Tallinn Secondary School of Science

The most suitable algae species for cleaning wastewater from fish farms

Research paper

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11. Grade

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Introduction

Over half of the fish products that end up on our dining tables today are grown in fish farms (NOAA Fisheries home page). Unfortunately, fish farming is causing sea or ocean pollution, because of fish faeces, secretion and feed residues that are released into the water. In addition, there are problems caused by fish diseases, parasites, chemicals and medications, mainly antibiotics. (Mereorganismide kultiv. home page) Wastewater from fish farms can be cleaned with various filters, but these processes are relatively expensive. The best solution would be to use natural resources, for example algae, which get essential nutrients for their survival from the nutrient-dense wastewater and thus work as "water purifiers". These algae could be additionally utilised for other purposes, like for developing new natural materials.

This research paper is relevant as it contributes to the development of environmentally friendly fish farming and aims to help keep the Baltic Sea clean. As an enclosed sea, the Baltic Sea is especially vulnerable, so all measures to reduce pollution and maintain ecological balances are extremely important. Fish is an important part of the human diet. So fish farming helps to keep the ecological balance of global oceans by sparing edible fish living in the wild from being caught.

There is only one fish farm in Estonia, where fish is grown in the sea, in Kesknõmme by Tagalaht bay in Saaremaa Island. However, the opportunities to farm fish in the sea would be much bigger when environmentally friendly technologies could be introduced. Currently, such technology is being developed in Kesknõmme, using algae cultivation to clean the wastewater.

The objective of this research paper is to evaluate which algae species would be the most suitable for cultivating in tanks and cleaning wastewater from the fish farms. The research questions are following:

- What would be the most suitable algae species to cultivate for cleaning wastewater from fish farms?
- Which algae species can be the healthiest and survive the longest in an artificial living environment in tanks?

The hypotheses made are the following:

- There are algae species in the Baltic Sea that can be successfully cultivated in artificial conditions inside tanks.
- The most suitable algae species for cleaning wastewater are thin and fast growing *Cladophora glomerata* and *Ulva intestinalis.*

This study has been made in cooperation with the science- and development project "Treatment of marine water-based fish farm waters by cultivating macro algae" run by Estonian Marine Institute of the University of Tartu. The head of the project is the lead research professor Georg Martin from the University of Tartu, who is also one of the supervisors of this study. During the project, different algae species are cultivated in tanks. Their oxygen production is measured and, based on the outcomes, their well-being and ability to clean wastewater is assessed.

This research paper consists of two parts: a theoretical and a practical part. The theoretical part gives a brief introduction about fish farming and about its negative impact on the environment. The algae species used in the Marine Institute project are presented more in depth: their physiology and their ability to clean water. In the practical part, the oxygen produced by the algae in the water tanks and by the algae grown naturally in the sea is measured and their biomass/dry mass is determined. From that the oxygen production is calculated ($mgO_2/(g \cdot h)$). Chemical samples taken from the tanks are also analysed. They show the change in the amount of chemical pollutants and the algae's abilities to bind those substances. After that the conclusion is made and the most suitable algae species for cleaning wastewater from fish farms and for surviving the longest in artificial environment are assessed.

1. Fish farming

1.1. Fish farming sector

Fish and fish products are one of the most traded and consumed foods in the world today. Millions of people work in the fishing sector; most of them are poor fishermen in developing countries. Competition and demand is high and the global ocean's fish population is decreasing rapidly. To satisfy the high demand for fish, aqua farming has been developed. (Liiber *et al*. 2017: 54-55)

Fish farming is a branch of aquaculture with the aim to grow fish for human consumption and to supply water bodies with young fish, which strengthen and restore existing fish populations (Kalapeedia home page).

The fish are grown in ponds, fresh water pools and in sea cages. Thanks to aquaculture technologies, the cultivating conditions of water organisms can be regulated and thus the yields can be significantly higher compared to natural conditions. Aquaculture fish production has grown rapidly in the last few years. In 2014, for the first time, the consumption of aquaculture products exceeded the consumption of products caught from natural water bodies. (Liiber *et al*. 2017: 55)

Aquaculture is most widely used in Asian countries, accounting for 88% of the total fish farming production. Unlike in Asia, this industry is not very developed in Europe, despite the strong demand. The main obstacles are lack of marine space, difficult financing conditions, great competition in the global market and harsher environmental protection requirements compared to other production areas. (Liiber *et al*. 2017: 56)

Estonia's coastline is about 3 780 km long, which means that fish production has a significant role on Estonian's food table. (FAO / UN).

1.2. Negative effects of fish farming on the environment

Fish farming is very resource intensive with the largest resource being natural water. Different types of fish farming require different amounts of water, but the quantity of nutrients released into the water from the farm is always a central issue, both in the case of reusing the farm water or returning it back to nature. (Kirm 2016) Fish farming waste can be very polluting for the environment. Excess biogens, especially phosphorus and nitrogen, released from fish cages affect the environment negatively. It is especially dangerous in shallow waters and in undulating bays where water does not mix or change quickly. (Trei 1991: 14)

Biogens get into the sea from faeces, feed residues and fish carcasses (emitted from fish cages), which in turn spread many fish diseases (Figure 1 and 2). In addition to biogens, also antibiotics used to treat fish diseases contaminate the water. These are harmful to both the aquatic ecosystem and for humans. (Liiber *et al*. 2017: 57)

Figure 1. Fish feed residue and faeces on the seabed under the fish farming cage, photo by Georg Martin

Figure 2. Fish carcass on the seabed under the fish farming cage, photo by Georg Martin

When the concentration of biogens is too high in the water and the nutrient elements balance is disturbed, certain plant species start to proliferate causing changes in the seabed's overall flora (Trei 1991: 14). The water body gets enriched with nutrients or, in other words, it eutrophicates and as a result stronger plants with faster growth start to proliferate (Liiber *et al*. 2017: 41). Some aquatic plant species may disappear from the natural community all together because they cannot compete with the more aggressive species (Liiber *et al*. 2017: 41). The rapid growth of algae mass causes changes in the chemical, physical and biological features of the water body. Light transmission through the water is impeded and oxygen concentration is rising in the surface level of the water. At the same time the oxygen conditions deteriorate. (Ojaveer 2014: 247) These changes can be dangerous to other water species as well (Liiber *et al*. 2017: 41). Because most of the oxygen is in the surface level of the water, the organisms living in the bottom of the

water body do not get enough oxygen and perish. Also, in eutrophic waters, fishes can no longer move freely or get sunlight.

Nutrient-rich surplus water used in fish farms must be cleaned before releasing back into the sea. All sorts of water purification filters, which have different efficiencies, can be used for this purpose, but they are very expensive solutions. Due to the low salinity of the Baltic Sea, the biological cleaning methods that are used elsewhere in the world cannot be used here. The plant species used normally in bioreactors cannot grow in the low salinity waters of the Baltic Sea. As a result, the new technology has been developed, which makes it possible to clean wastewater from fish farms efficiently in the low salinity conditions of the Baltic Sea. (Kirm 2016) This technology has been developed by Estonian Marine Institute of the University of Tartu and is the same that is studied in this research paper.

1.3. "Treatment of marine water-based fish farm waters by cultivating macroalgae" – project by University of Tartu Estonian Marine Institute

This research paper has been made in cooperation with the science- and development project "Treatment of marine water-based fish farm waters by cultivating macro algae" run by Estonian Marine Institute of the University of Tartu. The aim of the project is to develop a solution to clean wastewater coming from fish farms in a resource-efficient way. The project was implemented from 2017 to 2020 (Kirm 2016). The head of the project is the lead research professor Georg Martin from the University of Tartu, who is also one of the supervisors of this paper (University of Tartu's home page). The following project description is based on the Marine Institute's project application.

As part of this project, a cultivation system is designed and built on the coast. The system uses natural seawater currents, which allow algaes to be grown in them. The system is built for testing and improving, allowing to find the most favourable lighting conditions, water flow rate and suitable algae density. It is studied whether there is a need to enrich the flow of natural seawater with carbon or nutrients according to the vegetation period.

The growth rate of algae in different times is determined and based on that the most suitable length of the cultivation period is established. (Kirm 2016)

The methods used in this project are based on previous scientific research and on experiments worked out within the project. The experiments help to determine the most suitable conditions for cultivating the algae, to ensure the greatest biomass growth. (*ibid*.)

The project is innovative as there is no previous experience of intensive artificial macro algae cultivation in the Baltic Sea region. The development of the most suitable cultivation method allows to grow plants under conditions that are suitable for them and that promote their growth. (*ibid*.)

This research paper's author's role in the project was to measure the oxygen production of the three plant species studied and determine what species consume the most nutrients. In the summer of 2019, the author visited Saaremaa twice for fieldwork (18.06.19 and 22.07.19). During the first visit only the oxygen produced by the algae was measured. At the second time, much more was done. The author was able to go to sea with her supervisor, Georg Martin, watch how divers catch seashells (*Mytilus trossulus*) from the sea (which were later also put into the tanks), help the scientists pick algae in shallow sea water and observe the activities of the scientists and researchers more closely. In autumn, the author was worked on determing the biomass of the algae collected in the summer in the Estonian Marine Institutes laboratory in Tallinn. From the collected data the oxygen production of the species was calculated ($mgO_2/(g \cdot h$).

