

## Microorganisms in marine biotechnology – the great powers of the smallest inhabitants of the seas and oceans

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*Key words: marine microorganisms, marine biotechnology, microbial cultures, astrobiology, marine drugs, pigments, lipids, bioremediation*

### Abstract

In this study, we present five infographics created by thirteen students of the Marine Biology and Biotechnology course at the Klaipeda University (Lithuania). The topics of the works are related to the potential applications of marine microorganisms in biotechnology. The paper also includes an introduction illustrating the overall aim of the classes and survey results summarizing the completed work.

### Introduction

Biotechnology is a branch of applied science that uses biological systems or living organisms and their metabolites to develop various products and technologies. It is a broad field of science and therefore it is usually divided into different subfields (different colors represent different biotechnology branches). Of these, blue (or marine) biotechnology plays an important role and is characterized by its still undiscovered potential. Blue biotechnology, defined by the Organization for Economic Cooperation and Development (OECD), is the application of science and technology to living organisms from marine resources, as

well as parts, products, or models for the production of knowledge, goods, and services.

It is observed that society, secondary school teachers, school children who are about to enter higher education institutions, as well as first-year students of natural/environmental sciences, do not always know or are familiar with the potential of blue biotechnology, and, in fact, even with the term itself. Therefore, there is a need to intensify education, share knowledge, and inspire young people to study, conduct research, and plan their professional careers in this field.

In this work, we present infographics prepared by the second-year BSc Marine Biology and Biotechnology students at Klaipeda University (Lithuania). The task was a component of the overall grade from the course named Microorganisms in Marine Biotechnology (15 %). This new approach (group tutoring) to this part of the assessment was intended to replace the multimedia presentations prepared by students in previous years, which lecturers considered ineffective in terms of learning outcomes. The change was also intended to result in a more individual and partnered approach to the student, greater involvement, and broader competence development. At the beginning of the semester, students self-selected into groups and chose topics; lecturers provided some materials to get started. The blended learning approach included also e-mail and face-to-face consultations. One meeting was held mid-semester to discuss the progress of the work, licensing, and intellectual property issues. At the end of the semester, students presented their work to the whole group and the lecturers. Brainstorming during that meeting resulted in a final version of infographics.

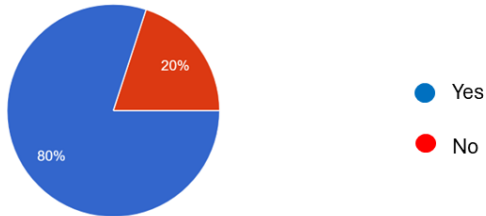
The themes of the infographics cover a variety of angles of marine biotechnology. Infographic no. 1 presents state-of-the-art approaches that help to grow marine microorganisms that were previously unculturable, which is a huge challenge for marine microbial biotech. To date, more than 99% of marine microbes have never been cultured under laboratory conditions. Infographic no. 2 is about the possible use of eukaryotic microorganisms called thraustochytrids. These enigmatic microbes have enormous potential for application in the field of human health and green chemistry. Two further works (no. 3 and 4) deal with specific compounds, dolastatin and astaxanthin. These are metabolites of marine microorganisms that have already found their place on the market. The last infographic (no. 5) describes the potential use of marine microorganisms in the future colonization of space.

For the majority of Students (80%) this was a new challenge, since they had not done infographics before, and the vast majority (70%) were unwilling to return to the previous form of the task (multimedia presentation) (Fig. 1A, and B). The challenge of learning the new software and designing graphic concepts did not pose difficulties for the students; the most difficult challenge was the synthesis of the available materials, and the selection of the final content (Fig. 1C). It seems, therefore, that through this task the students had the chance to develop new competences, which are extremely important in a world overloaded with information. The overall perception of the task was very positive (Fig. 1D), and the opportunity to learn to work in a group was specifically noted. One voice only questioned the purpose of the task, which required more time in an already overloaded semester.

# Tutoring Gedanensis

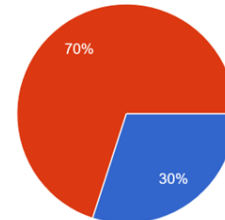
Was this your first time creating an infographic?

