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Biodegradation of substrates used for soil erosion prevention

Dalia Ambrazaitienė,

Olga Belous,

Audronė Žukauskaitė

Klaipėda University,
84 H. Manto St., LT-92294 Klaipėda,
Lithuania

Galia Shulga

Latvian State Institute of Wood Chemistry,
27 Dzerbenes St., Riga, Latvia

One of the main stages in the research of erosion prevention technologies is to determine the expedience of the substrate to the plants. The next equally important stage is to study substrate biodegradation. The investigation of substrates fell on the middle of the summer time. The coast sand and sandground samples were put into 10 l buckets. According to the experimental scheme, different layers of hydrolytic lignin (0, 2, 5 cm) were placed on the top of the soil $LSTM_2$ and $LSTM_1$ + promoter polymers were spread (200 ml m^{-2}) on the lignin layers. The buckets with the substrate were exposed to the influence of atmospheric conditions during the whole period of the experiment. Samples for microbiological research were taken at the beginning of the experiment and four, eight as well as forty five weeks later. Simultaneously drain water, soil and lignin chemical analyses were carried out.

At the beginning of the experiment, bacteria were active in the degradation of the coast sand organic matter, thus their number was high enough even without lignin. Later the thickness of the lignin layer influenced the fluctuation of bacteria amount. The micromycetes were the main factor in the hydrolytic lignin degradation process. The number of micromycetes in the coast sand substrate at the beginning of the experiment was statistically significantly influenced by the thickness of the hydrolytic lignin layer $r = 0.84$; after 4 weeks from the beginning of the research this coefficient decreased to $r = 0.64$, and 8 weeks later it was $r = 0.48$. In the sandground where carbon store was more abundant (C = 1.25%), bacterial microflora took the main part in the degradation process.

The C/N ratio of 25–30 indicated that the lignin degradation process in the substrates was going on. In other cases organic matters were already degraded and transformed into nutrients.

The research results indicate that the substrates made of lignin and polymers can be mineralized by natural microorganisms, however, they cannot be used for soil erosion prevention or growing plants without additional nitrogen compounds.

Key words: hydrolytic lignin, modified polymers, soil, biodegradation, bacteria, fungi, C/N ratio

INTRODUCTION

Most marine countries have researched into their coastlines and prepared coast protection programs (Nichols, 1992; Zeidler, 1992; Pethick, 1993; Diehl, 1998). The coastline stability insurance being one of the major objectives of such programs necessitates the search for coastline zone soil erosion reduction means with an emphasis on the preservation of stable vegetation in the forested parts of dunes. Fast formation of plant cover and its stability assures better growth and moisture conditions in the afforested plots which are strengthening the dunes.

There are technologies where synthetic polymers are used for sand strengthening, however, this method is neither effective nor environmentally friendly, therefore, the use of natural materials is suggested. Lignin, which appeared as a natural product of the evolution process, is one of them. When plant cells were infected with symbiotrophical or parasitical fungi, the evolutionary protection mechanism was evoked, with the formation of a triple complex aromatic alcohol polymer known as lignin (Сидоров, 2003).

On the other hand, the fermentative system for lignin digestion has developed in fungi, particularly in basidiomycetes. The mineralization of decayed plants

in the soil has the fermentative system of the same nature. Such an important part of the soil as humus consists of humine compounds which are products of lignin degradation. Berg et al. (1982) and Berg and Ekbohm (1991) have proved that lignin is the most degradation-resistant element of the soil organic matter. Fungi do not use lignin constantly as a carbon source for their growth and energy and can begin to degrade lignin only when other carbon sources such as cellulose and hemicellulose become unavailable (Kirk, Farrell, 1987). Lignin contains also large quantities of plant nutrients and acts as a slow-release nutrient storehouse for carbon and nitrogen.

