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The Potential Application of Effluent after Microalgae Anaerobic Digestion for Fertilization of Lettuce

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1. Introduction

Recently, it is observed growing trend in many EU countries to use of natural and organic fertilizers that are environmentally friendly. The societies' awareness resulting from dangers of using chemical fertilizers is still growing. Also, food industry noticed dynamic growth of organic food products. Therefore, demand for organic farming production will increase and role of different kind natural and organic fertilizers will gain importance. Worth noticing is fact that, the matter of third generation biofuels production is willingly discussed among scientists (Pawłowski, 2015). Thus, it should be highlighted that not only production of biogas is the ultimate goal but safe management of effluent after microalgae anaerobic digestion (algal effluent) as valuable organic fertilizer (Zabochnicka-Świątek, 2010; Zabochnicka-Świątek, 2013).

Due to the fact that food demand will be constantly growing, the maintenance of soil fertility will be key issue to face it. Currently soil degradation concerns approximately 50% of arable land in the world and it is projected to intensify in the future years (FAO, 2015). Despite that share of arable land in Poland equals 60% in total land use, the soil quality in Poland remain low. For instance, production properties of Polish soil from 1 hectare is comparable to 0.6 hectare of European Union arable land (Skłodowski & Bielska, 2009). The soil analysis indicates on decreasing level of organic matter content, with the average value of

1.94% in 2015 (EPI, 2017). Polish soils are characterized by high level of acidification – about 50% of arable land. Majority of soils belong to IV valuation class – 40%, with the surface area of 7 572 thousand ha (CSO, 2016). Diminishment of soil fertility may be the result of overusing chemical fertilizers. In 2014/15 the consumption of chemical fertilizers reached up to 39.2 and 123.2 kg/ha in terms of pure ingredient.

Taking into consideration negative effects of chemical fertilizers on the environment such as nitrogen leaching, depletion of nutrients, loss of organic matter and acidification it is desirable to explore for innovative, organic fertilizers which will not pose negative impact on environment. Algal biomass used for agricultural purposes will act in accordance with agricultural sustainable manner. Studies to date indicate that algae can be utilized as biofertilizers, seed primers, biostimulants and influence the growth of plants; improvement of physicochemical characteristic of soil (Lichner et al., 2013; Godlewska et al., 2016; Michalak et al., 2017). Different forms of algae such as dry biomass, extracts or post-fermentation residues are discovered to bring the value as effective fertilizer due to high micro and macronutrients content, phytohormones and vitamins (Blunden et al., 2010; Papenfus et al., 2013; Garcia-Gonzalez & Sommerfeld, 2016; Zabochnicka-Świątek, 2017). Organic fertilizers obtained from algal biomass are not toxic and do not pollute the environment unlike mineral fertilizers. The anaerobic digestion of microalgae provides biogas and effluent (Ward et al. 2014). Research on the impact of dry and living algal biomass on plant yielding and soil fertility was carried out, however algal effluent after microalgae anaerobic digestion as organic fertilizer has not been examined so far.

The main aim of the work was to assess the potential application of the algal effluent for fertilization of lettuce. Influence of the algal effluent fertilization on growth parameters of lettuce was also examined. The changes in physicochemical characteristics of sandy soil after application of algal effluent were determined, too.

2. Materials and methods

The pot experiment was conducted using 3 fertilization combinations in triplicate in the laboratory conditions. The experiment was set up on very light soil of valuation class VI, with granulometric composition of loose sand (PSSS, 2008). The soil samples were taken from the Czestochowa steelworks area. At laboratory soil was dried and mixed in order to homogenize the soil material and sieved through 2.0 mesh for further preparation for analysis. Prior application of fertilizers, soil samples were taken from soil layer of 15 cm and analyzed. All results are given on a dry mass content.

In the soil samples were determined: the dry matter content according to PN-ISO 11465:1999. pH was measured according to PN-ISO 10390:1997. Kjeldahl's nitrogen was analyzed according to PN-ISO 11261:2002. Total carbon was determined by means of Multi N/C 2100 Analytik Jena. Measurement of Total Organic Carbon (TOC) concentration was conducted according to PN-ISO 14235:2003, by means of Spectrophotometer HACH DR/4000 V. Macro elements (P, K, Ca, Mg) and microelements (Cu, Zn, Fe) were analyzed according to PN-ISO 11047:2001 by means of plasma spectrometer – Spectro Arcos ICP-OES. Determination of available phosphorus and potassium content was carried out according to Egner-Riehm method (Karczewska & Kabała, 2017). The available magnesium content was examined by Schachtschabel method (Karczewska & Kabała, 2017). Measurement of humic acids content was carried out by means of method described by Stevenson (1994). Hydrolytic acidity and sum of alkaline cations were analyzed according to modified Kappen method (Karczewska & Kabała, 2017).

