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Duckweed from a Biorefinery System: Nutrient Recovery Efficiency and Forage Value

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Abstract: This paper presents the results of an interdisciplinary study aimed at assessing the possibility of using duckweed to purify and recover nutrients from the effluent remaining after struvite precipitation and ammonia stripping from a liquid fraction of anaerobic digestate in a biorefinery located at a Dutch dairy cattle production farm. The nutritional value of duckweed obtained in a biorefinery was assessed as well. Duckweed (*Lemna minuta*) was cultured on a growth medium with various concentrations of effluent from a biorefinery (EFL) and digested slurry (DS) not subjected to the nutrient recovery process. The study's results showed that duckweed culture on the media with high contents of DS or EFL was impossible because they both inhibited its growth. After 15 days of culture, the highest duckweed yield was obtained from the ponds with DS or EFL contents in the medium reaching 0.39% (37.8 g fresh matter (FM) and 16.8 g FM per 8500 mL of the growth medium, respectively). The recovery of N by duckweed was approximately 75% and 81%, whereas that of P was approximately 45% and 55% of the growth media with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively. Duckweed obtained from the biorefinery proved to be a valuable high-protein feedstuff with high contents of α-tocopherol and carotenoids. With a protein content in duckweed approximating 35.4–36.1%, it is possible to obtain 2–4 t of protein per 1 ha from $EFL_{0.39\%}$ and $DS_{0.39\%}$ ponds, respectively.

Keywords: biogas plant; farm biorefinery; duckweed; P and N recovery; nutritional value

1. Introduction

The European Green Deal sets a new perspective for the socioeconomic development of European countries with respect to natural environment and rational resource management [1]. The reduction of greenhouse gas emissions assumed in this document requires the implementation of several solutions in the field of industrial production, energy, transport, and agriculture. The goals set therein impose the need to develop and implement innovative solutions that improve the functioning of existing production technologies and expand the production capabilities of economic entities. This phenomenon has been previously observed, but activities in this area should be intensified in the near future. In the

previous decades, European agriculture has specialized and concentrated its commercial production. Intensive animal farming has caused major environmental impacts. The main problem of farm management is the production of large amounts of animal manure. Intensive manuring and storage of fertilizers are the causes of greenhouse gase (GHG) emissions, acidification of the environment as a result of ammonia (NH_3) emissions, eutrophication of surface waters, and pollution of groundwater due to the elution and surface runoff of phosphorus and nitrogen from the fields [2,3].

The concentration of livestock production has allowed for the implementation and development of a branch of renewable energy, namely the agricultural biogas industry based on the availability of large amounts of animal manure. In the agricultural biogas plants, natural fertilizers serve as substrates for biogas production in the methane fermentation process [4]. In turn, biogas produced in agricultural biogas plants is used to produce electricity and heat. However, the environmentally justified and economically desirable biogas production process generates significant amounts of waste, i.e., digestate. Due to the content of plant nutrients, the digestate can be used to fertilize arable lands. However, a low dry matter content (approximately 3–5%) [4] and the dilution of nutrients in a large bulk of digestate generate high transport and application costs, exceeding, in particular cases, the value of digestate bulk components.

The digestate is often applied in doses exceeding the nutritional needs of plants on the fields in the vicinity of biogas plants. This leads to increased water and air contamination with substances emitted from soils fertilized with the digestate. One of the main problems in digestate management is its excessive hydration and a popular method for its treatment and conversion involves its mechanical separation into a solid fraction (SFAD) and a liquid fraction (LFAD). The SFAD is typically used for fertilization purposes, while the management of the remaining LFAD poses a serious problem.

Nevertheless, LFAD can be a very valuable substrate for the production of mineral fertilizers and feedstuffs. Its further processing, involving the recovery of minerals, ascribes to the idea of a circular bio-based economy, which promotes the construction of biorefineries producing biofertilizers, protein feed, bioenergy, and biochemicals from organic wastes. Moreover, it is in line with the new EU comprehensive approach, i.e., The Farm to Fork Strategy [5]. There are many technologies for the recovery of components from liquid organic materials, including liquid fraction of the digestate. They differ in terms of efficiency, costs, and the final product recovered. Technologies based on struvite precipitation, ammonia stripping, evaporation, or membrane separation have recently become popular in this respect [6–9]. Nutrients from LFAD can also be absorbed by aquatic plants, for example, duckweed. The study under the use of duckweed for the recovery of nutrients from domestic wastewaters has been carried out since the 1980s [10]. Duckweed production was also aimed at nutrient recovery from swine lagoon water [11]. After duckweed harvest, nutrients are removed from the waste and recovered as biomass [12]. The use of aquatic plants is cost-effective due to their high potential for nutrients recovery, easy effortless harvesting, and high productivity [13]. The harvested plants, as the value-added by-products, can be used as biofuels [14,15]. Duckweed is characterized by a high protein content, ranging from 15% to 45% of dry matter [16–18]. The entry of new high-protein products (e.g., duckweed) should support the supply of the European feed and food market [19]. The annual European import of soybean (containing 40% protein) for protein use is 20 million tons per year [20]. The development of a technology for nitrogen recovery to be used for fodder production, for example, duckweed production on LFAD, could make the EU more independent of external inputs. Sońta et al. [21] reported that duckweed could be used in the feeding of ruminants (cattle and sheep), poultry (laying hens, broiler chickens, and ducks), pigs, and aquatic organisms (fish and shrimp).