Lignin obtained as a waste of wood chemistry industry can be successfully used in planting as a vegetation substrate and support soil protection from wind erosion. The objective is to create a soil erosion prevention technology to ensure a better plant rooting in the substrate, which would be environmentally friendly and mineralized by the natural soil microflora. Hydrolytic lignin waste is light and has poor adhesive features, thus it is a problem to use it directly. However, better results were obtained by combining two substances – hydrolytic lignin and lignin–based modified polymers, which were used as binders and enhanced adhesion (Viksne et al., 2004). There is a lot of data on lignin biodegradation in literature (Kirk, Farrell, 1987; Eriksson et al., 1990; Tuomela et al., 2000; Vikman et al., 2002), however, data on the biodegradation features of lignin–based modified polymers and hydrolytic lignin affected by such polymers are insufficient.

Hydrolytic lignin, the main sources of which are wastes of feeding leaven industry, is the basic compound used in soil erosion prevention substrates. During the production process, waste can be polluted with sulphates, chlorides and heavy metals, which could also suppress lignolytic activity. The study results show variable lignolytic activity of different microorganisms during the degradation of different substrates (Rodriguez Couto et al., 2001). Prior to using the above–mentioned substrates in soil erosion prevention technologies, it is essential to determine how long hydrolytic lignin overspread on poor soil remains unmineralizated and whether the natural microflora is able to degrade it.

MATERIALS AND METHODS

One of the main stages in the research of erosion prevention technologies is to determine the expedience of a substrate to the plant. The research was carried out to estimate the effect of lignin–based polymers on seed viability (Belous et al., 2004). The next equally important stage is to study substrate biodegradation, therefore, an experiment was carried out to study the biodegradation of hydrolytic lignin, modified polymers and soil complex. Experiments of detection of substrate mineralization were set after the results of seed viability had been received. The investigation of these substrates fell on the middle of the summer time. Coast sand and sandground samples were put into 10 l buckets. According to the experimented scheme, different layers of hydrolytic lignin (0, 2, 5 cm) were placed on the top of the soil. $LSTM_2$ and $LSTM_1$ + promoter polymers were spread (200 ml/m^2) on the lignin layers. The substrate buckets were watered. The test was repeated three times. At the beginning of setting, the buckets were put in a greenhouse and after two weeks they were moved to an open plot of the Botanical Garden of the Klaipėda University. Samples for microbiological research were taken at the beginning of the experiment and four, eight and fourty-five weeks later with a special soil perforator at the full depth of the bucket. Simultaneously, chemical analysis of drain water, soil and lignin was carried out.

The following chemical ingredients were estimated in the substrate: carbon (Heraeus device, ISO 10694:1995) and total N by the Kjeldahl method (ISO 11261:1995).

Such microbiological parameters as total bacteria and fungi (micromycetes) (CFU-colony forming units) were determined for 1 g dry substrate. Evaluation of bacteria was made by applying the meat peptone agar media, and for fungi acidic beer mash agar (pH 3.5–4.0) was applied by the standard plate count method (Методы…, 1991).

Soils. Coast sand and sandground were chosen for the studies. Such optional selection was determined by the granulometrical composition, as these kinds of soil are least resistant to wind erosion and are dominant in the coastal forests and greenery. Chemical soil composition is presented in Table 1.

Table 1. **Chemical characteristics of hydrolytic lignin and soils used in the experiment**

Chemical indicators	Coast sand	Sand-ground	Hydrolytic lignin
pH	6.5	5.5	5.1
P_2O_5, mg/kg^{-1}	185	380	404
K_2O, mg/kg^{-1}	54	307	831
Total carbon, %	0.09	1.25	30.91
Organic carbon, %	0.05	1.25	30.91
Total nitrogen, %	0.005	0.143	0.314

Hydrolytic lignin is a feeding leaven production waste about one million tons of which are accumulated in a 20 ha area field near Kėdainiai (a town in Lithuania). The chemical characteristics of hydrolytic lignin are presented in Table 1.

Lignin-based modified polymers were produced at the Latvian State Institute of Wood Chemistry. Two polymers were employed in the study: $LSTM_2$ and $LSTM_1$+ promoter. The concentration of the polymers (50 g/l and 100+10 g/l, respectively) used in the biodegradation studies was selected depending on the effect on seed viability (Belous et al., 2004).

STATGRAPHICS Plus and Microsoft Excel software was used for data variance and correlation analysis. Multifactor ANOVA was conducted for evaluation of data probability.

