an array of non-specific, gas sensitive, chemical sensors that allow the identification of a biological volatile profile. The disadvantage of this instrumentation is that it is not able to provide a signal for each single volatile (Zhang and Li, 2010). This type of analyser is based on an uncoated quartz-based SAW (Surface Acoustic Wave), and on an uncoated piezoelectric quartz crystal that vibrates at a specific frequency, producing a signal that can identify the molecules thought a VOC retention times library (Rowan, 2011).

An additional device developed to improve sampling techniques is the micro-purge extractor (μPE) chip combined with a micro-scale GC (μGC) system. A miniaturized system, which provides rapid, on-field and real-time water sample analysis, being able to detect also low analyte concentrations. The device is composed by one inlet for the sample and another one for a pure inert gas. In the same way, the chip contains two outlets dedicated separately to water waste and to the purged analytes leading to the micro-thermal pre-concentrator. Once the compounds have been trapped, desorption (thanks a resistive heating) and subsequent transfer and separation on a μGC column, takes place, followed by the identification of analytes through a micro-thermal conductivity detector within the column (Soria et al., 2015).

The research on VOCs is not only focused on the collection of qualitative and quantitative data, but it is also aimed at understanding the physiological and ecological meaning of these compounds to extract possible useful bio-information for various purposes in different sectors. There are a variety of tools to manage and interpret the high number of experimental data allowing the identification of the biological VOCs, such as: Artificial Neural Network (ANN), Discriminant Factorial Analysis (DFA), Hierarchical Cluster Analysis (HCA), Partial Least Squart (PLS), Principal Component Analysis (PCA), *etc.* (Zhang and Li, 2010). The discrimination extent that each approach can achieve may be diverse (Goulitquer et al., 2012).

VOC production may be affected by the physiological stage of the samples as well as their genetic background or in response to environmental conditions. However, the identification of volatile emissions associated *e.g.*, to certain biotic or abiotic stress responses or biotic interactions, is often technically challenging, as it requires the differentiation between the constitutive metabolites and non-constitutive metabolites in large datasets. The R package "gcProfileMakeR", allows an automatic data analysis using the CAS numbers (Chemical Abstract Service Number) to group constitutive and non-constitutive (by quality or frequency) metabolites from large datasets (Perez-Sanz et al., 2021).

Another essential tool is the metabolite and mass spectral database that enables identification of VOCs, such as the National Institute of Standards and Technology (NIST) and the Seaweed Metabolite Database (SWMD) (Goulitquer et al., 2012).

Finally, also bioindicators can be used to identify fluctuations in VOCs within aqueous environments by monitoring changes in biological processes, species, or communities over time, including variations in plants, planktons, animals, and microbes. Biomonitoring refers to the quantitative determination of a response, such as changes in the chlorophyll content of species in response to pollution (Tandlich, 2011). Biomonitoring approaches apply both to individual indicators such as morphological changes, the emergence of resistant species, or survival of indicator species as well as population assessments, considering patterns in community changes, or population fluctuations (Zolkefli et al., 2020).

While chemical measurements only characterize conditions at the time of sampling, bioindicators add a temporal component to the measurement, as the observed changes depend on the life span or residence time of an organism in a particular system. Furthermore, while highly sensitive technologies are needed to detect low concentrations of chemicals in environmental systems, the levels of pollutants, even so very low, may be reflected by the tolerance range of bioindicators (Holt and Miller, 2010).

One example of a bioindicator for VOCs contamination in aquatic systems is the monitoring of benthic foraminifera assemblage in marine sediments of the Naples harbour with *Elphidium* spp. being the most sensitive to VOC contamination (Ferraro et al., 2009). Pollution of the groundwater

with the VOCs benzene or trichloroethylene can be assessed by analysing plant tissue concentrations of this organic pollutant in deep-rooted species of trees (Tandlich, 2011). The flagellated, microalga *Euglena gracilis* was shown to be a useful bioindicator for the genotoxic pollution with semi-volatile organic chemicals of the Taihu Lake in China. Biomonitoring may be performed by measuring several parameters responsive to varying concentrations of organic extracts to which *E. gracilis* was exposed, such as contents of photosynthetic pigments, activities of superoxide dismutase (SOD) and peroxidase (POD) or DNA damage by the comet assay (Li et al., 2014).

In summary, bioindicator monitoring, apart from individual enumeration or identification of species, include several other techniques such as spectrophotometry, flow cytometry, the quantification of antioxidant enzymes but also molecular techniques such as determination of DNA breaks, DNA fingerprinting or high-throughput sequencing (Zolkefli et al., 2020; Li et al., 2014).

3. VOCs produced by marine microorganisms

The information on aquatic VOCs, compared to the data available for terrestrial environments, is still limited. Despite the difficulty in finding and analysing data, it was demonstrated that aquatic organisms are able to produce a broad range of bioactive compounds, which are involved in numerous processes (*e.g.*, competition, predator defence, environmental stress response and resource foraging) with important ecological repercussions (Leflaive and Ten-Hage, 2009; Fink, 2007; Achyuthan et al., 2017; Lawson et al., 2020).

A wide spectrum of VOCs classes were identified in aquatic environments such as terpenoids, sulphur compounds, alkanes, alkenes, alcohols, aldehydes, carboxylic acids, esters, furans, halogenated, hydrocarbons, ketones, and derivates (Yu and Li, 2021; Santos et al., 2016; Zuo, 2019). Among the various groups of VOCs analysed thus far, we report here on some of the most frequently identified volatiles in aquatic environments.

3.1. Halogenated compounds

Are a large group that includes different classes of VOCs. In aquatic environments they are produced by microorganisms like phytoplankton and algae. In particular, this compounds appear as trace gases containing chlorine (*e.g.*, $CHCl_3$, chloroform and CH_2ICl, chloroiodomethane), iodine (*e.g.*, CH_2ICl, diiodomethane and CH_3I, iodomethane) or bromine (*e.g.*, $CHBr_3$, tribromomethane; CH_2Br_2, dibromomethane, $CHBrCl_2$, bromodichloromethane; $CHBr_2Cl$, dibromochloromethane and CH_2BrCl, bromochloromethane) (Yu and Li, 2021; Achyuthan et al., 2017; El Hattab, 2020; Paul and Pohnert, 2011). They are considered VOCs of special interest because of the various environmental impacts that they can cause, based on the delivering of reactive halogen atoms to the atmosphere. For example, bromine atoms usually destroy ozone in the atmosphere, while chlorine atoms can cause ozone destruction or formation by reacting with ozone or other VOCs, respectively (Yu and Li, 2021).