2. Algae in the sea

2.1. Morphology and characteristics

Algae are the most primitive plants. They have no distinct plant organs, like roots, stems, leaves or vascular tissue. They are found all over the world in saltwaters, freshwaters and on land in wetter areas. Most algae are microscopic or invisible to the naked eye. (Lenntech home page)

By morphology, algae are mainly divided into unicellular, filamentous and carboncyte (mõikjad/tsöenosüüdid) algae. Most algae are unicellular. They can often occur closely together as colonies. In this case, there is no connection between the cells as there is no direct exchange of nutrients/matter or information between them. Many unicellular algae are immobile. (Olli 2010: 8-9)

Filamentous, or thread-like, plants are very common among algae. This structure develops when cells are split in one direction but do not separate from each other, forming a cell chain. The threads can be branched or unbranched, and single or multicellular. (Olli 2010: 9)

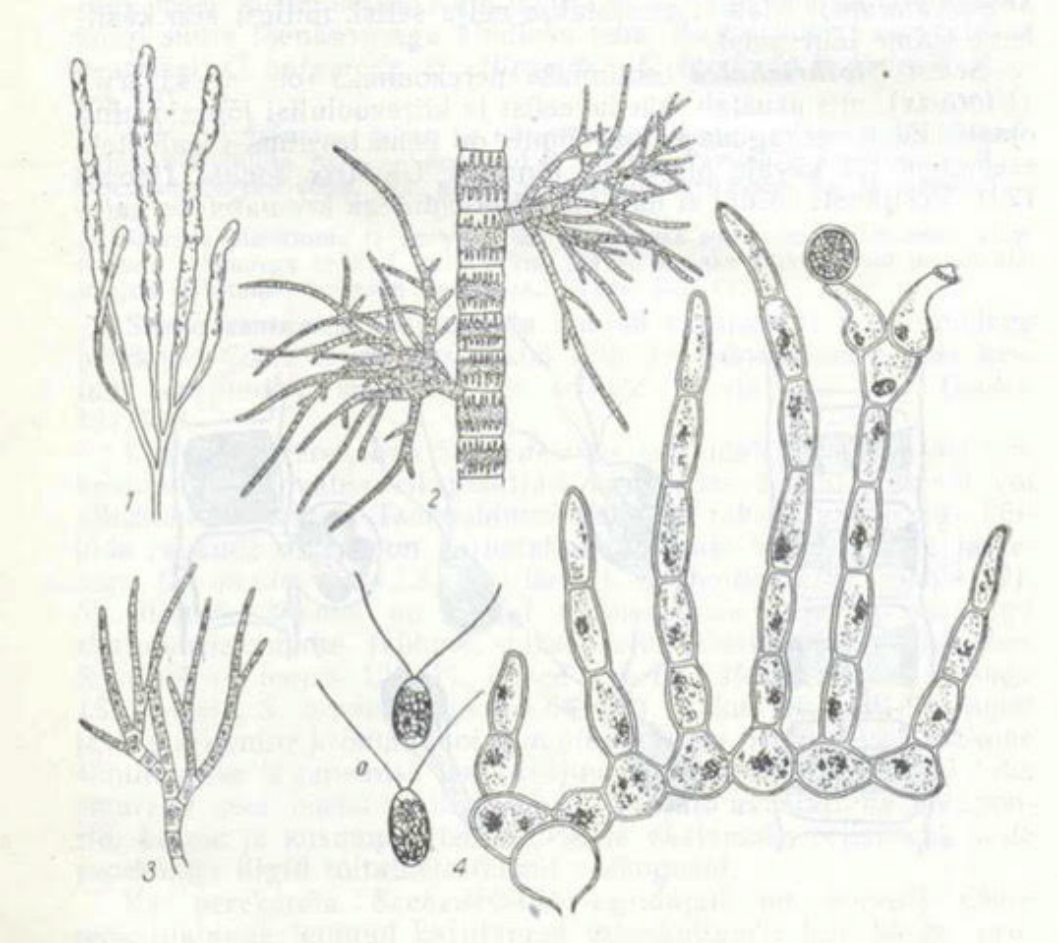

Figure 3. Examples of filamentous algae thalli

Source: Kalda, 1970, page 236

A carbonocyte algae essentially consists of a single large cell containing cell plasma with all organelles, including multiple nucleuses (Figure 4). According to morphology, one algae can belong to two different groups at the same time, for example a filamentous carbonocyte. In this situation, the organism does not have only one cell, but has simply very few cell septums. This specific group also includes the *Cladophora* genus, which is very widespread in Estonian waters and which was also used in this research. (Olli 2010: 9)

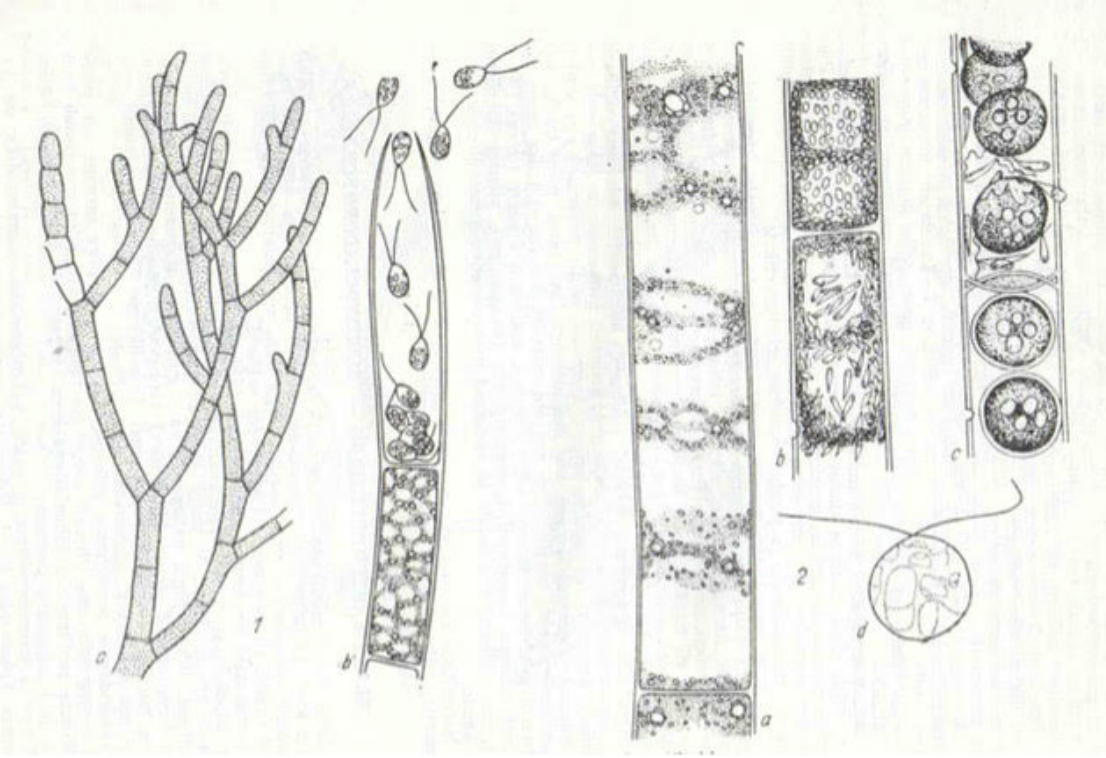

Figure 4. Carbonocyte algae's thalli

Source: Kalda, 1970, page 242

2.2. Algae's ability to clean contaminated water

Algae absorb nutrients and water with the entire surface of their thallus (Trei 1991: 36). Algae survive by consuming light, water, and nutrients. Photosynthesis takes place with the help of light or solar energy, carbon dioxide, water and mineral salts. Carbon dioxide in water enters the sea as a result of the metabolism of aquatic organisms, inflowing waters, and precipitation and diffusion from the air. (*ibid*.: 12)

To synthesise organic matter, algae mainly need carbon, oxygen, hydrogen, but also nitrogen, phosphorus, silicon, iron, magnesium, manganese, and copper. From these, two elements – nitrogen and phosphorus – are named biogenic elements or biogens, because they are the basis of life processes. These are elements that directly affect photosynthesis and the development of plants. The content of biogens in the water is fairly small and usually measured in micrograms per litre of water. (Trei 1991: 12)

Nitrogen enters the sea by inflowing rivers and by air. Rivers bring nitrogen into the sea mainly as nitrites, nitrates and ammonium ions, but they also contain other insoluble and dissolved organic nitrogen compounds. Nitrogen of local origin can also be found in the seawater. When dead organisms decompose, ammonia (NH_3) is formed, which in the water is converted to an ammonium ion ($-NH_4^+$), which is acidified under aerobic conditions. (*ibid*.: 12)

The other important biogenic element is phosphorus, which is mainly absorbed by plants as phosphates. Phosphorus enters the sea through the decomposition of organisms, sediments, inflowing rivers, rainwater from fields, and domestic and industrial wastewater. (*ibid*.: 13-14)

The consummation rate of the biogens depends on the shape and structure of the algae thalli. Nutrients are absorbed the fastest by annual algae, which have thin and thread-like thalli that are branched. Algae with thicker, less branched and flatter thalli have slower metabolisms and thus absorb fewer nutrients. It has been determined that macro algae can also take advantage of temporary increases in the concentration of biogens in the water and can store them in stock. Later, when conditions are favourable, the stored biogens are used for growth and development. (*ibid*.: 14)

The method of using algae to purify water is based on their ability to bind biogens in their thalli. Therefore, species with a fast metabolism and high biomass growth are the most suitable.

2.3. Algae and plant species used in the research paper

2.3.1. Algae specie *Cladophora glomerata*

Cladophora glomerata is a green algae species, which belongs to the *Cladophora* genus and is widely spread in the Baltic Sea (Figure 5). Compared to other similar algae belonging to the same genus, it is much more difficult to recognise, because the specimens vary in size and may grow attached onto rocks, plants, or even float in open water. *Cladophora glomerata* can be found in the waterfront and up to a depth of 5 m. It grows in salt- and freshwaters, but prefers more shady areas. (Trei 1991: 39-44)

Figure 5. *Cladophora glomerata*, photo by Georg Martin

In figure 6, it can be seen that the thallus of the algae is branched, formed like a thin thread and each thread consists of only one row of cells (Trei 1991: 39-44).

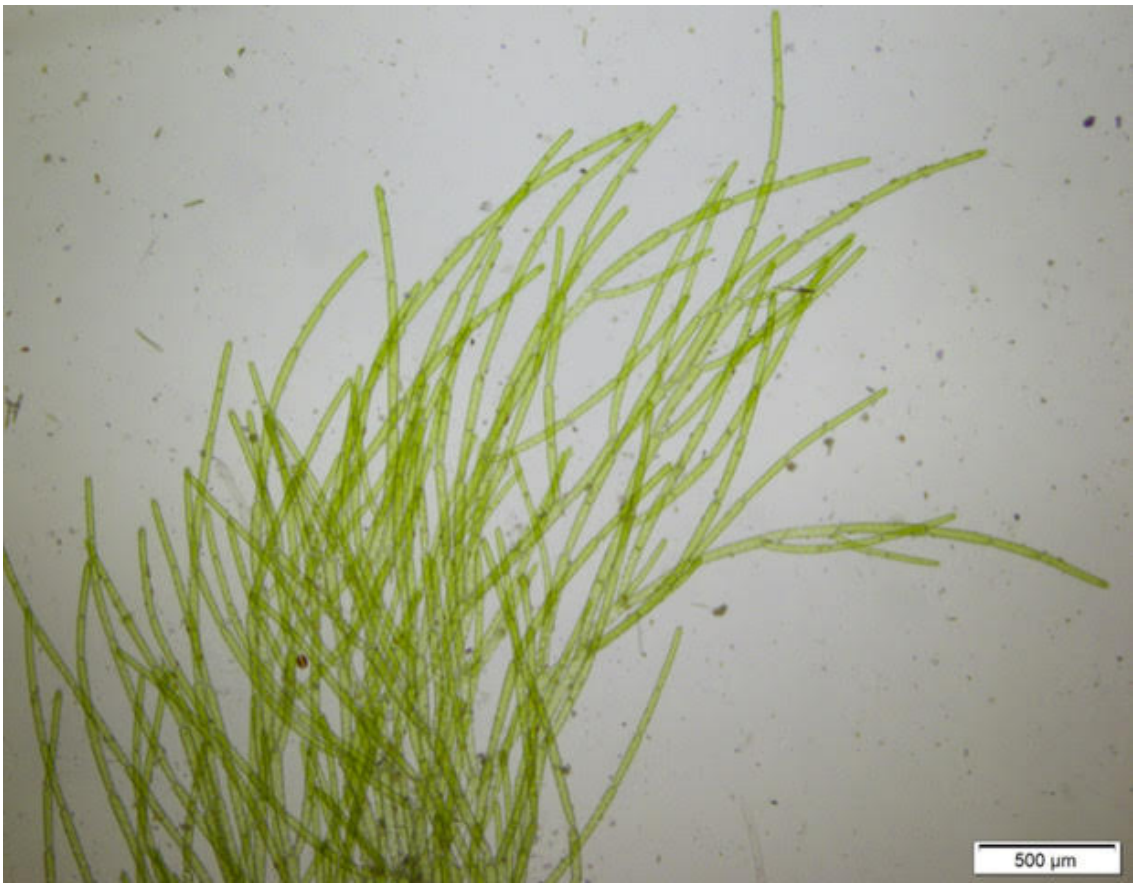

Figure 6. *Cladophora glomerata*'s thallus, photo by Georg Martin

2.3.2. Algae species *Ulva intestinalis*

In figure 7, the algae species *Ulva intestinalis* can be seen. It belongs to the Chlorophyta taxon (a group of green algae) and as the group name implies it has a brightly green coloured thallus, which is unbranched. (MarLIN home page).

Figure 7. *Ulva intestialis*, photo by Georg Martin

It is tubular, hollow, wrinkled in appearance, and can grow to about 20 cm in length. Its thallus is made up of only one cell layer. The main differences between *Ulva intestinalis* and *Cladophora glomerata* come from their thallus's morphology. Both of the species' bodies are only one cell thick, but *Ulva's* cells form a hollow plastic-film-like tube and *Cladophora's* cells are arranged simply one after the other in branched chains. *Cladophora's* cells are long and thin but *Ulva's* cells are smaller in size and lie closely against each other (Figure 8). *Ulva* can grow in salt- and freshwaters. (Kipp *et al*. 2019)

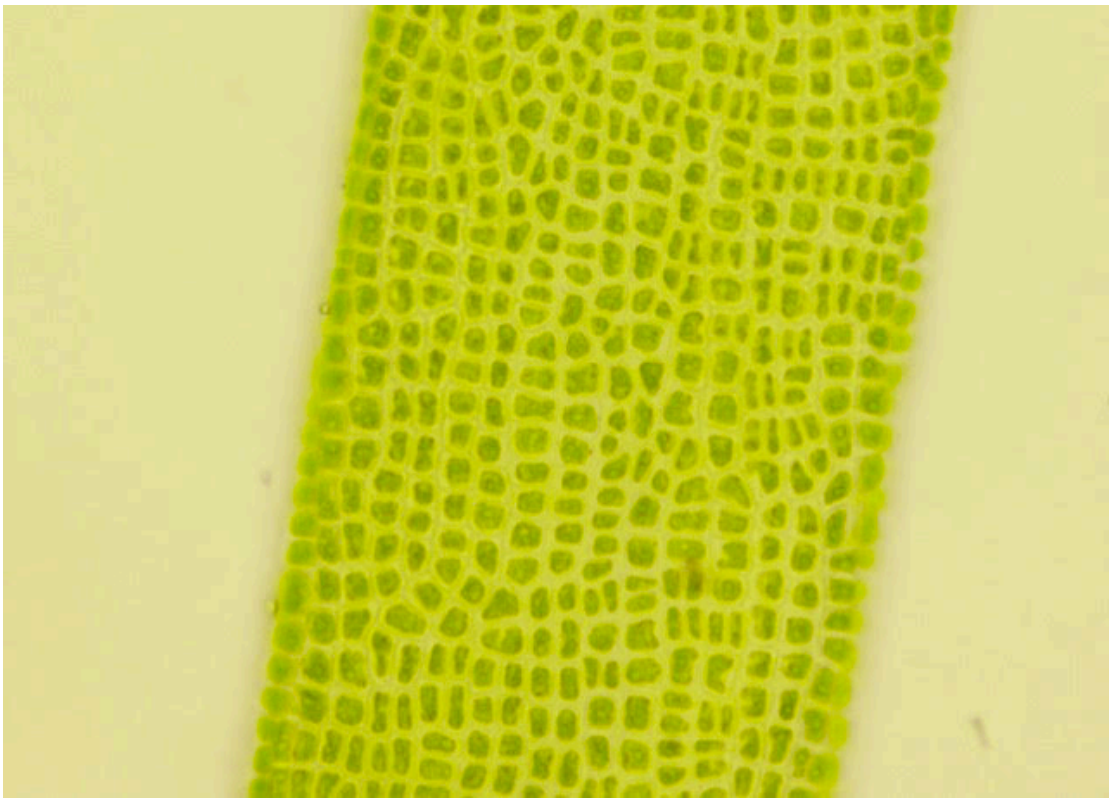

Figure 8. *Ulva intestialis*, photo by Georg Martin

This species is widely spread all over the world, especially in Europe – on the shores of the Arctic Sea and the Baltic Sea (MarLIN home page).

2.3.3. Plant species *Ceratophyllum demersum*

Beside algae, a flowering plant species *Ceratophyllum demersum,* commonly known as hornwort, was also tested (Figure 9). Hornwort is peculiar, because it has no roots at all and it grows in the water. The fact that it is without roots and that it is really light allows it to swim freely in the water. The plant can bloom profusely in Estonia, but it cannot produce fruit, because it needs warmer weather. In the Baltic Sea they reproduce vegetatively. (Trei 1991: 89-92)

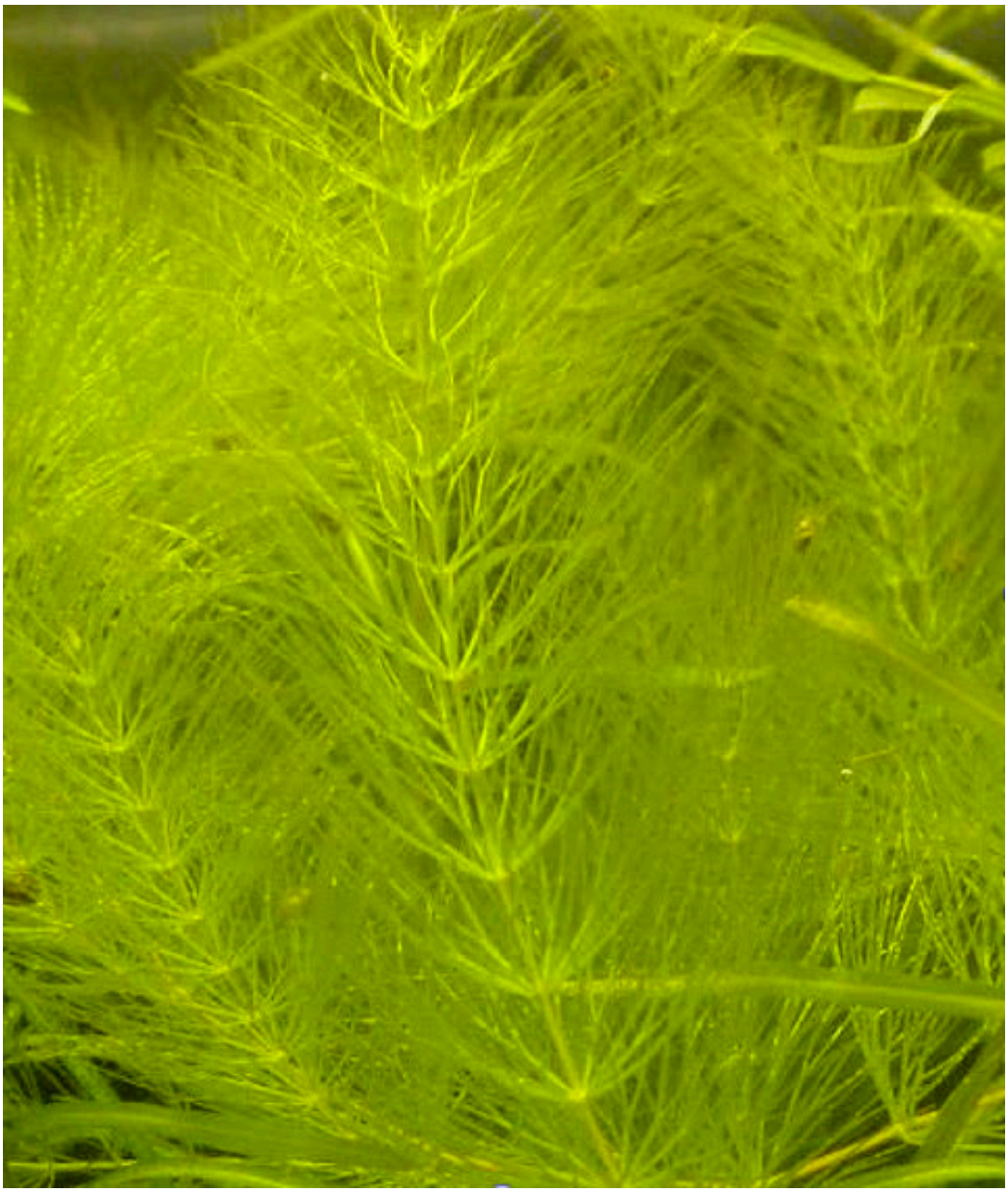

Figure 9. ***Ceratophyllum demersum***

Source: Wilson, (i.a)

Hornwort prefers shallow and warm (+20 °C) coastal waters for growth. The plant is most abundant in inland waters, but it also survives very well in the Baltic Sea, because of its high salinity tolerance. It also prefers biogen-rich waters, and the main reason for its spreading is thought to be eutrophication. (Trei 1991: 89-92) Hornwort is also commonly also used in aquaristics, because it does not need to grow attached to something (Martin, 2020).

3. Algae production measurements

In the practical part of the research, it is described how the author measured the oxygen produced by the algae in the water tanks and by the algae grown naturally in the sea, determined their biomass/dry mass, and calculated their oxygen production or, in other words, the oxygen amount (in mg) that 1g of algae produces in 1 hour. The bigger the algae's oxygen production is, the greater their ability to bind bio-compounds from the water. Chemical samples taken by Georg Martin from the water tanks were also analysed. After the measurements and analysis, the conclusions were made and the most suitable algae species for cleaning wastewater from fish farms and for surviving the longest in artificial environment were assessed.

