fermentation

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Article

Algal Biomass Accumulation in Waste Digestate after Anaerobic Digestion of Wheat Straw

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Abstract: Cultivation of microalgae in waste digestate is a promising cost-effective and environmentally friendly strategy for algal biomass accumulation and valuable product production. Two different digestates obtained as by-products of the anaerobic fermentation at 35 °C and 55 °C of wheat straw as a renewable source for biogas production in laboratory-scale bioreactors were tested as cultivation media for microalgae after pretreatment with active carbon for clarification. The strains of microalgae involved were the red marine microalga *Porphyridium cruentum*, which reached 4.7 mg/mL dry matter when grown in thermophilic digestate and green freshwater microalga-*Scenedesmus acutus*, whose growth was the highest—7.3 mg/mL in the mesophilic digestate. During cultivation, algae reduced the available nutrient components in the liquid digestate at the expense of increasing their biomass. This biomass can find further applications in cosmetics, pharmacy, and feed. The nitrogen and phosphorus uptake from both digestates during algae cultivation was monitored and modeled. The results led to the idea of nonlinear dynamic approximations with an exponential character. The purpose was to develop relatively simple nonlinear dynamic models based on available experimental data, as knowing the mechanisms of the considered processes can permit creating protocols for industrial-scale algal production toward obtaining economically valuable products from microalgae grown in organic waste digestate.

Keywords: anaerobic digestion; waste digestate; microalgae cultivation; modeling

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1. Introduction

Microbial fermentation processes in biosphere are responsible for the greater part of the biologically driven hydrogen and methane. Biomethane could be produced by processing various types of waste. Anaerobic digestion (AD) is a well-known biological process used for the utilization of organic waste for green energy production [1]. Increasing biogas production worldwide, rich in biomethane, that could be used for heat and/or electricity generation, will meet the energy supply needs with renewable alternatives [2]. The improvement in various aspects and parameters in AD, such as pretreatments, reactor types, co-digestion, process modeling, and control, promotes reaching a better insight into the process and improving its stability and efficiency [3].

Great quantities of digestate are thus produced after anaerobic digestion of organic wastes, which causes problems related to transport costs, gas emissions, and sludge accumulation. At the same time, nutritional substances, such as nitrogen and phosphorus, remain available therein. It is necessary to find alternative pathways for valorization with the aim of reducing the environmental impact and improving the economic profitability of anaerobic installations [4]. Waste digestate from biogas production can be used for direct treatment of agricultural crops instead of fertilizing, but in most cases, the organic matter

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BIOLOGY
Cell biology

EXAMINATION OF EXOPOLYSACCHARIDES FROM *Porphyridium cruentum* FOR ESTIMATION OF THEIR POTENTIAL ANTITUMOUR ACTIVITY IN VITRO

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Abstract

Globally, research is being conducted focusing on new biologically active substances and technologies for their application in various sectors, in the hope that their biological and medical applications will be widely used in the next decade.

The introduction of therapeutic agents of biological origin isolated from certain species of bacteria and algae and their potential for cancer treatment is based on their lower toxicity and hence greater safety compared to synthetic pharmaceuticals. In this respect, the potential of microbial producers and microalgae is enormous. Among the biologically active components isolated from algae of the greatest importance for biology and medicine are complex polysaccharides, as well as some pigments.

In the present work, the antitumour properties of two fractions of polysaccharides from the red microalgae *Porphyridium cruentum* were observed by determining their effect on cell viability of four different cell lines.

New data from the study indicate that samples treated with *P. cruentum* polysaccharide have significant and dose-dependent antiproliferative and cytotoxic effects. Changes in nuclear morphology in MCF-7 tumour cells induced by polysaccharide treatment, including chromatin condensation, nucleus fragmentation, and apoptotic body formation, have been demonstrated. Polysaccharides show obvious pharmacological effects in the treatment of tumour cells without side effects.

Key words: polysaccharides, *Porphyridium cruentum*, antitumour activity, fluorescent microscopy

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Biological and biochemical oxidation processes; medical image processing; neural networks and fuzzy logic techniques

POLYSACCHARIDES PRODUCED BY TWO RED ALGAL STRAINS GROWN IN DIGESTATE WITH POTENTIAL ABILITY OF TUMOR CELL INHIBITION

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ABSTRACT

Research on the biological activities and applications of polysaccharides as active biocompounds synthesised by marine unicellular algae, which are, in many cases, released to the surrounding environment – exopolysaccharides, has revealed the possibility of many applications. We have investigated the growth and development of two red microalgae *Porphyridium cruentum* and *Rhodella reticulata*, where the used medium was decolourised with active carbon digestate from anaerobic digestion of agricultural waste – wheat straw. The biomass accumulation for both strains reached a dry weight of 5.2 to 5.5±0.2 mg ml^{-1}, starting from 0.85 mg ml^{-1} m initial concentration for 6 days. Production of heteropolysaccharides was estimated to show increased yield of 0.35 mg ml^{-1} on the 6th day of cultivation for the *Rhodella reticulata* and 0.28 mg ml^{-1} for *Porphyridium cruentum*, compared to 0.25 mg ml^{-1} of the control – growth in a standard medium. The two isolated bioactive substances were then tested using 3-(4, 5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulphophenyl)-2H-tetrazolium, inner salt – (MTS test), as potential antitumor agents on two cancer cell lines and a normal one to establish diminishing of cell vitality, mostly when applied to the high metastatic cell line MDA-MB 231- 47%, when electroporation was applied by the heteropolysaccharide synthesised by the *Porphyridium cruentum,* grown in digestate.