3.2. Terpenes or terpenoids

Are one of the largest groups of emitted secondary metabolites with important ecological functions in chemical communication (Yu and Li, 2021; El Hattab, 2020; Watson, 2003). Generally, they are known for their olfactive properties and are of economic interest due to their possible industrial applications, especially in the food, beverage, and perfume/essential oils sectors. In the aquatic environment, terpenoids are often associated with their ability to influence the smell of water generating odours (El Hattab, 2020; Watson, 2003). An abundant VOC belonging to the terpene group is isoprene (C_5H_8). Due to its presence in large amounts and its reactivity with hydroxyl radicals (•OH) that cause a reduction of the oxidizing capacity in the atmosphere, this is considered a significant compound for atmospheric chemistry studies (Yu and Li, 2021; McGenity et al., 2018). Moreover, it was observed that the concentration of

tropospheric ozone can increase *via* photolysis, as a consequence of nitrogen dioxide (NO_2) production by the ability of isoprene to react also with nitric oxide (NO), when present at high concentrations (McGenity et al., 2018). Besides the chemical atmospheric influences, the function of these biogenic compounds in aquatic environments are not fully understood. So far, their main ecological role is proposed to be a defence mechanism. Terpenes could act as antioxidants, protecting against damages by reactive oxygen species (ROS) that may be produced by different stressors like light intensity, that also seems to be an influencing factor for isoprene production (Santos et al., 2016; McGenity et al., 2018; Srikanta Dani and Loreto, 2017). The possible correlation between light intensity and isoprene production was reinforced thanks to the detection of a linear relationship between photoprotective carotenoids and the emission of this volatile in marine phytoplankton, reinforcing the possible photoprotective function of these compounds (Hackenberg et al., 2017).

3.3. Monoterpenes

Are a lesser-known group of compounds found in aquatic environments that are known to be able to have very low volatility oxidation products (Yu and Li, 2021) and to be used by some microorganisms like cyanobacteria, under specific conditions, to poison other competitors (Liu et al., 2021a). Many volatile aroma and flavours compounds are found in monoterpenes such as camphene, eucalyptol, limonene, α-pinene and β-pinene, (Spinelli et al., 2011; Meskhidze et al., 2015; Liu et al., 2021a). Moreover, other terpenoids derived from carotene degradation were identified in aquatic environment, some known examples are β-cyclocitral, geranylacetone, α-ionone and β-ionone, that are also some of the toxic volatile compounds used by cyanobacteria (Liu et al., 2021a).

3.4. Acetone, acetaldehyde, and methanol

Are part of the oxygenated volatile organic compounds (OVOCs) that in marine environments are mainly emitted by phytoplankton, probably as a consequence of algal cells lysis (Yu and Li, 2021).

In the atmospheric processes, these three reactive gasses are supposed to play a critical role, influencing the oxidation capacity, and the global ozone budget. Besides that, in the marine environment, these OVOCs are considered as a labile carbon source to the surface waters microbial community, and to stimulate the particle formation and growth in the remote marine atmosphere (Yu and Li, 2021; Beale et al., 2015). Methanol is mainly consumed in the troposphere thanks to the reaction with OH, which generates formaldehyde and reactive hydrogen radicals (HO_x) as reaction products. Besides that, acetaldehyde, through an oxidative reaction, can produce formaldehyde, HO_x and the stable by-product peroxyacetyl nitrate (PAN, involved in sequestering reactive nitrogen). Lastly, there is acetone that undergoes oxidation forming hydroperoxide, and subsequently acetaldehyde, formaldehyde, and methyl glyoxal. Photolysis occurs in both acetaldehyde and acetone, generating acetic acid, carbon monoxide, PAN, peracetic acid and other radicals (Beale et al., 2015).

3.5. Aquatic sulphur VOCs

Are considered an important subject of study because of their implications in the field of global sulphur cycle and climate. Several organisms have been identified (such as microalgae and diatoms) capable of producing dimethylsulfoniopropionate (DMSP) that, together with methanethiol, are precursors of dimethyl sulfide (DMS), a climate-active gas in the oceans' surface. These types of VOCs are considered very important as they can have significant climatic consequence (Galí et al., 2015; Achyuthan et al., 2017). In detail, DMS is considered as one of the most emitted sulphur compounds to the remote oceanic troposphere mainly thanks to phytoplankton (Yu and Li, 2021). In terms of ecological aquatic functions, both DMS and DMSP work as antioxidants, protecting against the damages caused by ROS.

Other sulphur compounds of potential climate impact include: dimethyl di- and trisulfide (DMDS and DMTS), isopropylthiol and diisopropyl di- and trisulfide (Achyuthan et al., 2017).

Besides the previously mentioned examples, there are other types of VOCs produced by aquatic microorganisms with known important functions. These include the polyunsaturated aldehydes (PUAs) and their precursors, the polyunsaturated fatty acids (PUFAs). These compounds seem to be involved in predator defence strategies and in intra- and interspecific interactions (Bacellar Mendes and Vermelho, 2013; Leflaive and Ten-Hage, 2009).

4. VOCs produced by microorganisms in aquatic non-marine environments

Among the various VOCs detected in aquatic environments, it is important to mention that the already cited metabolites and related ecological processes are not exclusive to seawater organisms. Some of them can also be emitted in freshwater environments (*e.g.*, lakes and rivers) and *vice versa* (Fink, 2007; Watson, 2003). However, the data available until now about aquatic VOCs emission in non-marine environments is relatively limited, which complicates an extensive understanding of the ecological impact of these compounds in freshwater environments and more extensive studies are still needed for a comprehensive ecological knowledge of these systems (Fink, 2007; Steinke et al., 2018a).

4.1. Isoprene and DMS

These compounds are present in marine environments but they have been also found to be produced in freshwater by organisms like cyanobacteria and cryptophytes (Seco et al., 2020; Steinke et al., 2018a). Some examples are: *Scenedesmus obliquus* that emits both isoprene and DMS; *Anabaena variabilis*, *Cryptomonas* sp., *Microcystis aeruginosa* and *Synechococcus elongatu* that produce isoprene only, and *Aphanizomenon flos-aquae* and *Chlorella vulgaris* that were found to only produce DMS. Regarding the possible functions of these VOCs in freshwater and considering the multiple roles in terrestrial and marine environments (*e.g.*, response to oxidative or light stress), it is assumed that the production of these VOCs, in the same or similar form as the marine volatiles, may assist in physiological acclimation to environmental conditions of freshwater phytoplankton (Steinke et al., 2018a).

4.2. Geosmin and 2-methylisoborneol (2-MIB)

They are volatiles belonging to the class of terpenoids that have been largely detected in aquatic non-marine environments. They are typically emitted by some benthic and pelagic aquatic microorganisms in environments such as lakes, reservoirs, running waters and slow-flowing rivers (Watson, 2003; Jüttner and Watson, 2007; Fujise et al., 2010). The biological importance of these two compounds is still unclear, besides that they are usually associated to events of algae blooms and are considered among the main causes of unpleasant odours and taste in the freshwater ecosystems. Geosmin and 2-MIB are an object of study of great interest for the beverage industry (*e.g.*, for the sale of drinking water), as their presence causes significant problems in drinking water supplies by deteriorating the quality of the product (Tucker, 2000; Wright et al., 2014; Watson et al., 2016; Fujise et al., 2010).

4.3. β-Cyclocitral and β-ionones

They are abundant VOCs in freshwater lakes which belong to the class of terpenoids resulting from the oxidation of carotenoids. These volatiles are known to be produced by algae and cyanobacteria, and have inhibitory effects on the growth of different organisms such as the green alga *Chlorella pyrenoidosa* (Watson, 2003; Xu et al., 2017; Liu et al., 2021b). β-Cyclocitral is an odour compound, well-known for its effects on drinking water supplies and it is apparently attributed to a defence role against microscopic grazers

feeding on phytoplankton, even if further studies are needed to define its function (Santos et al., 2016). Another VOC produced in freshwater environment is β-ionone, a volatile derived from β-carotene through carotenoid cleavage by dioxygenases with supposed allelopathic inhibitory and damaging effects (Zhang et al., 2013; Shao et al., 2011).

5. Molecular basis of VOCs production by aquatic microorganisms

The production of VOCs involves multiple biochemical pathways and hundreds of genes. In plants, VOCs are involved in plant–plant interactions, pollinator attraction as well as predator repellence. The specific VOCs profile emitted by plant tissues including roots, leaves and flowers, depends on the genotype as well as environmental conditions (Maffei, 2010). The importance of plant VOCs led to the isolation of genes encoding candidate enzymes responsible for the biosynthesis and regulatory circuits of plant VOCs (Dudareva et al., 2013), while less information is available concerning the VOCs produced by aquatic organisms such as algae or cyanobacteria.

VOCs belonging to a specific chemical class may be produced by a wide range of different organisms. Volatile sesquiterpenes, *e.g.*, are typical metabolites produced by plants and fungi, but also algae and bacteria.

A comparison of terpene synthase genes between two red algae, *E. australicus* and *P. purpureum*, and land plants and bacteria revealed that red algal terpene synthases phylogenetically clustered with bacterial terpene synthase and not with land plant terpene synthases. Furthermore, red algae were found to rely solely on the plastidial methylerythritol phosphate (MEP) pathways for precursor supply, while other terpene-producing eukaryotes use the cytosolic mevalonate (MVA) pathway to produce sesquiterpenes. It was hypothesized that terpene synthases in red algae were acquired from bacteria through horizontal gene transfer (Wei et al., 2019). Precursor supply is also operating through the plastidial MEP pathway in green algae as indicated by transcriptome data (Lohr et al., 2012; He et al., 2018) including the green microalgae *Chlamydomonas reinhardtii* (Vavitsas et al., 2018).

Details about terpene synthase genes related to the terpenoids geosmin and 2-MIB are reported for cyanobacteria. These compounds are the origin of the earthy/muddy odour of freshwater. Following the identification of geosmin synthase gene in actinomycetes, two similar genes, *geoA1* and *geoA2*, were also identified in the cyanobacteria *Phormidium*. Geosmin synthase is a bi-functional terpene synthase that catalyses conversion of farnesyl-diphosphate into geosmin. Expression analysis showed maximal transcription at 20 °C and a light/dark cycle of 12 h, coinciding with peaks of geosmin production, while no expression occurred at continuous darkness by this phototrophic organism (Ludwig et al., 2007). Experiments conducted with the cyanobacteria *Anabaena circinalis* AWQC318 under different light regimes proposed that geosmin synthesis was not under circadian control, but rather coordinated with cell growth and metabolism (Giglio et al., 2011). The terpenoid 2-MIB is proposed to be generated from its precursor geranyl diphosphate (GPP) by a two-gene operon, one gene is a SAM (S-adenosyl-L-methionine)-dependent *C*-methyl transferase (GPPMT) and the other gene is a C_{11} homo-monoterpene synthase (MIBS) and sequences similar to GPPMT and MIBS were identified in a set of different cyanobacteria (Watson et al., 2016).

The transcription of genes involved in the production of VOCs by algae was shown to be highly affected by nitrogen content in the medium. In case of the cyanobacteria *Microcystis aeruginosa*, nitrogen starvation induced the expression of genes involved in the promotion of VOCs emission, specifically terpenoid and benzenoid precursors (Zuo et al., 2018). In case of the microalga *Lobosphaera incisa*, nitrogen deprivation induced both upregulation and downregulation of specific VOCs synthesis genes. Upregulation included genes for fatty alcohol, fatty aldehyde, and ketone synthesis, as *e.g.*, *LiLOX*, encoding a chloroplastic lipoxygenase which mediates oxidation of PUFAs. Downregulation affected genes putatively implicated in alkene biosynthesis (Kumari et al., 2020).

Filamentous brown alga, including *Ectocarpus siliculosus* and *Laminaria digitata*, emit volatile brominated and iodinated halocarbons to the

atmosphere. *Ectocarpus siliculosus* is characterized by an especially strong emission of methyl iodide, being this emission slightly increased under oxidative stress conditions. Halogenated hydrocarbons produced by macroalgae play an important role in climate functioning through its influence on earth radiation budget and oxidation power of the atmosphere (Paul and Pohnert, 2011). *Extocarpus* genome annotation proved the existence of a vanadium bromoperoxidase, but this enzyme cannot account for methyl iodide production, which is proposed to depend on *S*-adenosyl-methionine-dependent methyl transferase, although presently genome annotation has not uncovered such gene in *Ectocarpus* (Küpper et al., 2018).

6. Triggers and function of VOCs emission by aquatic microorganisms

Several aquatic organisms have been shown to produce a variety of bioactive compounds, which play a fundamental role in ecology, and can have multiple functions concerning inter and intra specific communication (Fink, 2007).

In order to detect, produce and/or release the chemical cues, the action and interaction of different biological components is required.

The three essential components for any biological communication system are represented by an emitter, a receiver and an environment where the information exchange may occur (Venuleo et al., 2017).

In aquatic ecosystems, there are different biotic and abiotic triggers that can influence and control the production of these metabolites. Inductors may be the presence of predators or competitors for nutrients or substrates or the necessity of a certain nutrient or substrate during reproductive phase. Other environmental cues that may function as determinants of VOC production by aquatic microorganisms are also light availability or excess of UV radiation, temperature, relative humidity, salinity conditions, oxidative stress and others (Venuleo et al., 2017; Korpi et al., 2009; Zuo, 2019; Shaw et al., 2010; Fink, 2007; Lawson et al., 2020).

6.1. VOCs emission for intra and interspecies communication

VOCs are a well-known media of inter and intraspecies communication occurring in a wide range of organisms, varying from bacteria, microalgae, fungi, to higher organisms (*e.g.*, animals, insects and plants).

Thanks to their characteristics described before, VOCs may play the role of infochemicals in marine and freshwater environments (Achyuthan et al., 2017).

