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Assessment of the possibility of using hydrolysis lignin
for biological reclamationVictoria M. Yurk^{ID}✉, Aleksandra A. Shashkova,
Vyacheslav A. Snegirev^{ID}, Natalia A. Tretyakova^{ID}

Ural Federal University, Yekaterinburg, Russian Federation

✉v.yurk@yandex.ru

Abstract. Hydrolysis lignin is a large-scale industrial waste that has no useful application. It is disposed in special landfills, causing alienation of valuable lands and having a negative impact on the environment. The aim of the work was to evaluate the possibility of using hydrolysis lignin formed as a result of the activities of the Ivdelsky timber processing plant for biological reclamation of disturbed lands. The object of the study is hydrolysis lignin from a lignin storage facility located in the city of Ivdel, the Sverdlovsk region. The study of the composition of the runoff accumulated in the lignin deposit showed that the content of lignin in it is 58.97 wt. %, also a significant part is the mineral fraction, represented mainly by oxides of silicon, aluminum and calcium. The total content of heavy metals does not exceed 0.15 wt. %. The experiments have shown that the toxic effect of the aqueous extract on the test culture (red clover *Trifolium pratense* L.) is absent, respectively, the waste is not dangerous to the environment. The substrates used for biological reclamation should not have a toxic effect on the crops grown. In this regard, a phytotoxicity assessment was carried out by growing test object (oats *Avena sativa* L. and red clover *Trifolium pratense* L.) on a substrate from the waste. The results of the experiment showed a lower percentage of germination and growth of plant biomass compared to the control, but the test objects developed normally, there were no damages on the leaves and shoots. Thus, the studies have shown the possibility of using the hydrolysis lignin as a substrate for biological reclamation without preliminary treatment.

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Оценка возможности использования гидролизного лигнина для биологической рекультивации

В.М. Юрк^{ID}✉, А.А. Шашкова, В.А. Снегирев^{ID}, Н.А. Третьякова^{ID}

Уральский федеральный университет, Екатеринбург, Российская Федерация
✉v.yurk@yandex.ru

Аннотация. Гидролизный лигнин является крупнотоннажным промышленным отходом, который не находит полезного применения и размещается на специальных полигонах, выступая причиной отчуждения полезных земель и оказывая негативное воздействие на окружающую среду. Цель исследования – оценка возможности использования гидролизного лигнина, образовавшегося в результате деятельности Ивдельского лесозавода, для биологической рекультивации нарушенных земель. Объект исследования – гидролизный лигнин из лигнинохранилища, расположенного в г. Ивдель Свердловской области. Изучение состава отхода, накопленного в лигнинохранилище, показало, что содержание лигнина в нем составляет 58,97 % масс., также значительную часть составляет минеральная фракция, представленная в основном оксидами кремния, алюминия и кальция. Суммарное содержание тяжелых металлов не превышает 0,15 % масс. Проведенные эксперименты показали, что токсический эффект водной вытяжки на тест-культуру (красный клевер *Trifolium pratense* L.) отсутствует, соответственно, отход имеет V класс опасности. Используемые для биологической рекультивации субстраты не должны оказывать токсический эффект на выращиваемые культуры. В связи с этим была проведена оценка фитотоксичности по выращиванию тест-культур – овса *Avena sativa* L. и клевера лугового красного *Trifolium pratense* L. – на субстрате из отхода. Результаты эксперимента показали меньший по сравнению с контролем процент всхожести и прирост биомассы растений, но при этом тест-культуры развивались нормально, на листьях и побегах отсутствовали какие-либо повреждения. Таким образом, исследования показали возможность использования рассматриваемого гидролизного лигнина в качестве субстрата для биологической рекультивации без предварительной обработки.

Ключевые слова: фитотоксичность, рекультивация, рекультивационный материал, переработка отходов

Вклад авторов. Юрк В.М. – научное руководство исследованием, проведение исследовательского процесса; Шаикова А.А. – проведение исследовательского процесса, отслеживание воспроизводимости результатов экспериментов; Снегирев В.А. – предоставление лабораторных образцов азотфиксирующих бактерий для проведения исследования; Третьякова Н.А. – курирование данных, подготовка черновика рукописи. Все авторы ознакомлены с окончательной версией статьи и одобрили ее.

