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BIODEGRADATION OF CELLULOSE IN BOTTOM SEDIMENTS OF TURAWA LAKE

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In the presented study the attempt at conducting biodegradation of bottom sediments of Turawa Lake was made. In the research cellulolytic bacteria strains were employed and the biodegradation assumed 19% reduction of the total cellulose content. At the beginning cellulolitic bacteria, originating from the bottom sediments, were isolated and selected. Next step involved evaluation of the cellulolitic activity of bacteria strains and selection of the three most vigorous, to be used in the process of bottom sediments biodegradation. The amount of decomposed cellulose was assessed with the use of anthrone method and changes in bacteria enumeration were determined with the index method. The most efficient group, regarding biodegradation of cellulose, was the mixture of the following bacteria species: *Cytophaga* and *Cellulomonas*. The reduction obtained was equal to 66%, and the bacteria enumeration increased 300-fold, to finally reach the level of the Most Probable Number equal to 10^5 NPL per 1g of the bottom sediment.

Key words: biodegradation, cellulose, bottom sediment, Cytophaga, Cellulomonas

INTRODUCTION

Organic matter, produced in lakes *in situ* in photosythesis processes by phytoplankton and aquatic higher plants and organic matter deriving from the outer sources delivered by the surface water containing cellulose, is decomposed in aerobic conditions (in water and surface layer of the sediments) as well as in anaerobic conditions (in deeper layers of the water and bottom sediments). Cellulose in its pure form is very rare, and most often it is associated with other polysaccharides and lignin complexes, which has a significant influence on its decomposition rate by microorganisms. Biological decomposition of cellulose and its derivatives requires specific enzymes, which catalyze the reaction of breaking it down to less complicated constituents (disaccharides and monosaccharides), that are easier absorbed by the microorganisms. Bottom sediments, accumulated in Turawa Lake for over 60 years, are mainly sapro-

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pelic muds containing up to 25% of organic matter. The thickness is varied and fluctuates between ca. 10 cm to almost 2 m, and the capacity is estimated of 4 mln sq metres (Teisseyre 1984). Polysaccharides such as: cellulose, hemicellulose and fibre are the main dominants in the content of organic matter in bottom sediments. Therefore, one of the methods for reducing their amount seems to be biodegradation conducted in natural conditions, by means of carefully selected cellulolytic bacteria strains.

The objective of the study was evaluation of the possibility to biodegrade cellulose in bottom sediments of Turawa Lake with the application of cellulolitic bacteria strains, isolated from the lake water and sediments.

MATERIALS AND METHODS

BOTTOM SEDIMENT

For the investigation, bottom sediment containing ca. 19% of cellulose and pH 6.5 was collected from the north-west part of Turawa Lake, from the depth of 4-5m. It was initially shredded and dried, obtaining 10kg of material for tests.

AUTOCHTONIC MICROFLORA ISOLATION

Cellulolytic bacteria were isolated in two stages. First, little amount of fresh bottom sediment was placed directly on solid Waksman medium with powdered cellulose (Burbianka et al., 1983) and incubated at 21^0C, for 7-14 days. In the second stage, typical mucous colonies of cellulolytic bacteria were inoculated into liquid Park medium (Górska and Russel, 1997; Vardavakis 1989) with filter paper discs. The bacteria cultures, prepared as above, were incubated at 21^0C for 14 days. Only cultures with distinct cellulose decomposition were classified for the further investigation. Strains presence of the following species: *Cytophaga*, *Cellulomonas* and *Bacillus* (Holt and Krieg 1984) was confirmed during microscopic, macroscopic and biochemical identification. Biochemical tests were performed with the use of traditional methods and microanalyser mini API.

EVALUATION OF ENZYMATIC ACTIVITY OF ISOLATED STRAINS

Six isolated bacteria strains marked respectively as: *Cytophaga* C1, *Cytophaga* C2, *Cytophaga* C3, *Cellulomonas* C4, *Cellulomonas* C5 and *Bacillus* C6 were subjected to the cellulolytic activity evaluation. Prior to marking, they were grown in a liquid Park medium with blotting stripes at 21^0C for 14 days. The evaluation was carried out by analyzing the weight loss in filter paper discs, during 28 days of culturing on Park medium. In order to achieve this, 150 cm^3 of Park medium, 250 mg of filter paper discs and 5 cm^3 of the cellulolytic bacteria culture were inoculated into a flask of 250 cm^3 capacity. The parallel procedure was applied to the 5 remaining isolated strains. Incubation was conducted at 21^0C on a rotation shaker and the weight loss in the discs was measured every 14 days.