Keywords: red microalgae, digestate, heteropolysaccharide, antitumor activity.

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Biomethane production using ultrasound pre-treated maize stalks with subsequent microalgae cultivation

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ABSTRACT

This study utilized a renewable energy source, agricultural waste, in anaerobic digestion (AD) at appropriate conditions to obtain biogas and biomethane as an energy carrier. Maize stalks underwent ultrasound (US) pre-treatment for better accessibility for microorganisms, as lignocelluloses have a stable structure, insoluble in water and resist both mechanical and enzymatic attack. The digestate after an anaerobic digestion process was used for cultivation of algae after adsorption with activated carbon for clarification. Photosynthetic microalgae have industrial and economic perspectives, so their low-cost cultivation has a great potential for many applications. The results showed the impact of US pre-treatment of maize stalks as a sole substrate and co-digested with algal biomass. The total yields were 1116 cm^3/L, 1350.5 cm^3/L and 1293.25 cm^3/L for the untreated, ultrasonically pre-treated and microwaved maize stalks. The possibility of accumulating algal biomass using anaerobic digestate as a medium was demonstrated. US pre-treatment (400 W) showed high efficiency with respect to the extractives obtained per unit of energy input. Addition of 4 g/L of microalgal biomass as a co-substrate led to an increase in the biogas yield compared to native stalks. A small closed circle system, starting from anaerobic digestion of lignocellulosic substrates followed by microalgae cultivation in the digestate and subsequent return of microalgal biomass back in the bioreactor as a co-substrate was realized, encouraging circular economy. The suggested scheme is a simple and low-cost technology, as the substrate used is freely available and renewable, and algae proved to grow in a waste effluent as medium.

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KEYWORDS
Ultrasound pre-treatment; maize stalks; anaerobic digestion; biomethane; microalgae

Introduction

Environmental protection and renewable energy sources have become important global concerns in recent years. The fossil fuels are constantly depleting while the available renewable sources possess a great economical potential. Energy produced from biogas after its purification and concentration can be used for electricity generation, heat production or as direct automobile fuel [1]. Biogas can generate electricity by using a combustion engine, fuel cell or gas turbine, with the resulting electricity being used on-site or sold. The production of biogas through anaerobic digestion offers significant advantages over other ways of agricultural waste treatment [2]. The technologies for biogas production are widely known and are the most convenient way of recycling bio-waste. The application of the obtained biogas depends on the quantity of methane in it. As a raw material for the production of heat and electrical energy, biogas may serve in many biogas plants to cover part of their electrical needs. Biomass has the advantage to be considered as carbon neutral because the quantity of CO_2 released during combustion is the same as that absorbed by the plant during photosynthesis [3]. The biomethane production from lignocellulosic wastes is a widely studied process because of the broad spread and easy access to materials such as wheat straw, maize stalks, etc. Harvesting residues and other agricultural waste represent available source of lignocellulosic material useful for energy production. Lignocellulosic biomass is one of the most abundant renewable resources in the world and can be

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Engineering in Life Sciences

RESEARCH ARTICLE

Characterization and potential antitumor effect of a heteropolysaccharide produced by the red alga *Porphyridium sordidum*

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Taking into account the rising trend of the incidence of cancers of various organs, effective therapies are urgently needed to control human malignancies. However, almost all chemotherapy drugs currently on the market cause serious side effects. Fortunately, several studies have shown that some non-toxic biological macromolecules, including algal polysaccharides, possess anti-cancer activities or can increase the efficacy of conventional chemotherapy drugs. Polysaccharides are characteristic secondary metabolites of many algae. The efficacy of polysaccharides on the normal and cancer cells is not well investigated, but our investigations proved a cell specific effect of a newly isolated extracellular polysaccharide from the red microalga *Porphyridium sordidum*. The investigated substance was composed of xylose:glucose and galactose:manose:rhamnose in a molar ratio of 1:0.52:0.44:0.31. Reversible electroporation has been exploited to increase the transport through the plasma membrane into the tested breast cancer tumor cells MCF-7 and MDA-MB231. Application of 75 μg/mL polysaccharide in combination with 200 V/cm electroporation induced 40% decrease in viability of MDA-MB231 cells and changes in cell morphology while control cells (MCF10A) remained with normal morphology and kept vitality.

KEYWORDS
algal heteropolysaccharide, antitumor effect, *Porphyridium sordidum*

1 | INTRODUCTION

Cancer is one of the most common reasons for human mortality. The treatment of cancer diseases requires a therapeutic approach with severe side effects. Recently, new sources of non-toxic natural substances with potential anticancer effects are under active investigation [1]. Marine resources attract the attention of many researchers focusing on the diverse biological activities of microalgal polysaccharides and the possibilities for their application [2]. There has been a tremendous interest in developing anti-cancer polysaccharide drugs over the last decade. Polysaccharides from renewable sources as fungi or algae belong to biomacromolecules in which residues of monosaccharides are connected to each other by glycosidic linkages. They offer some biological information due to the structural variability comparing with nucleic acids or proteins [3]. Polysaccharides are characteristic secondary metabolites of many algae. Although cultivation of microalgae seems easy, there are many challenges including minimizing contamination, efficient provision of carbon dioxide, controlled light supply following optimal cultivation

Abbreviation: DW, dry weight.

Specific Antitumor Effect of the Combined Action of Algal Heteropolysaccharide and Electroporation

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Abstract: *Marine organisms are potentially prolific sources of highly bioactive secondary metabolites that might represent useful leads in the development of new pharmaceutical agents. In this study the biological effect of the freeze-dried heteropolysaccharide, isolated and purified from the red microalga Rhodella reticulata strain using electroporation was evaluated. Two different types of cells – tumor and non-tumor were treated with the heteropolysaccharide alone or together with the application of electroporation. The effect of the treatment was evaluated in parallel: with proliferation test for estimating cell viability and with immunofluorescent cytoskeleton staining to establish changes in morphology. Evidence for cell line specific viability reduction (70% from the control in case of cancer cell line treatment and only 30% in non-tumor cells) in a dose dependent manner was presented. These findings will arouse further interest in heteropolysaccharide as a new anticancer drug suitable for clinical trials.*

Keywords: *Antitumor effect, Rhodella reticulata heteropolysaccharide, Electroporation, Immunostaining.*

Introduction

Marine resources have attracted worldwide attention in many research fields [8-10]. The focus is in the search for bioactive substances for development of new drugs, because of their relatively low toxicity and high bioactivity [24]. Cancer is one of the most lethal diseases that threaten human life. Therefore, it is more than important to find novel, effective and nontoxic compounds from natural sources to be used together with the generally used chemotherapy. The great potential of marine microalgal polysaccharides to be used as anti-inflammatory, antiallergic, analgetic, antitumor agents was recently reviewed [6, 19, 21]. Red algal heteropolysaccharides (hPSHs) have attracted much attention in the biochemical and medical areas because of their anti-cancer effects. The mechanism of the anti-cancer activity is related to the ability of hPSHs to inhibit the growth of cancer cells (cytotoxic or cytostatic effect), to stimulate the immune response, to inhibit tumor angiogenesis and to induce apoptosis

PROTECTIVE EFFECT OF *DIXONIELLA GRISEA* (RHODOPHYTA) POLYSACCHARIDE AGAINST MYELOID GRAFFI TUMOR IN HAMSTERS

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Summary

Immunomodulators of natural origin have raised a considerable interest in red microalga as a producer of high molecular weight polysaccharides because these biopolymers often show advantages over the polysaccharides that are currently in use. We investigated the protective effect of *Dixoniella grisea* polysaccharide (DgPSH) on Graffi myeloid tumor in hamsters (GTBH). Various doses and ways of application of polysaccharide administered before and after tumor transplantation were studied. The mean survival time, inhibition of tumor growth, mortality percent and transplantability were determined. We further investigated the in vitro effect of DgPSH on the functional state of peritoneal macrophages by macrophage spreading and phagocytosis. The obtained experimental data demonstrate the protective effect of *Dixoniella grisea* polysaccharide (DgPSH) on the survivability of GTBH as expressed by decrease of the tumor transplantability, inhibition of tumor growth, elongation of mean survival time and reduced mortality percentage. The DgPSH increases both spreading and phagocytic ability of peritoneal macrophages in healthy and GTBH. The stimulation followed a dose-dependent pattern. The observed protective effect at the beginning stage of tumor progression can be partly explained by the temporary immunostimulating and immunorestorating action of the polysaccharide on the immune cells (macrophages, lymphocytes, NK-cells) or by direct cytotoxicity on Graffi myeloid tumor. Studies aimed at elucidation of immunomodulating activities of red microalgal polysaccharides in Graffi tumor bearing hamsters are currently in progress.

Key words: Dixoniella grisea, polysaccharide, Graffi myeloid tumor, macrophages.

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Environmentally friendly oxidation processes

BIOCHEMICAL COMPOSITION OF *Porphyridium cruentum* CULTIVATED IN WASTE DIGESTATES

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ABSTRACT

The bioremediation of digestate using microalgae is perspective solution to decreasing the eutrophication from organic waste. Cultivation of microalgae in waste anaerobic residue leads to a reduction in production costs of valuable bioactive metabolites.The red microalga *Porphyridium cruentum* is a representative of Rhodophyta, which is a producer of many useful bioproducts. The present study aimed to determine how the biochemical characteristics change when this microalga was grown in two undiluted digestates obtained as by-products of the anaerobic biodegradation at 35°C and 55°C of wheat straw, as a renewable source for biogas production.

The results showed that the growth of the control was comparable to that in the thermophilic digestate, but the biochemical characteristics of the three variants (control, termophilic digestate, mesophilic digestate) were quite different. Accumulation of proteins and lipids is significantly higher in the algae cultivated in digestates (over 1.1 times for thermophilic variant), while carbohydrates reached 51% in the control, 41% in the thermophilic digestate and 38% in the mesophilic one, respectively.

In conclusion: *Porphyridium cruentum* grew well in thermophilic digestate. The strain could be used simultaneously for bioremediation of agricultural waste as well as for the production of valuable lipids and proteins.

Keywords: *Porphyridium cruentum*, waste digestate, bioremediation, lipids, carbohydrates.

