



Enhancing duckweed cultivation for sustainable energy and wastewater management in wastewater treatment plant

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ABSTRACT

Duckweed, a perennial and one of the smallest vascular plants, possesses significant potential as a co-substrate in methane fermentation. Nevertheless, the challenges associated with cultivating and breeding this vegetation on an industrial scale remain a subject of scrutiny. In this article, the authors present findings from a series of laboratory and outdoor tests aimed at formulating a comprehensive solution for the cultivation of duckweed on an industrial level. The proposed solutions encompass technical innovations and a year-round cultivation system for duckweed. These solutions are specifically tailored for small water and sewage management companies, with the primary objective of converting 100% of sludge into a valuable product and halting 100% of water circulation, by utilizing treated sewage. The overarching goal is to generate cost-effective, green energy, thereby providing the potential for energy independence for enterprises. This innovative approach not only addresses environmental concerns but also aligns with the economic viability of small-scale water and sewage management operations.

Keywords: Biomass; Co-substrate; Duckweed; Energy potential; Methane fermentation

1. Introduction

Biomass is defined as organic material of plant origin obtained from special energy crops or as products [1]. Another definition can be found in the Regulation of the European Commission where biomass means the biodegradable fraction of products, waste, and residues from agricultural production (including substances of plant and animal origin), forestry, and related industries, including

fisheries and aquaculture, as well as biogases and the biodegradable fraction of industrial and municipal waste [2]. The industry press defines biomass differently, describing it as biodegradable products, parts of products, waste, and residues of biological origin. In a broader sense, biomass is all the organic matter existing on our planet, both of animal and plant origin, undergoing biodegradation. Nowadays, biomass is primarily discussed as a renewable energy source [3,4]. Biomass is indeed the oldest, easiest to obtain, and

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most widely used renewable energy source. It mainly comes from agriculture, as well as forestry and related industries, such as fisheries and aquaculture [5]. Without delving into further interpretations of the term biomass, it is necessary to identify its characteristic features, which appear in each interpretation. Certainly, it is organic material resulting from the biodegradation of various fractions of products or waste. According to different classifications, phytomass (plant biomass) and zoomass (animal biomass) can be distinguished. The further part of the article will consider plant-origin biomass as a co-substrate in the methanogenic fermentation of wastewater treatment plant wastes. The authors experience gathered in the course of managing a water and sewage company allows the conclusion that biomass produced based on wastewater treatment plant waste has strategic importance in seeking energy independence for such a company.

Algae (Latin: *Algae*, Greek: *Phykos*) - a morphological-ecological group, traditionally consisting of several unrelated evolutionary lines of thallus organisms, that is, tissue-free. They are autotrophic organisms, subject to rapid growth, and can be very efficiently cultivated in agriculturally and forestry-unfavorable areas. Their energy efficiency is many times greater than that of rapeseed, corn, or soybeans. Algae have high photosynthetic and lipid accumulation properties, making them a very valuable source of biomass, a carbon dioxide absorber (as well as various heavy metals), and a high-energy raw material. Thanks to their properties of binding nitrogen and phosphorus from wastewater, they become a key bioremediator of aquatic environments.

Algae are considered an alternative source of biofuels due to their simple cellular structure, rapid growth, ability to accumulate large amounts of carbohydrates, and relatively low lignin content [7]. Algae can produce 30–100 times more energy per hectare compared to terrestrial crops. However, algae used for bioenergy production are too small to be collected, which would significantly increase the cost of separation. Duckweed is the common name for the family Lemnaceae, which is the fastest-growing angiosperm in the world. They note that compared to algae, water duckweed is relatively large in size, which facilitates their separation. It has strong adaptive abilities to the surrounding environment. These characteristics mean that duckweed is widely spread in ponds of still or slightly moving natural waters around the world. The seasonal growth rate of duckweed ranges from 3 to 9.5 tons ac^{-1} year $^{-1}$. In stagnant ponds with organic matter, duckweed is characterized by a faster growth rate due to its capabilities to hyperaccumulate water pollutants and to efficiently absorb nitrogen (N) and phosphorus (P) in domestic wastewater.

Lesser duckweed, small duckweed (*Lemna minor* L.) is a species of perennial belonging to the family of Araceae (according to the Reveala system) or a separate family of Lemnaceae. It is one of the smallest vascular plants. As found in the literature on Lemnaceae, commonly known as water duckweed, it has 36 species in five genera and is found worldwide. Similar to the algae described earlier, duckweed is characterized by high adaptability to the environment, rapid growth rate, and large accumulation of dry mass. Water duckweed, as a hydrophyte, grows without occupying land and can accumulate large amounts of starch, which

is a raw material for biofuel production, through phototrophic autotrophy.

Li et al. [8] note that with the growing shortage of traditional energy sources and accompanying serious environmental challenges, biomass-based biofuels are preferred as the most promising alternative [AQ2]. As one of the primary energy raw materials from biomass, research into its production methods and synthesis mechanisms is emerging. In recent years, duckweed has been used as a high-quality new biomass raw material due to its advantages, including rapid biomass accumulation, high starch content, high biomass conversion efficiency, and wastewater reclamation. According to the authors, the advantage of water duckweed is its high starch content and 'lack of competition with humans for food and land', making it a new type of non-grain starchy raw material with high energy efficiency. In summary, the author additionally states that establishing an efficient and stable genetic transformation system for duckweed could allow for the use of duckweed as a new type of biomass energy.

The average annual growth rate of duckweed (in favorable climatic conditions) can reach the level of weight doubling per day, and from 1 hectare of duckweed cultivation, about 28 tons of starch can be accumulated annually [9]. Duckweed can be converted into bioethanol with a conversion efficiency of 94.7% of the starch in duckweed and a theoretical ethanol yield approximately eight times higher than that of corn. Additionally, duckweed can remove contaminants from wastewater through enrichment and can combine water pollution control with bioenergy production. Therefore, water duckweed is a promising strategic new non-food biomaterial that can solve the raw material problem encountered during the development of the biofuel industry.

Chen et al. [10] also confirmed the potential of duckweed, stating that according to the results of average biomass production, the annual yield of duckweed could be 32.8 t ha^{-1} . The biomass production of wheat grain was estimated at 2.80 t ha^{-1} annually. Furthermore, as a benchmark of practically utilized bioenergy crops, corn could produce 1.47–7.90 t ha^{-1} annually. According to statistics from the Food and Agriculture Organization of the United Nations, the annual corn yield in China in 2016 was 5.95 t ha^{-1} . Compared to other crops, duckweed was therefore a suitable candidate for bioenergy raw material.

Experiences in the circular economy at an urban wastewater treatment plant led to some significant conclusions. First, excess sludge (in the case of the enterprise from Sława) is not calorific enough to optimize the production process of high-methane biogas and thus electrical and thermal energy. Second, the sludge after fermentation is not fully sanitized (the temperature of 37°C is insufficient to eliminate all bacteria and parasite eggs). Third, lime sanitization is a costly operation and does not provide proper business results. The aim of the research was to reduce the negative impact of a biological wastewater treatment plant on the natural environment by increasing the quality of the substrate supplied for methanogenic fermentation and increasing the utilization of waste from biogas production, as well as to study the degree of additional cleaning of treated wastewater in WWTP.

2. Methods and materials

2.1. Characteristic WWTP

The water and sewage company, where large-scale research was conducted, belongs to the category of small enterprises. The wastewater treatment plant serves 30,000 PE (Population Equivalent), and the average daily inflow of wastewater is 2100 m³/d. The company started its journey towards energy independence in 2016 when a 200 kWh photovoltaic farm was established on its premises [6]. The comprehensive modernization and expansion of the company, which took place between 2018–2022, introduced a comprehensive management of sludge processing and control and monitoring of all processes at the wastewater treatment plant. The photovoltaic farm operating since 2016, after the company's expansion, did not meet all energy needs. Photovoltaics, for those familiar with the topic, is an installation supporting the energy processes in the company. Its seasonal operation and exposure to variable weather conditions do not allow for stable planning of all the company's energy needs. The water and sewage company operates continuously, and many processes are carried out at night and in the early morning. The energy demand is highest at these times. Midday and afternoon in the summer period mean high sunlight, and therefore maximum photovoltaic work. Therefore, from the observation of the distribution of energy production from photovoltaics, it was possible to read that at peak times, the farm was able to produce more energy than the company needed at that time, but at night and in the morning, the company had a high energy demand, which it had to purchase from an external operator.