BIODEGRADATION OF BOTTOM SEDIMENT

Biodegradation of the sediment was carried out in a laboratory conditions, in 5 glass containers filled with 2 kg of the bottom sediment and 5 dm^3 of the water from Turawa Lake. Each of the three containers was inoculated with 25 cm^3 of the one most vigorous cellulolytic bacteria strain. The fourth one contained the mixture of all three strains. Last container was a control treatment. Biodegradation was carried out at 21^0C, for 6 months and every 2 weeks the microbiological and chemical analysis of the sediment was performed. Water which evaporated from the container was refilled regularly with the lake water.

MICROBIOLOGICAL AND CHEMICAL ANALYSIS OF THE SEDIMENTS

In the microbiological quantitative analysis, the cellulolytic bacteria enumeration was determined with the index method on Park medium with blotting stripes. The Most Probable Number of bacteria (MPN) per 1g of sediment was stated according to the Mc'Crady's tables (Grabińska-Łoniewska, 1996). Chemical analysis included determination of cellulose content, with the aide of anthrone method, by stating the amount of liberated glucose after hydrolysis (Kłyszejko-Stefanowicz, 1980).

RESULTS AND DISCUSSION

The results of cellulolytic activity evaluation of the strains isolated from the bottom sediments, are presented in Table 1 and Figure 1.

Table 1. Cellulolytic activity evaluation of isolated strains[1]

Bacteria strains	Cellulose weight [mg]		
	1 day	14 days	28 days
Cytophaga C1	250.0	144.2	84.9
Cytophaga C2	250.0	194.2	159.3
Cytopgaha C3	250.0	196.1	111.7
Cellulomonas C4	250.0	201.2	118.6
Cellulomonas C5	250.0	205.1	123.5
Bacillus C6	250.0	218.8	175.4

[1] – cellulolytic activity evaluation was based on the cellulose weight loss in 28 days' strains cultures.

Six identified strains, belonging to the species: *Cytophaga*, *Cellulomonas* and *Bacillus* were subjected to the analysis. In the first 2 weeks the highest enzymatic activity characterised aerobic *Cytophaga* rods, marked as C1. Cellulose reduction amounted 42.32%, and its content was reduced from 250.0 mg to 144.2 mg. The enzymatic activity of the other *Cytophaga* strains (C2 and C3) and *Cellulomonas* (C4 and C5) was about 2-fold lower. The amount of cellulose ranged between 205.1 mg and 194.2 mg, which corresponded to the reduction level between 17.96% - 22.32%. The lowest cellulolytic activity characterized *Bacillus* strain (C6) and the cellulose decrease amounted 12.48%. After 28 days of cultivation, again the most vigorous strain was C1 of *Cytophaga* species. Recorded cellulose reduction amounted 66.04% and its content dropped to 84.9 mg. Lower activity (by ca. 10%) was stated for C3 strain of *Cytophaga* species – after 4 weeks cellulose content amounted 111.7 mg. It was slightly lower in the bacteria cultures with *Cellulomonas* (C4 and C5) and ranged between 118.6 mg and 123.5 mg, while cellulose reduction was 52.56% and 50.60% respectively. After 28 days (similar to the period of 14 days) the lowest activity characterized the strain of *Bacillus* species (C6). The cellulose content dropped to 175.4 mg, which corresponded to the reduction of 29.84%.

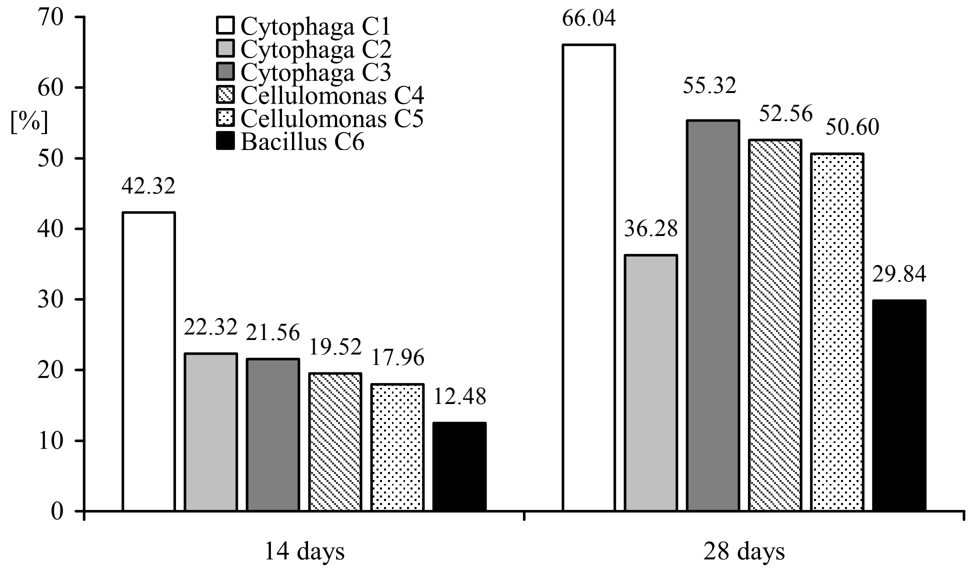

Fig. 1. Reduction of the cellulose content by the strains isolated from bottom sediments