A



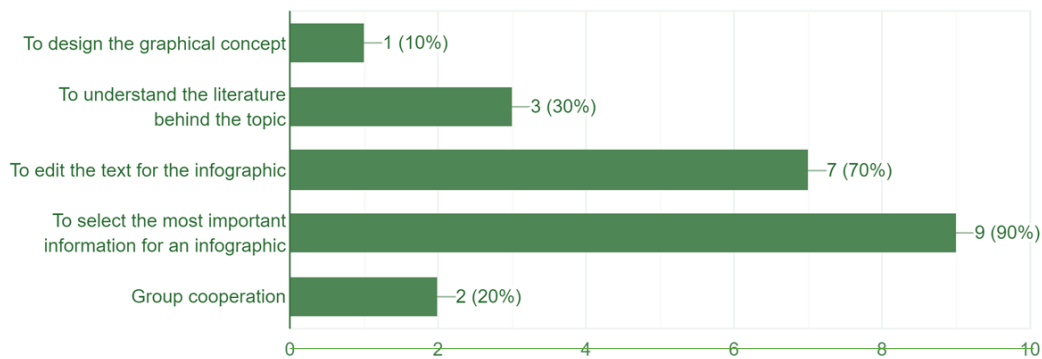
Would you prefer to go back to the previous version of this assignment, the preparation of a power point presentation by each student separately?

B



What was the most difficult part of making the infographic ?

C




List the benefits that the task has given you.  
New competencies, new knowledge, technical knowledge, others.

D

- 😊 I've learned more about licenses and about the topic itself.  
Had to learn how to work with a new program, new formatting and accessories, it was fun to create a new design.  
Train the abilities of working in a group.  
I better understood how to put together an infographic both visually and with putting together the information from different sources.  
Increased creativeness.  
New experience in preparing an infographic, learning more about the topic that was required to prepare, working in a group.
- ? It was an interesting task, however the amount of work needed in preparation for the publishing and such was a bit much keeping in mind that it is the end of the school year and we have many other papers to write and exams to prepare for, so this task has sometimes become an extra addition on an already stressful period. Overall it was nice to try something new, but I don't really see the need for it.

Fig.1. Results of the anonymous post-class survey

INFOGRAPHIC no. 1

 Klaipėdos universitetas

## NOVEL STRATEGIES TO CULTIVATE UNCULTURED MARINE MICROORGANISMS

Pareigyte Martyna and Dukauskaitė Karolina  
Klaipėda University, Marine Research Institute, Biology and Marine Biotechnology

Owing to the extensive metagenomics studies, that are based on the analyses of the environmental DNA, we know that marine microbial diversity is huge.

It is estimated that uncultivable genera constitute 81% of the Earth's microbiome (Liu et al., 2022). In this work, we present some new ideas that are likely to increase the number of cultured marine bacteria.

### 1. Enrichment culturing

Involves usage of non-standard media components (e.g. extract from marine sediment, or from marine macroorganisms) to provide unknown to science components, essential for bacterial growth (Cardoso et al., 2023).

Enrichment culture techniques are mainly used to increase a small number of the desired microorganisms to detectable levels (Madhuri et al., 2019).

### 2. Co-culturing

Involves growing two or more microorganisms together in the same culture medium to create a relationship beneficial for their growth (Kapoore et al., 2021). Bacteria in the marine environment are never alone, they are found in numerous habitats, not only in water sediments, but also in association with algae and other macro-organisms.

For many years, microbiologists have instead focused on pure cultures. The co-culturing approach thus creates conditions closer to the natural environment (Caudal et al., 2022).

## 4. Metagenomics

These days, we are 'flooded' with a plethora of metagenomic data. Can they also help to increase the success rate in marine bacterial cultivation?

Yes! Metagenomic data can provide information on primary metabolism, which further can help to adjust media for selected microbes. Shotgun sequencing may also reveal rare taxa, on which further cultivation could be focused (Liu et al., 2022).

## 5. High-throughput culturing

This involves culturing microorganisms in high-throughput format, using miniaturized culture conditions and automated systems that allow to grow many microorganisms at the same time.

This facilitates the screening of large numbers of microorganisms and the identification of optimal culture conditions for each one.

System works when you dipping a plate with multiple through-holes mixed environmental cells leads to capturing a single cell. It has a membrane that covers arrays of through hole from each side and there are screws that provide sufficient pressure (Nichols et al., 2010).