The second source of its own energy was methanogenic fermentation. Therefore, the focus was on strengthening and improving the quality of the substrate supplied for methanogenic fermentation. As there are large meat processing plants in the Slawa municipality, an agreement was signed with them to receive and utilize the floatation (excess sludge from the on-site wastewater treatment plant). This increased the calorific value of the substrate supplied for fermentation. The biogas production process significantly increased. The methane content in biogas is 68%–70%. The post-fermentation sludge after centrifugation (there was a change in the sludge dewatering technology from dewatering work to the use of centrifuges) contains up to 22% dry mass and still retains the organic properties of the post-fermentation. To further increase the production of biogas and its calorific value, research began on the possibility of using waste biomass.

2.2. Growth of algae and duckweed on a laboratory scale

Biomass production began in July 2022 on a laboratory scale in 5L and then 20L glass bioreactors. The work started with the inoculation of the *Chlorella* algae, which, according to literature data, was a promising material for industrial biomass production. In the reactors, algae were cultivated in: 1) water with additional supplementation, 2) treated sewage with additional supplementation, and 3) treated sewage without additional supplementation.

Parallel to the laboratory experiments on algae, experiments were conducted on another hydrophyte, namely water duckweed. The choice of water duckweed was natural,

as duckweed is an integral part of municipal sewage treatment plants. On secondary settlers, spontaneous growth of this plant can be observed, which does not require any care for its growth. Research on duckweed in terms of reproduction and energy properties was conducted in a similar way as with algae. However, the properties of duckweed for many reasons led to its selection as a strategic biomass for fermentation processes in small WWTPs.

The biomass increase was assessed visually and by weight on a laboratory scale, and its energy potential on a laboratory scale in the studies of biogas production in eudiometer after 21 days and 55 days of fermentation under mesophilic conditions (temperature 35°C).

3. Results

Water duckweed as a strategic biomass has its evident advantages. First, it is an organism that can spontaneously cultivate itself in the treated sewage of any wastewater treatment plant. Wherever sewage appears (municipal, industrial, agricultural, etc.), water duckweed fulfills its absorptive role. An aquatic environment contaminated with nitrogen, phosphorus, and heavy metals, or in the presence of general environmental pollution is conducive to the growth of water duckweed.

The growth rate of duckweed under laboratory conditions was visible (Fig. 1). Over two weeks (September 2022), the duckweed increased its mass more than fourfold, and in the next period (October 2022), it increased more than sixfold. This means that the mass doubles every 48 h. This result demonstrates, on a microscale, the incredible rate of biomass growth regardless of the medium used. Water duckweed grown on treated sewage does not need supplementation or additional 'feeding'. Under stress conditions caused by a lack of nitrogen and phosphorus, it multiplies on its own. To stimulate the rate of multiplication, it is sufficient to create a natural aquatic environment for the duckweed with appropriate lighting and temperature. In optimal environmental conditions, such as protection from wind, appropriate concentration of nutrients in the water, and optimal density, duckweed can produce biomass at a yield of 10–30 tons/ha annually [11]. They add that the intensity of light and the growth rate of duckweed show a direct relationship unless the light intensity is too high. Most importantly, in the natural environment, duckweeds generally grow in the range of 6°C to 33°C; the optimal water temperature for the development of duckweed is 19°C–30°C.

However, duckweed is sensitive to increased chloride content. The effect on the growth and chemical composition of duckweed biomass was found at a concentration of 4 g NaCl/L [12]. The occurrence of chlorides at a concentration of 100 millimoles of NaCl/L (5.85 g NaCl/l) in water may also reduce the removal of nitrogen and phosphorus by duckweed or even cause a negative removal efficiency of these nutrients [13]. According to our research, the percentage inhibition of biomass growth was almost 70% at a concentration of 6.35 g NaCl/L [14].

