„Stockholm Junior Water Prize, 2025”

Microalgae produced in circular economy as an alternative protein source

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1. Short Summary

A possible solution to the increasingly urgent protein deficiency could be green biorefining, which is a process to get edible proteins out of plants. But one of the produced by-products called brown juice is harmful to the environment in large quantities, for example, it can cause eutrophication. In my research we wanted to get advantage of this situation by using the brown juice as an additive in the growing media of microalgae to increase the production and the nutritional value of the harvested biomass. Recycling brown juice this way helps to fight with protein deficiency because the harvested biomass contains high amount of protein, suitable for human or animal consumption.

2. Introduction

My name is Dóra Százvai and I am a student of the György Bessenyei Secondary Grammar School in Kisvárda, I am currently in 11th grade. I grew up in the countryside in a village and I always had a great interest in biology and chemistry, these had been my favourite subjects for a long time. A childhood dream of mine is to become a veterinarian, but in the recent years I got interested in the life of a researcher. Fishing had always played a curtail role in my family's life, since they lived close to river Tisza. I enjoy this activity with my father, and the last year as an exchange student I got to experience what is like when people living depends on the natural water around them. People in Southern Louisiana heavily rely on the bayous for food (as fish, crab, and shrimp), transportation, and many families' business is based on them. But unfortunately, these waters are polluted which I only realized when I first volunteered to help clean up section with my friends. Since then, I am really interested in water management and environmental protection.

3. Statement

Our research was carried out at Institute of Applied Plant Biology, at the University of Debrecen under the supervision of assistant professor Nóra Bákonyi Ph.D. The microalgae were grown in the experimental greenhouse called Biodrome and extra measurements were taken in the laboratory. I conducted every step of the research project myself, with the

guidance of my mentor. In the preparation for this competition, I got help from both of my supervisor and the teachers at my high school.

4. Summary

The rapid growth of human population significantly contributed to global protein deficiency, in the past years. The production of leaf protein concentrate through green biorefining has proven to be promising in overcoming protein deficiency. However, during this process a large amount of by-product called brown juice (plant extract, phytoserum), is generated. The safe management of brown juice causes more problems because the large-scale disposal of it to the environment holds great risks. One of these effects is the contribution to the eutrophication of natural waters because of its nitrogen and phosphorous content. On the other hand, brown juice is proven to be useful in other areas, for example, in the right concentration it can be a great nutrition source in different growing media. But these properties combined can be used to our advantage, by adding brown juice to the growing media of microalgae. With this process we can utilize the by-product from the production of an alternate protein source by recycling it in microalgae production to produce more protein suitable for human consumption.

In this study our goal was to investigate the applicability of brown juices extracted from different plants in various concentrations to increase microalgae biomass production and nutritional value of biomass.

During the research, *Arthrospira platensis* (SAG 257.80) microalgae was grown in cost efficient-spirulina media treated with brown juices of purple sweet potato (*Ipomoea batatas L.*) and wheat sprout (*Triticum aestivum L.*) separately, in different concentrations. The study involved 5 treatments, along with a control group. The cultures were maintained for 12 days in a grow tent, where environmental factors were monitored. At the start and then every 4th day, the following measurements were taken: optical density, pH, electrical conductivity. Besides these, photographic documentation was used to record the growth of the cultures. On the 12th day, cell morphology of the microalgae was examined under a microscope, and the fresh weight of the harvested algae biomass was measured. Then after lyophilization the dry weight was also recorded.

Based on our results, it can be concluded that the investigated microalgae behaved differently because of the different concentrations of brown juices.

5. Introduction

5.1 Topic introduction, research aims

The rapid growth of the human population heavily contributes to protein deficiency, because the fertile land that ensures the food supply for people has been decreasing due to environmental pollution and global climate change. This affects other branches of agriculture like livestock breeding. As a result of all this the demand for high quality protein supplementation has increased, especially for an alternative that could partially or potentially completely replace animal proteins. The production of plant proteins through green biorefining has proven to be promising in overcoming protein deficiency. During this process, proteins are extracted from plant parts that have a high protein content such as soybeans or alfalfa. However, during this process a large amount of by-product, called brown juice is generated. The safe management of brown juice causes more problems, because the large-scale disposal of it to the environment holds great risks. (BÁKONYI et al., 2020) One of these effects is the contribution to the eutrophication of natural waters because of its nitrogen and phosphorous content. As of right now there is only one trial factory operating in Hajdúnánás, Hungary. During operation it produces 20 m^3/day of brown juice, which gets 1000 times diluted and then it is used to water the crops at the factory's fields. But this is not sustainable because of the overproduction of this by-product. It cannot be stored at room temperature because it begins to deteriorate within hours. Possible solutions of this problem are freezing or fermenting the brown juice, but these procedures are not cost effective. Studies have shown that brown juice can be used in as an additive in growing medias in the right concentration. (MAKLEIT et al.,2018) During our research we wanted to find an innovative solution for recycling brown juice in the perspective of circular economy, by combining two already known process: green biorefinery and microalgae cultivation.

Nowadays the cultivation of microalgae is becoming more popular, and the interest for microalgae-based food supplements are increasing due to its nutritional value. The microalgae biomass has high protein content, and it can supplement 30% of a person's daily protein intake. Microalgae cultivation has many advantages, microalgae have one of the quickest growth rates among microorganisms, their cultivation does not depend on the seasons and can be done in areas that are infertile for agricultural use, other than that their by-product can be 100% recycled. It is cost-effective because the growing media can be partially or fully substituted. Because of all these microalgae can be implemented into circular farming systems.

Because we tend to see algae thrive in eutrophic waters, we wanted to test whether application of different concentrations of brown juices from different plants affect the growth of microalgae. Our goal was to increase the quantity and the quality of the harvested microalgae biomass.