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Biotechnology

OPTIMIZING THE PRODUCTION OF VALUE-ADDED SUBSTANCES DERIVED FROM *CHROOCOCCUS* SP. R-10 (CYANOPROKARYOTA)

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(*Submitted by Academician A. Galabov on December 16, 2019*)

Abstract

The cyanoprokaryote *Chroococcus* R-10, which was isolated from a hot spring in south-west Bulgaria (28 °C), was introduced to laboratory cultivation for the first time. In this research we tried to estimate the optimal conditions for the best growth and accumulation of its extracellular polysaccharides and the light-harvesting phycobiliproteins. The results showed that high biomass yield was observed when the alga was cultivated in the range of 26–35 °C regardless of the light intensity. The most suitable conditions for growth and polysaccharide accumulation are a temperature of 26 °C and unilateral irradiation of 132 μmol photons $m^{-2}s^{-1}$. High light intensity in combination with high temperatures reduced the total amount of phycobiliproteins as expected. The oxygen evolution properties were used as a sensitive signal for PSII operation which confirmed once again the physiological advantage of the optimal growth temperature conditions. Using the newly estimated optimum growth parameters, we also managed to achieve maximal biomass yield of 10.3±0.3 $g.dm^{-3}$, polysaccharide viscosity of 4.9±0.2 m $Pa.cm^3g^{-1}$and phycobiliprotein yield of 0.8±0.1 $g.dm^{-3}$. The large quantities of the produced polysaccharide increase the strain's potential for future biomedical studies such as anticancer, antibacterial, and antifungal.

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polymers

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Article

Bacterial Natural Disaccharide (Trehalose Tetraester): Molecular Modeling and in Vitro Study of Anticancer Activity on Breast Cancer Cells

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Abstract: Isolation and characterization of new biologically active substances affecting cancer cells is an important issue of fundamental research in biomedicine. Trehalose lipid was isolated from *Rhodococcus wratislaviensis* strain and purified by liquid chromatography. The effect of trehalose lipid on cell viability and migration, together with colony forming assays, were performed on two breast cancer (MCF7—low metastatic; MDA-MB231—high metastatic) and one "normal" (MCF10A) cell lines. Molecular modeling that details the structure of the neutral and anionic form (more stable at physiological pH) of the tetraester was carried out. The tentative sizes of the hydrophilic (7.5 Å) and hydrophobic (12.5 Å) portions of the molecule were also determined. Thus, the used trehalose lipid is supposed to interact as a single molecule. The changes in morphology, adhesion, viability, migration, and the possibility of forming colonies in cancer cell lines induced after treatment with trehalose lipid were found to be dose and time dependent. Based on the theoretical calculations, a possible mechanism of action and membrane asymmetry between outer and inner monolayers of the bilayer resulting in endosome formation were suggested. Initial data suggest a mechanism of antitumor activity of the purified trehalose lipid and its potential for biomedical application.

Keywords: bacterial natural disaccharide; trehalose tetraester; *Rhodococcus wratislaviensis*; breast cancer; anticancer activity; molecular modeling

1. Introduction

The isolation, characterization, and study of the properties of new bioactive compounds are a crucial part of the fundamental research in the field of biology and medicine and provide unlimited opportunities for new drug discoveries [1,2].

Biosurfactants are naturally occurring surface-active biomolecules produced by a variety of microorganisms [3–6].

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BIOTECHNOLOGICAL PERSPECTIVES OF THE RED MICROALGA *PORPHYRIDIUM CRUENTUM*

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Abstract: The red microalga *Porphyridium cruentum*, a potential source of bioactive substances, was the subject of a study on the effect of nutrients and different CO_2 concentrations (0.04 and 2%) on the growth, biochemical composition and synthesis of phycobiliproteins and polysaccharides. For this purpose the alga was grown on two nutrition media, referred in this manuscript as M1 and M2 (M1 is richer in chloride and nitrogen substances). We have estimated that the abovementioned parameters varied under different cultivation conditions. Best yield was observed in M1 in the presence of 2% CO_2, with a peak of the measured biomass equal to 4.0±0.1 g L^{-1}. On the other side, compared to M1, M2 stimulated the pigment synthesis when the culture was enriched with 2% CO_2. The highest amount of carbohydrates and pigments was observed when the alga was grown with 0.04% CO_2. These results confirm that *Porphyridium cruentum* posesses great adaptive capabilities that can be used in future biotechnological practices.

Keywords: growth, biochemical composition, polysaccharide, phycobiliprotein, CO_2 concentration

INTRODUCTION

Microalgae are a vast group of aerobic photosynthetic microorganisms that thrive in various habitats (Sharma, 2007; Phang, 2008). They are suitable producers of biomass which is a valuable raw material for different industries (pharmaceutical, cosmetic, food, etc.), because of the compounds that can be extracted from it. Such commercially applicable compounds include pigments (β-caroten, astaxanthin, phycocyanin, phycoeritryn), polysaccharides, unsaturated fatty acids and proteins. *Porphyridium cruentum* is a subject of great interest due to its high levels of productivity of sulphated polysaccharides (Gouveia et al., 2008), which have been proved to possess different biological activities – antioxidant (Tannin-Spitz et al., 2005), anti-inflammatory (Matsui et al., 2003), anti-tumor (Gardeva et al., 2009), antiviral (Minkova et al., 1996; Raposo et al., 2014), immune stimulating (Sun et al., 2012) and many others. Phycobiliproteins are deep-colored, water-soluble proteins that are present mainly in Cyanobacteria and Rhodophyta. They capture light energy, which is then passed on to the chlorophylls during photosynthesis. Also, they can neutralize the reactive oxygen species due to their chemical structures and chelating properties, thus reducing oxidative stress (Christaki et al., 2015).