Most of the studies conducted so far have focused on the possibility of using duckweed for the recovery of minerals from natural fertilizers [22,23] or sewage sludge [24]. In contrast, there are no reports on the feasibility of its production using effluent from a biorefinery that recovers nitrogen and phosphorus by struvite precipitation and ammonia stripping from the liquid fraction of the digestate. These processes use significant amounts of NaOH and $MgCl_2$ [25], which may inhibit the growth, development, and yielding of duckweed.

Considering the aforementioned current trends in pursuit of a closed circulation of fertilizer components in the agriculture, an interdisciplinary study was carried out to assess the possibility of using duckweed to purify and recover nutrients from the effluent remaining after struvite precipitation and ammonia stripping from the liquid fraction of anaerobic digestate in a biorefinery located at a Dutch dairy cattle production farm. The nutritional value of duckweed obtained in the biorefinery was also assessed.

2. Materials and Methods

2.1. The Farm Biorefinery and Duckweed Culture

The farm-scale biorefinery was located at the Experimental Dairy Farm "De Marke" in Hengelo (Gld), the Netherlands. The scheme and description of the biorefinery and the physicochemical properties and the fertilizing value of the recovered products (struvite and ammonium sulphate) were presented in other authors' works [26–29].

The experiment with duckweed was carried out using 8640 mL (36 × 10 × 24 cm) plastic boxes, each filled with 8500 mL of the growth medium. It was conducted in a greenhouse under controlled CO_2 concentration (800 ppm), temperature conditions (24 ± 1 °C), and 16:8 h light/dark cycle, with light intensity of 7000 luxes. The growth medium was prepared with the addition of the effluent from the biorefinery (EFL) and digested slurry (DS) not subjected to nutrient recovery processes. The chemical composition of EFL and DS is provided in Table 1. The content of nutrients in DS was significantly higher than in EFL (Table 1). Therefore, lower amounts of DS than EFL were added to the growth medium. Various amounts of EFL and DS were mixed with water to produce the following growth media with different concentrations: $DS_{0.1\%}$, $DS_{0.2\%}$, $DS_{0.39\%}$, $DS_{0.6\%}$, $DS_{0.78\%}$, $DS_{1\%}$, and $DS_{1.56\%}$, as well as $EFL_{0.39\%}$, $EFL_{0.6\%}$, $EFL_{0.78\%}$, $EFL_{1.56\%}$, $EFL_{2.5\%}$, $EFL_{3.5\%}$, $EFL_{5\%}$, $EFL_{7.5\%}$, and $EFL_{10\%}$. The selection of the concentrations of growth medium (EFL and DS) resulted from previous studies [30,31]. Tested dilutions covered a wide range of nutrients content starting from deficient through optimal to very high concentrations. Duckweed (*Lemna minuta*, LM) was cultured in boxes; each inoculated with its 20 fronds. In turn, duckweed cultured in a box with pure water (W) served as the control. Duckweed growth dynamics was evaluated over 15 days of the experiment, by counting the number of duckweed fronds every 5 days. The duckweed growth yield was calculated based on the number of duckweed fronds harvested after 15 days of growth. Samples of plants were collected from the boxes with the highest duckweed yield and subjected to in-depth analyses. The experiment was carried out in triplicate.

Table 1. The physicochemical properties of digestate (DS) and effluent from the biorefinery (EFL) [26].

	DS	EFL
pH	7.8	12.2
D.M. (%)	6.8 ± 0.2	2.1 ± 0.1
N_{tot} (g·kg^{-1} FM)	3.4 ± 0.01	1.8 ± 0.1
NH_4-N (g·kg^{-1} FM)	3.0 ± 0.1	1.4 ± 0.05
P_{tot} (g·kg^{-1} FM)	0.4 ± 0.01	0.2 ± 0.0
K_{tot} (g·kg^{-1} FM)	4.7 ± 0.2	3.8 ± 0.2
Mg_{tot} (g·kg^{-1} FM)	0.6 ± 0.05	0.3 ± 0.02
Ca_{tot} (g·kg^{-1} FM)	0.9 ± 0.1	0.4 ± 0.01
Na_{tot} (g·kg^{-1} FM)	0.7 ± 0.06	9.2 ± 0.2
P_{ws} (mg·kg^{-1} FM)	212.5 ± 9.8	127.7 ± 11.5
Mg_{ws} (mg·kg^{-1} FM)	293.7 ± 19.9	178.8 ± 20.9
Ca_{ws} (mg·kg^{-1} FM)	230.8 ± 15.6	173.7 ± 19.7
Cu (mg·kg^{-1} FM)	15.3 ± 2.3	4.4 ± 0.9
Mn (mg·kg^{-1} FM)	11.0 ± 0.1	7.5 ± 0.4
Zn (mg·kg^{-1} FM)	16.4 ± 0.7	10.7 ± 0.9

Data (means ± standard deviation, n = 3). DS, digested slurry; EFL, effluent from the biorefinery after struvite precipitation and ammonia stripping; DM, dry matter; FM, fresh matter; tot, total; ws, water soluble.