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Introduction

Many primary processing plants generate large-scale wastes. Such wastes are inert, unreacted, associated or auxiliary materials with no valuable use. Currently, most of these wastes are disposed of in landfill sites or heaps near the facilities where they were generated. The negative impact of these facilities is manifested in the alienation of land potentially useful to the national economy, as well as in the toxicity of impurity compounds in the run-off that can migrate into the environment and poison it. Therefore, the problem of recycling large-scale waste is one of the current environmental challenges. Large-scale waste includes hydrolysis lignin.

Hydrolysis lignin is a waste product produced in the hydrolysis industry as a result of the delignification of timber. It is a non-hydrolysable residue of wood processing diluted with sulfuric acid solution and is a complex mixture of lignin, hard-to-hydrolyse polysaccharides, resin, various ashes, monosaccharides, residues of sulfuric and organic acids, more often acetic and formic acids, and moisture [1]. It is insoluble in organic solvents, alkalis and acids [2].

Currently, hydrolysis lignin is practically not used and is disposed of in lignin storage facilities and dumps [3]. Despite the fact that the material is difficult to dissolve in various conditions, the objects of its disposal can have a negative impact on the environment. For example, some impurities contained in the composition of the waste can migrate from the landfill site together with surface runoff. Such compounds include sulfuric acid and its salts, water-soluble organic acids, phenolic compounds. Also, as noted by the authors of the study [4], the surface of hydrolysis lignin contains a large number of radicals, which, when interacting with air oxygen, show a high ability to self-ignition, so not seldom focal ignitions are formed at lignin storages.

Since hydrolysis lignin has quite a lot of organic fractions, it is sometimes used as a fuel in hydrolysis plants [2; 5]. However, waste incineration is not always a

rational way of waste management, as it contributes to the emission of large amounts of pollutants and the loss of potentially useful raw materials.

At present, alternative ways of hydrolysis lignin processing are being searched for. For instance, this waste can be used to produce lignin-aldehyde resins [6], lignin plastic [7], sorbents [8; 9], and other valuable products. But technical lignin is characterised by heterogeneity of composition and variability of chemical properties, so its use in production processes is difficult [10]. It requires constant control and adjustment of the technological process, which can lead to a decrease in product quality, increase in production costs and deterioration of the environmental situation.

One of the promising and simple ways of useful utilisation of multi-tonnage non-toxic waste is its application for reclamation of disturbed lands.

In addition to a large amount of organic matter, hydrolysis lignin can contain a sufficient amount of nutrients, which makes it an attractive raw material for fertiliser production [11], as well as a potential substrate for biological reclamation of disturbed lands.

There are known ways when large-tonnage organic waste was used as a raw material for obtaining fertilisers and soils. For example, the authors of [12] developed a special two-component soil substrate from forestry processing wastes and sewage treatment facilities for reclamation of landfills. In [13], lignin sludge was added to excess activated sludge to obtain a fertile substrate.

The aim of the research is to assess the possibility of using the waste hydrolysis lignin, formed as a result of the Ivdel'sky timber processing plant activity, for biological reclamation of disturbed lands.

Materials and methods

The hydrolysis lignin waste tested in this work was sampled from a lignin storage facility in Ivdel, the Sverdlovsk region. The appearance of the waste is presented in Figure 1. The waste was sampled from the upper part of the landfill using the square method in accordance with GOST 12071–2014¹.

According to FWCC² hydrolysis lignin belongs to the group 3 01 212 00 00 0 “Lignin from processing of agricultural raw materials” without specifying the waste hazard class. In this regard, it is necessary to establish the hazard class in order to determine possible ways of its further utilisation. The hazard class of the waste was determined in accordance with the methodological recommendations³ on the effect

¹ GOST 12071-2014. *FOUNDATIONS. Selection, packing, transport and storage of samples. Intro.* 2015-07-01. Moscow : Standartinform, 2015. 12 p.

² Federal Waste Classification Catalogue. *Rosprirodnadzor: Federal Service for Supervision of Natural Resources Management*. Available from: <https://rpn.gov.ru/fkko/> (accessed: 15.10.2024).

³ Rusakov NV, Kryatov IA, Pirtakhia NV, Tonkopii NI, Kartseva NY, Starodubov AG. *Justification of the hazard class of production and consumption waste on phytotoxicity: methodological recommendations, MR 2.1. 7.2297-07*. