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Effects of organic compounds on the macroalgae culture of *Aegagropila linnaei*

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Abstract: The effects of the impact of four organic compounds (ascorbic acid, biotin, glucose and sucrose) on ash, protein, fiber, fat and amino acid contents in the freshwater *Aegagropila linnaei* biomass were examined in 7 and 14 days of cultivations in high concentrations of tested compounds (100 mg L⁻¹). The presence of examined organic compounds had a negligible effect on the development of algae and their biomass composition. There were no significant differences in the amino acids composition in the biomass in the presence of organic compounds compared to the test system. However, the increase in ash content was observed irrespective of the cultivation time in the case of all used organic compounds. Only slight differences in crude fat concentration were observed in the case of 7 days cultivation with ascorbic acid, biotin and sucrose, while the highest increase of ash content was observed after 14 days of supplementation with glucose. None of the compounds affected changes in amino acid content in the *Aegagropila linnaei* biomass. The results suggest that an environment enriched with the test organic compounds had only minimal, or at most short-term, effects on the algal biomass composition.

Keywords: remediation, sucrose, glucose, biotin, vitamin C

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1 Introduction

Bioremediation is a well-known technique which involves a degradation of toxic pollutants to non-toxic products. In this process different organisms are used to remove or neutralize hazardous chemicals from a contaminated site, such as soil, sediment, groundwater, or surface water [1]. Phyco-remediation is a special type of bioremediation which uses algae to uptake pollutants. Most of the research on phyco-remediation concerns the use of dried and dead forms of algal biomass to remove heavy metals from soil and water systems [2-4]. The effect of the presence of metals in the biomass of macroalgae is already well known, but there are no reports relating to the assessment of the effects of various organic compounds on the properties of algae.

Algae are able to bind heavy metals due to the presence of specific compounds in their thallus, such as polysaccharides, lipids, proteins and polyphenols, which generally bond metal ions by chelation [5]. Hence, biosorption process using algae as biosorbents for metal ion removal has been frequently studied in recent years [6-9]. Depending on algae species, their morphology and habitat, water temperature, its salinity, pH and other factors, algae are able to cumulate various metals, e.g.: lead, zinc, arsenic, nickel, cadmium, mercury, copper, chromium, iron and others. Thus, the literature shows many examples of the use of phyco-remediation for heavy metal removal, mostly from wastewaters [2,10-14]. Apart from marine species, freshwater algae have also been widely studied in the bioremediation process. The uptake of metal ions from wastewater has been reported using *Oscillatoria limnetica* Lemm. [*Pseudanabaena limnetica* (Lemm.) Komárek], *Anabaena spiroides* Kleb., *Eudorina elegans* Ehr. and *Chlorella vulgaris* Bey. The results showed that the freshwater algae species have great sorption capacity for Cu, Cd and Pb [13]. Also the biosorption of different inorganics by freshwater *Cladophora* species has been extensively reported [2,10,12,14-16]. Summarizing, different species of algae seem to be promising biosorbents

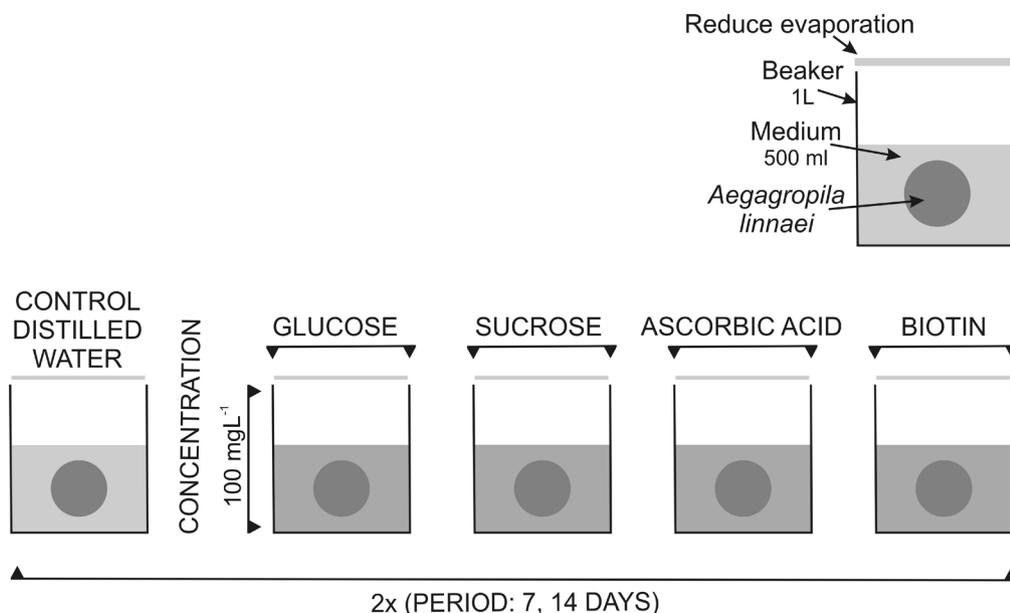


Figure 1: The laboratory experiments

for the treatment of wastewater inorganics, due to low-cost of biomass and their considerable high sorption capacity. However, following a literature survey, there is no extensive study on the biosorption of organic compounds using algae biomass in the literature, with the exception being aromatic compounds and pesticides [17-19]. The aim of our studies was to find out if the freshwater alga *Aegagropila linnaei* Kütz., cultured in a water medium with a high concentration of the following organic compounds: ascorbic acid, biotin, glucose and sucrose, is able to cumulate these ingredients and whether it affects the biomass production (dry biomass) and its composition (ash, total protein, crude fiber, crude fat and amino acid content).

2 Experimental procedure

2.1 Macroalgae culture in the medium supplemented with organic compounds

Balls of *Aegagropila linnaei* were used to determine the ability of vitamins and carbohydrates to be cumulating by macrogreen algae. In order to mobilize the culture samples, the macroscopic green algae were cleaned and sterilized using deionised water. Afterwards, glassware (1 liter beakers) were sterilized. Then algal balls (± 15 g of wet weight) were placed for 48 h in distilled water at a room temperature (the process of acclimatization).

Distilled water was filtered through Whatman GF/F glass fiber filters and sterilized. Algae were cultured in medium (modified Wang's medium) for a period of 7 and 14 days in a constant temperature (294.15 K) and light intensity ($250 \mu\text{mol photons m}^{-2} \text{sek}^{-1}$) in phytothron (CONVIRON model CMP 6050) [20]. Ascorbic acid, biotin, glucose and sucrose were used as a source of organic compounds.

The experiment was composed of the rank of control samples with distilled water and four ranks with the medium enriched with ascorbic acid, biotin, glucose and sucrose at the same concentrations (100 mg L⁻¹). For each rank there were 3 repetitions performed. Glass containers were filled up with 500 ml of medium and ± 15 g of fresh macroalgae biomass (Fig. 1). Two series of experiments were performed; the first lasted for 7 days, and the second for 14 days.

Each time, after completion of the experiment series, the biomass of *Aegagropila* balls was dried in a laboratory oven (2 hours at the temperature of 378.15 K) to constant weight. Next, the components characterizing nutritional properties of biomass were determined.

2.2 Analytical methods

The analysis of biomass concerned the dry biomass, ash, total protein, crude fiber, crude fat and amino acids. The samples of algae biomass were analyzed according to AOAC (2007) for dry matter (method no. 934.01) and ash (method no. 942.05) [21]. Crude protein was determined

by Kjel-Foss Automatic 16210 analyzer (method no. 976.05), crude fat by Soxtec System HT analyzer (method no. 2003.05), and crude fiber by Tecator Fibertec System I (method no. 978.10). The content of N-free extractives was calculated as 1000 – (crude protein + crude fat + ash + crude fibre) and expressed as g kg⁻¹ DM.

The ground algae samples were subjected to acid hydrolysis with 6 M HCl at the temperature of 383.15 K for 23 hours. Amino acids were determined using an AAA 400 amino acid analyser (INGOS, Czech Republic) with ion exchange chromatography. Post column ninhydrin-based detection and sodium citrate buffer were used. The ninhydrin amino acid derivatives were detected using packing of column OSTION Lg ANB (column height: 35 × 0.37 cm) at 570 nm for primary amino acids and at 440 nm for secondary amino acids.