In our previous study biogas production from algal biomass digestion was performed and algal effluent was obtained. The effluent after 3-week anaerobic digestion of algal biomass *Scenedesmus acutus* was utilized for fertilization of lettuce. The macro elements as well as microelements contained in algal effluent could be more available to plants than in raw dry algal biomass. Measurements of pH, Kjeldahl's nitrogen, Dissolved Organic Carbon (DOC) concentration and macro elements (P, K, Ca, Mg) and microelements (Cu, Zn, Fe) in algal effluent were done.

The widely used mineral fertilizer Azofoska in this experiment was used as reference fertilization with dose of 1.1 g/kg of the soil recommended by manufacturer.

Plants for the experiment were selected based on short vegetation period and need of rational fertilization. *Lactuca sativa* chosen for the pot experiment exhibits sensitivity for salinity in the soil and nitrates accumulation.

Fertilization was carried out based on nitrogen content in the tested soil as the most significant macronutrient for plants growth and nutritional requirements for plants. The experiment was conducted based on the principles of good agricultural practice (GAP) and protecting plants against over-fertilization.

Before foundation of experiment, homogenous seeds were selected and used to produce the seedlings of lettuce which lasted around 15 days. Fertilization of plants took place in the time of seedlings transferring for the 0.5 kg pots. Plants were watered regularly throughout the entire experiment and leachate was collected in plastic bottles. Lettuce seedlings were provided with artificial conditions with the lightening of 12h per day with appropriate temperature of 18°C. While experiment was over, the plants were harvested and measured. The fertilized soil was analyzed in terms of pH, Kjeldahl's nitrogen, total carbon, total organic carbon, C/N ratio, macro (P, K, Ca, Mg) and microelements content (Zn, Cu, Fe), available forms of P, Mg and K.

The fertilization treatments for experiment are following:

- Mineral fertilizer Azofoska with N/P/K ratio of 13/6/17 in the dose of 1.1 g/kg,
- Algal effluent in the dose of 33.61 g/kg,
- Control sample – tap water without any additive.

3. Results and discussion

3.1. Characteristics of soil and fertilizers

The examined soil exhibits alkaline character in KCl – 7.60, which exceeds the maximum value of pH for Polish soils which is 7.4 (IUNG, 2017). The value of pH measured in H_2O is the same as maximum pH measured in Polish soils. TOC measured in soil is in the level of 7493 mg/kg which is placed at the lower limit in the range for Polish soils (3600-38400) mg/kg. Similarly, the value of Kjeldahl's nitrogen is assessed as low (N_{Kjeld} – 7493 mg/kg) because the value is close to the lower limit of range for Polish soils 400-3600 mg/kg. The content of macro elements in examined soils considerably below the average level of Polish soil, with exception of calcium. Particularly the soil suffers from potassium deficit, 21.3 times less than the average value recorded for Polish soils. The available forms of macro nutrients, phosphorus and potassium content place the soil in the average soil fertility class. In the light of above mentioned properties of tested soil can be concluded that soil needs to be fertilized in order to restore nutritional values.

Mineral fertilizer Azofoska is characterized by specific content of nutrients presented in the Table 1. pH of this fertilizer was amounted to 5.6, which indicates on acidic character.

Table 1. Characteristics of mineral fertilizer Azofoska
Tabela 1. Charakterystyka nawozu mineralnego Azofoska

Nutrient	Compound name	[%]
Nitrogen	Total nitrogen	13.3
	Nitrate nitrogen	5.5
	Ammonium nitrogen	7.8
Phosphorus	Phosphorus pentoxide soluble: in neutral ammonium citrate and water	6.1
	Phosphorus pentoxide soluble: in water	4.0
Potassium	Potassium oxide soluble in water	17.1
Magnesium	Total magnesium oxide	4.5

The characteristic of the algal effluent is presented in the Table 2. The algal effluent effluent has a basic character (pH − 8.30). Kjeldahl's nitrogen of tested additive is at the level of 79938 mg/kg. The measured parameter of dissolved organic carbon indicated on high level of organic matter content (15151 mg/kg). The content of macronutrients is varied but the highest amount is recorded for calcium − 24030 mg/kg. There is twice predominance of phosphorus to potassium content (17084 mg P/kg and 8007 mg K/kg).