2.2. Analytical Procedures

The collected duckweed samples were divided into two portions. One portion was determined for dry matter content and, after drying, for contents of total protein, crude ash, crude fat, crude fiber, and minerals. The second portion was frozen and lyophilized. Then, the lyophilizate was determined for contents of α-tocopherol and carotenoids.

The chemical composition of the duckweed was determined according to AOAC [32]. Dry samples of duckweed were homogenized and mineralized with HNO_3, H_2O_2, and HCl using a Model DK 20 digestion unit (VELP Scientifica, Usmate, Italy). The P content in the duckweed was determined with the vanadomolybdophosphoric method using a Genesys 10 UV-VIS (ultraviolet and visible light region) spectrophotometer (Thermo Electron Corporation, Madison, WI, USA). Contents of Ca, Mg, K, Na, Zn, Cu, Cd, Pb, Al, and Cr in the duckweed were measured using a SOLAAR atomic absorption spectrometer (AAS) (Thermo Elemental, Cambridge, UK).

The content of α-tocopherol was determined using HPLC accordance the ESA Application note [33]. Carotenoids were separated and their contents were determined using the HPLC system (Dionex) equipped with a CoulArray electrochemical detector (ESA Inc, Chelmsford, MA, USA) [34].

2.3. Estimation of Nutrient Use Efficiency

An apparent nutrient recovery (ANR) was calculated according to the formula by Cavalli et al. [35]:

$$\text{ANR (\%)} = \text{nutrient uptake on EFL + W or DS + W/nutrient content in EFL + W or DS + W} \times 100$$

2.4. Statistical Analysis

Results obtained were developed statistically using the ANOVA procedure of one-way analysis of variance with Statgraphics 6.0 Plus software. The significance of differences between means was identified using F-test ($p \leq 0.05$).

3. Results and Discussion

3.1. Duckweed Yields

Under optimal conditions of access to light, carbon dioxide and nutrients, the growth of *Lemnaceae* can be exponential [36]. Among the nutrients, the content of nitrogen and phosphorus have the greatest influence on duckweed growth [16]. The organic waste analyzed in the present study seems to be a suitable growth medium for duckweed due to the high proportion of ammonium nitrogen in total nitrogen (88% and 78% for DS and EFL, respectively, Table 1). Duckweed prefers an ammoniacal form of nitrogen because NH_4^+ is easily converted into amino acids which form organic N compound [37]. In our study, the type of growth medium (Tables 2 and 3) used in the experiment affected duckweed growth (Figures 1 and 2). Duckweed culture on growth media with high contents of the digestate from a biogas plant or an effluent from a biorefinery was impossible. After 15 days of culture, the highest duckweed yield was obtained on the media with DS or EFL content at 0.39% (173 and 77 pieces of fronds, i.e., 37.8 g FM and 16.8 g FM per box, respectively, Figures 1 and 2). The yield of duckweed cultured on the growth media with $DS_{0.39\%}$ and $EFL_{0.39\%}$ was almost 5-fold and 2.5-fold higher, respectively, as compared with the yield obtained from the control box (W). After 15 days of culture, approximately 144–161 pieces of fronds were harvested from each box, i.e., 31.5–35.2 g FM per box for $DS_{0.2\%}$ and $DS_{0.6\%}$, respectively. In the case of boxes with DS added to the culture medium, the lowest duckweed yields were obtained in those with $DS_{0.1\%}$ and $DS_{1.56\%}$ (116 pieces of fronds, i.e., 25.4 g FM per box). In the box with $DS_{0.1\%}$, duckweed growth could be inhibited by nitrogen deficiency (3.5 mg N_{tot} L^{-1}), whereas in the box with $DS_{1.56\%}$ it could be inhibited by excess nitrogen (54.6 mg N_{tot} L^{-1}) and potassium (75.5 mg K_{tot} L^{-1}) (Table 2). Bergman et al. [30] and Caicedo et al. [31] proved that the best duckweed growth was obtained when the total nitrogen concentrations ranged from 3.5 to 40 mg N L^{-1}. A higher concentration of nitrogen than 60 mg N L^{-1} resulted in inhibition of

duckweed growth which was probably associated with the presence of free ammonia in the growth medium [38]. According to Körner et al. [39], the NH_3 toxicity on duckweed occurred with an NH_3-N concentration greater than 1 mg L^{-1}; the maximum tolerance for unionized ammonia was observed at 8 mg NH_3-N L^{-1}. Ammonia is the main nutrient limiting duckweed growth, but further research is needed to determine its optimal level for duckweed growth [38].

Table 2. Initial nutrient concentrations in growth media with different concentrations of the digestate from the biogas plant (DS).

Nutrients	$DS_{1.56\%}$	$DS_{1.00\%}$	$DS_{0.78\%}$	$DS_{0.6\%}$	$DS_{0.39\%}$	$DS_{0.2\%}$	$DS_{0.1\%}$
				mg L^{-1}			
N_{tot}	54.6	35.0	27.3	21.0	**13.7**	7.0	3.5
NH_4-N	48.2	30.9	24.1	18.5	12.0	6.2	3.1
P_{tot}	6.4	4.1	3.2	2.5	1.6	0.8	0.4
K_{tot}	75.5	48.4	37.8	29.0	18.9	9.7	4.8
Mg_{tot}	9.6	6.2	4.8	3.7	2.4	1.2	0.6
Ca_{tot}	14.5	9.3	7.2	5.6	3.6	1.8	0.9
Na_{tot}	11.2	7.2	5.6	4.3	2.8	1.4	0.7
P_{ws}	3.4	2.2	1.7	1.3	0.8	0.4	0.2
Mg_{ws}	4.7	3.0	2.4	1.8	1.2	0.6	0.3
Ca_{ws}	3.7	2.4	1.8	1.4	0.9	0.5	0.2
				µg L^{-1}			
Cu	0.25	0.16	0.12	0.09	0.06	0.03	0.02
Mn	0.18	0.11	0.09	0.07	0.04	0.02	0.01
Zn	0.26	0.17	0.13	0.10	0.07	0.03	0.02

Table 3. Initial nutrient concentrations in growth media with different concentrations of the effluent from the biorefinery (EFL).

Nutrients	$EFL_{10\%}$	$EFL_{7.5\%}$	$EFL_{5\%}$	$EFL_{3.5\%}$	$EFL_{2.5\%}$	$EFL_{1.56\%}$	$EFL_{0.78\%}$	$EFL_{0.6\%}$	$EFL_{0.39\%}$
					mg L^{-1}				
N_{tot}	183.6	137.7	91.8	64.3	45.9	22.9	14.3	11.0	7.2
NH_4-N	142.8	107.1	71.4	45.0	35.7	17.8	11.1	8.6	5.6
P_{tot}	20.4	15.3	10.2	7.1	5.1	2.5	1.6	1.2	0.8
K_{tot}	387.6	290.7	193.8	135.7	96.9	48.4	30.2	23.3	15.1
Mg_{tot}	30.6	22.9	15.3	10.7	7.6	3.8	2.4	1.8	1.2
Ca_{tot}	40.8	30.6	20.4	14.3	10.2	5.1	3.2	2.4	1.6
Na_{tot}	938.4	703.8	469.2	328.4	234.6	117.3	73.2	56.3	36.6
P_{ws}	13.0	9.8	6.5	45.6	3.3	1.6	1.0	0.8	0.5
Mg_{ws}	18.2	13.7	9.1	63.8	4.6	2.3	1.4	1.1	0.7
Ca_{ws}	17.7	13.3	8.9	62.0	4.4	2.2	1.4	1.1	0.7
					µg L^{-1}				
Cu	0.45	0.34	0.22	1.57	0.11	0.06	0.04	0.03	0.02
Mn	0.77	0.57	0.38	2.68	0.19	0.10	0.06	0.05	0.03
Zn	1.09	0.82	0.55	3.82	0.27	0.14	0.09	0.07	0.04

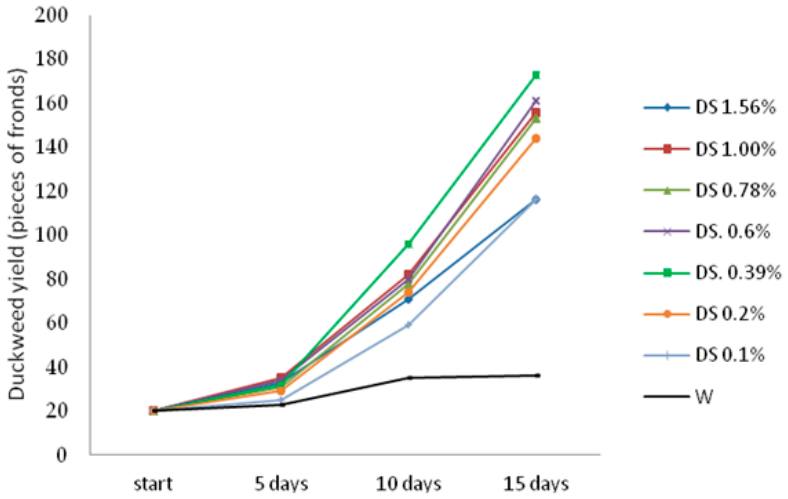

Figure 1. Yield of duckweed cultured on growth medium with different concentrations of the digestated from the biogas plant (DS).

