Biodegradation products of natural and styrene-butadiene rubbers using the Lactiplantibacillus plantarum strain and their use in the food industry

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Abstract

A global problem of environmental protection is storage and disposal of automotive industry waste, which contains rubber components, including tires. The storage of rubber waste requires large areas and their natural decomposition takes several dozen years (Duda, 2016). Among different methods of rubber parts' disposal, biological solutions are the most environmentally friendly, although they require a long time.

The urgent need to solve the increasing amount of rubber waste has become a reason to analyze and describe a biological method that would effectively utilize natural and styrene-butadiene rubbers. These are the basic components of rubber products. The method uses the common bacteria strain - *Lactiplantibacillus plantarum.* Considering the presented environmental problem, several research goals were formulated. First, the ability of bacteria to be active on a substrate, which contains natural and styrene-butadiene rubbers was tested in laboratory conditions. Moreover, the most optimal conditions for biodegradation process of the above-mentioned waste were established. The aim of the dissertation was also to use, in the food industry, bacterial cellulose which was produced during the rubber waste utilization process.

The experimental work began with the cultivation of several types of microorganisms. Some of them belonged to the Collection of Pure Cultures of Lodz University of Technology and were given a collection number. One bacterial strain, which was used for tests, did not have collection number. The bacterial strains were selected for their ability to produce biomass and the possibility of growing on a substrate, that contained various carbon sources.
At the initial stage of the research, selected strains were activated and their ability to grow on substrates, that contained rubbers was checked. Preliminary analyzes have shown that the unclassified bacterial strain caused decomposition of rubbers. Other strains showed growth problems on standard substrates and they also were unable to grow on culture medium, which contained a rubber waste as a carbon source.

Further research was continued using the *Lactiplantibacillus plantarum* strain and various mixtures of natural rubbers, styrene-butadiene rubbers and EPDM (ethylene-propylene-diene monomer) blends as the primary carbon source. Cultures were carried out under different pH and different temperature conditions. After their completion, a mass balance was carried out. Also, an analysis of the elemental composition was carried out.

The properties of bacterial cellulose obtained during the cultivation process were also tested and various types of layered composites were prepared for their innovative use in the food industry.

It was proven that the *Lactiplantibacillus plantarum* is able to grow on a medium in which rubber waste is the basic carbon source for the bacteria. It has also been shown that this strain has cellulolytic properties, which was has not been mentioned in the literature before. It has been proven that the above-mentioned bacteria were able to grow in a liquid, glucose-free medium with the addition of various types of rubber mixtures and they obtained carbon from rubbers, leading to mass loss and thus partial biodegradation of the used rubber waste. The success of the experiments and mass losses, after 10 days of cultivation, amounting to 1-5% (after one cultivation cycle) were observed for sulfur vulcanizate of rubber (NR), a mixture of natural rubber with the addition of carbon black (NR + CB), sulfur vulcanizate of natural rubber with sulfur cross-linking unit (NR + cross-linking unit), NR + CB + cross-linking unit, styrene-butadiene rubber with the addition of carbon black (SBR + CB), vulcanizate of styrene-butadiene rubber (SBR), sulfur vulcanizate of styrene-butadiene rubber (SBR + unit), sulfur EPDM (ethylene-propylene-diene) rubber vulcanizate filled with soot (EPDM + CB), and rubber dust from end-of-life-tires and used transmission belts.

Other mixtures used in the research, it could be assumed that they contained components harmful to bacteria, or because of their complicated structure, the bacteria were not able to obtain carbon from them.

In the samples where bacterial activity and weight losses were observed, the average carbon content before the cultivation process was from 65.12% to 98.06%. After the cultivation, this content ranged between 51.17% and 78.03%, which meant that the bacteria obtained carbon for themselves from rubber waste. The activity of bacteria during the cultivation process, apart from carbon loss, was evidenced by the growth of bacterial cellulose on the surface of bioreactors and the presence of other elements in microscopic analysis. Carbon and oxygen were present in the pre-cultivation samples (some measurements also showed trace amounts of zinc and nitrogen). Traces of sulfur and rhenium were also found in deeper layers of rubber waste . Detailed results and statistical analysis of carbon losses are presented in chapter 9.