Table 2. Characteristic of the algal effluent after anaerobic digestion
Tabela 2. Charakterystyka odcieku pofermentacyjnego z glonów

Parameter/Nutrient	Unit	Algal effluent
pH_{H2O}	–	8.30
$N_{Kjeld.}$	mg/kg	79938
DOC	mg/kg	15151
P	mg/kg	17084
K	mg/kg	8007
Mg	mg/kg	3969
Ca	mg/kg	24030
Cu	mg/kg	150
Zn	mg/kg	579
Fe	mg/kg	7841

3.2. Effect of fertilization on soil

The applied fertilizers influenced on physicochemical characteristic of the soil. Application of mineral fertilizer caused decrease of pH from 7.60 to 7.40, while pH after addition of the algal effluent did not change. In both cases the fertilized soil has not change the alkaline character. In general, recommended pH for most of the plants is within the range 5.5-7.2 (Dyśko et al., 2014) It is documented that acidic and alkaline soils may disturb nutrients uptake by plants, particularly influence on deficiencies of phosphorus. In very acidic soils, at pH (in 1M KCl) below 4.5-5.0 the absorption of phosphorus rapidly decreases and phosphorus turns into practically insoluble compounds with plant-toxic aluminum. Inversely, when pH is above 6.8, absorption of phosphorus decreases. Systematic organic fertilization promotes stabilization of soil pH due to high organic matter content and increased buffering properties. Mineral fertilizers such as Azofoska used in this experiment exhibit acidifying properties and therefore should be used deliberately to not pollute the environment by toxic aluminum ions (Barak et al., 1997).

The Kjeldahls' nitrogen has increased in fertilized soil compared to control but the change was inconsiderable. Upward trend was noticed in case of mineral fertilization, change of Kjeldahl's nitrogen by 133 mg/kg compared to control. Other literature sources obtained results confirming that biogas digestate is the source of easily available forms of nitrogen for plants uptake and influenced on the greater biomass growth by approximately 30% compared with mineral fertilizers (Nabel et al., 2014). It is worth noticing that plants can absorb only mineral forms of nitrogen (NO_3^-, NH_4^+) which is several percent of total nitrogen (Bednarek & Tkaczyk, 2003; Zawadzki, 2003). Additionally, long-term application of the algal effluent as organic fertilizer prevents total nitrogen loss and thus prevents surface and groundwater against nitrates contamination. In this experiment, similar values of nitrogen achieved by two different types of fertilizers confirms that application of the algal effluent may constitute good source of nitrogen as well as commonly used Azofoska.

There is observed increase of TOC after application of the algal effluent 1.2 fold increase of TOC and decrease of the same amount of TOC in case of mineral fertilization. It resulted from the fact that the algal effluent is significant source of organic matter content. According to the literature, with the increase of organic matter, the sorption capacity also increased up to 40.10 mol/kg due to seaweed extract application (Habashy & Abdel-Razek, 2011). On the contrary, reduction of organic carbon by mineral fertilizer is in accordance with other literature sources (Dziadowiec et al., 2003; Rynowska-Hryńczuk, 1992). Crucial is fact that organic fertilizers decompose slowly in the soil, allowing for continuous release of nutrients and sustain the microbial activity for long time in comparison to mineral fertilizers (Murphy et al., 2007). Rural Development Program 2014-2020 assumes that soil analysis should be extended to twice TOC analysis which is regarded as necessary as well as providing positive organic matter balance due to fertilization (RDP, 2017).

C/N ratio has increased after treatment with the algal effluent, from 9.08 to 11.22. Differently, decrease of C/N ratio was recorded for mineral fertilization, change by 2.3 unit compared to control. Widening the C/N ratio is found to have positive effect on the balanced mineralization and immobilization processes in the soil. The application of mineral fertilizers favors mineralization processes and increase the availability of nitrogen and subsequently may be the reason of nitrate leaching deep into the soil profile and groundwater.

The obtained results of macro elements analysis revealed that both organic and mineral fertilization caused increase of potassium, phosphorus, calcium and magnesium content compared to control. The highest increase was recorded for application of mineral fertilizer, although the change is regarded as not significant. The growing trend was also observed for the content of micro elements. The increase of zinc, copper and iron has inconsiderably increased after organic and mineral fertilization. It can be explained by short experiment time. The summarized physicochemical characteristic of fertilized soil is given in Table 3.