5.2 Green biorefining

Green biorefining is a process to get edible protein out of plants. During this process the fresh green biomass is being pressed to separate it into two fractions, green juice (GJ) and fibres. After that the leaf protein concentrate (LPC) is being coagulated from the green juice, a possible way for that is the application of a microwave heat treatment. (FÁRI, SZABOLCSI-DOMOKOS et al., 2022) The LPC is a gel-like substance, therefore it is easier to handle than protein extracted by other methods and it is easy to separate it from the by-product called brown juice (BJ) **(Figure 1)** To implant this process into circular economy we need to use plant parts that are unused by other branches of agriculture like parts of the crops that are left on the field, or parts that had been cut done to make a plant more desirable for customers at markets. In the future systems can be established where plants are specifically cultivated for biorefining with this we can minimalize the waste.

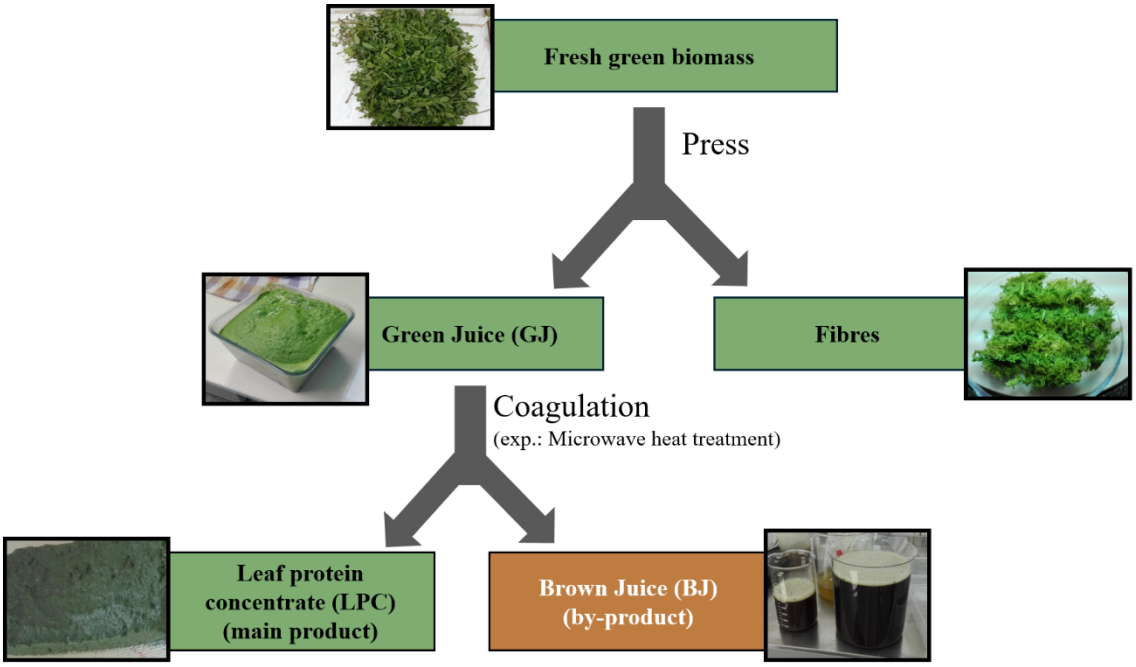


Figure 1: Sematic figure of green biorefining
Source: Bákonyi et. al., (2018), own edit

5.3 Brown Juice (BJ)

Brown juice is a brown or yellowish-brown liquid, which nutritional value is high because it still contains carbohydrates, amino acids, pigments, vitamins, and enzymes. Many research is aimed to get advantages of these, it is already proved that it can be used in yeast production, it can be a nutrition source in different growing media, and a milk substitute for young calves and an excellent bio stimulant. During the biorefining of different plants, we obtain BJ-s with different compositions, therefore BJ-s of different origins have different effects. **(Figure 2)**

Figure 2: BJ-s obtained from different plants
Source: own picture (2025)

5.4 Introduction of *Arthrospira platensis* (SAG 257.80)

Arthrospira platensis, more commonly referred to as Spirulina has high tolerance towards environmental factors as demonstrated by its widespread natural occurrence (FURMANIAK et al., 2017) Its morphology is characterized by a filamentous, multicellular, cylindrical structure **(Figure 3).** It has high nutrient content, consisting of all essential amino acids, vitamins, and minerals. The harvested biomass serves as a rich source of carotenoids, fatty acids, and proteins, which makes it suitable for both human and animal consumption (SAFI et al., 2014). Consumption of this microalgae is associated with numerous health benefits, including anti-inflammatory effects and cholesterol-lowering properties. In my research we used the *Arthrospira platensis* strain SAG 257.80, obtained from the Algae Collection of the University of Göttingen, as the inoculum.

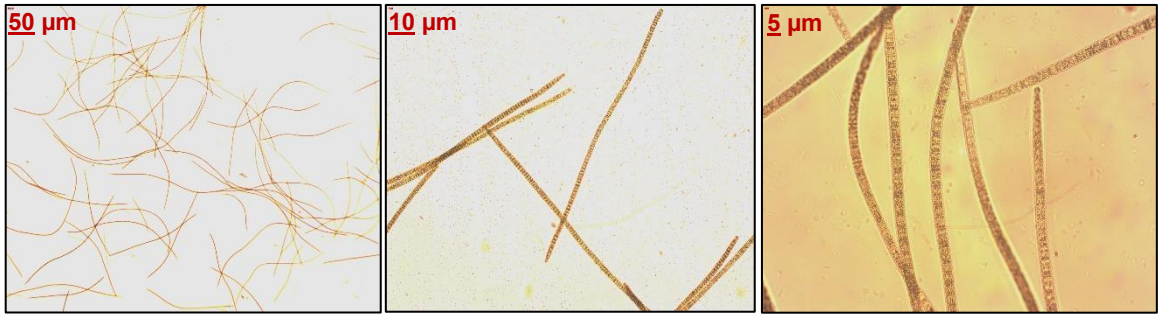


Figure 3: Microscopic examination of *Arthrospira platensis* (SAG 250.80)
Source: own picture (2025)

5.6 Introduction of plants used in the study

I wanted to study the brown juice of the following two plants because, in addition to their popularity over the past few years, wheat is the most widely cultivated crop worldwide, while various types of sweet potatoes rank fifth. Moreover, wheat is a monocot, while purple sweet potato is a dicot, meaning they have different nutrient uptake mechanisms, which results in different compositions of the brown juice extracted from them.