3.1. Production measurement methodology

The production measurement methodology used in this research paper was developed from the Gaarder and Gran's Light and Dark Bottle Method, which was first used in 1927. In the Gaarder and Gran method, bottles (approximately 200-400 ml) are filled with micro-algal water that is currently being examined, and the oxygen levels in the water are measured. Then, the bottles are closed and placed in a water-rich environment. Half of the bottles are placed in the dark and the other half in the sunlight. After a certain amount of time, which is usually a few days, the amount of oxygen in the bottles is measured again. Thanks to this method it is possible to determine a certain amount of micro-algae's oxygen production. (Pratt, Berkson (i.a): 328)

In this research a similar method was used to determine how much oxygen do the algae used in the project produce and how it differs from the oxygen production of algae growing in a natural environment. Firstly, the bottles used in the experiment were filled with water taken from a certain specified water tank. Subsequently, the amount of oxygen in the water (that was in the bottles) was measured with an oxygen sensor. The algae, previously incubated in the tanks, were placed into the bottles. The amounts of algae were chosen to be as similar as possible. Next, the bottles were placed into the same

water tank, so that the algae taken from the tanks would be in the same environment (under the same temperature conditions). After an hour (the incubation period was registered), the bottles were taken out and a black opaque/lightproof plastic bag was placed on them so that the algae could not continue the photosynthesis process. Then the oxygen in the bottles was measured once more, one by one. After the experiment, the water from the bottles was poured through a sifter, and the remaining algae chunk that was left in the sifter was packed into aluminium foil and each little pack was numbered so its biomass could be later determined.

3.2. Measurements on 18.06.19

On 18 June the author of this research along with research professor Georg Martin travelled to Saaremaa, Kesknõmme, where the Marine Institute's project was taking place. Right next to the shore a round pool filled with seawater had been built, which would later be filled with fish. The seawater was pumped through an aerator, which enriched it with oxygen. Water from the pool was pumped through a coarse filter (which removed some larger grains and bits) and then on to the water tanks, which were placed into four columns. In each column there were 12 tanks (Figure 10).

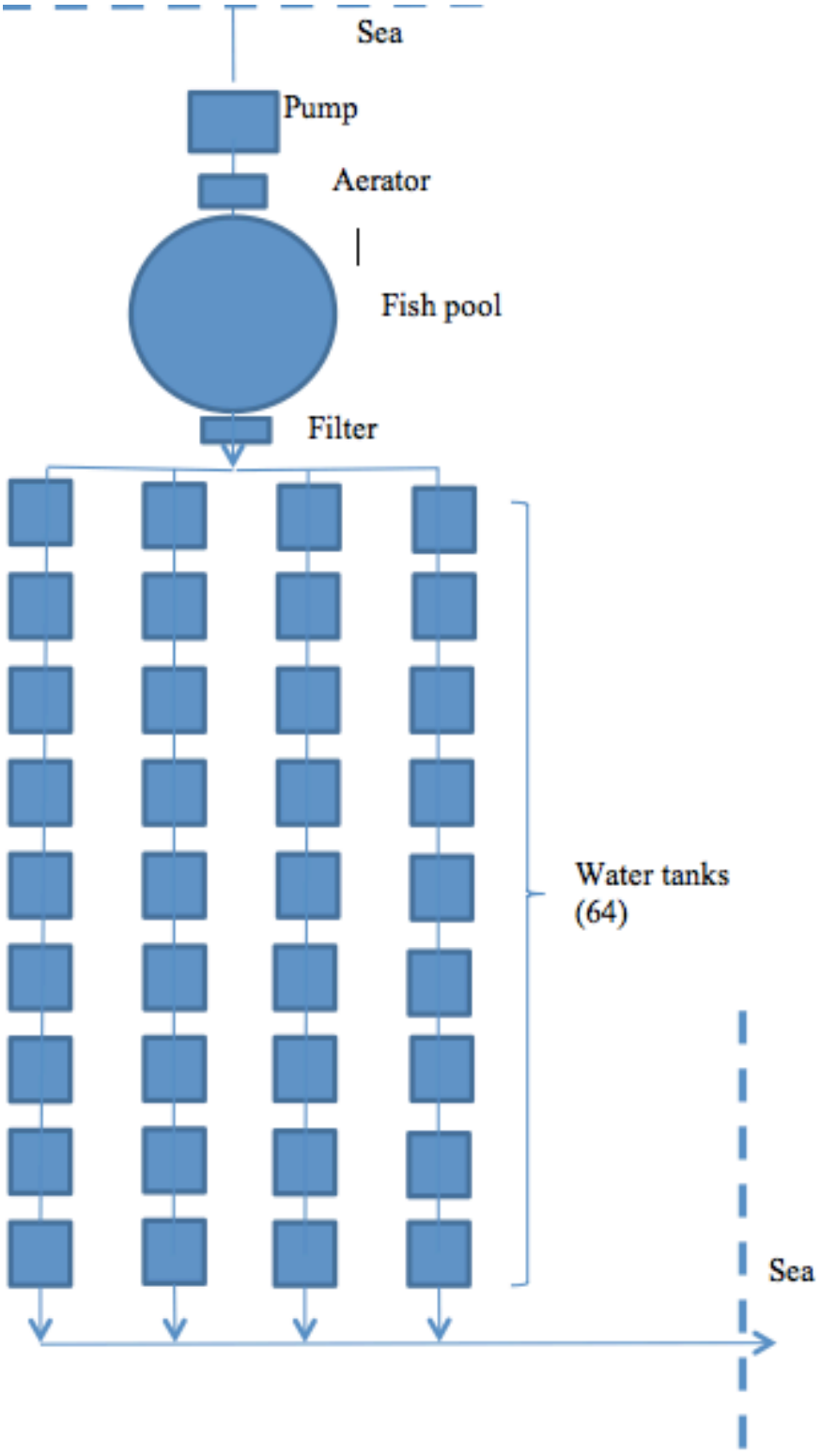

Figure 10. The scheme of the test equipment in Saaremaa, Kesknõmme

Source: Martin, 2019

The first column (on the left side) was for control and no algae were placed into those tanks. Into the remaining three columns the algae specie *Cladophora glomerata* was placed to grow along with seashells (*Mytilus trossulus*) (Figure 11). The seashells were placed in the tanks that were right next to the fish pool (in the first row) because they are active filters that consume thicker undissolved particles from the water, so that only dissolved nutrients would remain for the algaes. The environment created in the tanks was made as similar as possible to the environment of the Baltic Sea.

Figure 11. Weighing seashells (*Mytilus trossulus*), photo by Georg Martin

At that time, *Cladophora glomerata* had been growing in the tanks for a few weeks and much of the algae mass had already coloured brown, which means it was already dead (Figure 12). The reason was probably that the seawater pumping system was not working at its full capacity, therefore the water exchange in the tanks was too slow and the water in the tanks warmed up too fast. *Cladophora glomerata* is a species that is quite demanding of the environment, so the majority died and started to decompose. One of the research author's tasks was to measure how much oxygen the survived algae produced.

The measurements were conducted according to the methodology described in the previous chapter, but for accuracy are described in more detail below.

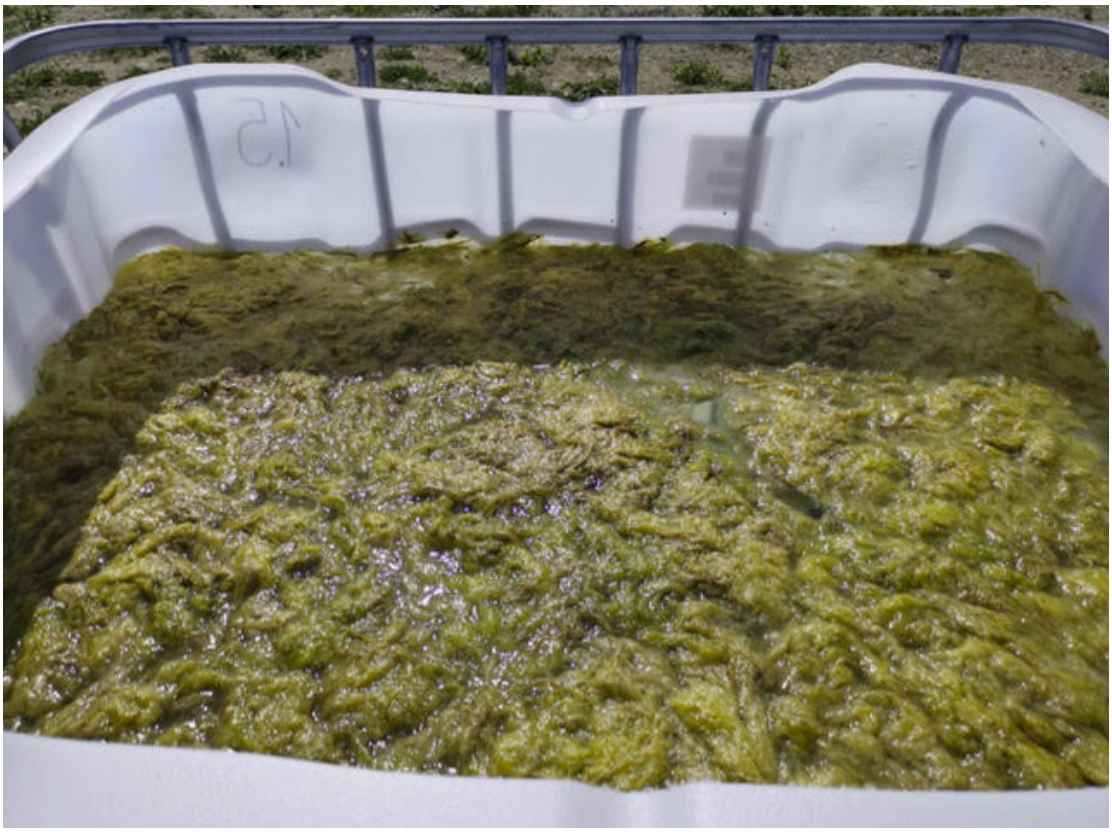

Figure 12. Partly decomposed algae mass (*Cladophora glomerata*) in the tanks, photo by Georg Martin

The following is the description of the algae oxygen production measurement process. The following items were used in the experiment: six bottles, an oxygen measuring sensor, a timer, a sifter, aluminium foil, fresh algae taken from the sea, and algae that were already growing in the tanks for a few weeks. The measurement took place from four to seven o'clock in the afternoon. Firstly, nutrient rich water taken from one water tank was put into the bottles and the oxygen contained in them was measured with a sensor. Next, a small bottle-cap sized amount of algae (*Cladophora glomerata*) was placed into two bottles. The same amount of algae (*Cladophora glomerata*), taken freshly from the sea, was placed into the next two bottles. The last two bottles were left empty for control, so it would be possible to determine the algae photosynthesis activity based on the difference in oxygen production. The bottles were secured into a special frame and

placed in the same water tank (where the algae were taken from) for approximately an hour (Figure 14). After one hour the amount of oxygen in the bottles was measured again (Figure 15).

