

Anaerobic fermented algae *Furcellaria lumbricalis* tablets as a dual-purpose fertilizer and substrate for sustainable seedling growth

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Abstract. This article presents a study focused on the creation, evaluation of anaerobic fermented algae *Furcellaria lumbricalis* pressed biomass tablets as a dual-purpose fertilizer and substrate for sustainable seedling growth. The research aims to address the need for more sustainable agricultural practices by exploring alternative solutions to chemical fertilizers. The methodology involved collecting and preparing *Furcellaria Lumbricalis* algae from the Baltic Sea coast, followed by anaerobic fermentation in a small-scale bioreactor. The resulting digestate was formed into tablets using a designed press and template. A growth experiment was conducted using basil seeds, comparing the performance of seedlings grown with the algae tablets to those grown with traditional substrate. The results showed that the seedlings grown using the anaerobic fermented algae tablets exhibited comparable growth rates and biomass to those grown with conventional methods. Effectiveness of the tablets can be attributed to the breakdown of organic matter during the fermentation process, which makes nutrients more accessible to plants and improves their uptake. However, further research is necessary to optimize the concentrations and formulations of the anaerobic fermented algae tablets. The effectiveness of these tablets should be evaluated in different plant species and growing conditions. In conclusion, this study provides promising evidence that anaerobic fermented algae *Furcellaria Lumbricalis* tablets could serve as an effective and sustainable solution for fertilizer and substrate needs in seedling growth.

1 Introduction

An essential component of aquatic ecosystems is sea algae. They possess a unique and diverse future potential. Algae have a wide range of economic applications, and their biological and functional characteristics make them excellent additions and supplements in the manufacturing of food, such as thickeners and nutritional boosters. Pharmaceuticals and the treatment of diseases both use algae. Algae are used as antioxidants in cosmetics, and their elements may be found in sunscreen and anti-aging treatments. Algae are employed in agriculture as biostimulants and organic fertilisers. A recent breakthrough is the production of biomaterials from algae, including biopolymers, composites, and bioplastics.

1,800 species of brown algae, 6,200 species of red algae, and 1,800 species of green algae are estimated to exist in marine and oceanic settings. About 200 species are used economically, including 10 algal types being widely cultured [1].

The underutilization of marine algae which recently washed ashore in Latvia and is a natural resource that is underutilised in Latvia, demonstrates the significance of this study.

The red algae *Furcellaria lumbricalis*, also referred to as *furcellaria*, and the brown algae *Fucus vesiculosus*, often known as bladderwrack, are the two types of algae most used commercially along the Latvian coast. *Furcellaria* has been utilised in Latvia since 1965 to produce furcellaran (food additive E407) [2]. By utilizing washed-up sea algae biomass, the eutrophication of the Baltic Sea would be reduced, leading to a decrease in nitrogen and phosphorus pollution [3].

The Liepaja Municipality officials frequently classify the debris of sea algae that has washed up on the coast as rubbish. According to preliminary estimates, 9,300 m³ of macroalgae biomass waste are hauled from the Liepaja shore with disposal expenses of 45,792 euros in 2022. The Liepaja Municipality Municipal Administration recognises it as a nuisance. The decomposed biomass might not be seen as waste but rather as a resource, such as for the creation of fertiliser pressed tablets.

2 Materials and methods

Collecting and preparing *furcellaria lumbricalis* algae from Baltic seacoast, fermenting the algae anaerobically in small scale 25l bioreactor, digestate chemical composition testing, forming digestate solid fraction in

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to tablets with designed forming press and template, conducting the growth experiment with basil seeds, analysing the data, and interpreting the results.

2.1 Collecting materials

The methods of harvesting *Furcellaria lumbricalis* depend on various factors such as the location of the algae, environmental conditions, and available resources of harvesters. It should be noted that the red algae *Furcellaria lumbricalis* has two subtypes - free-floating and fixed. It is recommended to harvest free-floating red algae using agricultural or specialized equipment, or manually. Fixed red algae are attached to rocks in the seabed, making their extraction complex and beyond the scope of this discussion [4]. The collection of washed-up sea algae on the shore serves as a potential source of methane gas in the environment. As the algae decompose on the coastal areas, greenhouse gas emissions such as CO₂ and CH₄ are generated. Depending on the conditions, the decomposition of sea algae can result in emissions of up to 38.73 t CH₄/t [5]. Macroalgae *Furcellaria lumbricalis* was collected from seashore to produce a fertilizer. The algae were manually collected on February 6, 2023, using rakes and human resources at the coastline of the city of Liepaja. The coordinates of the algae collection site are 56°30'33.1"N 20°59'21.6"E. Prior to further processing, the algae were rinsed in seawater.

2.2 Anaerobic fermentation

In a controlled laboratory setting on February 6, 2023, the production of a algae pressed fertiliser tablets was conducted. Purified algae material amounting to 25 litres was placed in a sealed biogas anaerobic fermentation system with a capacity of 30 litres. The sealed container, equipped with electric heating and a thermostat (precision ± 0.5°C), housed the input materials consisting of purified seaweed without inoculum. The operating temperature was maintained at 50°C. To measure the volume of exhaust gas (precision ± 1%), a Qianwei Kromschroder G-1.6 electronic gas flow meter was employed. The methane content was measured using a gas analyser comprising a methane sensor MQ-4 and a data processing device with data storage capability (precision ± 2%). A gas storage tank, connected to the gas outlet pipe and featuring a valve for closure. Setup installation included a pressure relief valve/siphon [6]. On February 20, 2022, a biomass collecting, and drainage procedure was carried out, extracting biomass from the anaerobic fermentation equipment. The liquid fraction of the digestate displayed a distinct plant residue aroma, which should be considered when scaling up fertilizer production.

After separation and preparation, the resulting fraction of the digestate was dried and prepared for agrochemical testing and pressing into tablets.

2.3 Agrochemical testing

In the field of agricultural science, the assessment of agrochemical properties is crucial for optimizing the use of organic-derived products. The testing aimed to provide valuable insights into the composition and suitability of this digestate as a potential agricultural resource. The Total Nitrogen content of the *Furcellaria lumbricalis* digestate was determined using the Kjeldahl method, a widely accepted technique for nitrogen analysis. This method involves the digestion of organic material with concentrated sulfuric acid, resulting in the conversion of nitrogenous compounds into ammonium sulphate. The resulting ammonium sulphate is then quantified using titration or spectrophotometric methods. In the case of the *Furcellaria lumbricalis* digestate, the analysis revealed a Total Nitrogen concentration of 560 mg/l. To assess the Exchange Acidity of the digestate, a specialized methodology designed for organic-derived products was employed. In this procedure, a 1:5 ratio of 1 M potassium chloride solution was mixed with the digestate, followed by agitation for an hour. This process ensures the transfer of weakly bound hydroxyl and aluminium ions responsible for acidity exchange into the solution. After the agitation period, electrodes were immersed in the resulting solution, and the pH was measured. The Exchange Acidity of the *Furcellaria lumbricalis* digestate was determined to be -6.10 pH using the aforementioned methodology. This measurement provides valuable insights into the potential impact of the digestate on soil acidity levels, which is crucial for determining its suitability as a fertilizer or soil amendment. The agrochemical testing of *Furcellaria lumbricalis* digestate yielded significant findings related to Total Nitrogen content and Exchange Acidity. With a Total Nitrogen concentration of 560 mg/l, this digestate exhibits considerable potential as a nutrient-rich organic fertilizer. Furthermore, the Exchange Acidity measurement of -6.10 pH suggests that the digestate may contribute to the reduction of soil acidity levels, enhancing soil health and fertility. These results underscore the value of *furcellaria lumbricalis* digestate as a sustainable agricultural resource and provide essential scientific insights for its application in farming practices.

2.4 Formation of Tablets

The template, crafted from metal, allows for the precise placement of the digestate material at the end of the press tip, facilitating the compression process. The process of forming digestate tablets involves the utilization of the custom press template. Shown in Figure 1.



Fig. 1. The template, crafted from metal. Source: author.

First, the dried digestate solid fraction is prepared for compression. The solid fraction is obtained through the removal of moisture from the digestate, resulting in a more concentrated form suitable for tablet formation. Once the solid fraction is ready, the press template, consisting of a metal structure, is positioned at the end of the press tip. This template acts as a guide and support for the compression process. The dried digestate solid fraction is then carefully added onto the template, ensuring even distribution and proper alignment. Next, the press is engaged, exerting pressure on the digestate material. This pressure, applied through the press tip, compacts the solid fraction and facilitates the formation of tablets. The compression process is carefully controlled to ensure consistent tablet size and density. See Figure 2.