5.6.1 Purple sweet potato (*Ipomoea batatas, L.*)

Purple sweet potato (PSP) (*Ipomoea batatas, L.)* is mainly cultivated for its tuberous roots, which are excellent sources of carbohydrates. Recently it became more popular in Hungarian kitchens as well and it is considered a superfood due to its high nutritional value and antioxidant content. Although less common, its young leaves are also edible and can typically be found in salads. After harvest, a significant amount of green biomass - stems and leaves- is left on the fields which remains unused. This can be used for plant protein production, in green biorefining that is implemented into circular economy. **(Figure 4)**

Figure 4: PSP as ornamental plant
Source: own picture (2025)

5.6.2 Wheat sprout (*Triticum aestivum*, *L.*)

Wheat sprout (WS) is the young wheat (Triticum aestivum, L.), harvested at a stage when only the cotyledons appear above ground. Its most known form is wheat sprout juice, extracted through cold pressing of a few-day-old wheat. Consumption of it is proven to have numerous health benefits such as boosting the immune system, its detoxifying effects, help in digestion. Furthermore, it is an excellent protein source, making it suitable for alternative protein production, because it can be cultivated in small crates, and it can be harvested for green biorefining after a few days. **(Figure 5)**

Figure 5: WS cultivated in crates for juice production
Source: https://frissbuzafu.hu (2025)

6. Materials and methods

6.1 Coagulation process of LPC from different GJ-s

The plants were grown in a controlled environment and previously harvested and pressed to obtain GJ. In the case of WS the whole green biomass was used while only the stems and the leaves of PSP was pressed. After that we used 800 W output microwave to coagulate the LPC from the GJ-s. Many differences were observed, first PSP needed 5 minutes and 100 °C to coagulate while WS only needed 3 minutes and 82 °C. The LPC obtained from the GJ of PSP was on the bottom, it was a darker grey colour and its texture was sandy, the BJ was on the top and it was cloudy and greenish brown. **(Figure 6)** On the other hand, the LPC obtained from the GJ of WS was on the top, it was green coloured and the texture of it was sandy as well, the BJ was on the bottom, first it was reddish brown and then it turned to dark brown and translucent. **(Figure 7)**

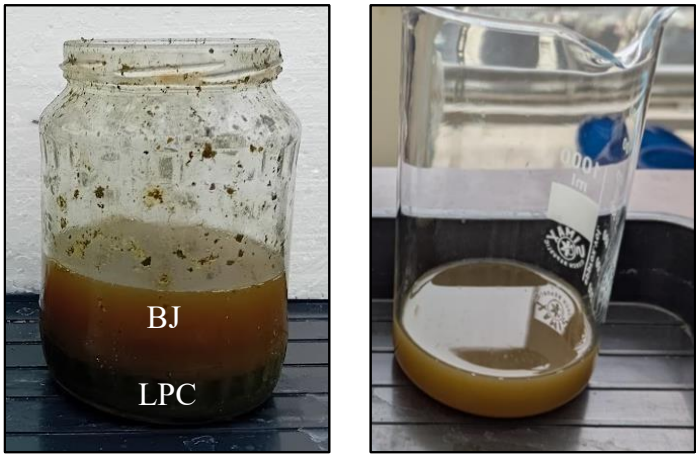


Figure 6: LPC and BJ separated from the microwave heat treatment of PSP GJ
Source: own picture (2025)

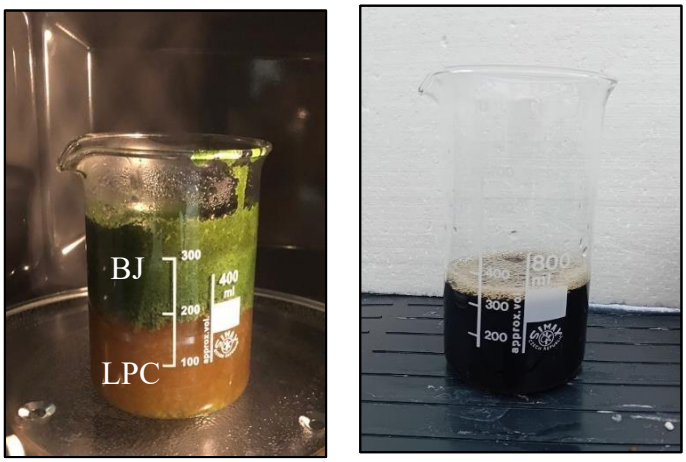


Figure 7: LPC and BJ separated from the microwave heat treatment of WS GJ
Source: own picture (2025)

6.2 Inoculation process of culture media

We prepared the cost-efficient spirulina liquid growing media according to an American recipe previously modified at by the university. **(Table 1)** We grew microalgae in a unique, innovative, integrated grow tent cultivation system for 12 days **(Figure 8)** The tent walls were covered with reflective foil, and the plant-growing lamp had individually adjustable light strips, ensuring that all cultures received equal amounts of light, which we verified through measurements before starting the experiment. Cultures were grown autoclavable polycarbonate reactor tubes that volume was 1.7 L. BJ-s from different plants were added at various concentrations—1%, 2.5%, 5%, 7%, and 10%—to the liquid medium. **(Table 2)** Including the control, 11 reactor tubes were inoculated with microalgae culture, each with a final volume of 0.5 litres. **(Figure 9)** Air was pumped into the polycarbonate reactor tubes to ensure the mixing of the cultures, while an Extech CO_2/Humidity/Temperature Datalogger monitored

environmental factors, saving data onto an SD card. The collected data was recorded and evaluated in Microsoft Excel.