Infochemicals act as a communication method and involve chemicals that transmit information between individuals, functioning as messengers and allowing communication within and across species (Fig. 1). They stimulate in the receiver a behavioural or physiological response, which is adaptive to either one or both interactants.

They are classified in two main categories: pheromones, which mediate an intra-species interaction and allelochemicals, which mediate an interspecies communication. In addition, according to which organism benefits from the interaction, allelochemicals are divided into three subcategories: allomones, kairomone and synomones.

An allomone is an allelochemical that evokes in the receiver a response adaptively favourable to the emitter. The term kairomone refers to a chemical substance that causes in the receiver a reaction adaptivity favourable to the receiver but not to the emitter. Synomones represent chemical substances that evoke in the receiver a favourable response to both the donor and the receiver organism (Venuleo et al., 2017; Dicke and Sabelis, 1988; Nordlund and Lewis, 1976).

An example of inter-species communication *via* VOCs can be found in cyanobacteria. It was demonstrated that *Microcystis aeruginosa* NIES-843, when co-incubated with *Tychonema bourrellyi* CHAB663, experiments a growth inhibition and cell death. Analysis on excreted compounds under this co-culture conditions evidenced that β-ionone represented the major volatile ingredient in the cultures of *T. bourrellyi*, pinpointing to a possible inhibitory effect of this compound on *M. aeruginosa* growth. In this case the action of the allelochemical released by *T. bourrellyi* can be classified as kairomone, because it favours *T. bourrellyi* under resources competition

and promotes it to become the dominant species (Venuleo et al., 2017; Shao et al., 2013).

Another example of VOCs utilised as kairomones by marine herbivorous organisms can be found in the interactions between the gastropod grazer *Radix ovata* and the benthic diatom *Achnanthes biasolettiana*. The analysis of the results of Fink et al., 2006 shows that, when subjected to cell damage, *A. biasolettiana* produces a blend of volatile compounds mainly composed by lipoxygenase products that are considered attractive and can be used as food-finding cues by *R. ovata*. The VOCs bouquet produced by *A. biasolettiana* comprised mono- and diunsaturated alcohols and ketones, mainly with C8- but also with C9-compounds, highlighting the possibility that the lipoxygenase production is not always adaptive or exclusively associated to defence mechanisms activation, but it can be also used as a possible food-finding cues, evidencing the incredible complex ecological role of these VOCs and their dependency on different chemical interactions and ecological context. Besides that, still more investigation has to be done in order to have a more comprehensive and clear understanding about the function of this exudates (Fink et al., 2006).

In contrast, an example of allomone was identified in two bacteria, *Azospirillum brasilense* and *Bacillus pumilus*, whereby specific VOCs emitted, in particular 2,3-butanediol and acetoin, had a positive effect upon the microalgae *Chlorella sorokiniana* by promoting its growth and increasing the production of lipids, carbohydrates, and chlorophyll *a* (Amavizca et al., 2017).

Finally, an example of synomone is DMS emission, an important VOC produced by different marine microorganisms during grazing events. These volatiles, in addition to its direct defence functions, operate by attracting species such as procellariiform seabirds (Nevitt, 2008), providing in this case a mutual benefit for both emitter and receiver (Venuleo et al., 2017).

6.2. Stress induced VOCs emission

Even though aquatic environments are relatively stable compared to terrestrial ones, as mentioned above, aquatic organisms are easily subjected to different possible stresses that can induce the production of VOCs. Under these conditions, VOCs perform important ecological roles, that are essential survival strategies for the emitters and their population (Zuo, 2019).

Overall, these chemical signals are involved in many processes, such as defence, growth, competition and environmental stress response (Achyuthan et al., 2017; Fink, 2007; Leflaive and Ten-Hage, 2009). The following paragraphs briefly summarize the main triggers that can lead to the production of infochemicals in this kind of ecological environments.

6.2.1. Abiotic stress induced VOCs emission

As mentioned above, stress factors that mostly can influence and control the production of VOCs by aquatic microorganisms due to environmental changes include variations in salinity or the induction of oxidative stress, the presence/absence of nutrients, the light and temperature variation and the change in humidity rate (Venuleo et al., 2017; Korpi et al., 2009; Zuo, 2019; Shaw et al., 2010).

An example of volatile signalling between marine microorganisms under salt stress condition can be found in *Chlamydomonas reinhardtii*. The study of Zuo et al., 2012 demonstrates that the composition of VOCs emitted by this microalga, under either sodium chloride (NaCl) or sodium carbonate (Na_2CO_3) stress conditions, can affect the unstressed cells, transmitting the proper information in order to help them to deal with the upcoming stress.

The emitted VOCs under both salt stress experiments were aldehydes, esters, alkanes, and terpenoids, with the addition of ketone and alkenes classes for the Na_2CO_3 condition exclusively. Some of the volatiles contained in this group of VOCs were responsible for the increase of antioxidant enzyme activity in *C. reinhardtii* (superoxide dismutase, peroxidase and catalase, SOD, POD and CAT respectively), probably acting as a combined signal (Zuo et al., 2012).
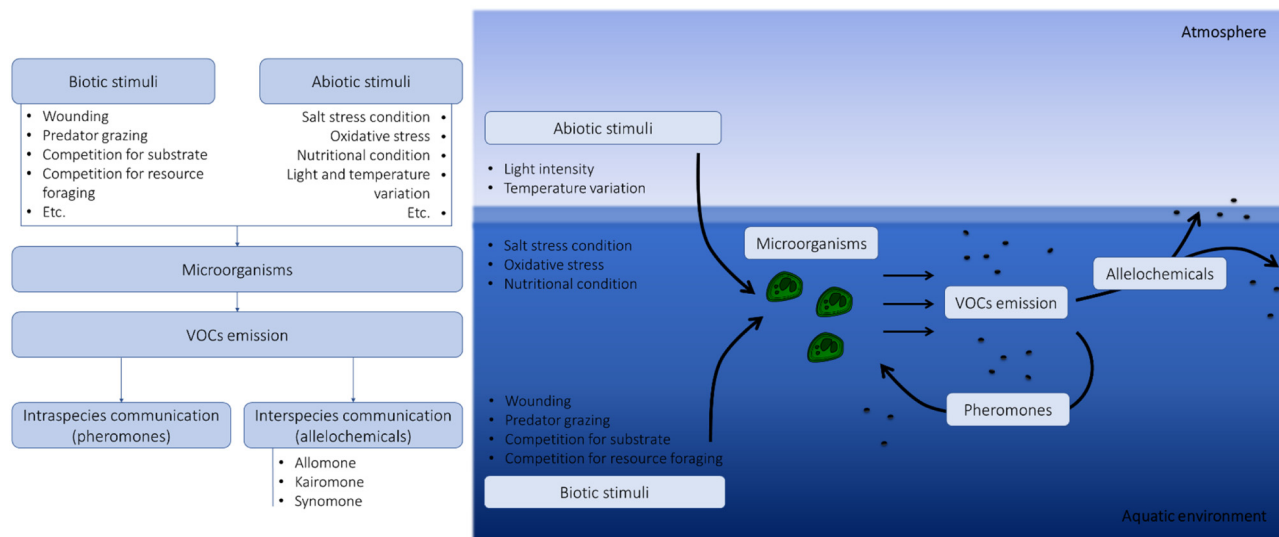
Fig. 1. Summary of the process of induction of VOCs emission and their different roles as aquatic chemical communication method. As a consequence of biotic and/or biotic stimuli, the aquatic microorganisms are induced to produce and emit different VOCs according to the specific trigger. VOCs as infochemical have roles that can be classified in two main categories: pheromones for intraspecies interaction and allelochemicals for interspecies communication. This category can be divided in other tree subcategories related to interspecies communication: allomones, kairomone and synomones, that differentiates on the type of adaptatively response related to the emitter and receiver.