Hight cellulolytic activity of *Cytophaga* and *Cellulomonas* species was confirmed in many authors' researches (Bujak and Targoński, 1988; Land et al., 2002; Liebert et al., 1984; Trojanowski, 1973) and can be explained by the fact that mentioned bacteria have the ability to synthesize both cellulase and cellobiase enzymes, that perform hydrolysis of cellulose, fibre, starch, chitin and many other polysaccharides. According to Janas et al. (2004) the highest rate of cellulose decomposition takes place in the neutral environment and is stimulated by the presence of Ca^{2+}, Mn^{2+}, Fe^{2+} and Cu^{2+} ions. The authors confirm also inductive influence of the substrate (cellulose) on the biosynthesis of cellulolytic enzymes. In the presented study, enzymatic activity of the rod bacteria of *Cellulomonas* species was lower after 14 and 28 days when compared to *Cytophaga* strain (C1), by 22-24% and 13-16% respectively. Obtained results are in accordance with the results obtained by Gołębiowska (1992), who noted 50-60% lower activity of *Cellulomonas* strains in the process of cellulose degradation when compared to *Cytophaga*, after 15 and 20 days of cultivation. The author also stated that bacteria *Cellulomonas* do not mineralize cellulose completely, but are able to produce side products, e.g. uronic acid and pigments. *Bacillus* strain (C6) employed in the analysis, was characterized by over 2-fold lower efficiency in the process of cellulose decomposition, when compared to the most vigorous strain. It is commonly known, that cellulose decomposition requires enzymes (among others: endoglucanase, exoglucanase and β-glucosidase) acting together in synergism (Bujak and Targoński, 1988; Górska and Russel, 1997; Land et al., 2002). The researches conducted by Beguin and Aubert (1994) indicate, that most *Bacillus* rods have the ability to synthesize endo-β-1,4-glucanase which is necessary only for decomposition of soluble cellulose derivative, such as: carbomethylcellulose. Decomposition of natural cellulose, which also contains crystallin areas, is catalyzed by exo-β-1,4-glucanase followed by cellobiase. However, disability to synthesize these enzymes restricts the participation of *Bacillus* bacteria in the process of cellulose mineralization.

In the next stage of the research, the attempt at conducting biodegradation of bottom sediments of Turawa Lake was made with the use of the most vigorous strains of isolated cellulolytic bacteria. In many authors' opinion (Gostkowska et. al., 1996; Latała et al., 2004) natural microflora supplemented with the biopreparation containing strains of high enzymatic activity, accelerates the process of biodegradation significantly.

Four prepared experimental combinations were inoculated with the respective bacteria of *Cellulomonas*: *Cytophaga* C1 (combination 1), *Cytophaga* C3 (combination 2), *Cellulomonas* C4 (combination 3), and a mixture of all strains (combination 4). Results of biodegradation are presented in Table 2 and Figure 2.

Table 2. Chemical analysis of the sediment throughout biodegradation process

Type of treatment[1]	Amount of cellulose [g/kg dry matter]			
	1st day	14th day	28th day	42nd day
control treatment	188.00	178.00	171.61	182.51
combination 1	188.00	146.00	120.94	83.99
combination 2	188.00	157.39	136.94	102.79
combination 3	188.00	167.88	161.06	148.65
combination 4	188.00	130.09	99.86	63.84

[1]combination 1 – *Cytophaga* C1,
combination 2 – *Cytophaga* C3,
combination 3 – *Cellulomonas* C4,
combination 4 – the mixture of *Cytophaga* C1, *Cytophaga* C3 and *Cellulomonas* C4.