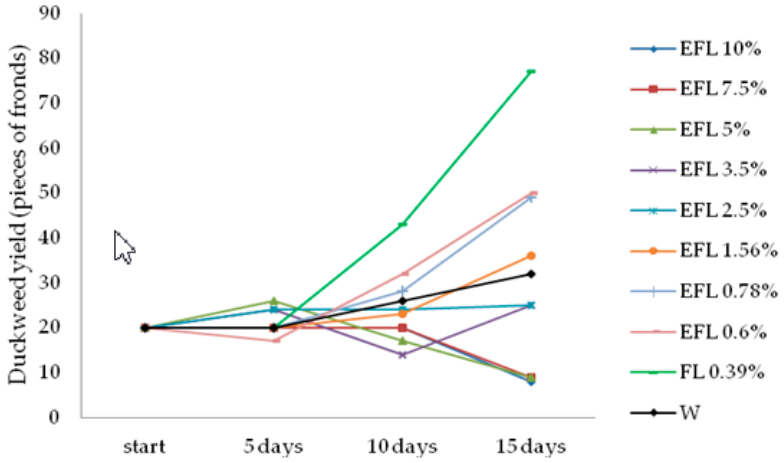

Figure 2. Yield of duckweed cultured on growth media with different concentrations of the effluent from the biorefinery (EFL).

In the present experiment, the 5% and higher EFL addition to the growth medium had a negative impact on duckweed yield. In boxes with these media, duckweed decay was observed from the 10th day of culture (Figure 2), while no duckweed growth was observed in the boxes with growth media containing 2.5% and 3.5% of EFL. In contrast, in the boxes with 0.78% and 0.6% of EFL in the media, duckweed yield reached approximately 50 pieces of fronds, i.e., 10.8 g FM per box. Although in the growth media with $EFL_{0.78\%}$ and $EFL_{0.6\%}$ the contents of nitrogen and phosphorus approximated those optimal for duckweed growth (10–40 mg N L^{-1} and 1.5–2.2 mg P L^{-1} [38,39]), duckweed yields in these boxes were ca. 36% lower than in those with $EFL_{0.39\%}$. The above results indicate that high concentrations of the effluent from the biorefinery inhibit duckweed growth. The growth limiting factors could be the excessive contents of potassium and sodium ions in the growth medium (23.3–30.2 mg K_{tot} L^{-1} and 56.3–73.2 mg Na_{tot} L^{-1} in $EFL_{0.6\%}$ and $EFL_{0.78\%}$, respectively). The high potassium content of EFL (3.8 g K_{tot} kg^{-1} FM, Table 1) was because it was obtained from the liquid fraction of the digestate (featuring a high potassium concentration) subjected to the processes of nitrogen and phosphorus recovery. During separation, a large part of potassium ions contained in the digestate migrates to its liquid fraction because, unlike nitrogen and phosphorus, they are not bound with the organic matter [4]. In turn, the high sodium content of EFL was due to NaOH addition during earlier struvite precipitation and ammonia stripping from the digestate's liquid fraction.

Study results demonstrate that potassium content (approximately 48 mg L^{-1}) and, simultaneously, a high sodium content (approximately 117 mg Na L^{-1}) in the medium inhibited duckweed growth, and that approximately 291 mg K L^{-1} and 704 mg Na L^{-1} made it impossible (Figure 2). However, literature data have indicated the feasibility of duckweed culture on media with a high salt content [38]. Sea salt has been applied in pilot studies of duckweed farming in Bangladesh. Leng [38] showed, however, that duckweeds tolerated high salt levels when additional nutrients were added into the growth medium.

The literature provides considerable data on the potential growth rates of duckweeds. Landolt and Kandeler [40] reported that, under optimal conditions, 73 tons dry matter (DM) of duckweed biomass ha^{-1} yr^{-1} could be produced, i.e., 20 g DM m^{-2} d^{-1}. Under less than optimum conditions, it was possible to obtain between five and 20 tons DM of duckweed biomass ha^{-1} per year. In this experiment, the duckweed yield produced was approximately 1.5 and 3.1 g DM m^{-2} d^{-1} (i.e., 31 and 70 g FM m^{-2} d^{-1}) on the media with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively. Similar yields of duckweed (i.e., 52–96.2 g FM m^{-2} d^{-1}) were obtained by Stadtlander et al. [41] on household sewage and diluted cattle slurry, respectively. In the present study, assuming that the duckweed production cycle was 15 days, it would be feasible to achieve yields approximating 5.4 and 11.1 tons DM ha^{-1} per year on a growth media with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively.