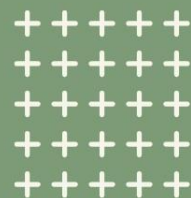
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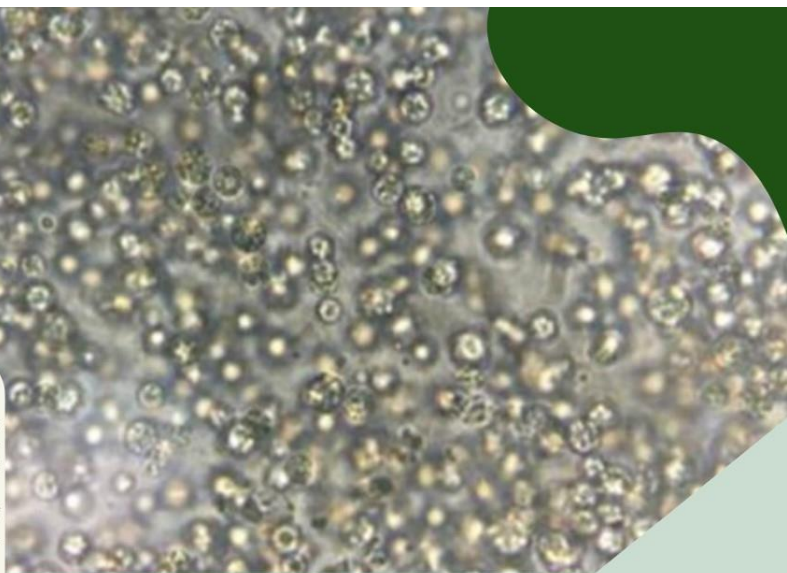
## INFOGRAPHIC no. 2

Thraustochytrids can biodegrade and detoxify organic pollutants like PAHs (Polycyclic Aromatic Hydrocarbons), DDT (Dichlorodiphenyltrichloroethane), and PCBs (Polychlorinated Biphenyls), making them potential candidates for bioremediation. They can use these pollutants as a carbon source and break them down into less toxic compounds. Using these protists for bioremediation could be a cost-effective and environmentally friendly way to clean up marine environments (Contreras and Oviedo, 2023).



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## THRAUSTOCHYTRIDS

### MARINE PROTISTS WITH BIOTECHNOLOGICAL POTENTIAL

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### Meet the thraustochytrids:

Thraustochytrids are common in marine and estuarine environments, including sediment, water, algae, and mangroves. They are unicellular and heterotrophic protists that aid in decomposing leaf litter in mangrove ecosystems, which helps with nutrient enrichment and food web enhancement (Kalidasan et al., 2021).

Discovered 80 years ago, thraustochytrids have recently gained considerable attention due to their biotechnological potential, fast growth, specific lipid metabolism, and their potential applications in various fields such as human health and green chemistry (Morabito et al., 2019).



HELLO  
 My Name Is  
 Thraustochytrid

Thraustochytrids' rapid growth and ability to grow on various carbon sources such as molasses make them economically interesting for lipid production. Their high biomass can be collected in a few days, and using low-cost carbon sources from agro-industrial waste can reduce the cost of lipid production. (Morabito et al., 2019)



FUN FACT: Some species of Thraustochytrids are bioluminescent, meaning they can produce light.

Thraustochytrids' life stages heavily rely on nutrition. They can accumulate high amounts of lipids and biomass when grown in artificial media with yeast extract or peptone, making them ideal for biotechnological applications. *Aurantiochytrium* and *Schizochytrium* thraustochytrids can accumulate lipids up to 30-50% of their dry weight, with DHA (docosahexaenoic acid) accounting for about half of the total fatty acids (Morabito et al., 2019 and Marchan et al., 2017).

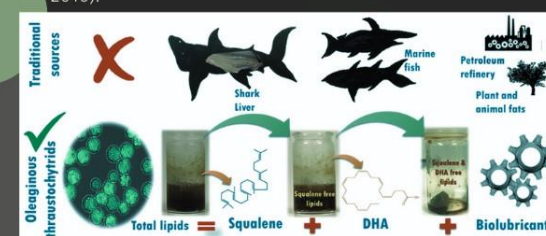
Bioactive metabolite production in thraustochytrids depends on the strain and growth substrate. Strains with extensive substrate preference and high utilization efficiency for cheap and widely available substrates are desirable for biotechnological and industrial applications (Lyu et al., 2020).