The mere increase in biomass does not testify to its quality and energy potential. Studies conducted in laboratory-scale fermenters (20 L) confirmed that water duckweed also has enormous energy potential. The trials



Fig. 1. Growth of water duckweed under laboratory conditions.

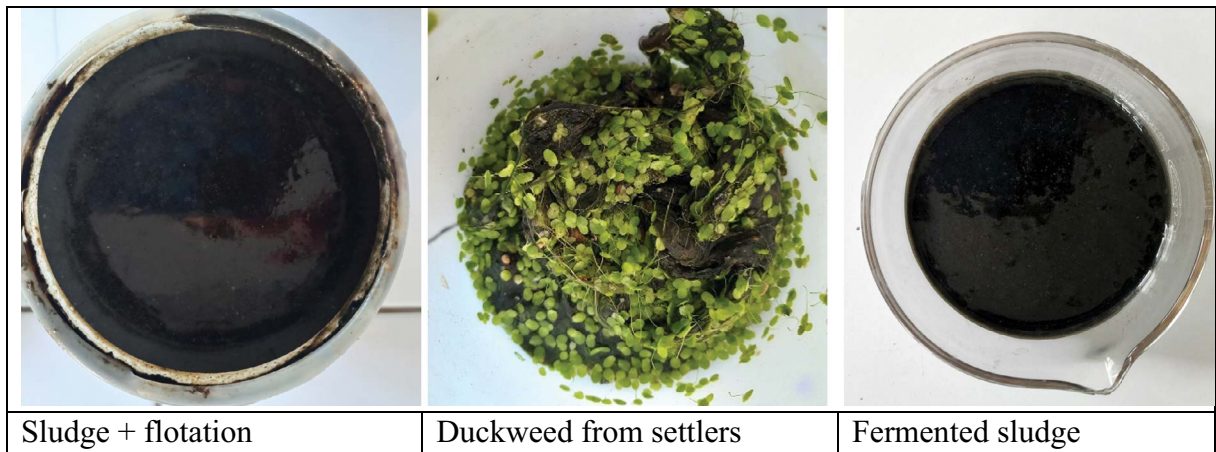


Fig. 2. Material accepted for study.

examined the potential of the duckweed, the potential of excess sludge mixed with flotation, and excess sludge with the addition of duckweed (Fig. 2). The basic parameters of the studied substrates are presented in Table 1. The results were surprising. Water duckweed alone has a greater energy potential than the excess sludge from secondary settlers, and greater than the mixture of excess sludge with flotation. But its potential was the highest in the mixture of all three substrates (Table 2). Biogas production from the tested mixture of three substrates (683 L/kg DM) turned out to be higher than the literature data for cow manure (200 – 300 L/kg DM), sewage sludge (350 – 500 L/kg DM) or grass (350 – 400 L/kg DM) [15].

Fig. 3 shows changes in the content of nutrients (C, N, P, and K) in relation to the initial values after 3, 7, and 10 days of duckweed growth. Values (+) indicate nutrient uptake, while values (-) indicate appearance in the aquatic environment

3.1. Growth of duckweed on a big scale

Based on the promising results of the energy potential study of water duckweed obtained in the laboratory, two open ponds were built on the site of the municipal wastewater treatment plant located in southwestern Poland, and the same phenomena were studied, but under natural conditions. As the construction of the ponds fell in the autumn period, the main studies were undertaken in early spring. The size and shape of the ponds reflected the filtration fields at a scale of 1:100, that is, the length of the pond was 20 m, and the width of the pond was 4 m. The water surface of the filled pond was 30 cm.