For 1 litre of media		
Ingredient:	**Quantity:**	**Price:** (In the case of buying industrial quantities)
Baking Soda (Sodium Bicarbonate) ($NaHCO_3$)	15.79 g	220 Ft/kg ~ 3,57 Ft/1l **0,59 €/kg ~ 0,0096 €**
Saltpeter (Potassium Nitrate) (KNO_3)	1.97 g	336 Ft/kg ~ 0,66 Ft/1l **0,91 €/kg ~ 0,0018 €**
Sea Salt(NaCl)	1.00 g	249 Ft/kg ~0,249 Ft/1l **0.67 €/kg ~ 0.00067 €**
Diammonium Phosphate ($(NH_4)_2HPO_4$)	0.10 g	170 Ft/kg ~ 0,017 Ft/1l **0.46 €/kg ~ 0.000046 €**
Iron supplement (Fe_2SO_4) (mixed with lemon juice or green tea)	0.115 ml (Fe_2SO_4: 0,04 g)	538 Ft/kg ~ 0,0215 Ft/1l **1.45 € / kg ~ 0.000058 €/l**
Ion changed water	Fill it up till 1 litre	

Table 1: Ingredients for cost-efficient spirulina liquid growing media
Source: American recipe modified by the Institute of Applied Plant Biology, University of Debrecen, own edit (2025)

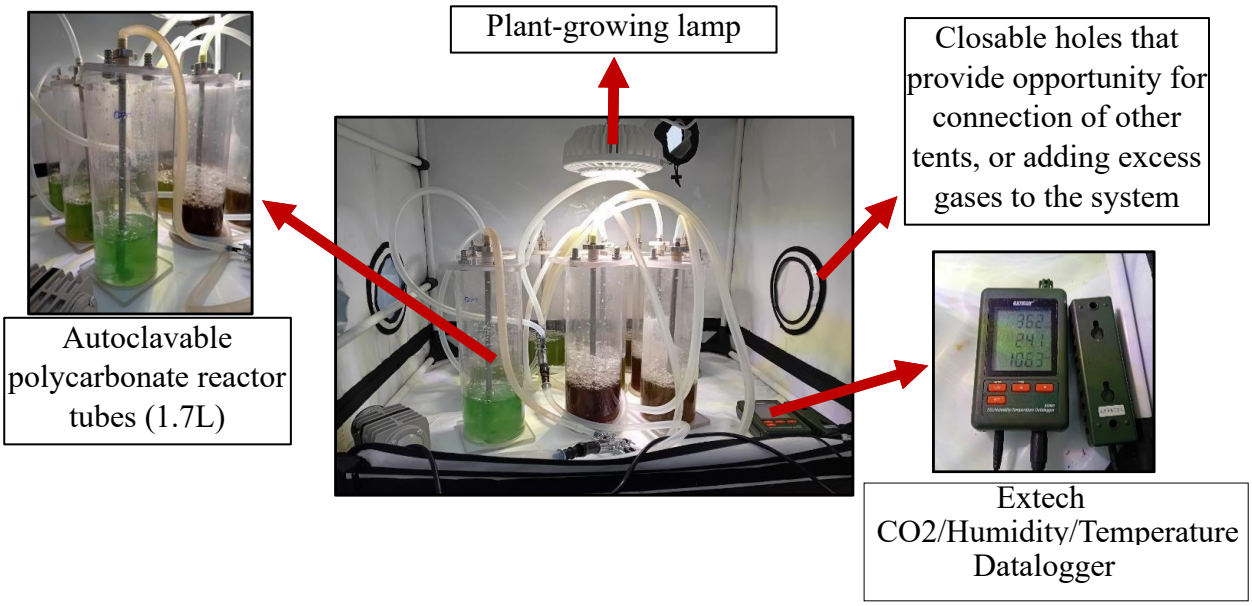


Figure 8: Growth tent cultivation system
Source: own pictures (2025)

BJ	BJ concentration	Treatment			
		Inoculum(ml)	BJ (ml)	Cost-efficient Spirulina media (ml)	Total (ml)
Control	0%	25.0	0.0	475.0	500.0
PSP	1%	25.0	5.0	470.0	500.0
PSP	2.5%	25.0	12.5	462.5	500.0
PSP	5%	25.0	25.0	450.0	500.0
PSP	7%	25.0	37.5	437.5	500.0
PSP	10%	25.0	50.0	425.0	500.0
WS	1%	25.0	5.0	470.0	500.0
WS	2.5%	25.0	12.5	462.5	500.0
WS	5%	25.0	25.0	450.0	500.0
WS	7%	25.0	37.5	437.5	500.0
WS	10%	25.0	50.0	425.0	500.0

Table 2: Composition of treatments (PSP-purple sweet potato, WS- wheat sprout, BJ-brown juice
Source: Own edit (2025)

Figure 9: Preparation of growth tent
Source: own pictures (2025)

6.3 Measurements that were taken every 4th day

At the beginning of the experiment and then every fourth day, we took 4 ml samples from the reactor tubes for measurements. One of the most important elements of biomass production is continuous, uniform growth, which is also essential for the industry to achieve economic and competitive algae production. To monitor the growth of the cultures, we measured the optical density (OD) using an Amersham Biosciences Ultrospec 2100 pro spectrophotometer. For accurate measurements, a reference must first be set, which in our case was distilled water. According to the literature, measurements were performed at 580 nm. The spectrophotometer uses a light source capable of creating specific wavelengths. We took four measurements to minimalize error, considering that algae cells settle quickly if left undisturbed. pH was

measured using a METTLER TOLEDO SevenEasy pH meter, which provides quick and simple measurements due to its glass membrane electrode. The electrical conductivity (EC) of the media was measured with a STEP Systems COMBI 5000 device. This provided information about the concentration of the dissolved ions in the media **(Figure 10)**