Table 3. Effect of the algal effluent fertilization on physicochemical characteristic of soil (Mean ± SD)
Tabela 3. Wpływ nawożenia odciekiem pofermentacyjnym z glonów na właściwości fizykochemiczne gleby (Odchylenie standardowe ± SD)

Parameter	Unit	Control sample	Azofoska	Algal effluent
pH_{KCl}	mg/kg	7.6±0.02	7.4±0.04	7.6±0.01
pH_{H2O}	mg/kg	7.8±0.02	7.4±0.01	7.6±0.01
$N_{Kield.}$	mg/kg	939±61	1072±31	944±36
TOC	mg/kg	8527±835	7261±380	10593± 2607
C/N ratio	–	9.1	6.8	11.2
P	mg/kg	140±1	175±4	167±11
K	mg/kg	574±17	762±15	629±47
Mg	mg/kg	895±8	990±16	952±57
Ca	mg/kg	4278±38	4350±110	4461±202
Cu	mg/kg	20±0.4	24±0.5	22±1.4
Zn	mg/kg	403±5	419±7	425±28
Fe	mg/kg	13841±110	13983±92	13655±524

Regarding the available forms of nutrients (P_2O_5, K_2O, Mg), its values show changes to a relatively great extend in soil (Fig. 1).

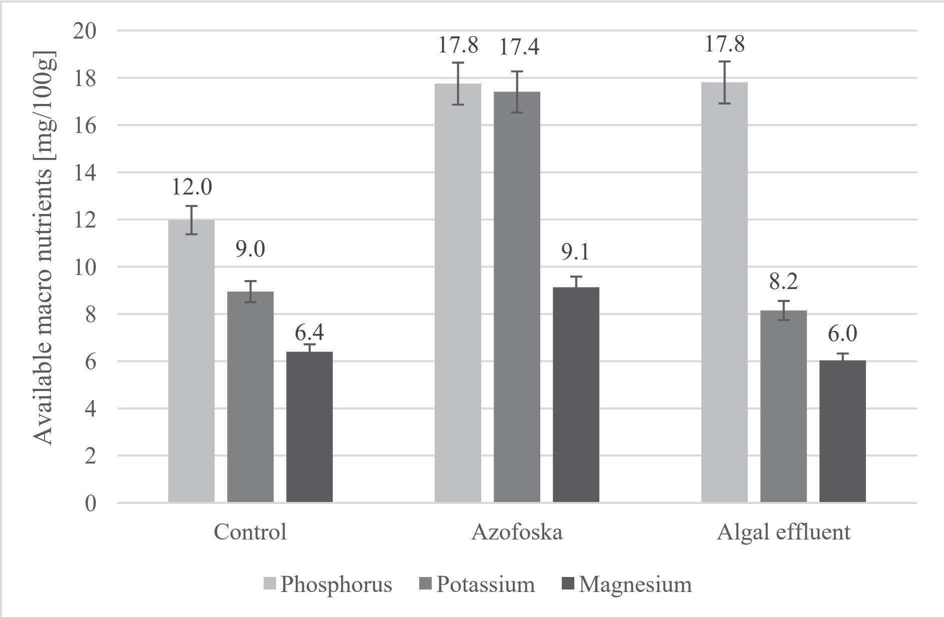

Fig. 1. Changes of available macro nutrients in soil after organic and mineral fertilization
Rys. 1. Zmiany zawartości łatwo przyswajalnych form makroelementów w glebie po zastosowaniu nawożenia

Considering available phosphorus content, its value was the same after treatment of the algal effluent and Azofoska − 17.8 mg P_2O_5/100 g. The limit numbers established for available phosphorus content are: 0.1-15 (average fertility); 15.1-20.0 (high fertility); above 20.1 mg P_2O_5/100 g (very high fertility). The soil fertility class for both organic and mineral treatment increased from average to high. Completely different effect was observed in case of potassium fertilization. The limit numbers for available K_2O content are as following: 7.6-12.5 (average fertility); 12.6-17.5 (high fertility); above 17.6 mg K_2O/100 (very high fertility). According to these intervals, application of mineral fertilizer resulted in the highest increase of available potassium with the value of 17.4 mg K_2O/100 g and changed soil fertility class from average to high.