## GROWS ON CHEAP AND WIDELY AVAILABLE SUBSTRATE !

Why did the Thraustochytrid go to the comedy club?  
 To tell jokes about its cheap carbon sources and how it's always trying to find ways to pinch a penny, but nobody laughed because they were all too busy being impressed by how much lipids it could produce!

### Products, that can be produced by thraustochytrids:

- **PUFAs** (Polyunsaturated Fatty Acids) are essential components of cell membranes and deficiencies can lead to cellular function defects. Thraustochytrids are a promising option for cheaper PUFA production as compared to fish oils (Lewis et al., 1999). PUFAs have potential applications in human health, aquaculture, and nutraceutical sectors (Kalidasan et al., 2021). Efforts are being made to improve and engineer the lipid production of thraustochytrids, including mutagenesis and metabolic engineering approaches (Morabito et al., 2019).
- **Squalene** is a primary metabolite used in the production of steroids, bile acids, hormones, and vitamin D. The main source is deep sea shark liver, but due to declining populations, a sustainable alternative is needed. Thraustochytrids are a potential source of squalene (Aasen et al., 2016 and Morabito et al., 2019).
- **Carotenoids**, divided into xanthophylls and carotenes, are used as colorants in food and feed, and as supplements due to their antioxidant and anti-free radical activities. Thraustochytrids can produce carotenoids like astaxanthin, phoenicoxanthin, canthaxanthin, echinone, and  $\beta$ -carotene. The amount of each carotenoid varies with growth period and strain, and the efficiency of  $\beta$ -carotene conversion to xanthophylls varies between strains (Aasen et al., 2016).



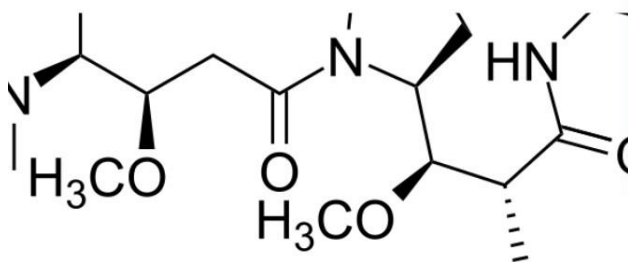
USE OF THRAUSTOCHYTRIDS FOR SUPPLEMENTS (Patel et al., 2020)

## Dolastatin 10 - what is it?

Dolastatin 10 is bioactive, cyclic peptide, and cytotoxin.

It disrupts cell function, leading to death in tumors. Currently, its analogs are used as a treatment for cancers.

This bioactive marine metabolite was first extracted from the sea hare *Dolabella auricularia* (1972) which lives in the Indian Ocean. Years later, it turned out that the real producers are symbiotic cyanobacteria (Singh, 2022).



FROM SLUG TO DRUG

Lileikytė Austėja, Litvinkovičiūtė Viktorija, Blakunova Inesa

Klaipėda University, Marine Research Institute, Biology and Marine Biotechnology

## DOLASTATIN 10

SUCCESSFULLY MARKETED NATURAL MARINE PRODUCT IN PHARMACEUTICAL INDUSTRY

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## From discovery to innovation

1972

DOLASTATIN-10 WAS DISCOVERED AND EXTRACTED FROM SEA HARE

1987

29 MG OF DOLASTATIN 10 WAS EXTRACTED FROM 1 TONE OF MOLLUSK DOLABELLA AURICULARIA

1989

THE FIRST TOTAL SYNTHESIS OF DOLASTATIN 10, THE PROCESS DID NOT DEPEND ON THE NATURAL SOURCE

2008

TWO DERIVATIVES (TASIDOTIN, SOBLIDOTIN) HAD BEEN ADVANCED IN II CLINICAL TRIALS

2011

BRENTUXIMAB (ANTIBODY-DRUG CONJUGATES) IS BEING APPROVED FOR TREATMENT OF HODGKIN'S LYMPHOMA

NOW

FROM BIOACTIVE CYANOBACTERIUM INTO THE DRUG, TO THE LAB AN TOTAL SYNTHESIS

Singh, 2022



## TESTED ON 7 TYPES OF CANCER

Dolastatin 10 can successfully induce apoptosis in various tumor cells, such as leukemia, small-cell lung cancer, prostate cancer, and lymphoma (Gao et al., 2021).