Figure 10: Measurements taken every 4th day: OD, pH, EC
Source: own pictures (2025)

6.4 Harvesting the cultures and determination of the fresh weight

The cultures were maintained for 12 days. The contents of the reactor tubes were filtered through a 32 µm filter paper to separate the algal biomass from the filtrate. **(Figure 11)** The filtrate was collected in 50 ml Falcon tubes for future use. We plan to reuse this filtrate for fertilizing soils, aiming to replace the nutrient loss from the removed green plant parts. Fresh weight was determined using an OHAUS PIONEER analytical balance.

Figure 11: Harvesting the microalgae biomass
Source: own pictures (2025)

6.5 Lyophilization and determination of the dry weight

We froze the harvested microalgae cells at -20°C and then the samples were lyophilized (freeze-dried). Lyophilization is a widely used method for determining the dry weight. To remove the water, the samples must be frozen and then the machine creates vacuum and produces heat to remove water molecules. Then, the dry weight was measured using an OHAUS PIONEER analytical balance. **(Figure 12)**

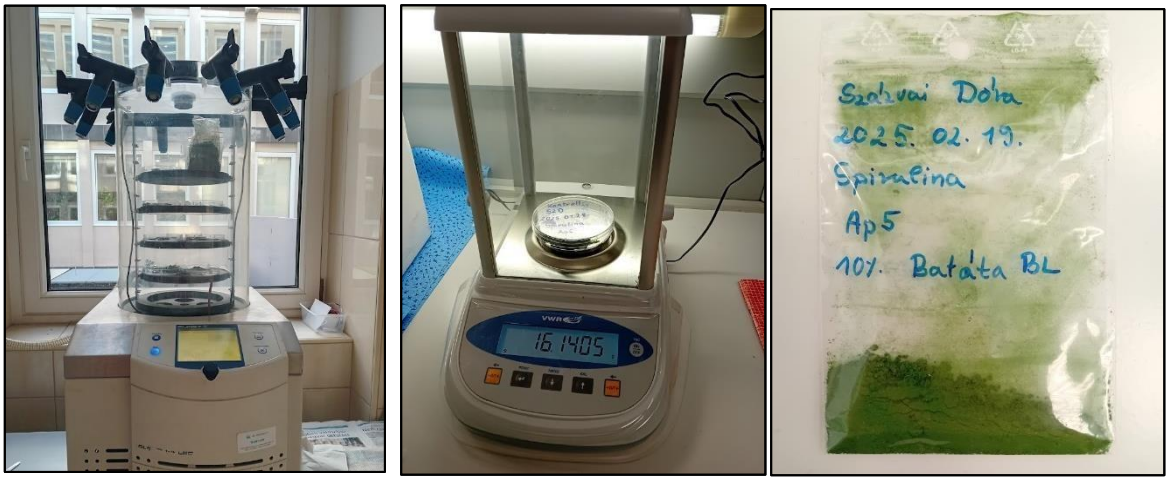


Figure 12: Lyophilization, measuring the dry weight of the samples, and dried algae biomass

Source: own pictures (2025)

7. Results, conclusion

7.1 Results of measurements taken every fourth day

Photographic documentation was used to record the growth of the cultures. We took pictures of the reactor tubes every fourth day. The cultures treated with PSP BJ started adapting to their changed environment on the 4th day, and they have completely stabilized by the 8th day, which can be seen by their deep green colour. **(Figure 13)** On the other hand, the cultures treated with WS BJ started producing different pigments by the 4th day. I would like to highlight the cultures treated with the 2.5% and the 5% BJ-s, because first they both started producing yellow and orange pigments - probably xanthophyl and carotenoids-, but on the 8th day they started to adapt, and they completely stabilized by the 12th day. In contrast, the mediums containing 7% and 10% BJ-s initially produced orange and red pigments – probably carotenoids and astaxanthins-, but they could not adapt to changes in their environment and mostly perished by the 12th day. **(Figure 14)**