3.2. Nutritional Value of Duckweed

Duckweed produced had a low content of dry matter, i.e., 43.98 g kg^{-1} and 47.10 g kg^{-1} when grown on the media with $DS_{0.39\%}$ and $EFL_{0.39\%}$, respectively. Investigations conducted by other authors with various duckweed species showed their dry matter contents ranged from 3 to almost 8% [18]. Considering the low dry matter content in the fresh matter of duckweed (approximately 4%), its addition to a diet will not significantly increase the energy or protein value of the diet. This is an important factor limiting the applicability of high amounts of duckweed fresh matter in feed ratios for animals from the intensive production systems. The nutritional value and usability of duckweed might be increased when it is dried and added in a dried form to complete feed mixtures. The potential of biomass to be used for feedstuff purposes is determined by protein content in its dry matter. In this study, the protein content of duckweed reached 360.7 g kg^{-1} DM and 353.7 g kg^{-1} DM when it was grown in the media with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively (Table 4). Dried duckweed with a protein content of approximately 350 g kg^{-1} DM can be considered to be a potential source of protein for farm animals and aquaculture. The content of protein in the studied duckweed was lower than in extracted soybean (46%), but higher than in rapeseed cake (30%), maize DDGS (25–30%), or legume seeds (21–35%), except for yellow lupine (38%) [42,43]. Investigations conducted by Rusoff et al. [44] and Appenroth et al. [18] pointed to a good amino acid composition of duckweed protein, resembling that of legume seed protein. However, Appenroth et al. [18] emphasized that its culture conditions significantly determined the total protein content of duckweed. In their study addressing the feasibility of duckweed culture on swine wastewater, Zhao et al. [23] demonstrated protein content in the biomass which ranged from 280 to 320 g kg^{-1} DM. In studies carried out at the University of New England, the crude protein content of duckweed, growing on a diluted effluent from housed pigs, increased with effluent levels of N which increased from about 150 g kg^{-1} DM crude protein with low levels of N (1–4 mg N l^{-1}) to 370 g kg^{-1} DM at between 10–15 mg N l^{-1} [38].

The high protein content and biological value of dried duckweed make it an interesting high-protein feedstuff on the condition that costs of its drying are low. The broad utilization of this feedstuff in animal feeding may be limited by its high fiber content (86.92 g kg^{-1} DM and 94.87 g kg^{-1} DM when duckweed was cultured on the media with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively, Table 4), which is higher than in such high-protein raw materials as extracted soybean, pea meal, or lupine meal. Therefore, dried duckweed can be used in higher amounts in the feeding of ruminants, such as Equidae or Leporidae.

Table 4. Proximate composition of duckweed.

Duckweed	Dry Matter g kg^{-1}	Total Protein	Crude Ash	Crude Fat	Crude Fiber
		g kg DM			
LM grown with $EFL_{0.39\%}$	47.1	360.7	224.8	30.35	86.92
LM grown with $DS_{0.39\%}$	43.98	353.7	220.3	29.26	94.87
P-value	0.033	0.37	0.487	0.532	0.196
SEM	0.765	5.122	4.354	1.159	3.356

LM, *Lemna minuta;* SEM, standard error of means.

Regardless of the growth medium type, the crude fat content of duckweed biomass was low and approximated 29.8 g kg^{-1} DM for $EFL_{0.39\%}$ and $DS_{0.39\%}$ (Table 4). In contrast, the analyzed duckweed had a high content of crude ash (224.8 g kg^{-1} DM and 220.3 g kg^{-1} DM when grown on the media with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively, Table 4) and, consequently, high contents of minerals. The mineral composition of duckweeds tested is presented in Table 5. The duckweeds differed significantly in the contents of Ca ($p \leq 0.05$) and P ($p \leq 0.01$), which were significantly higher in the plants cultured in the medium with $DS_{0.39\%}$ as compared with $EFL_{0.39\%}$, i.e., 4.32 g vs. 3.08 g Ca kg^{-1} and 4.54 g vs. 3.82 g P kg^{-1}, respectively (Table 5). Appenroth et al. [45] obtained higher contents of these elements in various species of *Wolffia* genus duckweed, i.e., approximately 20.4 g Ca kg^{-1} and 16.2 g P kg^{-1} DM on average.

Table 5. Contents of macro- and microelements, and heavy metals in the analyzed duckweeds.

Duckweed	Macroelements				Microelements			Heavy Metals			
	Ca	P	K	Mg	Na	Zn	Cu	Cd	Pb	Al	Cr
	g kg^{-1} DM				mg kg^{-1} DM						
LM grown with $EFL_{0.39\%}$	3.08	3.82	23.08	4.54	10.58	226.66	2.39	0.06	1.22	189.94	0.80
LM grown with $DS_{0.39\%}$	4.32	4.54	18.98	4.29	7.87	186.98	6.12	0.03	1.29	161.28	0.89
P-value	0.049	0.000	0.064	0.196	0.059	0.236	0.076	0.092	0.800	0.092	0.900
SEM	0.356	0.053	1.241	0.116	0.820	20.58	1.350	0.010	0.181	4.199	0.496

LM, *Lemna minuta;* SEM, standard error of means.