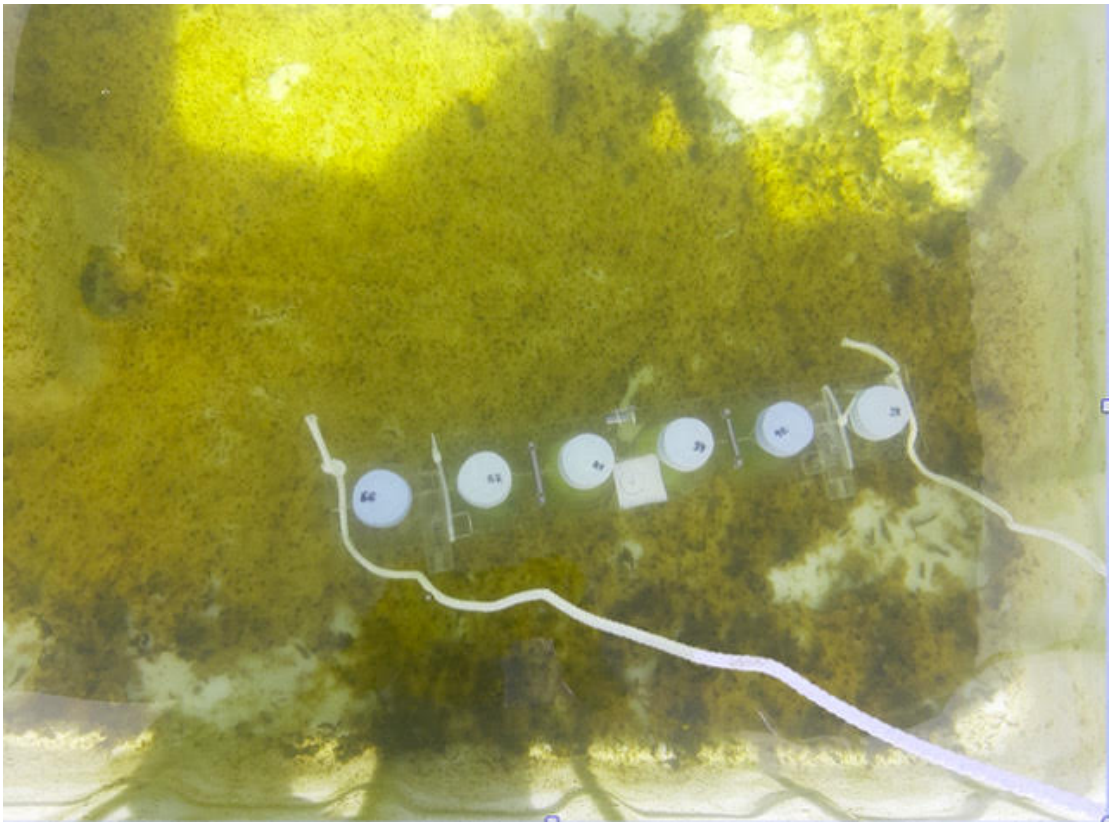

Figure 14. Bottled algae during the experiment, photo by the author

Figure 15. Measuring the oxygen produced by algae, photo by the author

The experiment was repeated twice more. After the experiments, the used chunks of algae were collected with the sifter and packed into aluminium foils and numbered. Each little algae package was taken back to Tallinn, to the Estonian Marine Institute's laboratory. The results of the measurements were written down in a table (Appendix 1) where it can be seen what species were used, where were they taken, the lengths of the experiment, and the amount of oxygen they produced that time. The exact volume of each bottle used was also written down, because they are later used in the oxygen production calculations. The according number of the package in which each algae chunk was stored in was also marked down.

3.3. Measurements on 22.07.19

On 22 July, the author travelled to Saaremaa again and carried out quite the same experiments, but with different species: an algae species *Ulva intestinalis* and a plant species *Ceratophyllum demersum* (Figure 16).

Figure 16. *Ulva intestinalis* in the tank, photo by Georg Martin

This time the subjects were also already growing in the tanks for a couple of weeks and most of them looked healthy and green. During these experiments only the oxygen produced by the algae (and the plant species) in the tanks was measured, so this time the same species were not taken freshly from the sea for comparison. On this day the measurements were repeated twice: six bottles with *Ulva intestinalis* and six bottles with *Ceratophyllum demersum.* The chunks of algae and plants were once again packed into aluminium foil and taken to the Estonian Marine Institute's laboratory. The results of these measurements were also written down in the table (Appendix 1).

3.4. The assessment of algae biomass

On 26 September the assessment of the algae biomass was carried out in the Estonian Marine Institute's laboratory.

The algae, from which the oxygen production was measured in the summer, were brought to the laboratory and put into a freezer. On 26 September the little numbered algae packages were defrosted. Thanks to the numbers marked on the packages it could be determined which algae species was in it, how much oxygen it produced, and on what date was it in the experiment. Next, the same amount of aluminium foils were cut out and numbered again. With a help of a small drinking glass the foils were formed into small basket-like containers. Each basket was weighed with a very precise scale and the weight was written down (Figure 17). Subsequently, each defrosted algae and the other plant chunks were placed into the baskets and then they were put into a drying machine/cabinet (Figure 18).

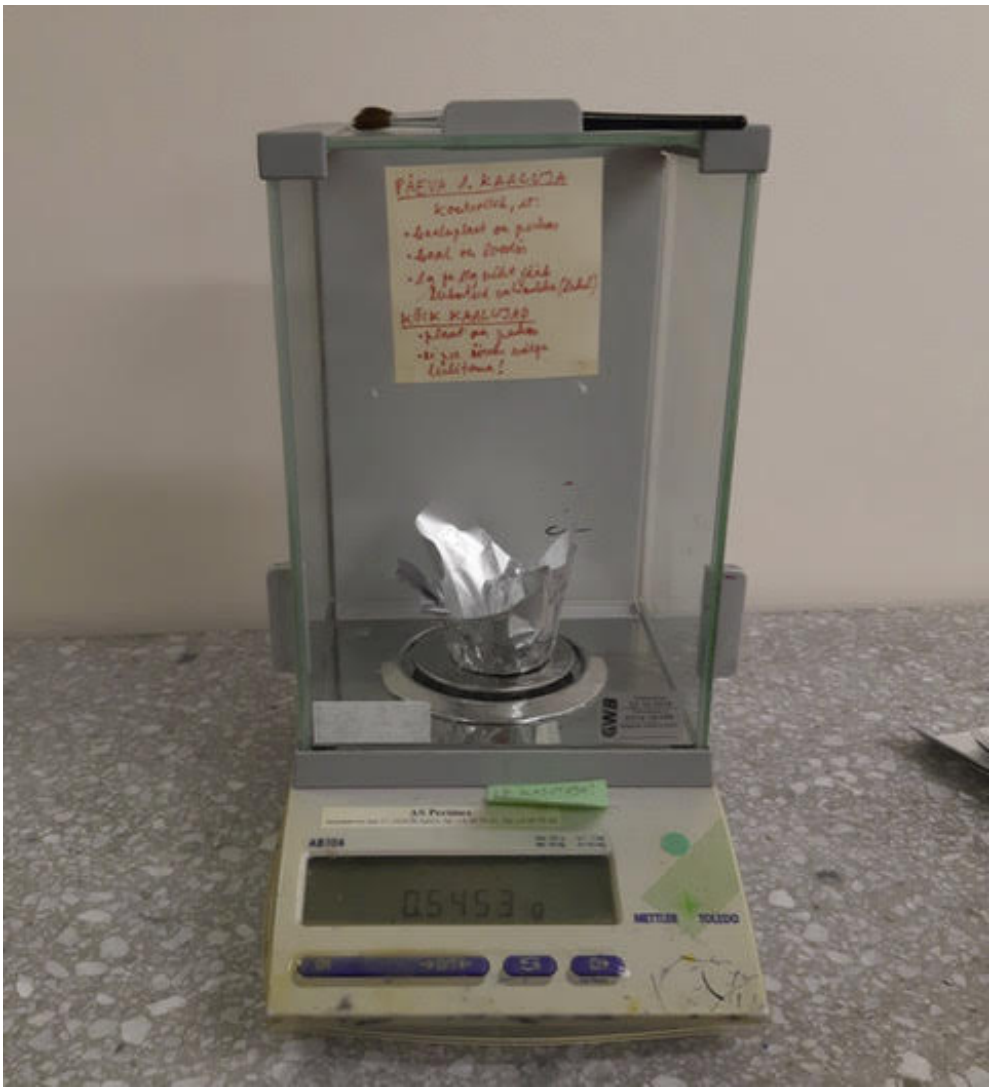

Figure 17. Weighing the aluminium foil baskets, photo by the author

Figure 18. Drying the algae, photo by the author

On 3 October (a week later), the dried algae were taken out of the dryer. Next, the algae along with the foil baskets were weighed again on a very precise scale. This process was very time consuming because the dried subjects had become very brittle and lightweight. From the weight of overall package the weight of previously measured foil basket was deducted, resulting in the algae (and the other plant species) biomass, or dry mass. The results were written down in a table (Appendix 1).

3.5. The results and analysis of chemical samples

During the project, some chemical samples were also taken from the water tanks, to see how much biogens the algae were able to bind. A chemist measured the amount of biogens in the fish pool, in the control tanks and in the algae filled tanks. From that data it could be determined which of the algae and plant species used in the project were the most efficient in cleaning the wastewater. The research paper's author analysed chemical samples taken on two different days: 02.05.19 *Cladophora glomerata* and 25.09.19 *Ulva*

intestinalis. Chemical samples concerning the plant species *Ceratophyllum demersum* were not analysed.

The following biogens were measured from the water in micromoles per litre (μmol/L): nitrogen, phosphorus, phosphate, nitrate, and nitrite. Firstly, the amount of biogens was measured from the fish pool and then from the tanks. Samples were taken from the first control column, where there were no algae or any other species, and then from the remaining three columns (from the second, fourth, eighth, eleventh and sixteenth row). From the algae filled tank rows the average measurement was calculated. The results are shown in the graphs below.

On 02.05.19 the biogen binding of *Cladophora glomerata* was measured from the water. The results show that the amount of nutrients in the control tanks and in the algae tanks are different but not remarkable. The chemist measured the changes in the amounts of phosphorus and nitrogen. The results of the measurement showed that the tanks filled with *Cladophora glomerata* contained less phosphorus than the fish pool, but the difference is small – approximately 0,4 μmol/L, which forms 26% of the phosphorus in the control tank (Figure 19). The measurements from the tanks in the eighth row seem to have a measurement error, because there is a lot more phosphorus in the water than in the control tank without algae and in the tanks before and after them. The discrepancy may have come from an error or from an accidental unknown piece of biogen-rich material (for example bird droppings). However, the measurements can be considered successful because the final results show that algae clean biogens, more specifically phosphorus, from the water, and therefore there is more phosphorus in the last control tank than in the last algae filled tanks.