The prepared ponds thus became an open research laboratory for biomass production. The inoculation of duckweed in open tanks took place in the autumn period. It was an unfavorable time, during which biomass growth was greatly limited. On one hand, the small initial sample size,

Table 1
Characteristics of the substrates accepted for the study

	Unit	Sample 1	Sample 2	Sample 3
Substrates used for sample preparation				
Fermented sludge (inoculum)	g	400	400	400
	g DM	12	12	12
	g VSS	7	7	7
Excess sludge + flotation	g	174	174	0
	g DM	8.0	8.0	0.0
	g VSS	5,5	5.5	0.0
Duckweed from settlers	g	0	87	87
	g DM	0	5	5
	g VSS	0,0	4.0	4.0
Total	g	174.0	261.0	87.0
	g DM	8.0	13.4	5.4
	g VSS	5.5	9.5	4.0
Distilled water refill				
Water	g	326	239	413
Final characteristics of the samples				
Percentage of dry weight (DW)	% DM	1.6%	2.7%	1.1%
Percentage of organic dry weight (ODW)	% VSS	68.1%	70.7%	74.6%

Table 2
Energy potential of the substrates

	Unit	Sample1	Sample2	Sample3
Biogas production results				
Biogas production after 21 days	mL	1365	3163	2309
	L/kg DM	171	236	428
	L/kg VSS	250	333	572
Biogas production after 55 days	mL	1834	4498	3688
	L/kg DM	229	336	683
	L/kg VSS	336	474	913

and on the other hand, the external atmospheric conditions meant that the cultivation and observation of duckweed began in the early spring period. This does not mean that research and observations were completely discontinued. During the winter period, a slow growth of duckweed was observed, and the temperature (external air, supplied sewage, and sewage in each plot) was measured. Measurements were taken three times at the same times of the day. During winter observations, the external temperature ranged from 4°C to –8°C, with occasional warmings even up to 10°C. The temperature of the sewage supplied to the ponds did not show such large variations, that is, it ranged from 10.3°C to 15.8°C. Meanwhile, the temperature of the sewage in the

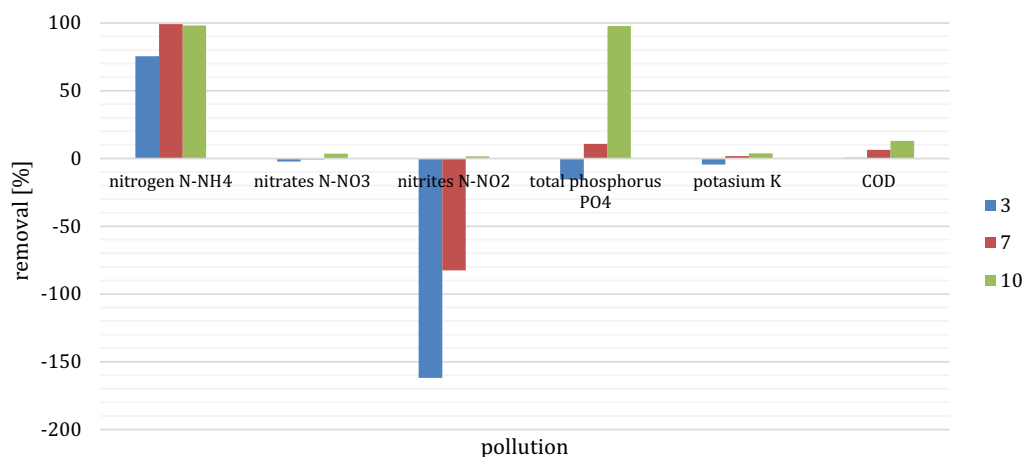


Fig. 3. Removal (+) of nutrients (C, N, P, and K) after 3, 7, and 10 days of duckweed growth and the appearance (-) as a result of biochemical processes in the aquatic environment.

individual ponds averaged 7.4°C–7.7°C. As can be seen, despite the negative external temperature, both the temperature of the supplied sewage and the temperature of the sewage in the plots were not very low. However, during the period of the strongest frosts at -8°C, icing appeared on the surface of the ponds (pond temperature, 3.6°C).

The sequence of sewage supply was 30 min/30 min over a 16/8 h period. In the early spring period, a clear increase in duckweed growth was noted (Fig. 4). Despite the fact that the external temperature was still relatively low (average in February was 3.1°C). Over 3-week periods, a several-fold increase in its mass was observed. From February to May, increased development of water duckweed was observed, so approximately every two weeks, the rate of its multiplication and partial collection of duckweed began. Its laboratory analysis was also repeated.