All of this is a premise for a further development of the algal biotechnology by studying the physiology and the biochemistry of the microalgae. In this study, we tried to determine how the changes in cultivation conditions of *Porphyridium cruentum* affect the composition of the biomass and the amount of the extracellular polysaccharides. The purpose was not only to acquire a higher amount of biomass, but to achieve better production of polysaccharides and/or pigments and at the same time to maintain stress-free conditions.

MATERIALS AND METHODS

Strain and growth conditions

Monoalgal, non-axenic cultures of *Porphyridium cruentum* (AG.) NAG Vischer 1935/107 (Rhodophyta, Porphyridiales, Porphyridiaceae) from the culture collection of the Institute of Botany ASCR, Třeboň, Czech Republic) were grown autotrophically in 200 mL flasks, at 22 °C under continuous illumination (white fluorescent light, 132 μmol photons m^{-2} s^{-1}) for 7 days (168h). The cultures were continuously supplied either with 0.04% (atmospheric air) or 2% CO_2. The initial culture density was 0.5 g L^{-1}. The content of the used nutritive media were shown on Table 1.

Tab. 1.
Content of the modified nutritional media for the growth of the red *Porphyridium cruentum* (based on the medium of Brody & Emerson, 1959).

	Elements	Medium 1 [g L^{-1}]	Medium 2 [g L^{-1}]
1	K_2HPO_4	0.8	0.66
2	KNO_3	1	-
3	KCl	-	16
4	NaCl	27	12.5

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Environmentally friendly oxidation processes

EFFECT OF HEAVY METALS ON THE GREEN ALGA *Scenedesmus incrassatulus*

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ABSTRACT

Green alga *Scenedesmus incrassatulus*, strain R 83, was treated by high concentrations of Cu, Cd and Pb added into the nutrition medium. Cd strongly inhibited growth – it was 60% decreased, followed by Pb – about 50% decreased, while the action of Cu appeared to be the less harmful. Cd and Pb impaired cell morphology and development, and worsen the algal culture.

It was found a progressive decrease in the amount of chlorophyll *a*, *b* and carotenoids corresponding to the duration of heavy metals action. Pb had the most pronounced negative effect on the pigment composition of *Sc. incrassatulus*. Meanwhile, Cu-treated variants remained less affected by the metal toxicity. These results were supported by the changes in malondialdehyde (MDA) content showing an enhanced lipid peroxidation under Pb and Cd pollution. Therefore, it could be suggested that Cu influenced in a different way metabolic processes in the algal cells, than Cd and Pb.

Sc. incrassatulus possessed a good adsorbing capacity for metal ions, especially for cadmium, which was absorbed in a much greater extent than lead and copper. For that reason, we assumed that the strain could be used in the treatment of wastewater, polluted by Cu, Pb and Cd.

Keywords: *Scenedesmus incrassatulus,* heavy metals, pigments, malondialdehyde.

AIMS AND BACKGROUND

A number of microalgal species are currently used for biological purification of industrially polluted environments including heavy metal contaminated areas[1–3]. It is

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Optimization strategies for improved growth, polysaccharide production and storage of the red microalga *Rhodella reticulata*

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To ensure good conditions for growth and heteropolysaccharide production by the red microalga *Rhodella reticulata,* optimization of the medium and conditions was performed. Stimulation of the growth of microalgal cells up to1.6-fold was achieved with a new medium. Maximal growth rate (μ=0.19 d^{-1}) was reached at the beginning of stationary phase of growth (72 h), together with the increase in the pigment content. The influence of the great quantity of humic acids and thiamin in the medium (proved by HPLC) on algal growth was also established. The amount of extracellular polysaccharide (0.365 mg ml^{-1}) produced by the algal cells cultivated in the new medium was 1.4-fold higher compared to the quantity in the standard medium. The immobilization of *Rhodella reticulata* cells into super-macroporous cryogel of 2-hydroxyethylcellulose lead to a prolonged period of storage. Algal cells were vital after three months and could be successfully used for the production of extracellular polysaccharide with values for immobilized cells 1.3 –fold higher than those for free cells.