3 Results and Discussion

Increasing use of algae biomass for industrial purposes, and as a valuable raw material in the food and animal feed production, causes the need to search for a new methods of enrichment or changing the composition of biomass. It has been shown that different inorganic molecules, especially metal ions [2-4,7-15], as well as inorganic phosphorus [22,23] and nitrogen from nitrates and ammonium salts [16], has a significant effect on the composition of algae biomass. In our studies, we focused on the impact of four organic compounds: ascorbic acid, biotin, glucose and sucrose, on ash, protein, fiber, fat and amino acid content in the freshwater *Aegagropila linnaei* biomass. High concentrations of the tested

compounds (100 mg L⁻¹) were used, because the lower tested concentrations of 1 and 10 mg L⁻¹ had no effect on the growth of algal biomass and its composition.

Tables 1 and 2 show the results of analysis of algae biomass obtained from the culture in water containing the bioactive organic test compounds. No significant increase in the dry biomass, total protein and crude fiber was observed after addition of tested organic compounds with respect to the control experiment (Table 1). The cultivation time of 7 or 14 days also did not matter. The only slight differences in the crude fat concentration were observed in the case of 7 days cultivation with ascorbic acid, biotin and sucrose. However, the increase in ash content was noticed, both after 7 and 14 days of cultivation, in the case of all used organic compounds. Moreover, the highest increase of ash content was observed after 14 days of supplementation with glucose. Kong et al. [24] noticed that the mixture of glucose and glycerol enhanced the production of *Chlorella vulgaris* biomass and stimulated the biosynthesis of lipids and soluble carbohydrates, whereas in our studies glucose did not affect the *Aegagropila linnaei* biomass production and only resulted in the increase in ash content. In contrast to our results, different observations were made by Bhatnagar [25] on various microalgae, where the supplementation with glucose and sucrose supported significant algal growth, and sucrose addition resulted in the highest increase in chlorophyll content in algae between others carbon sources.

Furthermore, Table 2 shows that there were no significant differences in the amino acid composition in the biomass in the presence of organic compounds compared to the test system. None of the compounds affected the changes in amino acids content in

Table 1: Analysis of *Aegagropila linnaei* biomass cultured in the laboratory in water with a nutrient medium supplemented with bioactive organic compounds

	Water with a medium		Water with a medium supplemented with 100 mg L ⁻¹ of ascorbic acid		Water with a medium supplemented with 100 mg L ⁻¹ of biotin		Water with a medium supplemented with 100 mg L ⁻¹ of glucose		Water with a medium supplemented with 100 mg L ⁻¹ of sucrose	
	After 7 days	After 14 days	After 7 days	After 14 days	After 7 days	After 14 days	After 7 days	After 14 days	After 7 days	After 14 days
Dry biomass (g)	94.47	94.54	94.31	94.39	94.52	94.25	94.27	94.61	94.17	94.34
Ash (g 100 g ⁻¹ biomass)	14.69	14.43	16.83	17.19	16.31	15.75	15.45	20.88	17.28	16.60
Total protein (g 100 g ⁻¹ biomass)	26.59	26.44	25.51	27.08	26.56	26.78	25.85	26.30	26.57	25.77
Crude fiber (g 100 g ⁻¹ biomass)	27.19	27.07	27.56	27.56	26.25	26.49	26.84	25.58	24.91	25.32
Crude fat (g 100 g ⁻¹ biomass)	1.87	1.87	2.19	1.65	2.37	1.83	1.22	1.85	2.66	1.37

Table 2: Amino acids content [g 100 g⁻¹ of total protein] in biomass of cultured *Aegagropila linnaei*