RESULTS

Bacteria and micromycetes biocenosis activity was selected as the key indicator in the biodegradation studies of hydrolytic lignin, modified polymers and different soil complexes. It was sought to detect the influence of lignin layer thickness and modified polymers on microorganisms involved in biodegradation. Furthermore, biodegradation features of a whole complex were evaluated.

The study of hydrolytic lignin biodegradation

The variation of microorganism number in coast sand covered with hydrolytic lignin during the 45 weeks of studies is shown in Fig. 1. The total number of bacteria during the research period in coast sand uncovered with hydrolytic lignin increased constantly, from 1248 thousand of CFU at the beginning of the study to 7014 thousand of CFU at the end. The thickness of the lignin layer on the sand surface was especially significant for the abundance of bacteria populations. The number of colony forming units fluctuated from 1121.3 thousand to 1934.7 thousand per 1 g of substrate with a 2 cm layer of lignin and from 4685.8 to 5879.1 thousand when a 5 cm layer of lignin was used.

Changes of micromycetes numbers (Fig. 1) in coast sand without lignin during the period of studies were insignificant, however, at the end of our studies the number of micromycetes increased to 79.93 thousand of CFU per 1 g of substrate. The number of micromycetes increased 5–8 times in the shudied substrate when the surface of coast sand was covered with hydrolytic lignin. The number of CFU was 6.28 thousand at the beginning and 70.03 thousand at the end of the research when a 2 cm lignin layer was applied, whereas when the lignin layer was 5 cm the number of CFU increased from 26.4 to 53.21 thousand per 1 g of substrate.

In the sandground without lignin, 6941.0 thousand CFU of bacteria were found at the beginning of the experiment and 15081.93 thousand at the end (Fig. 2). 10974,4–19568,4 thousand CFU were detected in one gram of substrate.

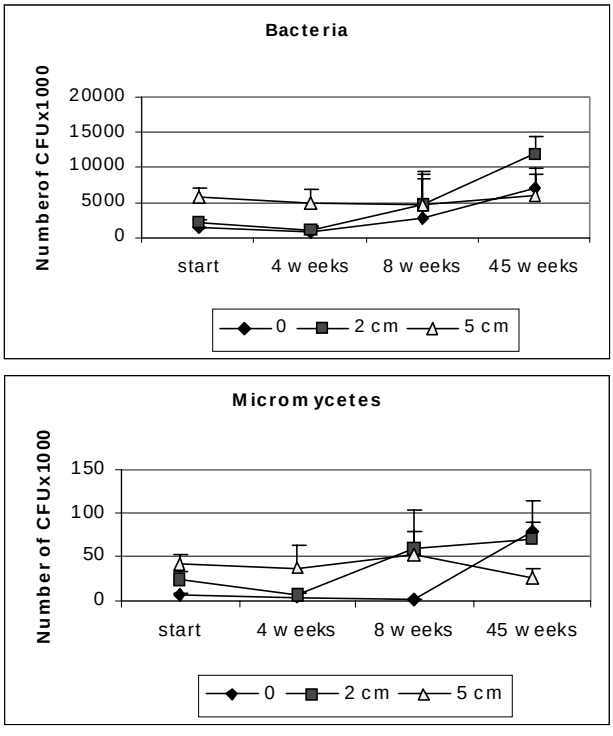

Fig. 1. Dynamics of microorganism number (thousand CFU 1 g dry substrate) in coast sand covered with different (0, 2, 5 cm) hydrolytic lignin layers