Key words: Algae; Bioactive substances; Growth medium; Immobilization

INTRODUCTION

A successful biotechnological process depends on the selection of a suitable organism capable to produce a desired product at optimal conditions. By 2015, the industrial biotechnology will have a 20% share in total chemical production [1]. The red microalgae of the genus *Rhodophyta* are potential sources of unique bioactive substances [2-3] that can find different applications - in medicine, pharmacy, cosmetics, food industry as food supplements, etc. [4-5]. Typical representative of the genus is the unicellular red microalga *Rhodella reticulata*, which has the ability to synthesize and release part of the polysaccharide material into the culture medium [6-7]. Its functions are mainly protective but its antiviral and antitumor effects are well known [8-9]. The growth of microalgae is primarily affected by abiotic environmental factors. Some biologically active organic compounds, namely vitamins, nucleic acids and organic matter such as humic substances and soil extracts, also stimulate the growth of algal cultures [10]. However, long cultivation of algae in a laboratory usually leads to contamination and decreased growth rates and densities, when cultured in traditional media [11]. It is suggested that the type of nutrient medium plays a critical role for the algal growth [12]. Therefore, the suitable balancing of the elemental composition of the growth medium is a tool to obtain high-density cultures. Different optimization strategies were applied to the media components in order to increase the productivity of algal cells, pigment synthesis and polysaccharide production [13-14]. An approach to keep the vitality of cells after long-term storage of the algal cultures in the laboratory is the immobilization technique. It is based on finding suitable carriers that can ensure preservation of the algal cultures for a long period under laboratory conditions. Immobilization of different algae had already been performed in alginate beads to increase the period of storage and preserve their physiological activity [15]. At the same time, immobilization facilitates both the extraction of the extracellular polysaccharide from the medium and the handling of the system [16].

Super-macroporous polymer cryogels are an interesting class of materials due to their unique heterogeneous open porous structure, which significantly increases the equilibrium sorption properties and allows unhindered diffusion of solutes, nano-particles and micro-particles. Usually, cryogels possess spongy-like structure of huge pores (50-200 μm) containing free water surrounded by thin walls and, therefore, they are often used for immobilization

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RESEARCH PAPERS

Temperature and Irradiance Effects on *Rhodella reticulata* Growth and Biochemical Characteristics[1]

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Abstract—The red microalga *Rhodella reticulata*, a potential source of bioactive substances, was the subject of study of the irradiance and temperature effects on growth rate and biochemical composition of algal biomass. The optimum temperature for growth decreased from 28 to 26°C with increasing light intensity from 260 to 520 μE/(m^2 s). The maximal growth rate was 0.21/day at 28°C and lower light intensity (260 μE/(m^2 s)). Variations in these parameters also affected the fatty acid productivity, and proteins and carbohydrates content. At 34°C and high light intensity the quantity of carbohydrates was 1.16-fold higher than the quantity at the optimal temperature and low light intensity. Protein content was the highest at lower temperatures for both light intensities. Fatty acid profile showed the highest percent for the polyunsaturated eicosapentaenoic acid (EPA) at 28°C and both light intensities (46% from the whole fatty acid content), an important feature for this strain. This is a prerequisite for use of EPA as a supplement in food industry.

Keywords: Rhodella reticulata, irradiance, temperature, biochemical composition, eicosapentaenoic acid

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INTRODUCTION

Over the past decade, algal biotechnology has grown steadily into a global industry with increasing numbers of entrepreneurs attempting to utilize its biochemical diversity for a wide array of applications [1]. The red microalgae of the division Rhodophyta are potential sources of unique bioactive substances that can find different applications in medicine, pharmacy, cosmetics, agriculture, and food industry as food supplements, etc. [2]. Microalgae are rich in carbohydrates, pigments, and essential fatty acids that could be used as functional ingredients. Valuable products in the red microalgae are the polyunsaturated fatty acids (PUFAs) which are essential for humans [3]. Higher plants and most animals lack the required enzymes to synthesize PUFAs from more than 18 carbons, although they are absolutely necessary for good functioning, conferring flexibility, fluidity and selective permeability properties of membranes. Consequently, lipid profiles in microalgae play a vital role in maintaining the integrity of the world's aquatic food webs [4]. Nutritionally, eicosapentaenoic acid (EPA) (20:5) is one of the most important fatty acids belonging to this group. These long-chain omega-3 fatty acids provide significant health benefits to the human population, particularly in reducing cardiac diseases, stroke and high blood pressure, depression, rheumatoid arthritis, and asthma [5]. They have also been reported to inhibit tumor growth [6].

The physiology and biochemistry of *Rhodella reticulata*, producer of the above mentioned biologically active substances, are poorly studied. To enhance microalgae production efficiency, it is necessary to optimize the growth conditions. Light and temperature are the major factors that affect the overall biomass productivity in nutrient unlimited culture conditions [7]. Temperature stress, in particular, influences the growth rate and chemical composition of microalgae and may limit nutrient interactions. Temperature has a major effect on the phase transition of membrane lipids, the kinetics of cellular enzymes, and active transport systems across membranes [8]. Meanwhile, light is also essential for the growth of phototrophic microalgae, as both light intensity and temperature affect their biomass productivity, metabolites formation, and production of some key enzymes associated with photosynthesis [9]. Understanding the combined effects of light and temperature on algal cultures will enable the optimization of growth in controlled production system by the implementation of temperature regulation as a function of irradiance [10].

The aim of this study was to explore growth and some biochemical characteristics of *R. reticulata*, pro-

[1] The article is published in the original.

Abbreviations: ADW—absolutely dry weight; EPA—eicosapentaenoic acid; PUFAs—polyunsaturated fatty acids.