Regarding oxidative stress, there are examples on the release of different VOCs under acetic acid stress conditions. Some studies demonstrated that *C. reinhardtii* cells tend to accumulate rapidly superoxide (O_2^-) and hydrogen peroxide (H_2O_2) and undergo programmed cell death (PDC) under acetic acid condition at pH 5.0. This type of biotic stress can lead to the release of abundant VOCs (Zuo et al., 2015; Zuo et al., 2012). In particular, C6 green leaf volatiles (GLVs, six carbon compounds which include alcohols, aldehydes and esters) and terpenoids, such as hexanal, longifolene, 3-methyl-2-pentanone. As a result of this emission, other cells (under normal culture conditions) reduce their growth or control the ROS level by increasing the activity of antioxidant enzymes. These studies indicate that VOCs work as agents of communication in order to transfer stress information from the dying cells to the other non-stressed cells, thus preparing them for the upcoming stresses.

An example of how the type of substrate and nutritional conditions can influence the production of VOCs can be found in studies on *Microcystis aeruginosa* and *Microcystis flos-aquae* (cyanobacteria belonging to the genus that is considered one of the mayors responsible for water blooms). These studies were conducted to investigate the effects, in terms of VOCs production, in the presence of different sources of nitrogen (N) and phosphorus (P), whose occurrence in natural conditions would lead to an acceleration of the eutrophication process (a kind of stress mainly caused by human activities). The VOCs blends emitted by these microorganisms depend on the P and N source and include furans, alcohols, aldehydes, benzenes, esters, hydrocarbons, sulfocompounds and terpenoids, with an increased emission under limited P and N nutrient condition. In eutrophicated waters, high nutrient content favours cyanobacteria growth, which than induces the emission of a variety of VOC blends in algae (Zuo et al., 2018; Ye et al., 2018).

Another example showing the influence of substrate and nutritional conditions on VOCs production can be found in the study conducted by Liu et al. (2021b) at Chaohu Lake, demonstrating how water eutrophication leads to cyanobacteria bloom events and the emission of a broad range of VOCs. In this study, alkanes and aromatics were shown to be the most abundant VOCs released, 23.1 to 63.7% and 16.6 to 46.3% of total emitted VOCs, respectively. These compounds may have consequences in both aquatic and atmospheric environments according to the type and quantity of emitted compounds (Liu et al., 2021b).

In relation to light availability stress, various studies suggest that isoprene production rates in different phytoplankton species are linked to changes in cellular chlorophyll content, photoprotective carotenoids content and photosynthetic activities, depending on the specific irradiance regime (Hackenberg et al., 2017; Shaw et al., 2010; Meskhidze et al., 2015). In species like *Pleurochrysis carterae*, *Rhodomonas salina*, *Thalassiosira weissflogii*, *Thalassiosira pseudonana*, and in the *Prochlorococcus* genus, a light promoting effect on isoprene production was shown, in particular at low irradiance levels beneath ~100/150 µmol m^{-2} s^{-1} (Meskhidze et al., 2015; Shaw et al., 2003).

Another possible biotic cue for VOCs emission by marine photosynthetic organisms is temperature (Meskhidze et al., 2015; Exton et al., 2013). In case of *Prochlorococcus*, maximum isoprene rates were observed at temperatures near 23 °C. Additional studies have to be carried out to clear the importance of light and temperature as triggers and understand whether or not the VOCs production is exclusively related to them or to other changes (Shaw et al., 2003; Lim et al., 2019).

6.2.2. Biotic stress induced VOCs emission

There are also several biotic stresses that can trigger the release of chemical signals with important ecological functions, such as predator defence, competition for substrate or resource foraging (Leflaive and Ten-Hage, 2009; Achyuthan et al., 2017; Fink, 2007; Zuo et al., 2018).

For example, the cell damage as a consequence of predator–prey interactions leads to the liberation of VOCs, acting as infochemicals that play an important role in protecting aquatic organisms, like algae and cyanobacteria (Fink, 2007).

A typical scenario involving VOCs released as a result of mechanical wounding or predator grazing is the DMS emitted from *Emiliania huxleyi*. Different studies informed that during grazing by the dinoflagellate *Oxyrrhis marina*, *E. huxleyi* activates a defence strategy producing DMSP, a chemical precursor that is subsequently converted to DMS by the enzyme DMSP lyase. The DMS acts as a warning signal towards predators, manipulating their food selectivity that may shift grazing pressure to other species reducing at the same time competition for nutrients (Wolfe et al., 1997; Wolfe and Steinke, 1996; Wolfe et al., 1994). Moreover, as mentioned above, DMS not only plays a defence role by acting as a repellent but additionally can act as an attractant for other species such as procellariiform seabirds (Nevitt, 2008).

Other volatiles, like geosmin and 2-MIB, which may reduce water quality by generating bad taste and odours, were shown to be released during cell lysis as a possible consequence of predator attack and environmental

stress, including variation in light intensity, temperature and intensity of water flow. Organisms, able to excrete these compounds, include cyanobacteria (Jüttner and Watson, 2007; Espinosa et al., 2020; Fujise et al., 2010), but the dynamics of production differ between organisms and, in some cases, between strains of the same species. Considerable research is still required to elucidate the reasons that cause their emission, the impact that the different producers can have and the physiological/ecological roles of these compounds (Jüttner and Watson, 2007; Espinosa et al., 2020; Watson, 2003).

The polyunsaturated aldehydes produced by diatoms, such as 2E,4E-decadienal, are considered as putative defence agents against copepods. These compounds have an inhibitory effect on grazer reproduction; thus, the action is not directed at adult individuals, but rather towards following generations. The emitted PUAs induce a reproductive failure acting on aspects related *e.g.*, to sperm motility, fertilization success, embryogenesis, *etc.* (Leflaive and Ten-Hage, 2009; Caldwell et al., 2004). Besides that, the factors that influence copepod reproduction, as well as the functions of PUAs in diatom–grazer interactions remain uncertain. There are numerous natural external factors that may also influence the copepod reproduction compromising the possible advantage analysed by the release of these secondary metabolites. Nonetheless, under controlled environment experiments, PUAs produced by diatoms have been found to inhibit the reproduction of many species that may consume them (Leflaive and Ten-Hage, 2009).