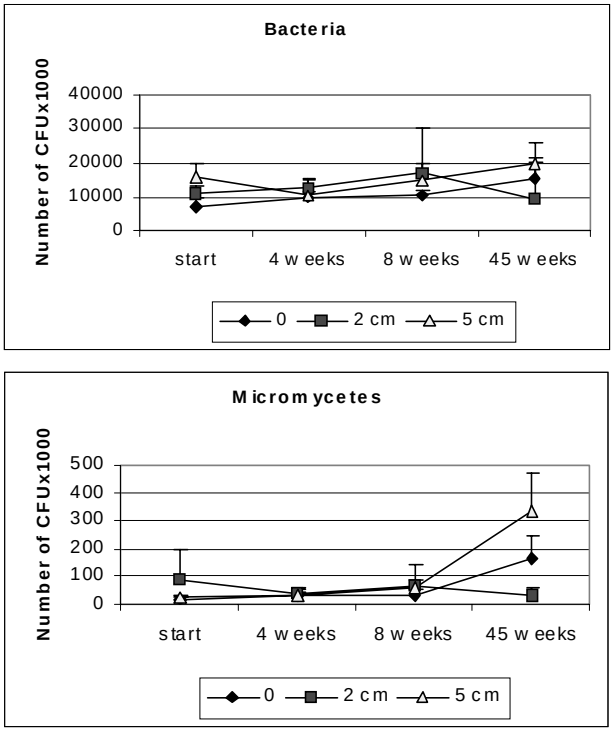

Fig. 2. Dynamics of microorganism number (thousand CFU 1 g dry substrate) in sandground covered with different (0, 2, 5 cm) hydrolytic lignin layers

The abundance of micromycetes in the substrate consisting only of sandground reduced from 5.57 thousand of CFU at the beginning to 1.79 thousand during the first weeks of study, but their number significantly increased at the end of the research period.

Analysis of biodegradation potentials of lignin-based modified polymers

1248.1 thousand of bacteria CFU were detected in the coast sand at the beginning of the study, while when the modified polymer $LSTM_2$ was sprayed on the surface the number of bacteria per 1 g of substrate increased to 25562.1 thousand, i.e. 20 times. The number of bacteria increased to 12093.5 thousand of CFU or

almost 10 times (Fig. 3) when the $LSTM_1$+ promoter was used. A reduction of the number of bacteria in all substrates was determined during the first weeks of the experiment. The population of bacteria abounded again at the end of the research, i.e. after 45 weeks. The greatest number of bacteria was determined in the substrate with the $LSTM_2$ polymer.

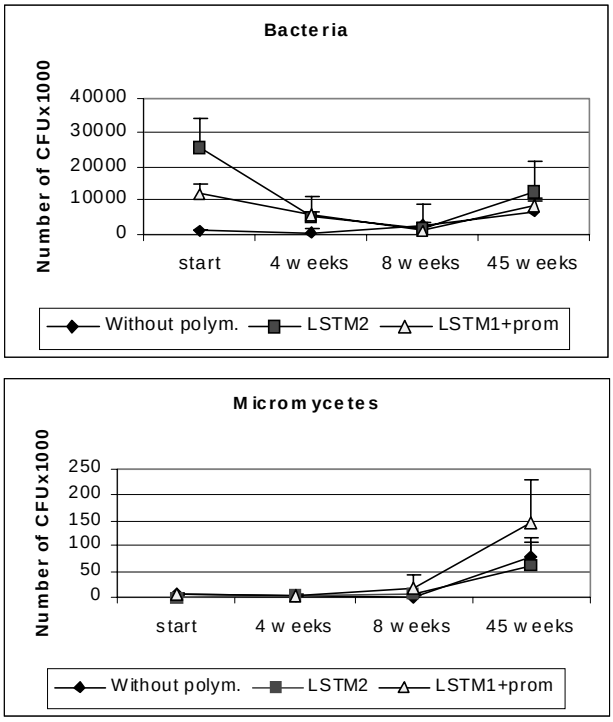

Fig. 3. Dynamics of microorganism number (thousand CFU 1 g dry substrate) in coast sand sprayed with different polymers

5.57 thousand of CFU of micromycetes per 1 g of substrate in coast sand were determined throughout the first year of study (Fig. 3). The $LSTM_2$ polymer reduced the abundance of this microorganism group 3 times, while the $LSTM_1$ + promoter nearly did not influence it. After four weeks of investigation it could be seen that in the coast sand the number of micromycetes decreased 3 times, but using polymers the number of micromycetes increased 2–3 times. An especially high amount of micromycetes per 1 g of substrate was observed at the final stage of the research.