Construction of Inorganic and Hybrid Biosorbents for Heavy Metal Ions Removal

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Abstract: *Hybrid biosorbents, synthesized by the sol-gel method on the basis of Tetraethylorthosilicate (TEOS) proved to be efficient for the removal of the heavy metal ions from aqueous solutions. The potential use of immobilized in TEOS algal cells of the red microalga Porphyridium cruentum and its products – low and high molecular heteropolysaccharides to remove Cu(II), Cd(II) and Ni(II) ions was evaluated. A laboratory bioreactor was involved in this process. Sol-gel sorbent with TEOS only was used as a control system to the hybrid biosorbents. Their structural characterization was performed using different methods. The maximum adsorption capacities were registered for the biosorbents with immobilized algal biomass and for the preparations with TEOS and high molecular algal heteropolysaccharide: for Cu(II), Cd(II) and Ni(II), they were 18.771 and 21.715 mg Cd·g^{-1} adsorbent; 16.662 and 17.545 mg Ni·g^{-1} adsorbent and 40.633 and 34.431 mg Cu·g^{-1} adsorbent, respectively. The adsorption of toxic Cu(II), Zn(II) and Ni(II) ions in the four types of sorbents proved to be effective. High percent of these ions removal was obtained during the first hours of the adsorption process.*

Keywords: *Hybrid biosorbents, Algae, Heteropolysaccharide, Heavy metal ions.*

Introduction

Decontamination of heavy metals in water around industrial plants has been a challenge for a long time. Presence of heavy metals even in trace amounts is toxic and detrimental to both flora and fauna. Finding a way for their removal is a serious task nowadays. Several methods have been used for the removal of heavy metal ions from aqueous wastes (chemical precipitation, ion exchange, adsorption on activated carbon, membrane technologies) [23]. When the metal ion concentration is below 50 mg·l^{-1} the technologies have been directed towards biosorption [1]. The mechanism of metal biosorption is a complicated process influenced by different factors and consists of two steps. In the first step metal ions are adsorbed to the cell surface by interactions between metals and the functional groups situated on the surface of the cells. All metal ions, before reaching the cell membrane and cytoplasm, get attached to the cell wall. The various polysaccharides and proteins with a number of active

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ORIGINAL PAPER

Lysis of Antarctic algal strains by bacterial pathogen

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Abstract The present paper describes the isolation, physiological and genetic characteristic of a bacterial agent which inhibits the growth of algae and causes death of laboratory cultures of Antarctic microalgal strains: prokaryotic cyanobacteria *Synechocystis salina* and green eukaryotic microalga *Choricistis minor*. The bacterial strain LB1 was isolated from algal damaged laboratory cultures of *S. salina*. It was established that this bacterium is obligate aerobic, Gram-positive, non-spore-forming, immotile, irregular rods with dimensions 0.3–2 μm. Our results showed that LB1 has algicidal effect to *S. salina* as well as to *C. minor*. Transmission electron microscopy observations confirmed the destruction of *S. salina* by the bacterium. Biochemical analysis of LB1 revealed positive reaction to D-glucose, catalase, hydrolysis of gelatin, acid production from: lactose, L-arabinose, L-ramnose, esculin and β-galactosidase. The partial sequence (1,404 bp) of the 16S rRNA gene of LB1 showed 99 % similarity with type strains of the genus *Microbacterium*. The results of the biochemical, antimicrobial and of 16S rRNA analysis of LB1 allowed us to identify LB1 as *Microbacterium* sp. Studying expression of pathogenicity of the bacteria to algal cultures will help to solve the problem of algal production for biotechnological purposes.

Keywords *Synechocystis salina* · Microbacterium · Lytic bacteria · Electron microscopy · Pigments

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Introduction

New algal strains are investigated for production of high-value molecules (e.g., fatty acids, pigments, polysaccharids, stable isotope biochemicals), that find wide application in medicine, pharmacy, agriculture and cosmetic (Spolaore et al. 2006).

The rich metabolite activity of microalgae determines their contamination with different microorganisms: bacteria, fungi, protozoa, viruses and others (Bassler 2006). These interactions could be indifferent, symbiotic and antagonistic. There are many examples of bacteria lytic activity to algae (Choi et al. 2005; Kang et al. 2005; Kim et al. 2009; Shi et al. 2009; Zhang et al. 2011). However, concerning exhibition of algicidical activity against the

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SYNTHESIS AND STRUCTURE OF SOL-GEL SILICA-POLYSACHARIDE HYBRIDS

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ABSTRACT

Sol-gel hybrids, containing different quantity of algal polysaccharide were synthesized. The silica-polysacharide hybrids structure was characterized, employing different methods. The results from the BET analyses revealed that introduction of a polysaccharide and formation of a hybrid structure leads to a decrease in the surface area, but to an increase in the pore size of the matrices. XRD analysis showed that the pure algal polysaccharide is partially crystalline. The obtained hybrid materials are in an amorphous state. The surface of the obtained hybrids was structurally investigated with Atomic Force Microscopy. Presence of a hybrid nanostructure with well-defined nanounits and their aggregates, with different designs formed by self-organizing processes, was observed.

Keywords: sol-gel, algal polysaccharide, structure, properties.

INTRODUCTION

Sol-gel is the prefered method for preparation of silica hybrid biomaterials. This technique provides an opportunity for control of the microstructure and properties [1]. The sol-gel silica and silica hybrid biomaterials provide many advantages as: mild experimental conditions of the synthetic method and the possibility to obtain many new materials with nanoscaled structure and extraordinary behaviour [2 - 5]. Biomaterials have to exhibit a large surface area and porosity and to ensure easy diffusion of bioreagents into the matrix [6]. These characteristics influence the immobilization of biomolecules, their biological activities and successful bioreactions on the surface and inside of the structure [7, 8].