Another example can be found in the interaction between *Microcystis* NRC-1 and *Daphnia magna*. *Microcystis* cell rupture activates the production of high amounts of β-cyclocitral around *Microcystis* colonies, influencing predator behavior of the grazer. β-Cyclocitral acts both as a signal of poor quality food and as a strong repellent, playing a role in aquatic chemical defence against grazers (Jüttner et al., 2010).

As mentioned before, the competition between organisms is another important trigger for aquatic VOCs production and knowing these compounds can be very useful, not only to detect ecological events and effects, that can occur in a specific natural environment, but also for different industrial sectors, which can use these compounds to increase the productivity of specific organism (*e.g.*, inhibiting undesirable pathogen growth or promoting desirable biomass production).

The concomitant growth of organisms belonging to the same or different species may lead to a depletion of nutrients and space, which then may act as a trigger for specific VOC emission and in turn may affect growth of the organism present (Zuo et al., 2012; Zuo et al., 2017; Zuo et al., 2018; Achyuthan et al., 2017).

An example of this can be found in *M. flos-aquae*, an aquatic cyanobacterial microorganism, which, under N limiting condition, releases terpenoids, such as eucalyptol and limonene. This VOCs production results in a strong inhibition of *C. vulgaris* cell growth, due to the inhibitory effect on photosynthetic pigment content and therefore photosynthetic efficiency, promoting *M. flos-aquae* as dominant species and leading eventually to blooms. The emitted VOCs here have allelopathic effects and are emitted as response to a situation of resources competition (Xu et al., 2017). A similar response occurs in *C. reinhardtii* cells during exposure to the VOCs emitted by *M. flos-aquae* under conditions of phosphate depletion (Xu et al., 2017; Zuo et al., 2017).

Another illustration of VOCs production affecting growth of competitor aquatic organisms is provided by the diatom *Nitzschia cf pellucida* that shows a pronounced allelopathic effect capable of inhibiting and killing the diatoms *Cylindrotheca closterium*, *Entomoneis paludosa*, *Navicula arenaria* and *Stauronella* sp. *N. cf pellucida* cells produce a blend of brominated and iodinated VOCs, such as cyanogen bromide (BrCN), which lead to the shrivelling of chloroplasts, loss of pigmentation and, consequently, a photosynthetic capacity reduction, growth inhibition and cell death. In particular, BrCN is a metabolite used as pesticide and fumigant with extremely inhibitory and toxic effects to *N. cf pellucida*'s competitors.

BrCN emission was demonstrated to be light-dependent and to be emitted for few hours after the onset of light, thus acting as a short-term signal. It is independent of the presence of competing diatoms but is targeted

specifically against competing algae. BrCN acts as a daily regulation of the biofilm's spatial organization and consequently enhances the access to light and nutrients. Compared with its natural rivals, *N. cf pellucida* is BrCN resistant, but it is still sensitive to the toxin at high concentrations. Therefore, the short-term toxin outbreak can be seen as an important mechanism for lowering the risk of autotoxicity (Vanelslander et al., 2012).

Another interesting element to evaluate is the influence of the growth phase of emitter microorganism on the type of released VOCs.

For example, the spectrum of VOCs composition emitted from cyanobacteria, analysed at Chaohu Lake, evidenced that these organisms operate significant changes in VOCs spectra production according to their life phase. These microorganisms emitted predominantly OVOCs and aromatics with volatile organic sulphur compounds during the apoptosis and decomposition phase respectively, and non-methane hydrocarbons during the stabilization and senescence phase (Liu et al., 2021b). Another example in freshwater environments, can be found in the analysis conducted in Taihu Lake, where microalgae showed a change in production of alkenes and OVOCs, with a reduction in quantity from the stationary to the apoptosis phase (*i.e.*, 55% to 12% for alkenes, 26% to 10% for OVOCs), and an increase of alkanes and volatile sulfide chemicals (*i.e.*, 17%–65% for alkanes, 2%–22% for volatile sulfide chemicals) between these phases. These different studies also evidenced how difficult are these types of investigations and how much these growth phase related data depend on the analysed organisms in combination with a variety of environmental factors (Zhou et al., 2016; Liu et al., 2021b; Yu et al., 2019).

One final example of the use of VOCs as infochemical can be found in the Coral reef where Symbiodiniaceae influence the volatilome production of their associated bacteria such as *Labrenzia* sp. 21p that can utilize the Symbiodiniaceae exudate to produce a wide variety of volatiles like acetone, camphor, DMS, dimethyl trisulfide (DTS), 2-ethylhexanal, and1,2-15,16-diepoxyhexadecane. The detected chemical functional groups were aromatic, halogenated, hydrocarbons and organosulfurs and some of them are known to be involved in inter-species communication, acting as antimicrobials and as antioxidants. In addition, also alcohols, aldehydes, esters, ethers, ketones and monoterpenes were identified (Lawson et al., 2020).

7. Technological applications of VOCs emission by aquatic microorganisms

Compared to the knowledge about VOCs emitted in terrestrial environments, their role in aquatic ecosystems, concerning quantity, composition and function in ecological interactions, remains still relatively unexplored (Rowan, 2011; Fisher et al., 2020; Steinke et al., 2018a).

In the last years, VOCs production and characterization in aquatic ecosystem has experimented an increased interest. The growing knowledge related to VOCs, the upgrading in detection and extraction methods, and the possibility to use them as valuable alternative source of chemicals, led this compounds to reach an immense importance in different industrial sectors (Achyuthan et al., 2017; Srivastava and Mazumdar, 2011; Herrmann, 2010; Gong et al., 2015; Santos et al., 2016; Berry et al., 2008). For example, marine and freshwater cyanobacteria have been discovered to produce a wide variety of bioactive metabolites. For the majority of these compounds, their functional role remains largely unknown. However, many of these metabolites seem to have the potential for a commercial development (Berry et al., 2008).

A possible application of VOCs technology in the production of metabolites from aquatic microorganisms is its utilization as biocontrol agents with the purpose to manipulate and control the biological interactions of pests and pathogens (Rowan, 2011). The use of aquatic VOCs as tools of biological control and regulation can be found in food industry sectors, aimed at avoiding infections by toxigenic and harmful pathogens *e.g.*, food and feed production and storage.

For example, the marine *Shewanella* strain YM8, at temperatures from 20 to 40 °C, produces a set of VOCs with a fungicidal effect. Both conidial germination and mycelial growth of aflatoxigenic *Aspergillus* pathogens is

inhibited, preventing the biosynthesis of aflatoxins, which have been found to be carcinogenic in a number of animal species. This fungicidal effect is active also against the phytopathogenic fungi Alternaria alternate, *Aspergillus niger*, *Aspergillus parasiticus*, *Botrytis cinerea*, *Fusarium graminearum*, *Fusarium oxysporum*, *Monilinia fructicola* and *Sclerotinia sclerotiorum*. Among the detected aquatic bioactive antimicrobial compounds, able to protect several crops and food and feed products from infections during storage, there are: butylated hydroxytoluene, DMTS, 2,4-bis (1,1-dimethylethyl)-phenol and 2,4-dimethyl-oxazole.

By applying specific marine VOCs as biocontrol agents, different improvements and benefits can be achieved, such as the possibility to avoid the usage of pesticides with their related possible environmental risks and consequence for human and livestock health, and cost-effectiveness in terms of biocontrol agents' production. Moreover, the extraction of VOCs from marine microorganisms, compared to extraction from plants, is easy to upscale under optimized conditions with shorter production times (Gong et al., 2015).

Another possible industrial application of aquatic VOCs is the design of a controlled approach that triggers allelopathy. This option could provide an efficient and financial favourable strategy to improve different industrial productions, such as the cultivation of microalgae for the generation of high value compounds.

For example VOCs may serve to improve algal biomass and lipid production or induct and enhance defence and/or survival strategies of the cultivated organisms against possible invasive organisms that may act as possible competitors/predators (Bacellar Mendes and Vermelho, 2013).

Moreover, this technology can also be used to control fast growing organisms, promoting an allelopathic inhibitory or damaging effect, for example in harmful bloom events.

Studies on nor-carotenoids showed that β-ionone has an impact on the growth of *Microcystis aeruginosa* NIES-843 at the EC_{50} of 21.23 ± 1.87 mg/L (Zhang et al., 2013; Shao et al., 2011) by targeting the reaction centre of PS II and the electron transport at the acceptor side of PS II. In addition, this compound is a lipophilic chemical, thus it has a highly bioaccumulative ability, a characteristic that facilitates the absorption and the passage through membranes of organisms, allowing, according to exposition time, the reaching of effective levels, even at a relatively low concentration (Shao et al., 2011).

Further research should be undertaken to clarify the relationship between these volatiles and the organisms they interact with. Despite all these possibilities, the allelopathic strategy has not been thoroughly explored yet (Bacellar Mendes and Vermelho, 2013).

Apart from all these technological applications of aquatic VOCs, they may also be used as non-invasive and non-destructive and financeable affordable real time bioindicators (Seco et al., 2020; Reese et al., 2019).

7.1. VOCs as bioindicators in natural habitats

Considering the different possible triggers for VOCs production by aquatic organisms, and their rapid response to external stimuli, such as changes in environmental conditions due to various factors, these compounds can be considered as possible bioindicators for the state of health of aquatic organisms or communities.

The research area of volatilomics is mainly applied to medical diagnostics, pest control and soil biological studies. However, it also has a great potential for aquatic ecological investigations, where it may be applied as a novel, relatively rapid, potentially highly cost-effective, non-destructive and non-invasive diagnostic tool for biomonitoring, allowing, among others, evaluations on health state, taxonomic composition and time change variation of aquatic environments. The possibility to have a clear *in vivo* metabolic footprint that can provide data on the totality of volatiles emitted in a specific environment, can supply a valuable continuous and real time information in ecological research.

The ability to identify and compare the quantitative and/or qualitative variation in volatilomes of a target ecosystem before, during and after disturbance, could offer new sensitive, fast and relatively inexpensive tools to detect the emergence of a disturbance, such as parasite infestation, harmful algal blooms, *etc.* (Steinke et al., 2018a). Moreover, the possibility of relying on an early warning system, could open doors to the development for robust technological sensors, biomonitoring and new management techniques for aquatic ecosystem, aimed at preventing or reducing its deterioration (Steinke et al., 2018a; Goulitquer et al., 2012).

Biomonitoring through VOCs may also be useful in the climatic change examining. Climate-relevant trace gases are exchanged between aquatic ecosystems and the atmosphere. In this exchange, VOCs play a vital role because of their effects on atmospheric chemistry and climate. Therefore, studying their emissions in response to climate change is crucial for effective predictions of possible future events (Seco et al., 2020).

Different aquatic VOCs (*e.g.*, isoprene, DMS, halogens, *etc.*) are well-known for playing an important role in the atmosphere chemistry affecting its processes (*e.g.*, the regional formation of ozone in the troposphere and the global carbon budget) and influencing climate (Colomb et al., 2008; Yu and Li, 2021; Shaw et al., 2010; Steinke et al., 2018a). Isoprene, for example, can react with OH, nitrate radical, ozone or sunlight in the atmosphere, with a consequential activation of an oxidation process and tropospheric ozone production (Yu and Li, 2021; Colomb et al., 2008; Shaw et al., 2010). Another example are the VOCs containing halogen (*e.g.*, CH_3Cl and CH_3Br) that are well known for their important role in ozone destruction in the stratosphere (Colomb et al., 2008).

There are several studies aimed at identifying the volatiles released in specific geographical areas of the world, in order to monitor and understand the cause-effect relationships that lead to the production of these compounds. The aim is to have a more comprehensive understanding of their ecological impact and the various factors that can influence their production and distribution, in order to allow the construction of possible predictive models aimed at monitoring the areas of interest (Han et al., 2021; Seco et al., 2020; Steinke et al., 2018b; Bravo-Linares and Mudge, 2009). Despite the efforts, the measurements of aquatic VOCs and their spatial-temporal distributions are still poorly developed and there is the necessity to investigate their role in altering and influencing the highly complex global climate system, including its important components such as the atmospheric chemistry and the marine aerosols (Yu and Li, 2021).

The main challenge to extend the volatilomics to aquatic ecological investigation is the compilation of vast datasets in publicly available databases, preferably using unified methodological approaches, in order to recognize certain volatilome patterns. This compilation would be necessary in order to identify key volatilome components to use as a potential monitoring and evaluation tool for complex environments (Steinke et al., 2018a).

An example showing the importance and the possible benefit provided by an increasing knowledge about these compounds, can be found in the discovery of the gene involved in the geosmin biosynthetic pathway that allows an advancement in detection strategies by identifying several geosmin odour emitting cyanobacteria, in different water sources, a considerable advantage in complex systems such as natural aquatic environments, especially for early detections and risk management strategies (Lee et al., 2017).

The knowledge of the role and the causes of VOC emission, in relation also to the type of producer organism, is essential for the development of these predictive models, especially in consideration of the development of remote VOC detection technologies.

For example, the possible use of satellite technology to analyse a large areas providing information about the spatially VOC concentrations and for the elaboration of maps useful to identify ocean domination by specific phytoplankton species, has already been reported (Colomb et al., 2008; Nichol and Wong, 2011).