## HOW IT WORKS IN CELL

Dolastatin 10 treats tumors by inducing cell death (apoptosis). It interferes with tubulin balance and microtubule assembly, which arrests the cell cycle and causes apoptosis (Gao et al., 2021 and Singh, 2022).

## CLINICAL TRIALS

Dolastatin 10 entered the I and II clinical trials, but due to strong side effects was dropped out from the clinical trials. However, in 2011 the analog of Dolastatin 10 conjugated with specific antibodies and approved for marketing (Gao et al., 2021).

## MIRACLE MOLECULE

New analogs have been developed based on dolastatin, conjugates of these analogs with antibodies can be found in 4 approved drugs, and more than 20 products that are in phase 1-3 clinical trials.

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Chemical structure of Dolastatin 10 is quite simple, therefore, the development of the Dolastatin 10 luckily did not depend on the natural source. Can you imagine that to extract such a small amount of active compound you would need every time 1 t of mollusks. The alternative is chemical synthesis.



## INFOGRAPHIC no. 4

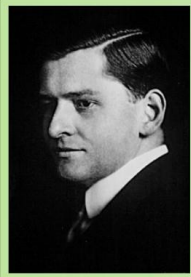
**Klaipėdos universitetas**  
Marine Research Institute,  
Biology and marine biotechnology,  
Pužinskas Lukas and Barauskaitė Modesta

### Astaxanthin production - advances and key challenges in the industrial use

Astaxanthin can be produced by many microalgae species, the best known and commercially used is *Hematococcus pluvialis*. Astaxanthin is a high-value carotenoid. When the algae are stressed by lack of nutrients, increased salinity, or excessive sunshine, they create astaxanthin.

#### History of astaxanthin

In 1938, Richard Kuhn (Austrian-German biochemist who was awarded the Nobel Prize in Chemistry in 1938 "for his work on carotenoids and vitamins") identified and isolated astaxanthin from lobster. <sup>[1]</sup>



#### Benefits of astaxanthin

Astaxanthin has a strong antioxidant capacity that leads to many positive health outcomes in protecting joints, post-workout recovery, protection from UV rays, and its many anti-inflammatory qualities. <sup>[2]</sup>

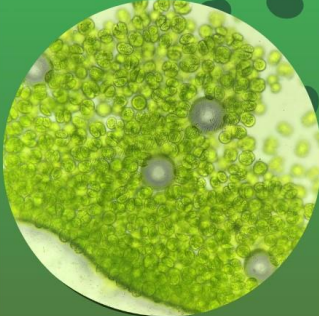
#### Growing potential

Currently, astaxanthin sold on the market is produced by chemical synthesis, but there is a great need to obtain it from other sources. For example, products containing astaxanthin are already available, most of them sold as food supplements containing 1–8 mg of astaxanthin. <sup>[3]</sup>

### Life cycle of *H. pluvialis* <sup>[3]</sup>

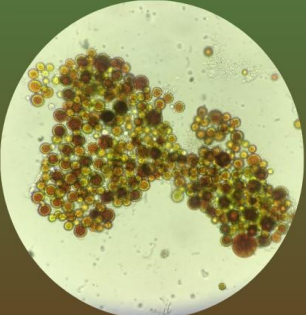
#### Green phase

In the Green Phase, the algae cells are cultivated under optimal growth conditions using pure water and high-quality nutrients to make the algal cells grow and multiply. The process starts in the clean room, where pure algae colonies are cultivated in petri dishes under controlled conditions. The cultures are then transferred to sterile Erlenmeyer flasks and then onto larger cultivation units. When high cell density is reached in photobioreactors (PBRs), 80-90 % of the culture is transferred to the next phase, and the remaining culture is recycled.



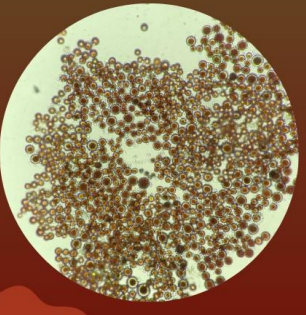
#### Starvation phase

In the Starvation Phase, the algae culture is gradually exposed to stress conditions (light, nutrient deficiency, etc.) to induce rapid astaxanthin synthesis.