Also, available magnesium content increased after application of mineral fertilizer, change from 6.4 to 9.1 mg Mg/100 g. The algal effluent added to the soil resulted in slight decrease of available potassium and magnesium content compared to control. It is mainly caused by nutrients utilization by plants and microorganisms during the experiment. However, it indicates that organic matter included in the algal effluent should be subjected to mineralization processes in order to become more available for plants uptake. Despite changes of available magnesium values, the soil fertility class maintained the same as in the control soil (very high fertility class above – 6.1 mg Mg/100 g).

3.3. Effect of fertilization on growth parameters of lettuce

The algal effluent influenced all growth parameters of lettuce. Number of leaves changed significantly from 5 to 7. Parameters such as leaves length and leaves width increased respectively, by 2.03 cm and 0.8 cm. However, roots length was inconsiderably shorter than in control lettuce. Plants looked healthy, without symptoms of over fertilization or plant diseases. Plants fertilized by mineral fertilizer did not survive until the end of pot experiment. The results of the experiment are presented in the Fig. 2.

Different authors draw attention to negative effects caused by using chemical fertilizers on the environment, e.g. nitrate pollution, loss of organic carbon and relatively high cost of given fertilizers. At the same time, literature sources provide alternatives which are organic fertilizers, such as compost, manure, sewage sludge and others (Diacono & Montemurro, 2010; Hatfield & Stewart, 2002; Kot & Frąc, 2014; Styszko et al., 2017; Ciesielczuk et al., 2015). Recently, scientists are interested in using alternative energy sources e.g. biogas, but utilization of biogas effluent and its impact on the environment has not been investigated (Möller, 2015). The application of biogas effluent and digestate as organic fertilizer and soil amendment is still taken under debate (Nkoa, 2014). Usually, feedstock of biogas production constitute animal manure or different kind wastes, therefore further experiments should be carried out to explore the potential use of algal effluent (Tambone et al., 2010; Murto et al., 2004). So far, obtained fertilization results within this experiment cannot be compared with other literature sources. The conducted experiment proved beneficial influence of the algal effluent on soil fertility and enhancement the growth of lettuce. Properly handled and managed biogas effluent has great but still untapped potential to be safely used in organic farming without inducing any negative aspects on the environment.

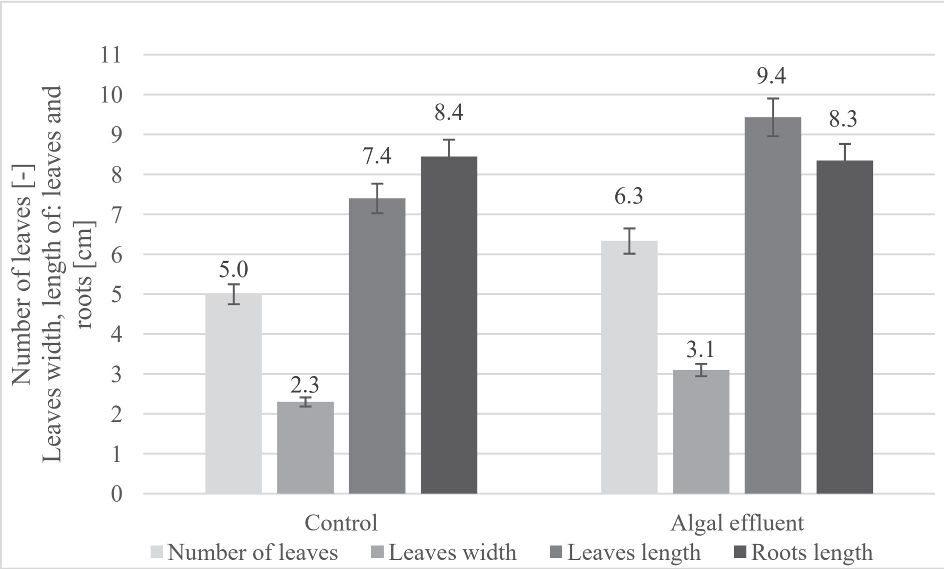

Fig. 2. Influence of the algal effluent fertilization on growth parameters of lettuce
Rys. 2. Wpływ nawożenia odciekiem pofermentacyjnym z glonów na parametry wzrostu sałaty

4. Conclusions

The conducted experiment draws attention on utilization of effluent after microalgae anaerobic digestion as a valuable fertilizer, characterized by high level of nutrients: N_{Kjeld} – 79938 mg/kg; DOC – 15151 mg/kg; P – 17084 mg/kg; Ca – 24030 mg/kg.