Moreover, the continuous technological advances, with the development of new and increasingly efficient equipment for remote monitoring in the aquatic environment, could gradually remove practical

obstacles, and, in addition, support the use of these compounds as real-time bioindicators of the health state of the environments for both commercial and ecological interest. One example is the measurement of surface–atmosphere VOC fluxes at a shallow subarctic lake and an adjacent graminoid-dominated wetlands in northern Sweden. Among the emitted VOCS, isoprene stood out for its strong dependence on temperature with diurnal and seasonal fluctuations. Understanding the response of VOCs emissions to climate change can help to accurately predict future climatic conditions (Achyuthan et al., 2017; Seco et al., 2020).

7.2. VOCs as bioindicators in controlled processes

Among all aquatic microorganisms, microalgae have recently gained a lot of attention around the world, because of their wide variety of applications and potential uses as a renewable, sustainable, valuable, and economical source for products such as biofuels, food ingredients and other specific compounds required for aquaculture, medical and cosmetic industry (Bacellar Mendes and Vermelho, 2013; Khan et al., 2018; Lee et al., 2017; Santos et al., 2016; de Souza et al., 2019). Moreover, these microorganisms can thrive in a variety of environments such as freshwater, marine, hypersaline, highly acidic ecosystems and wastewaters. These capabilities, along with their ability to fix atmospheric CO_2 and high lipid-to-biomass ratios, are key aspects in terms of industrial application range, allowing, for example, the production of biofuel and simultaneously bioremediation treatments, using different water sources and avoiding space competition with arable land (Reese et al., 2019; Khan et al., 2018; Rahman et al., 2020; Santos et al., 2016).

One of the main issues in microalgae cultivation is the existence of a variety of factors (*e.g.*, weather, grazing and parasitism) able of destroying a vast number of cells in a short time (days or hours) (Bacellar Mendes and Vermelho, 2013; Hannon et al., 2010; Reese et al., 2019).

Algae pond crashes are one of the most challenging problems to solve in industrial algal biomass production. Unpredictable contamination may cause considerable losses in terms of bioproduction and consequently economical profit (Reese et al., 2019). Current strategies to monitor and diminish these kinds of issues generally focuses on chemical treatments (Bacellar Mendes and Vermelho, 2013; Reese et al., 2019). The use of chemical countermeasures, despite their effectiveness at saving algal cultures when correctly applied, is excessively expensive for most algal industry business models. Moreover, to detect possible pathogens and contaminants, light microscopy is largely used. This method is a slow and labour-intensive monitoring strategy, which requires specialized and trained operators. To improve sensitivity and speed up analysis for daily algal culture monitoring, alternative methods involving automated and semi-automated technologies are under development.

Apart from the current detection methods, a possible strategy to avoid or efficiently manage contamination problems, is based on the use of non-destructive and non-invasive VOC sampling as biomarkers, which helps to improve the understanding and the ability to make prediction about the state of health of microbial cultures, preventing microalgae pond crashes (Reese et al., 2019). This technology has the potential to be used as an efficient discrimination method between infected organisms and uninfected ones, which are not displaying symptoms (Achyuthan et al., 2017; Reese et al., 2019).

For example, it has been observed that, during the rotifers *Brachionus plicatilis* active grazing period, *Microchloropsis salina* algal cultures release a set of specific VOCs (*e.g.* trans-β-ionone and β-cyclocitral), that can be used as possible monitoring biomarkers to identify high stress conditions and eventually indicate an imminent culture crash (Reese et al., 2019). Additional studies have been conducted on *M. salina* cultures, highlighting the correlation between VOCs and *B. plicatilis*. In detail, the most relevant compounds identified were pentane, 3-pentanone, 2-methylfuran and 3-methylhexane, that in combination with the ones derived by carotenoid breakdown, can be used collectively as a potential tool for the diagnostic evaluation of algal mass cultures.

The possibility of carrying out a rapid detection of the health state of controlled biological systems through non-invasive operations on biological organisms thanks to the use of VOCs as indicators, and/or as predictors, can be a fundamental help to monitor and eventually provide the necessary time to apply the appropriate treatments and to save an algae production system from collapse.

The use of VOCs as bioindicators and as a tool for biomonitoring controlled biological system still needs more research/data and a simultaneous development relating to the algal industry technology, in order to identify specific biomarkers of algal stress/death.

A helpful approach would be to establish a "chemical library" aimed to have better knowledge and understanding of the wide variety of metabolites produced *in vivo* conditions and to provide an early diagnostic tool for algal culture systems that are grazed or otherwise stressed. This is an essential step to give the basis for future works on developing predictive models based on VOCs detection and to help regulate complex biological systems (Fisher et al., 2020).

Some efforts have already been made in this direction, but further data needs to be collected. For example, studies on volatile composition in different growth phases from microalgae belonging to *Bacillariophyta*, *Chlorophyta* and *Chrysophyta* species were conducted, in order to provide informative reference data on choice of feeding period, cultivated organism flavours, ideal microalgae species breeding selection and natural water odour formation (Zhou et al., 2016).

Generally, the above explained uses of the microalgal VOCs may represent an improvement in the supply of a large volume of inputs to many different types of industries. Expanding the understanding of aquatic VOCs and their ecological role and metabolic pathways, can be an excellent opportunity also to exploit, in controlled industrial situations, the allelopathic role of some of these compounds towards possible predators (Bacellar Mendes and Vermelho, 2013).

It is clear that there is a need for further research to investigate the microalgae volatile profile to eventually use these compounds as biological control strategy and feedstocks (Santos et al., 2016).

8. Future perspectives

All research work carried out so far, has emphasized on the importance of VOCs in aquatic environments, also highlighting their economic and social potential. Up to now the studies conducted on VOCs produced by aquatic organisms are not enough to provide a complete and exhaustive knowledge on the subject and its different ecological impacts. Many unknowns are still to be resolved (Leflaive and Ten-Hage, 2009; Fink, 2007; Achyuthan et al., 2017; Steinke et al., 2018b).

However, the results obtained until now indicate that VOCs have an immense importance on several levels. The need for a precise investigation is required to avoid as much as possible the usage of sampling techniques that are destructive for organisms, which inevitably leads to offset data. The assessment of VOCs with procedures that can lead to cell lysis (a trigger event) must be avoided since it unfortunately could lead to the release of additional VOCs that would otherwise not be present, thus providing misleading or less reliable data, especially for ecological studies.

Further research needs to be done to identify and have a better understanding of the physiology of aquatic VOCs, of their producing organisms, and of the possible primary and secondary impacts. This knowledge will allow one to have a broad-spectrum understanding of the role and possible impact that these compounds have. Moreover, it could finally be exploited for industrial purposes, such as biomonitoring and managing of cultures, or the creation of specific control strategies and to develop useful predictive models (Seco et al., 2020; Reese et al., 2019; Rowan, 2011; Steinke et al., 2018a; Bravo-Linares and Mudge, 2009; Han et al., 2021; Bacellar Mendes and Vermelho, 2013).

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