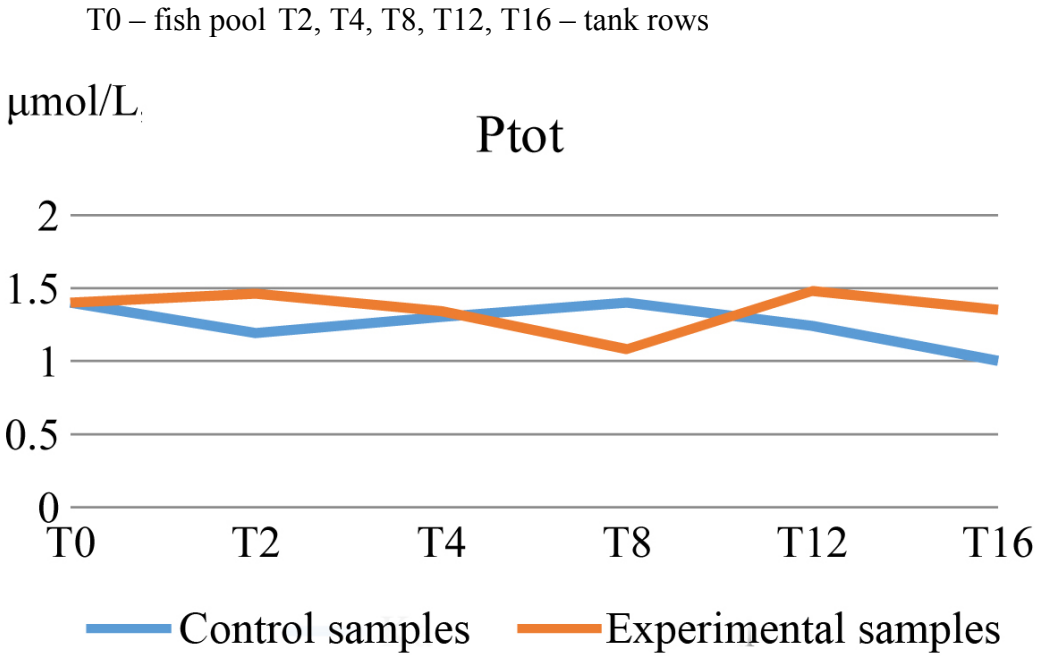

Figure 19. The content of phosphorus in the experimental and control water tanks, 02.05.19

The following figure describes the change of nitrogen biogens during the measurements (Figure 20). The change in nitrogen between the control tanks and the algae filled tanks was insignificant. It can be seen that once again there are less biogens in the eighth row control tanks than in the algae filled tanks. The reason for this could be the previously mentioned water contamination. There is a clear difference in the nitrogen consumption by algae in the last two rows. The difference is about 3 µmol/L, which forms 9% of the nitrogen in the control tank.

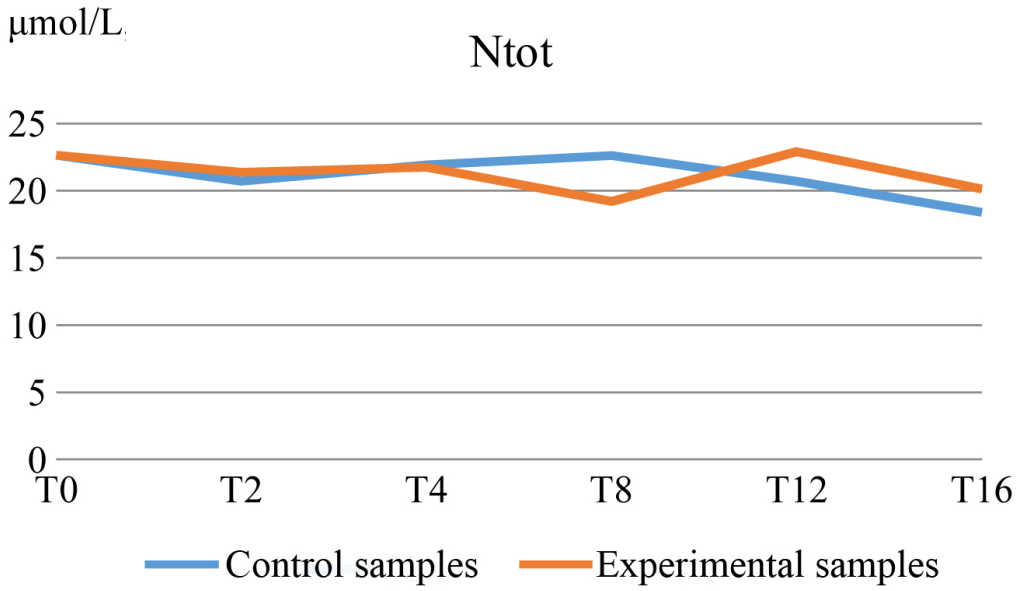

Figure 20. The content of nitrogen in the experimental and control water tanks, 02.05.19

On 25.09.19 the biogen binding of *Ulva intestinalis* was measured from the water. Compared to the previous measurements, it can be seen that the differences of nutrients between the control tanks and the algae filled tanks are much more significant, so it can be said that *Ulva intestinalis* had bound the nutrients much more actively. The following figures describe the changes of phosphorus, nitrogen, nitrate, and nitrite during the measurements.

The figure below shows that the amount of phosphorus decreased significantly in the tanks filled with *Ulva intestinalis* (Figure 21). Throughout the measurements the amount of phosphorus in the algae tanks remained lower than in the control tanks. The difference of phosphorus in the tanks in the last row was around 0,5 µmol/L, which forms 52% of the biogens in the control tank.

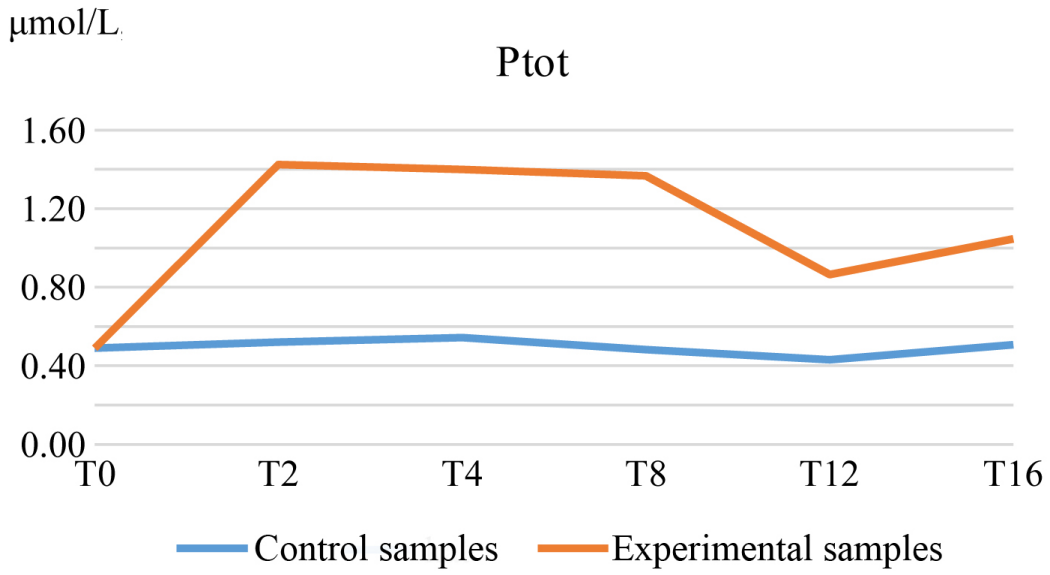

Figure 21. The content of phosphorus in the experimental and control water tanks, 25.09.19

The following figure shows that the algae consumed more nitrogen than phosphorus (Figure 22). Throughout the measurements the amount of nitrogen in the control tanks remained much higher than in the algae tanks. The difference of nitrogen in the tanks in the last row was around 4 µmol/L, which formed about 16% of the biogens in the control tank.

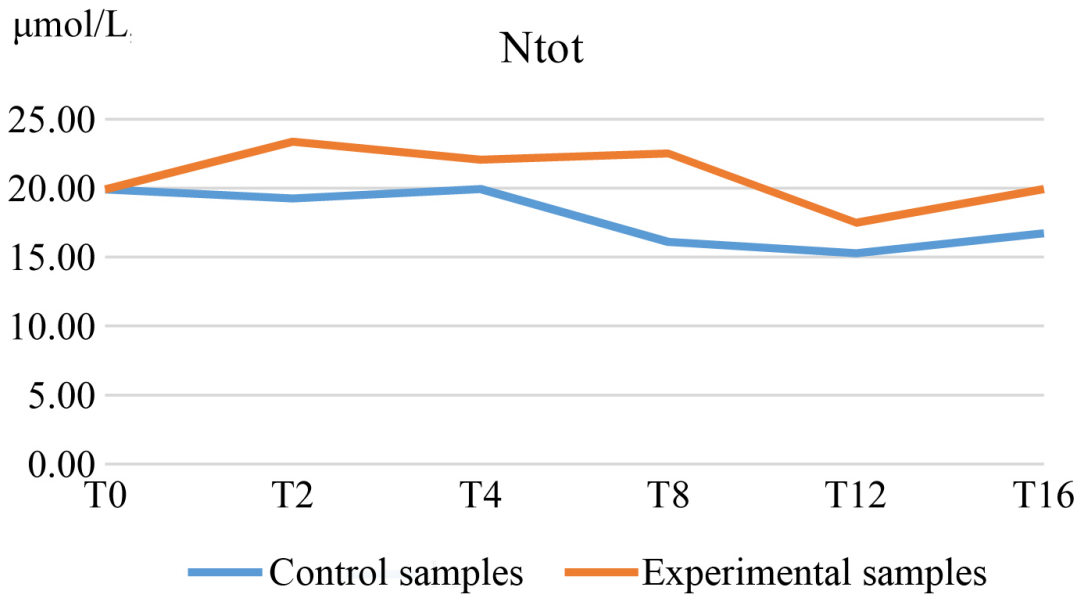

Figure 22. The content of nitrogen in the experimental and control water tanks, 25.09.19

During the chemical measurements taken from the *Ulva intestinalis* water, changes in the content of nitrate and nitrite were also measured. The figure shows that throughout the experiment the amount of the substances decreased gradually and that there is a big difference in the amount of nitrate and nitrite between the control and algae tanks (Figure 23). The difference in nitrates and nitrites in the tanks in the last row was around 0,3 µmol/L, which is about 60% of the substances in the contaminated water.