Samples of duckweed were blended to achieve a consistency similar to that of excess sludge. The crushed and liquefied (pulp) duckweed had a 5% dry mass content. The duckweed grinding system does not cause a loss of its mass, but there is a noticeable reduction in its volume. When blending 700 ml of duckweed weighing 342 g, the resulting pulp had a volume of 350 ml and a weight of 337 g. This means that blending duckweed significantly reduces the volume of the substrate, which can greatly reduce the costs of logistics and feeding duckweed for co-fermentation. Blending duckweed to achieve a similar structure to excess sludge has its clear benefits. First of all, blending duckweed protects the pump and mixer system from clogging by the duckweed's root system. From our observations, it appears that duckweed has the ability to cluster and bind together when pumped. Crushing and converting it into a pulp consistency also has additional benefits in the fermentation process. The crushed duckweed integrates better with the sludge and a clear synergy effect is observed between two or more substrates.

The study showed that blending duckweed allows obtaining a similar dry mass content (around 4.5%), which is a suitable structure for co-substrate with excess sludge and flotation. Feeding raw duckweed, ignoring technical conditions, could disrupt the fed co-substrates, sometimes with too high dry mass content, and other times too low. The pulp consistency has its clear benefits. First, the consistency of the substrates is at a similar level, the dry mass content can be regulated, and the crushing of duckweed has its benefits in inter-substrate integration and a clear synergy effect.

4. Discussion

The experience gained from laboratory research and open pond studies has led to the development of a ready

and comprehensive solution for future wastewater treatment plants. First and foremost, the universality of the proposed solutions should be noted. The biomass produced from sewage sludge is a desired solution and does not raise major practical doubts [15–17]. Using water duckweed for this purpose carries additional benefits of tertiary sewage treatment, which after minor purification techniques can be classified as water. The post-ferment obtained from the fermentation processes of co-substrates (sludge and duckweed) is assumed to have better mineral-organic properties due to its preliminary sanitization and stabilization in the methanogenic fermentation process [18]. Such post-ferment may not require additional sanitization with quicklime if the full sanitization process is carried out before introducing the sludge into fermentation. The most important thing is the produced biogas with a high methane content, which is converted into electrical and thermal energy through the cogeneration process. The amount of electrical and thermal energy is significantly greater thanks to co-substrate with its own biomass. An additional plus for the entire system is the production of biomass with known and predictable parameters and properties. The amount of biomass produced and fed into fermentation can be linear but also controlled. Intuitively, it can be stated that in the summer periods, biomass production will be increased, and feeding into fermentation can be increased, however, the system can be arranged so that fluctuations in this range do not affect the stable operation of fermentation, but only can cause the ferment to be held for longer or shorter periods.

According to the developed model, comprehensive solutions concern both methanogenic fermentation processes and biogas production based on co-substrate charges, but also the preparation of ponds for year-round cultivation of water duckweed. While cogeneration systems based on methanogenic fermentation processes are currently available market technological solutions, ponds for biomass production are an innovative solution.

First, it should be noted that ponds for cultivating water duckweed require quite large areas of land. It would seem that this is an unattainable condition for most companies, however, as the authors of these solutions point out, any wastelands and agriculturally unused surfaces, fields, meadows, or forest lands can be used for this purpose. In most small water companies, wastewater treatment plant locations are outside heavily populated urban areas. The location of treatment plants on the outskirts of cities is deliberate and brings measurable benefits. One of these benefits may be that in the vicinity of the treatment plant, there can be agricultural wastelands often at the disposal of local governments or the National Agriculture Support Center Acquiring



Fig. 4. Start of the vegetative period of water duckweed on open ponds in the early spring season.

(Poland) such wastelands for ecological purposes, with the assumption that they will be used for safe and ecological activities aimed at biomass production, but what is socially more important, additional sewage treatment and creating possibilities for its agricultural use seems to be possible. It is assumed that the amount of land designated for duckweed cultivation is equivalent to 1 hectare per 1,000 m³ of discharged sewage. This is another advantage of the conducted research.