#### Red phase

At the end of the Red Phase, the algal biomass is separated from the medium and dehydrated. The algal cells are cracked to produce a high-quality astaxanthin biomass. Astaxanthin is recovered using solvent-free supercritical CO<sub>2</sub> extraction, stabilized, and standardized to produce a range of astaxanthin products.





### Astaxanthin applications

#### Commercial potential of astaxanthin <sup>[2]</sup>


#### Aquafeed

Astaxanthin was first sold as an aquafeed ingredient, used to increase the astaxanthin content in farmed salmon and increase the characteristic orange/red color of their flesh. Animals who feed on the algae, such as salmon, red trout, red sea bream, flamingos, and crustaceans (shrimp, krill, crab, lobster, and crayfish), subsequently reflect the red-orange astaxanthin pigmentation.

#### Effect for farm animals



Astaxanthin production is not limited to microalgae only. This carotenoid has also been assessed as a feed ingredient for land animals. Dietary supplementation with *Phaffia rhodozyma*, which is a yeast rich in astaxanthin, led to a higher content of total free amino acids and improved texture and sensorial attributes on broiler chicken meat. The consumption of astaxanthin at 0.25 mg per kg of body weight per day led to increased milk yield in buffaloes.



*Phaffia rhodozyma*

#### Use in cosmetics

Astaxanthin is also being increasingly used in the cosmetics industry. Masks with natural antioxidants are highly appreciated. In a recent study, a mask containing astaxanthin was compared against another mask formulated using vitamin E and demonstrated a superior antioxidant capacity.

### Synthetic astaxanthin

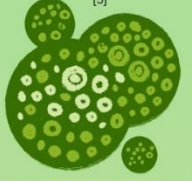
#### Production

Synthetic astaxanthin is produced by chemical synthesis that involves the reaction of certain organic compounds. The most common method used is the Wittig reaction, which involves the reaction of a ketone with an alkylidene triphenylphosphorane in the presence of a base catalyst. <sup>[4]</sup>

#### Synthetic vs. natural astaxanthin

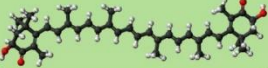
#### Composition

Natural astaxanthin is a complex mixture of different stereoisomers, while synthetic astaxanthin is typically a single stereoisomer. Therefore natural astaxanthin has a more diverse chemical composition than synthetic astaxanthin.



#### Bioavailability


Natural astaxanthin is generally considered to be more bioavailable than synthetic astaxanthin. While natural astaxanthin is around 95% esterified, the synthetic variant is completely free-form, making it less stable and much less bioavailable.



A general ball-and-stick model of the astaxanthin molecule. Black represents carbon, white - hydrogen, red - oxygen.


#### Safety concerns

Natural astaxanthin is considered to be safer than synthetic astaxanthin. Synthetic astaxanthin may contain impurities or other substances that could be harmful to human health.



#### Environmental impact

Synthetic astaxanthin is produced using petrochemicals, which can have a negative impact on the environment. Natural astaxanthin, on the other hand, is derived from sustainable sources like microalgae, which makes it a more environmentally friendly option.



#### Considerations

While synthetic astaxanthin may be cheaper and easier to produce, natural astaxanthin is generally considered to be the superior option due to its diverse composition, higher bioavailability, safety, and environmental sustainability.

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## MICROALGAE IN THE CONTEXT OF ASTROBIOLOGY

### BIOS-3

BIOS-3 - experimental closed ecosystem that has been explored for potential use of microalgae, specifically *Chlorella vulgaris*, to support life on lunar bases.

1972

**1** CHLORELLA CONTAINS ALL THE ESSENTIAL AMINO ACIDS, SUFFICIENT LIPIDS, AND NEARLY ALL THE ESSENTIAL VITAMINS (SALISBURY ET AL., 1997).

**2** A REACTOR SIZE OF 100 L PER PERSON IS ESTIMATED TO FULFILL 30% OF DAILY FOOD REQUIREMENTS (DETRELL G., 2021).

**3** ALGAE CULTIVATION IS RELATIVELY SIMPLE AND HIGHLY RELIABLE. IN ONE EXPERIMENT, THE INITIAL GROWTH RATE WAS SUPPRESSED BY 70% WITH ULTRAVIOLET RADIATION, BUT THE CULTURE RECOVERED ITS GROWTH RATE IN 24 HOURS (SALISBURY ET AL., 1997).