In the sandground, the number of bacteria was 5–6 times larger as compared with their number in the coast sand (Fig. 4). The usage of polymer $LSTM_1$+ promoter tripled the number of bacteria in the substrate. The number of bacteria in the substrate without polymer was constantly increasing during the study period.

The number of micromycetes in the sandground for the duration of the experiment both with and without polymers remained the same. However, an especially high abundance of this microorganism group was observed after the winter period, i.e. after 45 weeks.

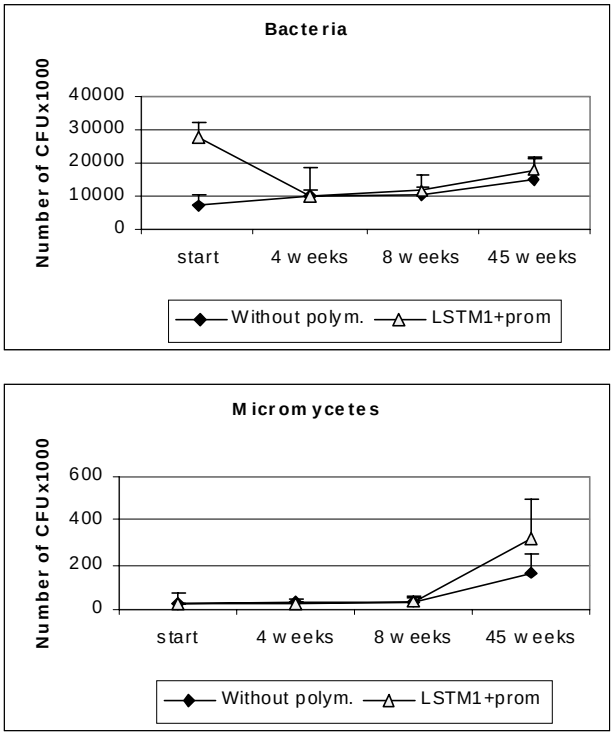

Fig. 4. Dynamics of microorganism number (thousand CFU 1 g dry substrate) in sandground sprayed with different polymers

The C/N ratio is especially important for the process of organic compound biodegradation.

Variations of fertilization, illumination and organism respiration during the vegetation enable labile C and N forms to quickly migrate from one part of the ground–environment–organism system to another (Kane et al., 1997). The practical significance of the C/N ratio becomes apparent if we compare the changes that take place in the substrate when lignin and polymers are added. The C/N proportion in the substrate complex was determined at the beginning and at the end of the experiment. In the coast sand (without polymers and lignin), the C/N ratio (Table 2) changed when the amount of nitrogen increased. In the substrate (coast sand and hydrolytic lignin) the C/N ratio increased significantly, however, a year later it considerably decreased. In the sandground the C/N index was constant. It happened mostly due to the fluctuations of the total amount of nitrogen.

DISCUSSION

Analysis of variance of the data using the F-ratio test with ($p > 0.95$) showed that different factors have a different influence on the microorganism number in coast sand. The number of bacteria was mostly influenced by modified polymers ($F = 106.0$), while hydrolytic lignin was less significant for them ($F = 31.4$). Micromycetes abundance was influenced mainly by hydrolytic lignin ($F = 74.5$) and only marginally by consumption of modified polymers ($F = 8,4$). The time of bio-

Table 2. **C/N ratio variation in biodegrading substrates**

Soil	Lignin layer	Polymers usage	Start	End
			C/N	C/N
Coast sand	0 cm	Without polymers	6.1	7.3
		$LSTM_2$	26.0	4.2
		$LSTM_1$+promoter	12.5	14.3
	2 cm	Without polymers	26.7	33.6
		$LSTM_2$	94.4	32.3
		$LSTM_1$+promoter	84.7	41.4
	5 cm	Without polymers	63.9	39.0
		$LSTM_2$	92.5	3.6
		$LSTM_1$+promoter	69.8	39.7
Sandground	0 cm	Without polymers	8.7	7.9
		$LSTM_2$	-	-
		$LSTM_1$+promoter	8.6	8.0
	2 cm	Without polymers	14.7	10.6
		$LSTM_2$	14.3	14.3
		$LSTM_1$+promoter	12.0	14.2
	5 cm	Without polymers	19.8	18.4
		$LSTM_2$	20.0	25.0
		$LSTM_1$+promoter	20.4	30.9

degradation was important for both studied groups – bacteria (F = 163.0) and fungi (F = 103.1).