It is important to remember that water duckweed multiplies quickly, but too large cultivation areas relative to the amount of inoculated duckweed must create the possibility of stable multiplication. This involves arranging the system of cultivation fields in such a way that there are seedling fields, whose sole purpose is the stable multiplication of duckweed, which in the next stage feeds a larger area of the next pond. Due to their function of stable multiplication, the seedling ponds have additional installations. First and foremost, the sewage supplied to them is additionally heated (using heat from cogeneration) and can be covered during periods of too low temperatures. Maintaining a constant temperature of the supplied sewage is possible with additional heating. Ensuring that the temperature in the seedling ponds does not fall below 10°C will result from the constant supply of sewage and its circulation. Excess supplied sewage will gravitationally flow to the pond below, and such an overflow system will prevent the sewage from cooling rapidly. However, it should be kept in mind that in the basic cultivation ponds of 0.5 ha – 1 ha, maintaining a constant temperature will be impossible, hence during summer periods, the pond should be filled with water duckweed, which will create a layer on the water surface, and the circulation below will cause constant mixing. Additionally, the system for collecting duckweed is designed to ensure that the daily harvest is proportional to the planned daily growth of duckweed. Although the optimal water temperature for the growth of duckweed is from 19 C to 30 C [19], the conducted research proved that it is possible to extend the vegetation time of water duckweed in the temperate zone.

In summer periods, this may be 20%–30% of the pond's surface, and in winter only 5% of the surface. During prolonged low air temperatures, duckweed will be successively harvested until favorable external temperatures return. Ultimately, the seedling ponds will be an element of maintaining the co-substrate fed into fermentation.

Another important issue is the illumination of the cultivation ponds. Water duckweed needs daylight for its growth. In autumn-winter periods, the time of daylight illumination shortens to 8–10 h. To maintain a 16/8 h light photoperiod, an additional lighting system that will further photostimulate the multiplication of duckweed should be introduced in the seedling ponds. From the literature, it can be read that the intensity of light and duration of illumination are important for the growth of water duckweed. Studies on light intensity and photoperiod showed that the biomass of duckweed and starch production increased with increasing light intensity and photoperiod, except for 200 and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$ [11]. Considering the cost of light, 110 $\mu\text{mol m}^{-2} \text{s}^{-1}$ were the optimal light conditions for starch accumulation with the highest maximum growth rate, biomass, and starch

production at 8.90 g m⁻² day⁻¹, 233.25 g m⁻², and 98.70 g m⁻², respectively. Furthermore, the results suggest that high light induction is a promising method for starch accumulation from duckweed. This study provides optimized lighting conditions for the future industrial cultivation of duckweed on a large scale. Besides the intensity of light and photoperiod for the growth of aquatic vegetation, light of the appropriate wavelength is important. Plants in the tank grow best if they are provided with light in the range of 400–480 nm (blue light) or 600–700 nm (red light). As for color temperature, optimal conditions are created by using light as close as possible to natural sunlight (about 6,500 K). In the seedling ponds, special LED lighting is planned to be installed, using blue and red colors for photostimulation.

It is evident that the choice of installation and its application should result in a favorable energy balance. This means that the traditional energy 'introduced' into the system cannot be higher than the benefits derived from obtaining the value of green energy from the system. Therefore, most solutions are based on the use of mechanics and elements of gravity between installations. The ponds for cultivating duckweed are arranged in a cascading manner with a slight lowering of each pond. Since the water surface in the ponds does not exceed 30 cm, the lowering of subsequent ponds is 20 cm relative to the primary pond. It is obvious that the seedling ponds, which start the process, are located at the highest. According to the developed model, the difference between the Pond 1 and 4 is 80 cm, which assuming that Pond 4 is built below ground level, and the height of Pond 1 does not exceed 60 cm above ground level. In the construction of ponds, an earth balance is maintained, meaning that the amount of earth excavated for the construction of the lowest pond compensates for the amount of earth for the highest pond.