Amino acid (g 100 g ⁻¹ of total protein)	Water with a medium		Water with a medium supplemented with 100 mg L ⁻¹ of ascorbic acid		Water with a medium supplemented with 100 mg L ⁻¹ of biotin		Water with a medium supplemented with 100 mg L ⁻¹ of glucose		Water with a medium supplemented with 100 mg L ⁻¹ of sucrose	
	After 7 days	After 14 days	After 7 days	After 14 days	After 7 days	After 14 days	After 7 days	After 14 days	After 7 days	After 14 days
Aspartic acid	11.50	11.40	10.68	10.57	11.04	9.40	10.35	9.86	11.50	11.11
Threonine	3.62	3.67	3.61	3.68	3.64	3.56	3.70	3.55	3.43	3.68
Serine	3.11	3.19	3.21	3.00	3.14	2.93	3.11	2.94	3.08	3.18
Glutamic acid	7.80	7.77	7.71	7.80	8.33	7.77	7.99	7.45	7.96	8.08
Proline	5.11	5.13	4.86	4.81	5.03	4.68	5.07	4.58	4.61	4.87
Cysteine	2.71	2.81	2.61	2.62	2.58	2.31	2.69	2.73	2.40	2.24
Glycine	3.78	3.79	3.81	3.79	3.79	3.66	3.86	3.72	3.70	3.84
Alanine	3.88	3.85	3.46	3.46	3.55	3.35	3.51	3.31	3.34	3.59
Valine	3.16	3.18	3.13	3.13	3.14	3.00	3.15	3.02	3.01	3.18
Methionine	0.80	0.81	0.72	0.71	0.71	0.76	0.77	0.71	0.71	0.80
Isoleucine	1.84	1.84	1.79	1.75	1.77	1.79	1.82	1.69	1.71	1.81
Leucine	3.82	3.85	3.90	3.86	3.75	3.64	3.82	3.70	3.60	3.89
Tyrosine	5.06	5.06	4.60	4.76	4.90	4.69	5.33	4.84	4.87	5.04
Phenylalanine	2.84	2.88	2.87	2.82	2.77	2.85	2.9	2.75	2.77	2.91
Histidine	3.65	3.60	4.33	4.41	4.19	4.41	4.64	3.91	3.98	4.36
Lysine	5.13	5.17	5.19	5.10	5.26	5.02	5.32	4.92	4.98	5.23
Arginine	7.92	7.94	6.54	6.86	7.83	6.04	6.24	7.34	6.24	7.61

Aegagropila linnaei biomass. It has been proved that salinity stress is one of the main factors, which might increase the free amino acid concentration in *Cladophora vagabunda* [26]. However, according to Kong et al. [24], the addition of glucose and glycerol to a nutrient medium can reduce the anabolism of proteins in algae. It may indicate that glucose supplementation, as well as other tested organic compounds, could result in inhibition of the synthesis of amino acids in *Aegagropila linnaei*.

The results obtained are somewhat surprising, however, it may indicate a different mechanism of bioremediation of organic compounds by algae with respect to the metal ions. The presence of the studied organic compounds had a negligible effect on the development of algae and their biomass composition in contrast to the metal ions [2-4,6-8,14,15], inorganic phosphorus [22,23] and nitrogen [16]. However, Berman & Chava [27] proved that also organic nitrogen sources might significantly stimulate the development of algae,

especially when urea was the only nitrogen source. There may be several factors responsible for the lack of an effect of the test organic compounds on algal biomass. First of all, due to the size of the particles, the organic compounds might not be absorbed by living algae. Probably, these compounds must be first degraded, e.g. by bacteria, to be able to be absorbed by algae, while there were no relevant bacteria in the laboratory experiment. Another factor may be the insufficient amount of feed in the nutrient medium available for the growth of algae. Generally, algae use available compounds present in a large excess of water. These results are very important in the case of the application of algae biomass for industrial purposes, abstracted from the natural habitat, as well as from breeding. Summarizing, according to our results, the chemical composition of biomass does not depend on the presence of tested organic compounds, while the impact of metal ions is far more significant [2-4,6-8,14,15].

4 Conclusions

In conclusion it should be said that the present experiment demonstrates that modifying the aquatic environment through organic compounds (ascorbic acid, biotin, glucose, sucrose) does not affect the development of macroalga *Aegagropila linnaei*, nor does it affect the chemical composition of its biomass.

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