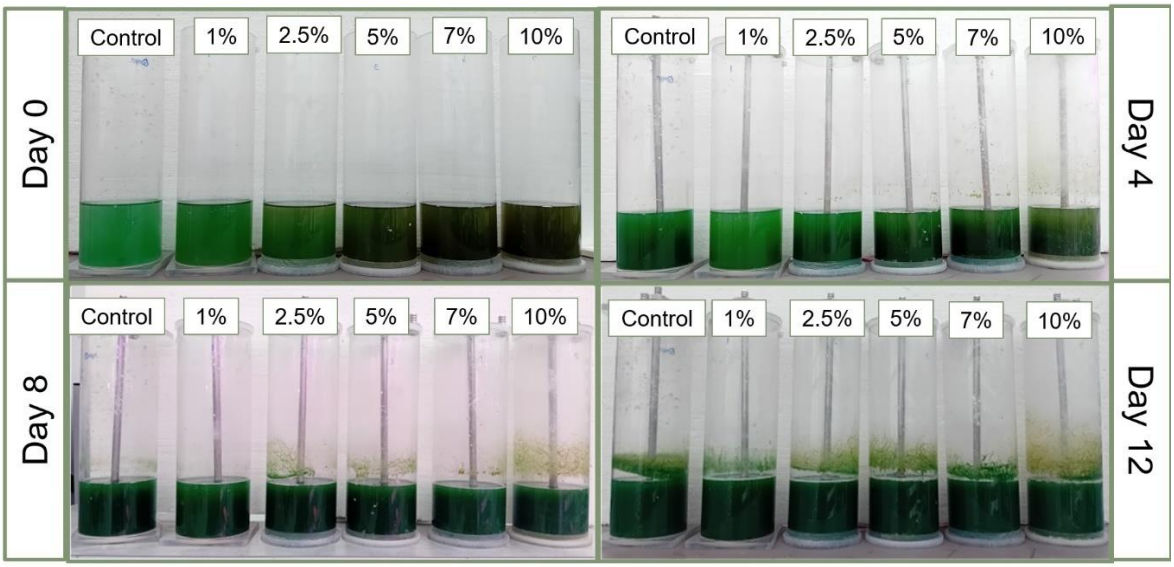


Figure 13: Growth of the cultures that had been treated with PSP BJ over the 12 days
Source: own pictures (2025)

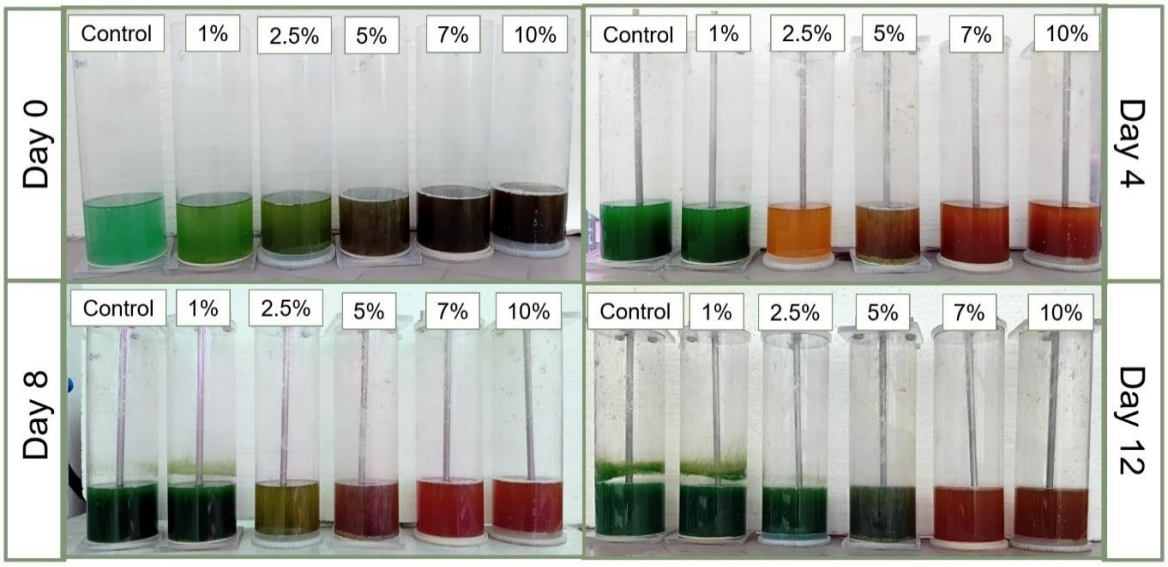
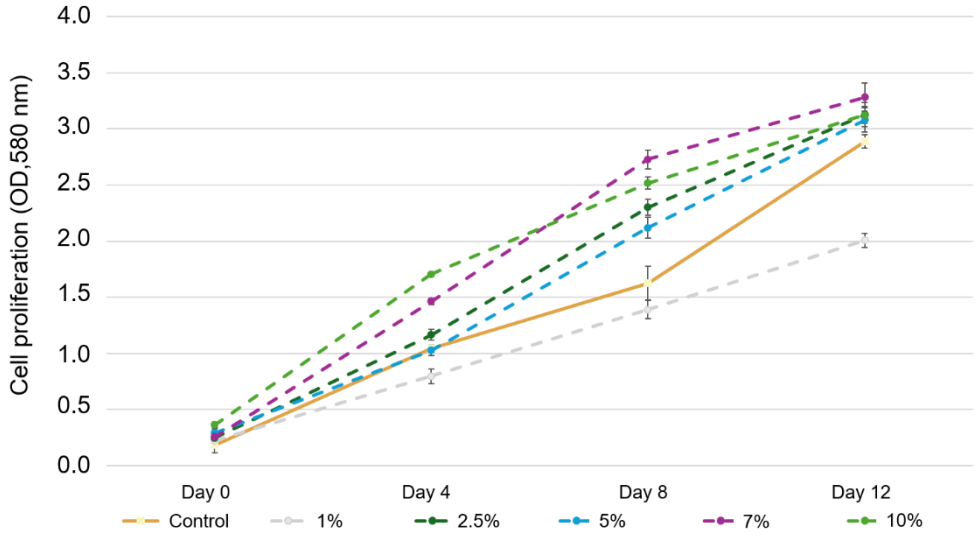


Figure 14: Growth of the cultures that had been treated with WS BJ over the 12 days
Source: own pictures (2025)

There were no significant differences in pH and EC between the cultures. The pH of all media increased, becoming alkaline, which is advantageous for larger-scale open cultivation systems because it lowers the risk of contamination.