Among all determined macroelements, duckweed featured the highest contents of potassium (23.08 and 18.98 g K kg^{-1} DM when grown with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively) and sodium (10.58 and 7.87 g Na kg^{-1} when grown with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively). These values differ from the literature data. According to Appenroth et al. [45], the potassium content of duckweed reached approximately 67.8 mg kg^{-1} DM and that of sodium reached 0.25 g kg^{-1} DM. Considering microelements and heavy metals analyzed in duckweed in the present study, worthy of attention are high contents of zinc (226.66 mg Zn kg^{-1} DM and 186.98 mg Zn kg^{-1} DM when duckweed was grown in the media with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively) and aluminum (189.94 mg Al kg^{-1} DM and 161.28 mg Al kg^{-1} DM for $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively). The determined content of zinc was nearly four times higher than its average content reported by Appenroth et al. [45], i.e., 53 mg kg^{-1} DM. The contents of minerals in duckweed are significantly influenced by their contents in the medium it grows in [46]. This is due to the high ability of this aquatic plant to absorb and accumulate metal ions, including heavy metals, from water [47]. Therefore, duckweed is used to remove minerals from water [48]. This may explain differences between the contents of elements (Ca, P, K, Na, and Zn) noted in this study and those reported by other authors [18,45].

Table 6 presents the contents of α-tocopherol and carotenoids in duckweed cultured in different growth media. Significant differences between duckweeds were demonstrated in their contents of β-carotene and zeaxanthin ($p \leq 0.05$). Higher contents of these components were determined in duckweed grown on the medium with $EFL_{0.39\%}$ than with $DS_{0.39\%}$. In turn, β-carotene content was higher by 43.4 mg kg^{-1} DM, and zeaxanthin content by 4.7 mg kg^{-1} DM. Regardless of the growth

medium type, the major carotenoid of duckweed proved to be lutein, followed by β-carotene and violaxanthin. The concentrations of the other carotenoids tested were significantly lower.

Table 6. Contents of α-tocopherol and carotenoids in duckweed cultured in different growth media.

Duckweed	α-Tocopherol	β-Carotene	α-Carotene	Violaxanthin	Zeaxanthin	Lutein
	mg kg^{-1} DM					
LM grown with $EFL_{0.39\%}$	63.7	346.1	22.0	252.8	33.2	583.7
LM grown with $DS_{0.39\%}$	59.5	302.7	21.1	279.9	28.5	568.5
P-value	0.132	0.037	0.563	0.633	0.045	0.652
SEM	1.76	11.25	1.09	18.68	1.30	23.30

LM, *Lemna minuta*; SEM, standard error of means.

Investigations conducted by Appenroth et al. [18,45] demonstrated similar contents of individual carotenoids and α-tocopherol in duckweed. The present study proved that duckweed, and in particular dried duckweed, could be a rich source of these compounds for animals. Alpha-tocopherol and carotenoids are both valuable antioxidants and serve important functions in the immune system of animals [49–51]. A comparison of α-tocopherol contents in dry matter of duckweed, grasses, silages, or hay has shown it was similar or higher [52–54]. In turn, contents of β-carotene, lutein, or zeaxanthin in duckweed dry matter were higher than in a dry matter of green forage, as well as grass and hay silages [55–58]. Due to low contents of α-tocopherol and carotenoids in maize silage, the addition of fresh or dried duckweed can complete contents of these bioactive compounds and improve feedstuff quality [53,56].

3.3. Nutrients Uptake

Depending on the species, N and P uptake by duckweed could vary between 150–1670 mg N m^{-2} d^{-1} and 15–300 g P m^{-2} d^{-1} [59]. Other authors reported that nutrient uptake rates of duckweed vary between 45 and 1670 mg N m^{-2} d^{-1} and between 8 and 220 mg P m^{-2} d^{-1} [39]. In this study, N uptake by *Lemna minuta* was 45.74 and 94.16 mg per box for $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively (i.e., 84.7 and 174.4 mg N m^{-2} d^{-1}) (Table 7). At the same time, the P uptake was 3.03 and 7.55 mg P per box (i.e., 5.61 and 13.98 mg P m^{-2} d^{-1}). Due to higher duckweed yields obtained at $DS_{0.39\%}$, nutrient uptake by *Lemna minuta* from $DS_{0.39\%}$ was higher than from $EFL_{0.39\%}$, i.e., by approximately 73, 98, 44, 73, and 437% for K, Mg, Ca, Zn, and Cu, respectively (Table 7). Landolt [16] demonstrated that the uptake of nutrients by duckweeds increased with their increased concentrations in the medium. Therefore, the *Lemna minuta* uptook larger amounts of nutrients from $DS_{0.39\%}$ than from $EFL_{0.39\%}$. However, $EFL_{0.39\%}$ contained more sodium than $DS_{0.39\%}$ (36.6 and 2.8 mg Na_{tot} L^{-1}, respectively, Tables 2 and 3), which explains the 15% higher uptake of this nutrient from $EFL_{0.39\%}$.

Table 7. Nutrient uptake by *Lemna minuta*.

Duckweed	N	P	K	Mg	Ca	Na	Zn	Cu
	mg per Box						μg per Box	
LM grown with $EFL_{0.39\%}$	45.74	3.03	18.29	3.60	2.44	8.96	179.65	1.89
LM grown with $DS_{0.39\%}$	94.16	7.55	31.57	7.14	3.51	7.82	311.05	10.18

LM, Lemna minuta.