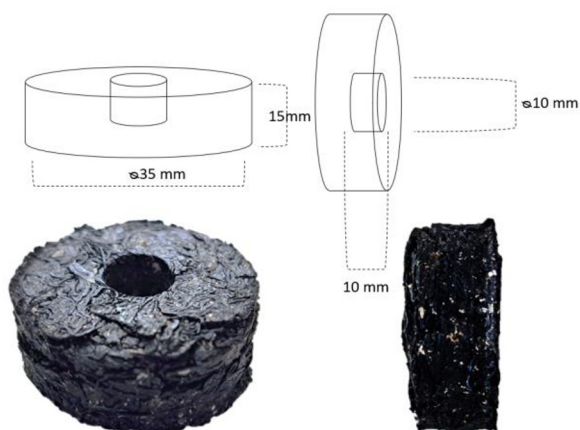


Fig. 2. The template, crafted from metal. Source: author.

2.5 Growth experiment

Seed growth experiment conducted in an automatic greenhouse, wherein the greenhouse is divided into two distinct sections Figure 3.

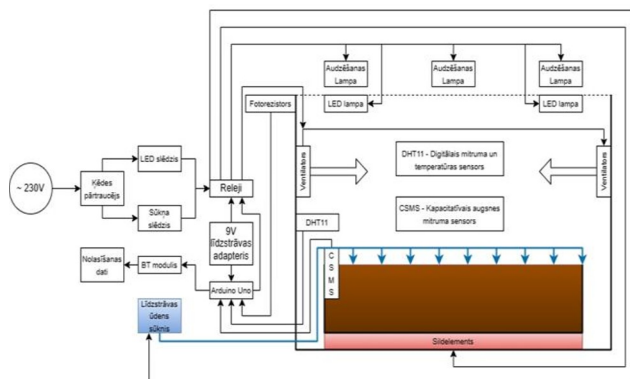


Fig.3. The automatic greenhouse scheme. Source: author.

Each section was sown with 150 seeds, allowing for a comparative analysis of growth patterns between the two sections. The automatic greenhouse provided an ideal environment for conducting the seed growth experiment. The greenhouse was divided into two equal parts, ensuring that each section had an equal number of seeds. This division allowed for a direct comparison of growth parameters and observations between the two sections. One section is reference section another section is with fermented algae tablets Figure 4.



Fig. 4. 150 basil seeds sowed each side, left side with algae tablets, right reference side. 8th day after sowing. Source: author.

A total of 150 seeds were sown in each section of the greenhouse. Care was taken to ensure uniformity in the sowing process, with each seed placed at an appropriate depth in the soil medium. Adequate spacing was maintained between the seeds to minimize competition for resources and optimize growth conditions. The automatic greenhouse provided a controlled environment for the experiment. Parameters such as temperature, humidity, and light intensity were monitored and adjusted as necessary to maintain optimal growth

conditions. This control ensured that any observed differences in seed growth between the two sections could be attributed to other factors and not environmental variations. Throughout the experiment, regular observations were made to assess seed germination, seedling growth, and overall plant development.

3 Results and discussion

The purpose of the experiment was to compare the growth patterns of seeds with and without the addition of fermented algae tablets.

The automatic greenhouse provided an ideal environment for the experiment. It offered controlled conditions such as temperature, humidity, and light intensity, which were continuously monitored and adjusted to maintain optimal growth conditions for the seeds. This ensured that any observed differences in seed growth between the two sections could be attributed to the presence or absence of the fermented algae tablets rather than environmental variations.

The left side was treated with algae tablets, while the right side served as the reference section. The tablets were added to the soil medium to provide additional nutrients and promote seedling growth. On the 8th day after sowing, observations were made and recorded. Table 1.

Table 1. Results of seed germination. Source author.

Day	With tablets	Without tablets
3rd	7	4
8th	119	105
13 th	145	132
18 th	145	132
23 th	145	133

Table 1 presents the results of seed germination. In the 3rd day, the section with algae tablets showed a germination rate of 7 seeds, while the reference section had 4 seeds germinated. This suggests that the addition of algae tablets had a positive impact on early seed germination. By the 8th day, the germination rates increased significantly, with 119 seeds germinated in the section with tablets and 105 seeds in the reference section. The higher germination rate in the section with tablets indicates that the additional nutrients provided by the algae tablets likely stimulated seedling growth. Continuing the observations, on the 13th, 18th, and 23rd day, both sections exhibited similar germination rates, with 145 seeds germinated in the section with tablets and 132 to 133 seeds in the reference section. These results suggest that the initial growth boost provided by the tablets may have levelled off, and other factors may now be influencing the growth patterns in both sections.