In the sandground, polymer usage was also more important for bacteria (F = 36.4), while the influence of hydrolytic lignin and time was less significant. Time was mostly important for abundance of micromycetes (F = 135.9).

Correlation analysis of the research data showed a relation between the thickness of the hydrolytic lignin layer in the coast sand substrate and the number of bacteria during the different research stages. At the beginning of the experiment, bacteria were active in the degradation of the coast sand organic matter, thus their number was high enough even without lignin. Later (after 4 and 8 weeks from the beginning of the study) the thickness of the lignin layer influenced the fluctuation of bacteria. This is confirmed by statistically significant correlation coefficients: r = 0.36 and r = 0.50, respectively.

Such a dependence is supported by publications stating that bacteria do not degrade lignin but only separate parts of its molecules (Kirk, Farrell, 1987; Gentry, Newby et al., 2001). According to these authors, the main role in lignin degradation is played by fungi. The results obtained confirmed that micromycetes were the main factor in the hydrolytic lignin degradation process. The number of micromycetes in the coast sand substrate at the beginning of the experiment was statistically significantly influenced by the thickness of hydrolytic lignin layer (r = 0.84); after 4 weeks from the beginning of the research this coefficient decreased to r = 0.64, and 8 weeks later it was r = 0.48. Probably it was caused not only by the decreasing amount of lignin but also by the variable conditions as indicated by some researchers (Vikman et al., 2002).

There was no significant correlation between the number of bacteria and the amount of lignin in the substrate with sandground at the beginning of the experiment. However, similarly to the coast sand substrate, the thickness of lignin layer influenced statistically significantly the number of micromycetes in the biodegrading substrate (r = 0.58). A similar correlation remained only during the first weeks of the research. Such results can be explained by the fact that lignin was the most important carbon source in the sand (C = 0,05%). In the sandground, where the carbon store was more abundant (C = 1,25%), bacterial microflora took the main part in the degradation process. The role of micromycetes in lignin degradation was confirmed by numerous studies of various researchers (Hartig, Lorberer, 1988; Deacon, 2005; Epperson, 2005).

Strong statistically significant correlation between the number of bacteria and polymer concentrations in the sandground was observed only at the beginning of the research (r = 0,71), and it decreased within 4 weeks (r = 0.39). A low statistically significant correlation between these indices was observed in the sand substrates during the mentioned period. This correlation was not detected in the further biodegradation stages. There was no statistically significant correlation between the number of micromycetes and polymer concentration in the substrates studied. Such results could be explained by the fact that the bacteria degrading compounds were dominant in lignin–based modified polymers.

The carbon–nitrogen ratio (C/N) changes indicate favorable conditions for lignin compounds degradation by microflora in the substrates studied. The situation is opposite in the case of compounds resistant to biodegradation (recalcitrant substrates): nitrogen is not needed for pure lignin degradation, unless it is performed by some groups of actinomycetes. In some literature sources it is noted that nitrogen does not stimulate lignin degradation (Eriksson et al.,1990; Sjöberg, 2003). Whereas, some fractions with a high amount of low–MW lignin type molecules are degraded only by bacteria whose activity is increased by the presence of nitrogen, and it indicates the dual role of nitrogen in lignin degradation. (Fog, 1987).

The results of our research show that lignin and polymers in the substrate were degraded without additional nitrogen. Therefore, evaluating C/N ratio changes using both hydrolytic lignin and polymer covered soil, we can conclude that in those substrates where the ratio was 4/1 an intensive organic matter degradation proceeded. The intensive nitrogen compound emission appeared in the substrate with the C/N ratio 20–25/1, while C/N > 25 shows that this organic material is accessible only to fungi. C/N ratio necessary for feeding and energetic needs of these microorganisms is 30/1 (Brady, Weil, 1999). Such a ratio of organic material was observed after 45 weeks only in the substrates with polymer and a thick lignin layer. Fungi have a possibility to absorb nutrients and thus can degrade materials whose C/N is 1000/1. In such occasions nitrogen fixation is possible, but often it is very slow (Larsen, Jurgensen et al. 1982).