Analyzing the above results, we could make a thesis that the percentage of carbon loss from the tested rubber sample is directly proportional to the thickness and mass of cellulose formed on the bioreactor surface, and thus to the activity of bacteria. In other words, the degree of

biodegradation of the rubber waste used in the cultivation process of the *Lactiplantibacillus plantarum* strain was directly proportional to the activity of bacteria.
Bacterial cellulose, formed during the cultivation and biorecycling of rubber waste, could be used in the food industry. Under the conditions of a typical papermaking process, bacterial cellulose alone cannot be used in papermaking due to its high water retention and unsatisfactory physical, chemical and strength properties. The best strength properties of the paper were obtained when bacterial cellulose was used as an applied (coated) layer on the layer of plant cellulose paper. By applying bacterial cellulose to sheets of paper made of ground or unground softwood, hardwood, or an equilibrium mixture thereof, the paper became completely air impermeable, which means that composite paper can be used to produce packaging materials with barrier properties.

The results of this work, as well as issues beyond its scope, are presented in the publications, communications, patents and awards listed below. I accomplished all my achievements under my maiden name – Kaźmierczak.

1. Kaźmierczak M., Olejnik T.P., Kmiotek M. - Natural paper-layered composites with barrier properties against air. Bioresources 15 (4), 9569-9574, 2020. (IF 1,369; 100 points of. MNiSW).
2. Kaźmierczak M., Olejnik T.P, Sielski J., Śliżewska K. - The process of natural and styrene-butadiene rubbers biodegradation with the help of a specific bacterial strain. Environmental Chemical Engineering, under review (100 points of. MNiSW).
3. Kaźmierczak M., Weronika Niwald- Jaźwińska, Tomasz P. Olejnik - Hodowla celulozy bakteryjnej przez szczep *Gluconacetobacter xylinus* jako sposób na utylizację odpadów. Interdisciplinary conference "Knowledge is the key to success", January 20, 2018, Łódź, book of abstracts, p. 17, ISBN: 978-83-949065-7-3. Oral presentation.
4. Kaźmierczak M. Hodowla celulozy bakteryjne jako potencjalny sposób na utylizację odpadów. II national conference "Scientific Research in Theory and Practice", 22/03/2018, Kielce. Oral presentation.
5. Kaźmierczak M. Olejnik T.P. Biodegradation process of natural and styrene – butadiene rubbers. National Scientific Conference ' Knowledge – Key to Success 2019, 3rd edition', 19.01.2019 r, Toruń. Oral presentation.
6. Kaźmierczak M., Olejnik T.P. – Proces biodegradacji kauczuków naturalnych i butadienowo – styrenowych przy udziale bakterii mlekowych. 5th National

Conference of Doctoral Students of Life Sciences Bioopen, 30-31.05.2019, Łódź, oral presentation.

7. Kaźmierczak M. – Proces biodegradacji różnego typu odpadów gumowych przy udziale bakterii mlekowych. 5th National Scientific Conference "Health Sciences in Theory and Practice", June 5, 2019, Kielce, oral presentation.
8. Kaźmierczak M., Olejnik T.P. - Biodegradation process of natural and styrene – butadiene rubbers with the help of Lactobacillus plantarum. National Scientific Conference ' Science and Young Researchers, 3rd edition', 15.06.2019r, Łódź. oral presentation.
9. Olejnik T., Kaźmierczak M., Bieliński D., Okraska M., title: 'Method for the production of bacterial cellulose'.
10. Olejnik T., Kmiotek M., Śliżewska K., Sobiecka E., Kaźmierczak M., Pat. 237035, title: 'Layered cellulose-paper composite and the method of producing the composite'.
11. Olejnik T., Śliżewska K., Sobiecka E., Kaźmierczak M., Pat.238266, title: 'Latobacillus plantarum LOCK 1145 strain of lactic bacteria and a method of producing bacterial cellulose by culturing the Lactobacillus plantarum LOCK 1145 strain'.
12. Award for the best oral presentation in the Natural Sciences panel at the National Scientific Conference 'Knowledge - Key to Success 2019, 3rd edition', 19.01.2019, Toruń.