The author used Table 1 to make comparative statistical analyse between the germination of seeds according to the days using student's test. The Student's t-test is a method of testing hypotheses about the mean of a small sample drawn from a normally distributed population when the population standard deviation is

unknown. Author first came up with two hypotheses to examine the development of seedlings containing and not containing seaweed tablets. Contrary to the null assumption (H0), which implied that there would be no appreciable difference in seedling development between the two groups, the other possibility (H1) stated that there would be a detectable difference. Author computed the variations in growth for each day between the groups to evaluate these predictions. These were the outcomes:

The third day revealed a growth difference equal 3 between seedlings with tablets and those without tablets. By the 8th day, the difference increased to 14, favouring the seedlings with tablets. The growth differences were constant at 13, 13, and 12, respectively, on days 13, 18, and 23.

Author then determined the mean of these differences, which equalled 11. This figure provided author with a picture of the two groups' general growth trends.

To determine the degree of data variability, we calculated the standard deviations of the differences. The result's standart deviation, illustrates the variance in the data points around the mean, was 4.53.

Using the mean and standard deviation, author obtained the t-value, that represents the difference between both groups in comparison to the range of variability within the groups. The t-value we obtained was 5.45.

After determining the degrees of freedom (df = 4), author looked up the critical t-value for a significance level (α) of 0.05. The critical t-value for analysis was approximately 2.776. Comparing the calculated t-value to the essential t-value (2.776), author found that the computed t-value (5.45) was greater. Considering this, author decided to choose alternative hypothesis (H1) over null hypothesis (H0). This indicates that the development of seedlings with tablets compared to those without differs considerably.

The results of this experiment demonstrate the potential benefits of using fermented algae tablets as a supplement for seed germination and early growth. The tablets appeared to have a positive impact on the germination, as evidenced by the higher number of germinated seeds in the section treated with tablets. However, as the experiment progressed, the effects of the tablets seemed to diminish, and the growth patterns between the two sections became more comparable.

Further research with a wider variety of plant species and longer observation periods would provide a more comprehensive understanding of the effects of fermented algae tablets on seed growth and plant development.

4 Conclusions

In conclusion, the paired t-test analysis provided evidence that the use of anaerobic fermented algae tablets positively impacted seedling growth compared to the conventional method without tablets. The tablets demonstrated their potential in enhancing seedling growth, making them a promising and sustainable solution for fertilizer and substrate needs in seedling

growth. Further research may focus on optimizing the concentrations and formulations of these algae tablets to explore their application in a variety of plant species and growing conditions.

Seed growth experiment conducted in an automatic greenhouse showed promising results regarding the positive influence of fermented algae tablets on seed germination and early growth. The tablets appeared to enhance germination rates and possibly stimulate initial growth. Further investigations are warranted to explore the long-term effects and potential applications of fermented algae tablets in agricultural practices. By harnessing the potential of algae in agricultural practices, we can promote sustainable and environmentally friendly farming methods while maximizing the benefits of this abundant natural resource.

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References

1. M. Syrpas, P.R. Venskutonis (2020), *Algae for the production of bio-based products*, In *Biobased Products and Industries*, 203-243 (Elsevier, 2020)
2. M.M. Monagail, L. Morrison, J. Appl. Phycol. **32**(2), 1287-1300 (2020)
3. C.J. Murray, B. Müller-Karulis, J. Carstensen, D.J. Conley, B.G. Gustafsson, J.H. Andersen, *Front. Mar. Sci.* **6**, 2 (2019)
4. P. Kersen, T. Paalme, L. Pajusalu, G. Martin, *Bot. Mar.* **60**(2), 207-218 (2017)
5. R. Lybæk, *Development, operation, and future prospects for implementing biogas plant : the case of Denmark*, Use, Operation and Maintenance of Renewable Energy Systems: Experiences and Future Approaches, 111-144 (2014)
6. U. Zaimis, R. Jurmalietis, A. Jansone, *Engin. Rural Dev.* 1916-1919 (2018)