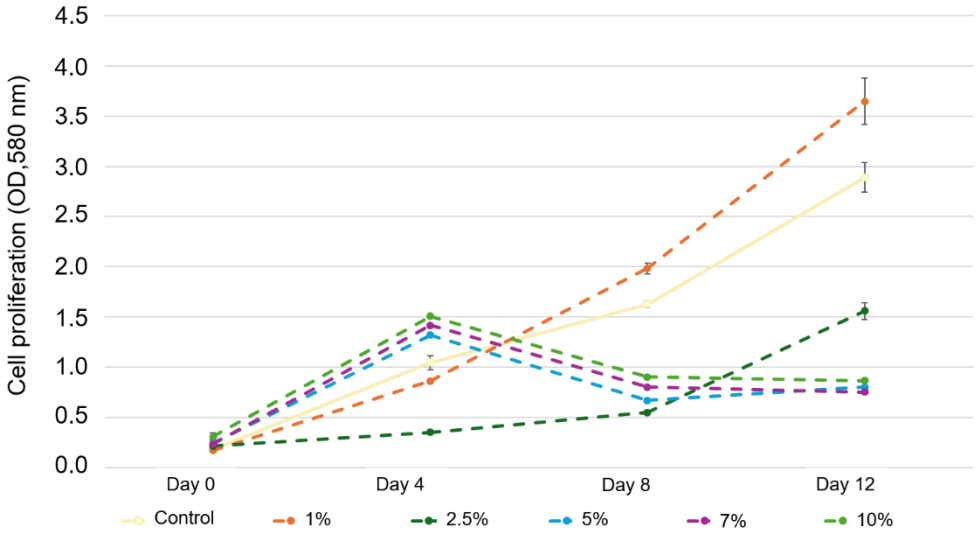
Based on OD measurements, cultures treated with PSP BJ showed continuous growth, except for the 1% treatment, which remained below the control. **(Graph 1)**



Graph 1: Changes of OD of microalgae treated with PSP BJ
Source: own edit (2025)

In contrast, WS BJ treatment only promoted growth at the 1% concentration. Higher concentrations (5%, 7%, and 10%) initially stimulated but later inhibited the growth of the microalgae after the fourth day.

The 2.5% WS BJ treatment showed adaptation starting from day eight. **(Graph 2)**

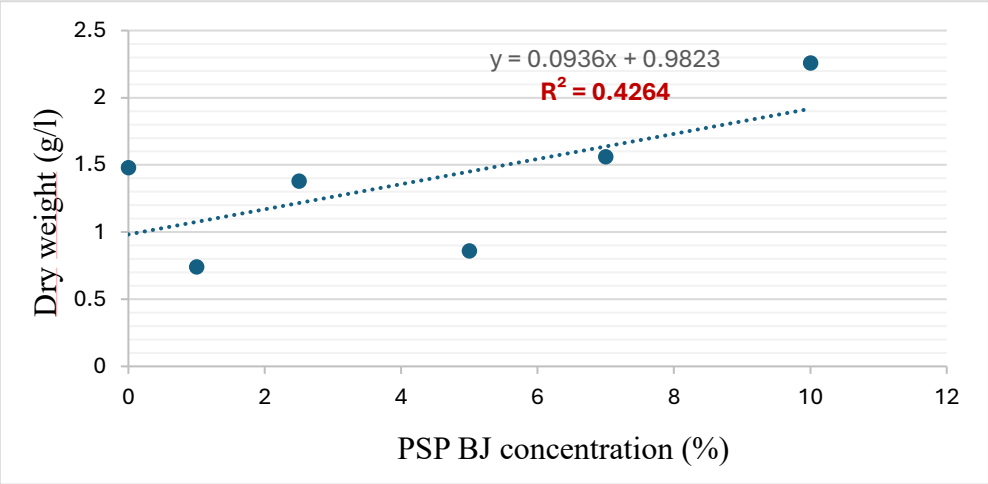


Graph 2: Changes of OD of microalgae treated with WS BJ
Source: own edit (2025)

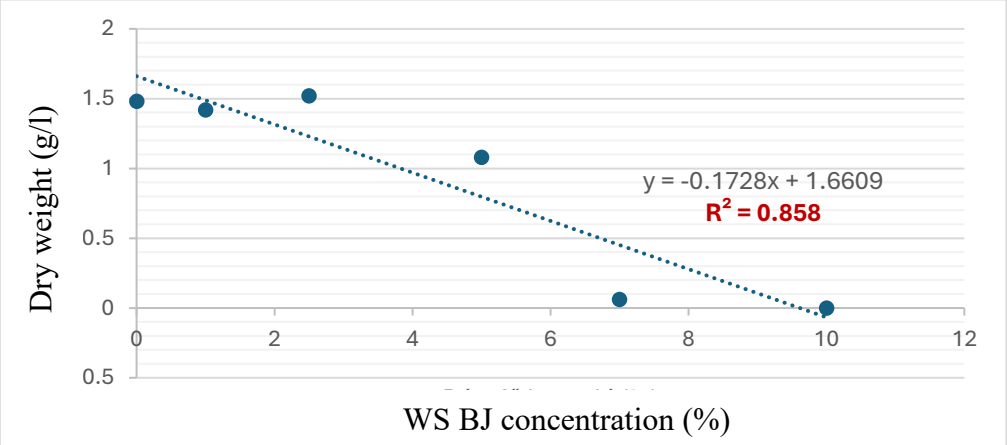
7.2 Dry weight measurements

Our experiment was essentially a preliminary study to assess whether the BJ-s of these two plants are suitable for supplementing microalgae culture media. As there were no previous data available, repeated experiments are necessary for conclusive results. Based on the current data, we calculated the linear regression of the treatments using the LIN.ILL function in Microsoft

Excel. There is correlation bet ween the data if the R^2 value is less than 1. For the PSP BJ treatments, R^2 was 0.4264 (**Graph 3)**, while for WS BJ treatments it was 0.858.**(Graph 4)**



Graph 3: Linear regression of data collected from the PSP BJ treatments
Source: own edit (2025)



Graph 4: Linear regression of data collected from the WS BJ treatments
Source: own edit (2025)

Both fresh and dry weight measurements showed that the microalgae responded differently to the BJ-s of the two plants at different concentrations. For industrial production, dry weight is more relevant, as the microalgae biomass is commercially valuable in its dried form. The most successful treatment was the 10% PSP BJ, where the dry biomass reached 2.26 g/l. Overall, PSP BJ can be used at higher concentrations, while WS BJ showed beneficial effects only at low concentrations. Further studies are necessary to determine why higher concentrations of WS BJ are inhibitory; we assumed that phytochemicals or bioactive substances are present in the BJ that could suppress microalgae algae growth. **(Graph 5)**