With reference to publications (Kogel–Knabner, 2002), when the C/N ratio is >30, during the degradation of organic materials rich in lignin, nitrogen compounds are incorporated and cannot leach out to the environment or be used by plants. Thus, our case shows that coast sand substrate with hydrolytic lignin is short of the mentioned biogens and nitrogen cannot be used for plant needs. In the sandground soil, the C/N ratio suitable for organic matter mineralization was preserved in samples with a 5 cm layer of lignin, and it was much higher in samples covered with lignin and polymers.

The C/N ratio of 25–30 indicated that the lignin degradation process was going on. In other cases, organic matter is already degraded and transformed into nutrients. The research results confirmed the highest NO_3 concentration exceeding PAC (permission available concentration) in the drainage water which was analyzed during the last two years of the experiment.

The research results indicate that the substrates made of lignin and polymers can be mineralized by natural microorganisms, however, they cannot be used for soil erosion prevention or growing plants, especially the ones consuming plenty of nitrogen, without additional nitrogen supply.

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Dalia Ambrazaitienė, Olga Belous,
Audronė Žukauskaitė, Galia Shulga

DIRVOŽEMIO EROZIJOS PREVENCIJAI SKIRTŲ SUBSTRATŲ BIODEGRADACIJOS TYRIMAS

Santrauka

Substratų tinkamumo augalų augimui nustatymas dirvožemio erozijos prevencijos technologijose yra vienas svarbiausių tyrimo etapų. Ne mažiau svarbūs ir substratų biodegradacijos tyrimai. Substratų biodegradacijos tyrimai buvo pradėti vasaros viduryje. 10 l talpos indai buvo užpildyti pajūrio smėliu ir smėlžemiu, o jų paviršius, remiantis tyrimų schema, buvo padengtas skirtingo storio hidrolizinio lignino sluoksniu (0, 2, 5 cm). Lignino paviršius buvo apipurkštas polimerų $LSTM_2$ ir $LSTM_1$+ aktyvatorius tirpalu, skaičiuojant 200 ml m^{-2}. Indai su substratais buvo laikomi atviroje aikštelėje ir visą tyrimo periodą veikti meteorologinių sąlygų. Substrato mikrobiologinei analizei mėginiai buvo imami eksperimento pradžioje bei po 4, 8 ir 45 savaičių. Lygiagrečiai buvo imami drenažinio vandens ir substrato mėginiai cheminei analizei.

Tyrimo pradžioje ardant pajūrio smėlio organinę medžiagą buvo aktyvių bakterijų. Jų buvo pakankamai daug netgi substrate be lignino. Vėlesniuose tyrimo etapuose bakterijų skaičiaus kaita priklausė nuo lignino sluoksnio storio. Vis dėlto grybai buvo pagrindiniai hidrolizinio lignino ardytojai. Mikromicetų skaičius pajūrio smėlio substrate tyrimo pradžioje esminiai priklausė nuo lignino sluoksnio storio (r = 0,84), po 4 savaičių koreliacijos koeficientas sumažėjo iki 0,64, o po 8 savaičių jis buvo lygus 0,48. Smėlžemyje, kuriame anglies atsargos buvo didesnės (C = 1,25%), svarbiausios biodegradacijos proceso dalyvės buvo bakterijos. Akivaizdu, kad substratuose, kuriuose C/N pasiekė 25–30, biodegradaciniai procesai vis dar vyksta. Kitais atvejais organinės medžiagos mineralizavimas jau pasibaigęs. Taigi tyrimo rezultatai rodo, kad tirti substratai natūralios mikrofloros gali būti ardomi, tačiau priešerozinėse technologijose be papildomo augalų tręšimo azoto junginiais jų naudojimas gali būti ribotas.

Raktažodžiai: hidrolitinis ligninas, modifikuoti polimerai, dirvožemis, biodegradacija, bakterijos, grybai, C/N santykis