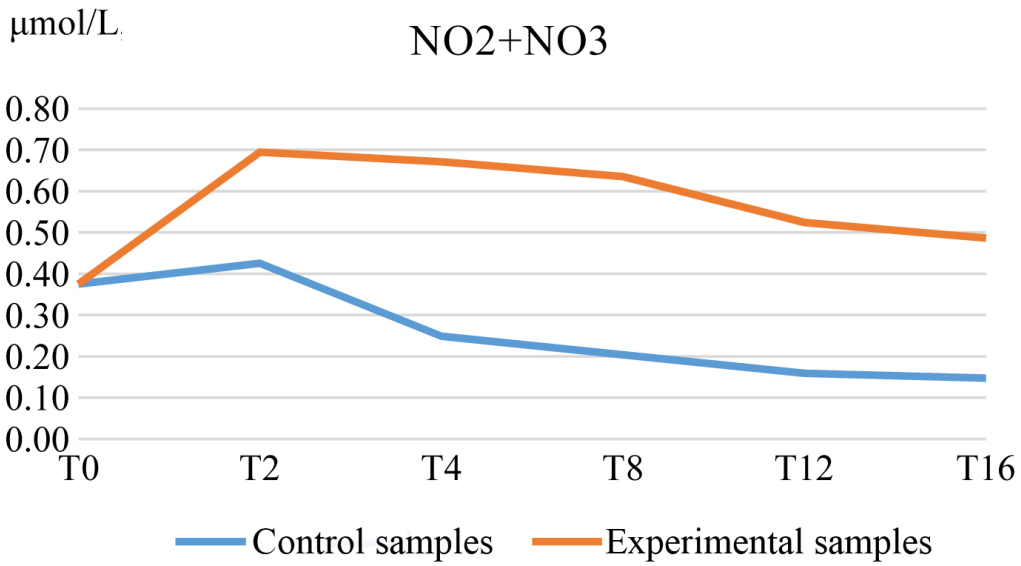

Figure 23. The content of nitrates and nitrites in the experimental and control water tanks, 25.09.19

Ulva intestinalis's phosphate consumption was also measured (Figure 24). Once again, throughout the measurements the amount of phosphate in the algae tanks remained much lower than in the control tanks. The difference of phosphate in the tanks in the last row was around 0,2 µmol/L, which is about 54% of the biogens in the control tank.

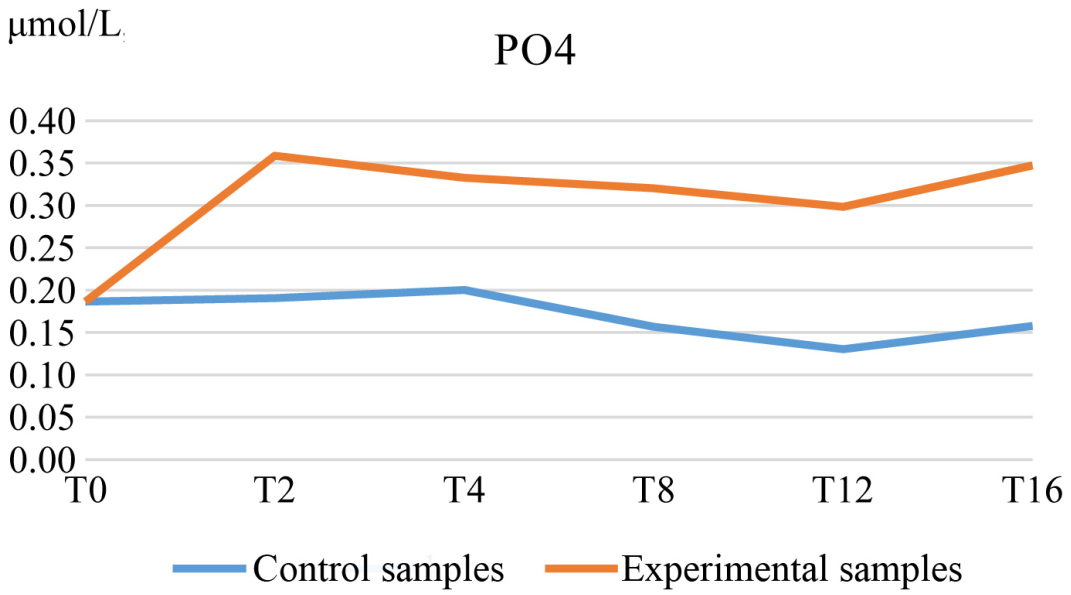

Figure 24. The content of phosphate in the experimental and control water tanks, 25.09.19

The performed chemical analyses show that *Ulva intestinalis* binds twice as much phosphorus and consumes nitrogen more efficiently than *Cladophora glomerata*. *Ulva* was able to bind more than half of the amount of phosphorus (52%) from the water, but only 16% of nitrogen. However, the amount of nitrogen in the water was much higher that the amount of phosphorus. *Ulva intestinalis* removed 4 µmol/L of nitrogen and 0,5 µmol/L of phosphorus. *Cladophora*, on the other hand, removed 9% of nitrogen (3 µmol/L) and 26% of phosphorus and (0,4 µmol/L). The amounts of biogens bound by different species cannot be directly compared because the samples were taken at different times (months).

3.6. Calculations and analysis of oxygen production

The best algae species to clean wastewater are the ones that are photosynthetically active, that is to say which are able to bind maximum nutrients per time unit. The photosynthetic activity of algae is indicated by the oxygen production of the plant, which is determined

by the ratio of algal biomass and the oxygen produced. The more oxygen the plant produces, the more biogens it binds from the water.

The oxygen production was calculated by the following formula:

$$P_O = \frac{(O_2 - O_1) \cdot V}{1000 \cdot m \cdot t}$$

where

P_O – oxygen production *($mgO_2/(g \cdot h)$)*

O_1 – the amount of oxygen in the water before the measurements *(mg/l)*

O_2 – the amount of oxygen in the water after the measurements *(mg/l)*

V – bottle volume *(ml)*

m – algae dry mass *(g)*

t – duration of the measurements *(h)*

The oxygen production of each used species was found and marked into the summarising table (Appendix 1). To analyse the results, the averages of the oxygen production from the measurements performed on the same date and time were marked into the table below (Table 1).

Table 1. The average oxygen production of the three species

	Species	Where were the species taken from?	Date + time	O_2 production ($mgO_2/(g \cdot h)$)
1	*Ulva intestinalis*	Tank	22.07_16:43	25.1
2	*Ulva intestinalis*	Tank	22.07_17:58	29.38
3	*Ulva intestinalis*	Tank	25.09_13:51	10.62
4	*Ulva intestinalis*	Closed tank	25.09_13:57	8.25
5	*Cladophora glomerata*	Sea	18.06_12:22	4.01
6	*Cladophora glomerata*	Sea	18.06_14:34	3.41
7	*Cladophora glomerata*	Sea	18.06_13:36	3.49
8	*Cladophora glomerata*	Tank	18.06_12:27	1.78
9	*Cladophora glomerata*	Tank	18.06_14:33	1.67
10	*Cladophora glomerata*	Tank	18.06_13:40	1.49
11	*Ceratophyllum demersum*	Tank	22.07_16:49	7.15
12	*Ceratophyllum demersum*	Tank	22.07_18:02	4.38

For the reader to get a better visual overview, a bar chart based on the data in the table was compiled (Figure 25). On the bar chart below it can be seen that *Ulva intestinalis* has the highest oxygen production.

$mgO_2/(g \cdot h)$

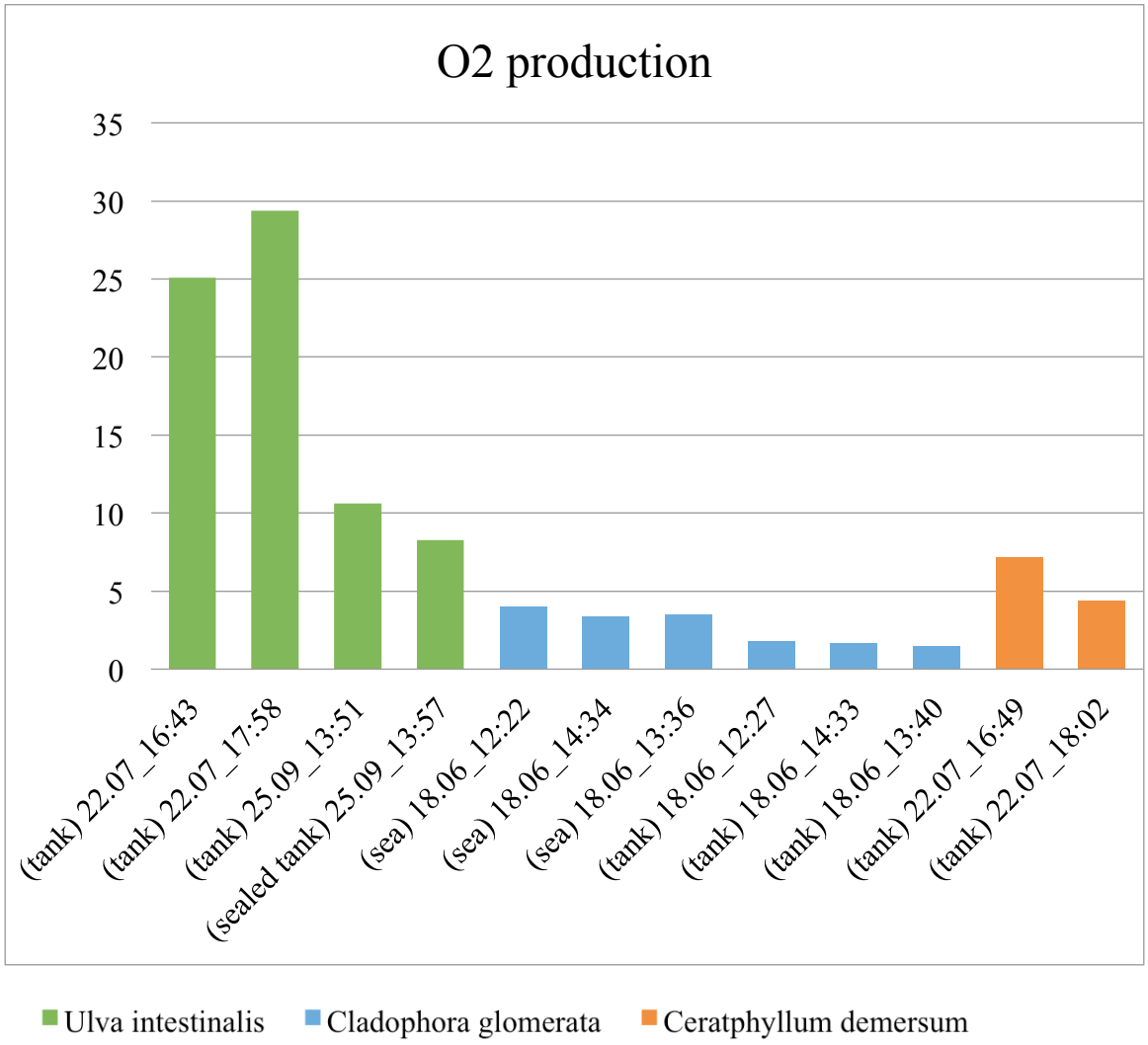

Figure 25. A bar chart about the average oxygen production of the three species, measured on three separate dates.