In combinations containing single cellulolytic bacteria strains, cellulose decrease corresponded to their celluolytic activity. However, the highest cellulose loss was noted in the 4th combination, comprising of *Cytophaga* and *Cellulomonas* strains mixture. It was confirmed in the own research (Latała et al., 2001; Wierzba and Nabrdalik, 2005), as well as by the other authors (Bujak and Targoński, 1988; Gostkowska et al., 1996), that the efficiency of biodegradation process was higher in the case when the microbe mixture was added in contrast to individual strains supplement. After 2 weeks of biodegradation, the decrease amounted 30.80% and cellulose content in sediment dropped to 130.09 g/kg of dry matter. Slightly higher results were recorded in combination 1, with the *Cytophaga* C1 strain – 146.00 g/kg of dry matter which accounted for 22.34% reduction. In combinations 2 and 3 cellulose loss rate shaped within the range of 16.28% to 10.70%. The highest rate was also noted after 28 days in sediment containing the mixture of strains. Cellulose content decreased to 99.86 g/kg of dry matter which accounted for 46.88% reduction.

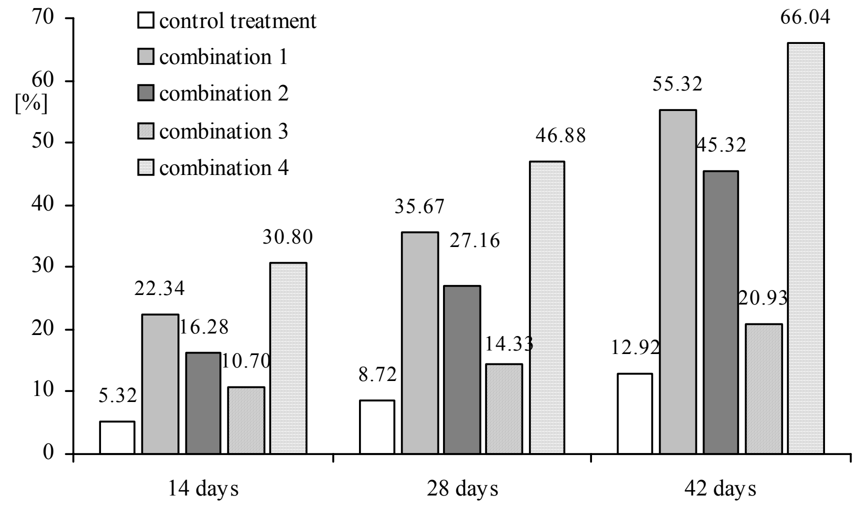

Fig. 2. Cellulose reduction rate throughout biodegradation of bottom sediments

The analysed loss in cellulose content, in the other examined combinations, was of 11.21% (combination 1) to 32.55% (combination 3) lower when compared to combination 4. Finally, the mixture of *Cytophaga* and *Cellulomonas* strains, caused the decrease in cellulose content in bottom sediment to the level of 63.84 g/kg of dry matter, which corresponded to 66.04% reduction. Slightly lower efficiency in cellulose decomposition (55.32%), characterised *Cytophaga* strain C1, employed in combination 2. Lower reduction (about 20%) was recorded in combination 3 with *Cytophaga* C3.

The lowest efficiency in biodegradation was stated for the third combination, containing *Cellulomonas* strain C4. Cellulose loss rate amounted 20.93% and was slightly higher in relation to the control treatment – 12.93% [Tab. 2, Fig. 2].

The same authors' group recorded much lower results during biodegradation of polysaccharides in residual waste with the amendment of biopreparation containing cellulolytic bacteria strains. The most intensive rate of polysaccharides reduction was noted in the first weeks of the experiment, and after 30 days was equal to 39% (Wierzba and Nabrdalik 2005). Also in the own experiments, hydrolysis of cellulose was the most intensive, in combinations 1 and 4, during the first 14 days of biodegradation. It amounted ca. 40% and over 45% of the total content of reduced cellulose, respectively.

The most probable reason for it, could be favourable conditions for the species *Cytophaga* in which cellulose is decomposed: slightly acid pH of waste (6.5) and facilitated oxygen access. According to Bujak and Taragoński (1988) cellulose decomposition is faster in soils of neutral or slightly acidic pH than in strongly acid ones. As many authors report (Eriksson and Pettersson, 1975; Land et al., 2002), the rate of hydrolysis is much higher in aerobic conditions than in nitrogen conditions.

During biodegradation, microbiological quantitative analysis proved growing tendency concerning the Most Probable Number (MPN) of bacteria in all combinations. The highest growth rate of bacteria, similar to cellulose reduction rate, were recorded in the first 14 days, in combination 1 and 4. In comparison to initial rate, bacteria enumeration increased by ca. 19-fold and ca. 35-fold respectively. Finally, after 6 weeks of biodegradation, the highest MPN of bacteria was stated in the combination containing strains mixture. Bacteria enumeration increased by 300-fold in relation to control treatment, and amounted over 10^5 MPN/1 g of sediment [fig. 3].