THE CULTURE OF CHLORELLA VULGARIS RECOVERED ITS GROWTH RATE IN 24 HOURS



**i** THE ABILITY TO RECYCLE WASTE, REMOVE CARBON DIOXIDE, AND PROVIDE OXYGEN, AS WELL AS THEIR NUTRIENT-RICH CONTENT, MAKES MICROALGAE REALLY USEFUL.

ATTEMPTS TO PURIFY THE AIR AND GROW FOOD FOR SPACE EXPLORATION IN A SEALED ENVIRONMENT BEGAN IN 1972. A SMALL, CLOSED EXPERIMENTAL ECOSYSTEM CALLED BIOS-3 WAS CREATED IN THE SIBERIAN CITY OF KRASNOYARSK. THE FACILITY WAS COMPLETELY UNDERGROUND. IT WAS POWERED BY A HYDROELECTRIC PLANT 30 KM AWAY. THE TOTAL GROWING AREA WAS 63 SQUARE METERS, WHICH PROVIDED AMPLE AIR-REGENERATION CAPACITY (SALISBURY ET AL., 1997).

IN THIS SYSTEM, PLANTS WERE GROWN IN A HYDROPONIC SOLUTION. STARCH-PRODUCING PLANTS AND OIL CROPS WERE GROWN AS WELL TO PROVIDE ENOUGH ENERGY (SALISBURY ET AL., 1997).

#### Challenges

ALGAE CONTAIN VIRTUALLY NO CARBOHYDRATES

FURTHER RESEARCH IS NECESSARY

SYSTEM AUTOMATION, MODULARITY, AND CONTINUOUS MONITORING WILL BE CRUCIAL DURING THE TECHNOLOGY DESIGN PROCESS.

RADIATION EFFECTS, OPERATION UNDER PARTIAL GRAVITY, HARDWARE SELECTION, SYSTEM AUTOMATION, AND LONG-TERM PERFORMANCE AND STABILITY NEED TO BE CONSIDERED (DETRELL, 2021).

## MICROALGAE IN THE CONTEXT OF ASTROBIOLOGY

### MELISSA

**1** A MICRO-ECOLOGICAL LIFE-SUPPORTING SYSTEM (MELISSA) WAS CREATED TO AID HUMANS IN LONG-TERM SPACE EXPEDITIONS. THIS PROJECT WAS SUPPOSED TO BE USED AS A TOOL THAT WILL HELP US UNDERSTAND CLOSED LIFE-SUPPORT SYSTEMS AND WILL EVENTUALLY LEAD TO REGENERATIVE LIFE-SUPPORT SYSTEMS FOR LONG-TERM MANNED MISSIONS (LASSEUR, 2008.)!

**2** IT IS INSPIRED BY AN AQUATIC SYSTEM AND IT CONSISTS OF FIVE MAIN COMPARTMENTS: FROM ANOXYGENIC THERMOPHILIC UP TO THE PHOTO-AUTOTROPHIC. THE DRIVING ELEMENTS ARE THE PRODUCTION OF FOOD, WATER, AND OXYGEN FROM THE ORGANIC WASTES GENERATED IN THE MISSION, LIKE URINE OR CO2. THE WASTEWATER COMPARTMENT USES FIBROBACTER SUCCINOGENES TO GET RID OF FIBROUS MATERIAL. THE FOOD PRODUCTION COMPARTMENT IS RESPONSIBLE FOR FIXATING CO2 AND MAKING OXYGEN AND, OF COURSE, PRODUCING FOOD (LASSEUR, 2008.).

**3** THIS PROJECT IS MANAGED BY THE EUROPEAN SPACE AGENCY AND IT INVOLVES AROUND 30 ORGANIZATIONS FROM EUROPE AND CANADA. THE PROJECT IS ORGANIZED IN 5 PHASES: RESEARCH AND DEVELOPMENT, PRELIMINARY FLIGHT EXPERIMENTS, GROUND AND SPACE DEMONSTRATION, TECHNOLOGY TRANSFER, AND COMMUNICATION AND EDUCATION. (LASSEUR, 2008.)

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