The most suitable algae species is the one that consumes the most biogens per unit time and can survive the longest in the artificial conditions in the tanks. The determined oxygen production shows which species bound the most biogens from the water, and the chemical analysis shows exactly how much nutrients were consumed.

Ulva intestinalis has the largest oxygen production 8 – 29 $mgO_2/(g \cdot h)$, *Ceratophyllum demersum* ranked second with oxygen production 4 – 7 $mgO_2/(g \cdot h)$. *Cladophora glomerata* showed remarkably smaller results with 1 – 4 $mgO_2/(g \cdot h)$. This shows that

Ulva is photosynthetically most active as it bound nutrients more and faster (and produces oxygen from them) than the other species. It can be seen that the *Ulva intestinalis* grown in ordinary tanks produced the most oxygen, and the ones grown in closed containers produced a little less. The bar chart shows that the best result were from July, because then the plants were the most active. The measurements were taken at around five o'clock in the day. It can be assumed that the results would have been even better if the measurements had been taken at midday, because then there is most sunlight and the algae are the most photosynthetically active.

The results from the chemical samples showed that *Ulva* bound biogens twice as efficiently compared to *Cladophora.*

Ulva intestinalis and *Ceratophyllum demersum* survived the best in artificial conditions and they also had the highest oxygen production. *Cladophora glomerata* was the least able to adapt under those conditions. *Ceratophyllum demersum* is a plant that is rather grown in aquaristics, and it was used more as a reference object in the measurements. As a result of the performed measurements, it can be stated that the best algae species to clean wastewater from fish farms is *Ulva intestinalis.*

*Ulva'*s suitability proves the hypothesis that thin and fast-growing algae are the most suitable for cleaning wastewater from fish farms. Like mentioned in a previous chapter, the amount of biogens depends on the shape and structure of the algae's thallus. It was also mentioned that algae which are thin, thread-like and branched absorb nutrients the best. These plants grow fast and their metabolism is fast as they absorb nutrients quickly and in large quantities. Thicker, less branched and flatter plants absorb less nutrients, because their metabolism is slower. (Trei 1991: 14).

Through the conducted measurements it is hard to say why *Ulva intestinalis* is better at adapting in artificial conditions than *Cladophora glomerata* because both species are thin and thread-like. Also, *Cladophora glomerata* is branching and *Ulva intestinalis* is not. It is likely that the adaptability and the ability to bind different biogens is due to their different thallus structure, which are compared in the chapter 2.3.2.. Both species' thalli are one cell thick/wide, but *Ulva'*s cells form a hollow tube and *Cladophora's* cells are arranged simply one after the other. *Ulva'*s cells are smaller and lie tightly next to each

other. So, *Cladophora* has fewer cells per the same surface area than *Ulva.* Maybe that is the reason why *Ulva* achieved better results than *Cladophora.*

The second reason could be that the artificial conditions created in the water tanks were more similar to the natural conditions of *Ulva'*s habitat than to *Cladophora'*s usual conditions. It is known that both species prefer more coastal areas, but perhaps the water temperature in the tanks was not suitable for *Cladophora.* (Trei 1991: 44)

Summary

Over half of the fish consumed by humans are grown under artificial conditions in fish farms. At the same time, aquaculture causes a lot of damage to the environment through pollutants that end up in our natural waters. The most environmentally friendly solution for wastewater treatment would be to use natural resources. This research focused on wastewater cleaning with algae.

The theoretical part of this research paper explains what fish farming is, its negative impacts on the environment, and presents the algae species that were used for the project and explains their ability to clean polluted seawater.

The practical part of this research paper was made in cooperation with the University of Tartu Estonian Marine Institute science- and development project "Treatment of marine water-based fish farm waters by cultivating macro algae".

In this project algae grown in artificial environments inside water tanks and algae grown freely in the nature were studied and compared. The oxygen production of the algae and the waters' chemical content was measured. Afterwards it was assessed which species were the most suitable for cleaning wastewater from fish farms, and which were the healthiest and survived the longest in an artificial living environment.

The proposed hypotheses ("*There are algae species in the Baltic Sea that can be successfully cultivated in artificial conditions in tanks,"* and *"The most suitable algae species for cleaning wastewater are thin and fast growing Cladophora glomerata and Ulva intestinalis")* turned out to be partly true. The most suitable species to clean wastewater is *Ulva intestinalis,* because compared to the other species it was much more photosynthetically active, and thus it bound the most biogens from the water. *Ulva* also turned out to be the most resilient to survive under artificial conditions. On the contrary, *Cladophora glomerata* did not survive as well in those conditions, and it was also significantly lower in photosynthesis activity. The third species analysed, *Ceratphyllum demersum*, adapted well in the artificial conditions but its oxygen production was quite low.

A question which should be further investigated would be: “What are the best artificial conditions in the water tanks for *Ulva intestinalis?”*. It would be important to know the most suitable temperature, amount of water, flow rate, concentration of biogens, etc.

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*i.a – without year (ilma aastata)

Appendix 1. Oxygen measurement results

Date	Algae species	From where were they taken?	Duration of the experiment (*h*)	O_2 concentration at the beginning of the experiment (*mg/L*)	O_2 concentration at the end of the experiment (*mg/L*)	Mass of aluminium foil (*g*)	Algae + mass of aluminium foil (*g*)	Algae's biomass (*g*)	The volume of the bottles (*ml*)	O_2 production ($mgO_2/g/h$)
18.06.19	*Cladophora glomerata*	Sea	0.92	7.7	10.9	0.605	1.227	0.622	608	3.41
18.06.19	*Cladophora glomerata*	Sea	0.92	7.7	12.2	0.6516	1.3046	0.653	611.95	4.60
18.06.19	*Cladophora glomerata*	Tank 3.13	0.92	7.7	9.2	0.6038	1.1334	0.5296	609.85	1.88
18.06.19	*Cladophora glomerata*	Tank 3.13	0.90	7.7	9.3	0.5694	1.2143	0.6449	609.1	1.68
18.06.19	*Cladophora glomerata*	Tank 1.12	0.85	8.4	9.2	0.619	0.9767	0.3577	611.95	1.61
18.06.19	*Cladophora glomerata*	Tank 1.12	0.78	8.4	9.5	0.6944	1.1923	0.4979	609.85	1.72
18.06.19	*Cladophora glomerata*	Sea	0.88	8.4	10.5	0.6232	1.036	0.4128	609.45	3.51
18.06.19	*Cladophora glomerata*	Sea	0.92	8.4	9.6	0.5497	0.7904	0.2407	609.1	3.31
18.06.19	*Cladophora glomerata*	Sea	0.78	8.2	9.8	0.58	0.9856	0.4056	608	3.06
18.06.19	*Cladophora glomerata*	Sea	0.78	8.2	11	0.6075	1.165	0.5575	611.95	3.92
18.06.19	*Cladophora glomerata*	Tank 2.13	0.78	8.2	10	0.5938	1.7155	1.1217	609.85	1.25
18.06.19	*Cladophora glomerata*	Tank 2.13	0.72	8.2	10.2	0.5992	1.5875	0.9883	609.1	1.72
22.07.19	*Ulva intestialis*	Tank	0.75	11.9	29	0.5556	1.0196	0.464	615.35	30.24
22.07.19	*Ulva intestialis*	Tank	0.75	11.9	30.5	0.4882	1.2264	0.7382	612.35	20.57
22.07.19	*Ulva intestialis*	Tank	0.75	11.9	30.6	0.514	1.1333	0.6193	608.05	24.48
22.07.19	*Ulva intestialis*	Tank	0.65	12	14.6	0.4792	0.6147	0.1355	608.4	17.96
22.07.19	*Ulva intestialis*	Tank	0.65	12	15.7	0.449	0.5445	0.0955	611.45	36.45
22.07.19	*Ulva intestialis*	Tank	0.65	12	16.1	0.5289	0.6434	0.1145	612.35	33.73
22.07.19	*Ceratophy-llum demersum*	Tank	0.90	11.9	14	0.4463	0.7654	0.3191	608.4	4.45
22.07.19	*Ceratophyl-lum demersum*	Tank	0.90	11.9	14.7	0.4679	0.7717	0.3038	608.9	6.24
22.07.19	*Ceratophyl-lum demersum*	Tank	0.90	11.9	15	0.4388	0.6339	0.1951	609.1	10.75
22.07.19	*Ceratophyl-lum demersum*	Tank	0.77	12.3	13.6	0.4809	0.7224	0.2415	613.2	4.31
22.07.19	*Ceratophyl-lum demersum*	Tank	0.77	12.3	13.4	0.4934	0.6469	0.1535	598.3	5.59
22.07.19	*Ceratophyl-lum demersum*	Tank	0.77	12.3	13	0.453	0.6248	0.1718	609.85	3.24

25.09.19	*Ulva intestialis*	Tank	1.02	14.8	19.7	0.5451	0.8116	0.2665	608.9	11.01
25.09.19	*Ulva intestialis*	Tank	1.02	14.8	20.6	0.4931	0.8311	0.338	609.1	10.28
25.09.19	*Ulva intestialis*	Tank	1.02	14.8	23	0.4935	0.9629	0.4694	614.9	10.57
25.09.19	*Ulva intestialis*	Tank (sealed)	1.30	14.8	20.9	0.4438	0.8301	0.3863	598.3	7.27
25.09.19	*Ulva intestialis*	Tank (sealed)	1.30	14.8	19.6	0.4359	0.7568	0.3209	609.85	7.02
25.09.19	*Ulva intestialis*	Tank (sealed)	1.30	14.8	22.5	0.4251	0.7715	0.3464	612.3	10.47

Abstract

The most suitable algae species for cleaning wastewater from fish farms

Wastewater from fish farms pollutes the sea, causing eutrophication. The Baltic Sea is an inland sea and due to that, very fragile. This research paper focuses on cleaning fish farming wastewater with algae.

The objective of this paper was to evaluate which algae species would be the most suitable for cultivating in tanks and cleaning wastewater from the fish farms.

The hypotheses were that there are algae species in the Baltic Sea that can be successfully cultivated in artificial conditions in tanks, and that the most suitable for cleaning water are thin and fast-growing algae species *Cladophora glomerata* and *Ulva intestinalis.*

The method used in the paper was to measure the oxygen production of algae growing freely in the sea compared to algae planted in the wastewater tanks and measuring the dry biomass of algae. From the biomass the oxygen production of the algae was calculated. Also, the changes of chemical components in the water and algae's ability to bind those components were measured.

Both research questions were answered, and the hypotheses partly confirmed. The experiments were carried out with local species *Ulva intestinalis, Ceratophyllum demersum* and *Cladophora glomerata.* From them, *Ulva* adapted best to artificial living conditions in wastewater tanks. *Cladophora,* on the other hand, could not adapt and died. *Ulva intestinalis's* oxygen production was also the highest, showing it used most of the biogens from the wastewater.

In conclusion, it can be said that the thin and fast-growing algae species *Ulva intestinalis* is most suitable for cleaning wastewater from fish farms.