According to the obtained results the following conclusions can be drawn:

1. Both fertilizers (algal effluent and Azofoska) increased in the soil key macronutrients essential for plants growth: nitrogen, potassium and phosphorus to similar degree, which means that post-fermentation residues can replace chemical fertilizers.

2. Despite of higher content of macronutrients and their available forms in the fertilized soil by mineral fertilizer, the utilization of the algal effluent is beneficial due to enrichment the soil in total organic carbon (10593 mg/kg), available phosphorus (17.8 mg P_2O_5/100 g), widening C/N ratio (11.2) and counteracting pH changes.
3. Application of algal effluent resulted in significant increase of lettuce growth parameters: increase of number of leaves up to 6, leaves width up to 3.1 cm, leaves length up to 9.4.
4. The algal effluent can act as organic fertilizer which slowly decompose and release nutrients in prolonged time in accordance to sustainable agricultural approach in comparison to fast-acting mineral fertilizers.

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Możliwości zastosowania odcieku pofermentacyjnego na bazie glonów do nawożenia sałaty

Streszczenie

W ostatnich latach obserwuje się wzrost stosowania nawozów pochodzenia naturalnego, które są przyjazne dla środowiska naturalnego. Użyty w badaniach odciek pofermentacyjny po beztlenowej fermentacji glonów zawierał substancje organiczne i związki mineralne niezbędne do uprawy sałaty. Dzięki ww. składowi odciek może stanowić konkurencyjny produkt dla nawozów naturalnych i alternatywę dla nawozów mineralnych. Badania prowadzono w warunkach laboratoryjnych. Głównym celem badań była ocena możliwości wykorzystania odcieku pofermentacyjnego z glonów jako nawozu organicznego pod uprawę sałaty masłowej. W pracy przeanalizowano również wpływ ww. odcieku pofermentacyjnego na właściwości fizykochemiczne gleby i wzrost roślin. Eksperyment założono na glebie bielicowej, lekkiej, klasy VI, o składzie granulometrycznym luźnego piasku. Roślinami użytymi w doświadczeniu była sałata masłowa, odmiana "Attractie". Doświadczenie przeprowadzono w trzech powtórzeniach. Dawki nawozów ustalono ze względu na następujące czynniki: zawartość azotu jako głównego makroelementu determinującego wzrost i rozwój roślin oraz wymagania pokarmowe badanych roślin i aktualną żyzność gleby. Przeprowadzona kompleksowa analiza gleby i eksperyment doniczkowy wykazały, że pofermentacyjny odciek z glonów można z powodzeniem stosować w celu poprawy żyzności gleby i zwiększenia wzrostu roślin. Dodatek pofermentacyjnego odcieku glonów spowodował wzrost całkowitego węgla organicznego o 2066 mg/kg, fosforu całkowitego o 27 mg/kg, całkowitego potasu o 55 mg/kg, całkowitego magnezu o 57 mg/kg, całkowitego wapnia o 183 mg/kg. Wskaźnik C/N wzrósł z 9,1 do 11,2. Wartość pH pozostała niezmieniona.

Abstract

In recent years, it is observed an increase in the use of natural fertilizers that are environmentally friendly. The effluent after microalgae anaerobic digestion (algal effluent) contained organic substances and mineral compounds necessary for lettuce cultivation, hence it is a competitive product for natural fertilizers and an alternative to mineral fertilizers. The experiment was conducted under laboratory conditions. The main objective of the research was to assess the possibility of using the algal effluent as an organic fertilizer for the cultivation of butterhead lettuce. The study also analyzed the effect of the algal effluent on physicochemical properties of soil and plant growth. The experiment was established on podzolic, light soil of VI fertility class, under granulometric composition of loose sand. Plants used in the experiments are butterhead lettuce, variety 'Attractie'. The experiment was conducted in three replicates. Doses of fertilizers have been established due to factors: the content of nitrogen as the main macro element determining the growth and development of plants, nutritional requirements of tested plants and current soil fertility. The performed complex soil analysis and pot experiment showed that the algal effluent can be successfully used to improve soil fertility and enhance plants growth. Addition of the algal effluent caused increase of total organic carbon by 2066 mg/kg, total phosphorus by 27 mg/kg, total potassium by 55 mg/kg, total magnesium by 57 mg/kg, total calcium by 183 mg/kg. C/N ratio has increased from 9.1 to 11.2. pH maintained at the same level.

Słowa kluczowe:
mikroglony, odciek pofermentacyjny, nawożenie

Keywords:
microalgae, algal effluent after microalgae anaerobic digestion, fertilization