The sewage supply installation system is located at the bottom of each pond in such a way as to force and control the circular movement of sewage in the appropriate sequence. The placement of the installation at the bottom of the pond is crucial for the constant feeding, oxygenation, and rotation of the duckweed. Sun et al. [20] pointed out that the growth rate of mixotrophic cultivation was respectively 4.98 and 6.22 times higher than under heterotrophic and photoautotrophic conditions. They add that mixotrophy produced more biomass than the simple sum of biomass accumulation during heterotrophy and photoautotrophy. Mixotrophy was also better in terms of starch and protein production, as well as the rate of nutrient and organic carbon removal from the growth medium. However, the starch content in heterotrophically cultivated duckweed was 2.06 times higher than in mixotrophy, suggesting a combination of mixotrophy and heterotrophy as an effective strategy for producing starch-rich biomass. This study thus provides a paradigm for future research supporting biomass production based on duckweed and organic wastewater treatment. Thanks to such a sewage supply system, duckweed undergoes successive stress caused by the supply of nutrients in a certain sequence. Since sewage supply is carried out by a water installation system, with an appropriate stream and sequence, excess sewage must be drained in the same cycle. To maintain a constant sewage level in the tank, an overflow system was created, causing

the sewage to flow to the tank below when exceeding the desired level. It is important that the overflow does not cause the freely floating water duckweed to overflow. The system is designed so that excess sewage is drained from below the root system of the duckweed. Overflowing excess to the pond below causes a build-up of sewage quantities in that pond, which consequently flow to the retention tank. The retention tank is located in the lowest layer of the installation, and all final sewage flows go there. In the tank are located pumping pumps, whose task is to pump additionally purified sewage to its final destination.

The intended use of sewage after tertiary treatment can vary. In the project assumptions, it is expected that the additionally purified sewage will meet the parameters of sewage that can be used for agricultural purposes. Depending on the degree of purification, and possibly with the use of an additional treatment system, its status could change from sewage to water suitable for food use. However, being aware that society is not ready to accept the status of water produced from sewage, such water could be used for agricultural needs, for livestock or technological purposes.

Another way to use the purified sewage could be its retention for firefighting or municipal services. Another option could be its use for forestry purposes.

An interesting solution is the creation of artificial water bodies, fish ponds, or recreational ponds, known as botanicals, which could be part of ecological education concerning aquatic vegetation. Creating an artificial reservoir, with walkways between low and high aquatic vegetation, could constitute a recreational center used throughout the year. Such natural water botanical gardens can be found in various places, and their creation 'from scratch' would have positive effects in systematizing knowledge about water, sewage, ecosystems, fauna, and flora in water bodies.

5. Conclusion

Duckweed is a potential source of biomass for alternative energy production. This article utilizes its research and indicates factors influencing the growth rate of water duckweed, methods of its cultivation, and systems for planned installations for its year-round cultivation. It also highlights the practical and business elements of a comprehensive solution, which aims to achieve energy self-sufficiency for enterprises, change their status from waste companies to production companies, and completely close the water cycle. The benefits of such a solution are for everyone. Here, one can cite the classic negotiation technique based on a win-win solution. Entities opting to apply the presented model of biomass production and its use for energy purposes create multiple winners.

The biggest beneficiary is the natural environment, which wins in several aspects: the purification of sewage discharged into the environment, the reduction of CO₂ emissions through the use of clean, green biomass to generate energy, and the improvement of soil conditions through the introduction of post-ferment with mineral-organic properties as a soil improver. The local community also wins, benefiting from not just the clean air but also gaining access to new places for resting and recreation. Education wins due to an interesting offer for year-round field lessons.

The enterprise wins by having a new product (soil improver, water), cheap energy, and no environmental fees. Science wins by implementing modern innovative solutions constituting a permanent research and process improvement center. Agriculture wins by having the possibility to obtain cheap water or soil improver to increase its agricultural yields.

Such a brief overview of the benefits can be a premise for following this direction and popularizing solutions that will soon become a necessity for the stable development of companies, countries, and entire economic systems.

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