3.4. Nutrient Recovery

The usefulness of duckweed for the purification of numerous wastewaters was analyzed by Körner et al. [60]. After 12–24 days of growth, duckweed removed 40 to 60% of the nitrogen from a solution. Goopy and Murray [36] reported that *Lemnaceae* usually used from 30 to 50% of dissolved phosphorus. Lower P recovery than N from organic waste may be because P can be absorbed by organic matter or precipitated with Ca^{2+}, Fe^{3+}, and Al^{3+} [61]. Alaerts at al. [62] showed that duckweed

utilized 74% nitrogen and 77% phosphorus from stabilized sewage. Zhou et al. [24] reported that over 93% of nitrogen and phosphorus was removed from municipal wastewater during 15 days of cultivation of four species of duckweed [24]. Similar data were presented by Yu et al. [63] and Cheng and Stomp [14] who studied duckweed growth on sewage water and wastewater from a hog farm, respectively. A higher level of nitrogen and phosphorus removal (98%) was recorded by Mohedano et al. [64] after duckweed cultivation on a pig-farm effluent. In the present study, N recovery reached approximately 75% and 81%, whereas P recovery reached approximately 45% and 55% from $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively (Table 8). The recovery of other components was lower, except for zinc, whose recovery level was similar to that of P. The tested *Lemna minuta* was able to remove more than 14% and 19% potassium, and 18% and 11% calcium from $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively. Magnesium recovery was similar for both growth media (i.e., approximately 35%). The lowest recovery was obtained for copper, reaching only 1.6% on average. Sodium recovery from $DS_{0.39\%}$ reached 33%, whereas it was only 2.9% from $EFL_{0.39\%}$ (Table 8). The results obtained show that after duckweed harvest, there was still a significant amount of sodium in the growth medium. Therefore, research should be continued to find a different species of duckweed that will absorb more sodium from the growth medium.

Table 8. An apparent nutrient recovery (ANR) from $EFL_{0.39\%}$ and $DS_{0.39\%}$.

Duckweed	N	P	K	Mg	Ca	Na	Zn	Cu
LM grown with $EFL_{0.39\%}$	75.1	44.8	14.2	35.5	18.0	2.9	49.7	1.3
LM grown with $DS_{0.39\%}$	81.1	55.3	19.7	34.8	11.4	32.7	55.5	1.9

LM, Lemna minuta.

3.5. Land Requirement

Due to the relatively high yield of duckweed with a proven forage value and the high degree of nitrogen and phosphorus removal from a growth medium, it can be concluded that this treatment of anaerobic digestate and effluent from biorefinery can be successfully carried out on a large scale. Pond depth intended for duckweed cultivation should be less than 0.5 m. Such ponds ensure that the plants grown there will have full access to the nutrients [65]. Assuming that the depth of the pond for duckweed cultivation is 0.5 m, the share of DS or EFL will be 0.39%, the duckweed cultivation cycle will last 15 days, and it will be possible to utilize about 468 m^3 of DS or EFL on 1 ha per year. This indicates that the proposed solution can be implemented preferably by small size biogas plants or biorefineries. The use of such low DS or EFL concentrations (0.39%) in the growth medium requires significant amounts of freshwater, and significant area, which may be a problem in large scale installations. In order to reduce this problem, it is necessary to find another species of duckweed that can tolerate higher concentrations of potassium and sodium in the growth medium than that of the tested *Lemna minuta.* A duckweed pond can produce approximately 5.4 and 11.1 ton DM ha^{-1} per year on $EFL_{0.39\%}$ and $DS_{0.39\%}$ ponds, respectively. With a protein content in duckweed of approximately 354–361 g kg^{-1} (Table 4), it is possible to obtain 2–4 t of protein per 1 ha yearly from $EFL_{0.39\%}$ and $DS_{0.39\%}$ ponds, respectively. For comparison, with an average alfalfa yield of approximately 8–15 t DM ha^{-1}, the protein yield is approximately 2–2.5 t [66–68]. The conducted research indicates that duckweed culture may be a promising source of protein.

4. Conclusions

This study demonstrated that duckweed culture, in a growth medium with a digestate from a biogas plant or an effluent after struvite precipitation and ammonia stripping from the liquid fraction of anaerobic digestate in a biorefinery, allowed for the effective recovery of minerals and the production of high-quality feedstuff for animals. The results obtained showed that N recovery reached approximately 75% and 81%, whereas P recovery reached approximately 45% and 55% of the growth media with $EFL_{0.39\%}$ and $DS_{0.39\%}$, respectively. However, after the duckweed harvest, there was still a significant

amount of sodium in the growth medium. Therefore, research should be continued to find a different species of duckweed that would absorb more sodium from the growth medium. Duckweed produced in the biorefinery proved to be a valuable high-protein feedstuff with high contents of α-tocopherol and carotenoids. With a protein content in duckweed of approximately 354–361 g kg^{-1} DM, it is possible to obtain 2–4 t of protein per 1 ha yearly from $EFL_{0.39\%}$ and $DS_{0.39\%}$ ponds, respectively, which is similar to protein yields from alfalfa cultivation.

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