Graph 5: Dry weight measurements (PSP-purple sweet potato, WS-Wheat Sprout)
Source: own edit (2025)

8. Future plans

In the future, we plan to repeat the experiment multiple times to refine the measurements. Additionally, we intend to carry out new analyses, such as measuring photosynthetic pigment content and protein content. After this, we can determine the most beneficial BJ concentrations for industrial applications. Ultimately, we aim to adapt the experimental setup for semi-industrial, then industrial-scale operations, starting with a 25-liter closed reactor tube. Also we would like to use the harvested biomass in food production. Our concept is either a protein bar which would use both the harvested purple sweet potato and the microalgae, therefore completely using the whole process, or microalgae gummies that could be a healthy alternative for kids.

9. Economic significance of the project

The cost-efficient spirulina liquid medium used in the experiment consists of fully commercially available components, with a production cost of approximately 0.01 EUR/l. BJ currently available at wastewater price, so it costs about 7.5 EUR/ m^3, 1 litre cost about 0.0075 EUR. In industrial contexts, microalgae biomass is typically marketed based on its weight in kilograms. For the production of 1 kg of microalgae biomass using the most effective treatment -10% application of PSP BJ - the cost of the growing media is approximately 0.62 EUR, while

the BJ supplement adds an additional 0.037 EUR. Thus, the cost of the microalgae inoculum, which currently is not available for comercial use. However, the strain used in our research is highly resilient, capable of being maintained in both liquid and solid media, so it would be a long time invesment. Microalgae are commonly cultivated in photobioreactors, which come in various shapes and sizes. Semi-industrial systems typically cost between 15,000 EUR and 100,000 EUR, while industrial-scale systems can range from 100,000 EUR to 1 million EUR. Although these systems would be one-time investments, and it can be recovered through biomass yield, because semi-industrial reactors can produce over 200 kg/year, while industrial-scale ones can yield up to over 30 tonnes/year. Thanks to its versatile applications - energy production, chemical industry, plant production, food industry- it has multiple potential target markets. Due to its high protein content, microalgae biomass itself represents a sustainable alternative protein source, which is increasingly in demand and offers competitive future investment opportunities. In the food industry 1 kg of *Arthrospira platensis* in powdered form can be sold for 50 EUR to over 100 EUR, depanding on the quality. In industrial scales this would mean that the initial capital costs would be recovered after 3.5 years. In the future, this research can serve as a basis for the development of a system that, in the approach of circular economy, recycles BJ, a by-product from the production of an alternative protein source, which is harmful to the environment in large quantities, and turns it back into farming in such a way that it produces even more protein suitable for human consumption.**(Figure 15)**

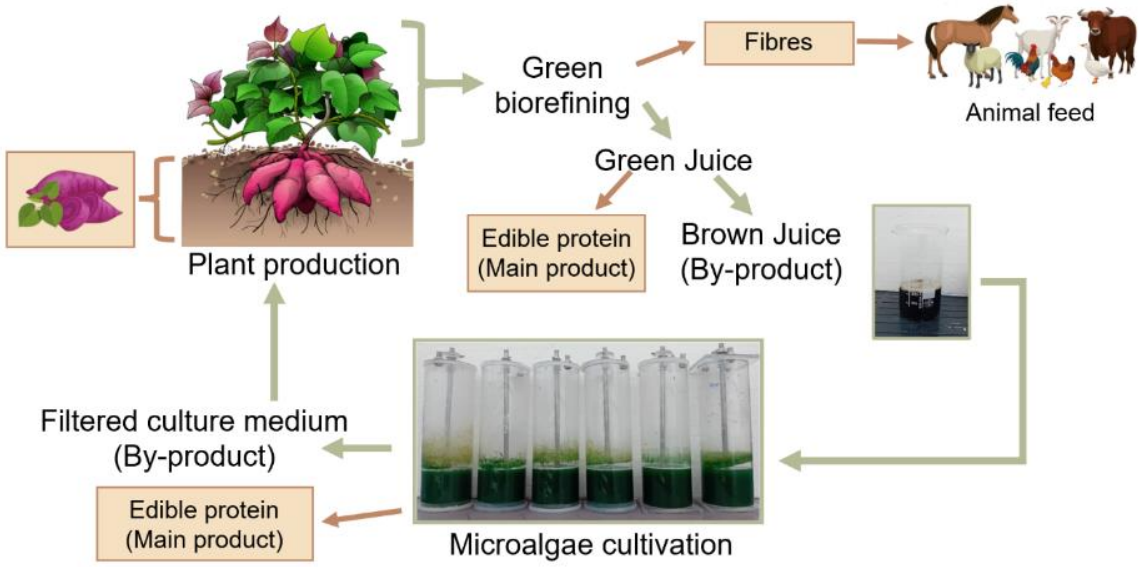


Figure 15: Microalgae cultivation implemented into circular economy
Source: own edit (2025)

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11. List of Abbreviations

- **BJ:** Brown Juice
- **EC:** Electrical Conductivity
- **GJ:** Green Juice
- **LIN.ILL:** Linear Trendline Function (Microsoft Excel) *
- **LPC:** Leaf Protein Concentrate
- **MÉK:** Faculty of Agricultural and Food Sciences and Environmental Management (University of Debrecen)
- **OD:** Optical Density
- **PSP:** Purple Sweet Potato
- **SD:** Secure Digital (SD card)
- **WS:** Wheat Sprout

** LIN.ILL refers to the Hungarian version of Microsoft Excel; in English versions, it is commonly known as "LINEST" or "Linear Trendline."*