Silicates are characterized with a stable structure at different conditions, presence of reactive groups, surface roughness and high bioactivity [9,10]. Combinations with biopolymers lead to development of hybrids with improved properties and expand applications [11, 12].

The use of materials, containing silica-synthetic polymers for biological purposes has been reported from many researcher groups [13 - 15]. Nature and properties of synthetic biopolymers can be modified depending on application [16, 17]. Investigations of these kinds of materials proved their biocompatibility, mechanical and chemical stability, and ability to form suitable matrices for biotechnology [18, 19].

Natural biopolymers in combination with sol-gel silica materials are innovative, extensively studied and applicable in many biological fields [20]. Natural biopolymers are known as biodegradable, biocompatible, hydrophilic and protective materials [21]. Chitin, chitosan, carrageenan, alginate are a small part of natural biopolymers [22, 23].

Many investigations based on silica-chitin and its

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BIOLOGIE
Biotechnologie

PREPARATION OF NANOMATRIX WITH CELLS OF RED MICROALGA *DIXONIELLA GRISEA* AND BIOSORPTION OF COPPER BY FREE AND IMMOBILIZED ALGAL CELLS

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(*Submitted by Academician V. Golemansky on November 28, 2007*)

Abstract

Nanomatrix with incorporated cells of red microalga *Dixoniella grisea* was prepared and the effect of copper biosorption by free and embedded in the matrix algal cells was studied. The results show satisfactory immobilization of the biocomponent within the sol-gel matrix. ICP-AES (atomic emission spectrometer with inductively coupled plasma) investigations demonstrated that as a result of copper accumulation the free algal cells increase production of polysaccharide and enhance the release of some light metals. *Dixoniella* cells retained capacity for copper biosorption after their incorporation in sol-gel matrix. Incubation of the biocers within Cu^{2+} solution showed that the content of copper was reduced 1.35-fold compared to that of control.

Key words: copper influence, sol-gel matrix, *Dixoniella grisea*, biosorption

Introduction. Sol-gel technology allows immobilization of various biomolecules and microorganisms without loosing their structure and activity.

On the basis of direct combination of living cells with synthetic materials [1] were developed biologically modified ceramics (biocers). Further biocers containing bacteria, fungi and yeasts for biocatalytic, biosorptive and functional materials were manufactured [2].

The microalgae have caught the scientific attention not only taking part in different biotechnologies, but also to their most promising role in nanotechnology.

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Interactions between the unicellular red alga *Rhodella reticulata* (Rhodophyta) and contaminated bacteria

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Aims: To define the role of the bacterial strains LR1 and LR3 in the *Rhodella* cell destruction caused by *Cytophaga* sp.LR2.

Methods and Results: The bacteria were obtained from algal culture with destruction. They were isolated in pure culture and tested for biochemical activities using Polymicrotest. The ability of bacteria to degrade and utilize the algal polysaccharide was investigated. The bacteria were grown in a media containing *Rhodella* polysaccharide as a sole carbon source. The level of the reducing sugars in the culture media was determined. Scanning electron microscopy (SEM) was used to define the location of bacteria in extensively and intensively cultivated *Rhodella reticulata* previously infected by *Cytophaga* sp. LR2.

Conclusions: The lysis of *Rhodella reticulata* cells is due to the joint action of the three bacterial strains with the former pathogen *Cytophaga* sp. LR2 playing the main role. The accumulation of the polysaccharide and the excreted metabolites of the strains LR1 and LR3 stimulated the development of *Cytophaga* sp. LR2. The adaptation of the strain to particular conditions of alga cultivation and the utilization of polysaccharide as a sole carbon source supported its stable growth in alga suspension and destruction of *Rhodella* cells.

Significance and Impact of the Study: The predominance of *Cytophaga* sp. LR2 over the two other contaminants and the lysis of *Rhodella reticulata* cells resulted from the ability of the bacterium to attach to the algal polysaccharide sheath. The formation of slime and extrusions facilitated the phenomenon of bacterial adhesion to the algal surface as well as the formation of colonial alga – bacterial spherules. The sedimentation of these aggregates decreased the ability of the algal strain to photosynthesize, led to the lysis of the cells and finally caused the death of *Rhodella*.

INTRODUCTION

Cells of *Rhodella reticulata* are encapsulated within sulphated polysaccharide, the external part of which dissolves in the medium. It is suggested that by its heterogeneity the *Rhodella* polysaccharide is similar to agar and carageenans produced by red macrophytes and is difficult to be degraded. It serves as a barrier of the cells against unfavourable environmental conditions and contamination (Ramus 1972; Geresh and Arad 1991). We have reported the isolation of a bacterial pathogen of *R. reticulata* (Toncheva-Panova and Ivanova 1997). Two other bacterial strains coexisting in *R. reticulata* cultures were isolated later.

The purpose of this research is to study the ability of the obtained three bacterial species to degrade and utilize the algal polysaccharide. The association between *Rhodella reticulata* and bacteria was observed with scanning electron microscopy (SEM) to define the location of bacteria in Rhodella cultures and to evaluate the role of the three member bacterial consortium in the algae disease.

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