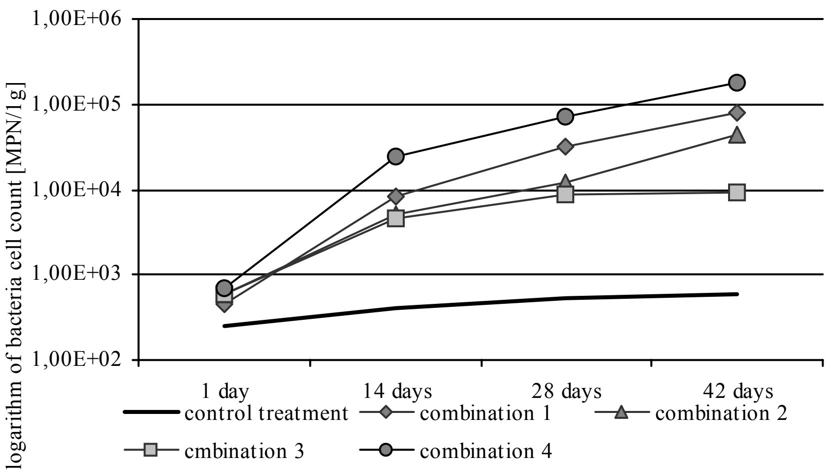

Fig. 3. Changes in bacteria enumeration throughout the biodegradation process of bottom sediments

In the study of the same authors concerning biodegradation of organic waste, slightly lower increase in cellulolytic bacteria enumeration was stated after 60 days of biodegradation, by ca. 240-fold when compared to the initial amount (Wierzba and

Nabrdalik, 2005). Distinct maintaining tendency for the growth of NPL of bacteria in combinations containing *Cytophaga* strains, supports the thesis about favourable conditions for mentioned bacteria growth, during bottom sediments biodegradation. Obtained results suggest, that the effectiveness of cellulose decomposition depended on both quantity and quality of bacteria employed. Also, active process of lignocellulose degradation is guaranteed by the application of the microbes' mixture that possess the ability to synthesise the cellulase complex of advantageous composition. According to Bujak and Targoński (1988), separate applications of two fractions of cellulolytic enzymes, extracted from the cellulolytic bacteria complex, was the reason why the hydrolysis of cellulose occurred at a rate by 2.5-fold slower than in the process of combined activity of both fractions. Many authors (Bujak and Targoński, 1988; Górska and Russel, 1997; Lynd et al., 2002) emphasise the important role played by the β-glucosidases, called cellobiase in biodegradation of lignocellulose. Its deficiency in cellulolytic complex during the process of cellulose hydrolisis, causes cellobiose accumulation, which inhibits endo – and egzoglucanases. The ability to synthesize significant amount of celobiase were ascertained among bacteria *Cytophaga*.

SUMMARY AND CONCLUSION

Bottom sediments of Turawa Lake contain significant amount of organic matter, including fibre and cellulose, accumulated by the bottom deposition of parts of plants, water animals and contamination that flows with the surface water and groundwater. Presented laboratory results indicate the possibility of polysaccharides decomposition in bottom sediments with the use of specially selected autochtonic microflora. Enzymatic hydrolysis of cellulose is a very complex process and it is performed in presence of the complex of enzymes. Therefore the best results of biodegradation were obtained when the mixture of *Cytophaga* and *Cellulomonas* bacteria were applied. Cellulose decrease rate was 10-45% higher as compared to single strains application and over 33% higher in relation to the control treatment. Biodegradation process was accompanied by significant increase in cellulolytic bacteria enumeration, especially in the combination containing the bacteria mix. Acidic pH of the waste and aerobic conditions of cultivation were favourable for the high enzymatic activity of *Cytophaga* strains.

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W pracy podjęto próbę biodegradacji osadów dennych Jeziora Turawskiego, o ok. 19% zawartości celulozy, z wykorzystaniem szczepów bakterii celulolitycznych. Na wstępie dokonano izolacji i selekcji bakterii celulolitycznych z osadów dennych jeziora. Następnie określono ich aktywność celulolityczną i wybrano trzy najaktywniejsze szczepy, które wykorzystano do biodegradacji osadów. Ilość rozłożonej celulozy określano metodą atronową, a zmianę liczebności bakterii metodą miana. Najskuteczniejsza w biodegradacji celulozy okazała się mieszanina szczepów z rodzajów: *Cytophaga* i *Cellulomonas*. Odnotowana redukcja celulozy po 42 dniach trwania procesu wynosiła ok. 66%, a liczebność bakterii zwiększyła się ok. 300-krotnie, osiągając poziom ponad 10^5 NPL/1g osadu dennego.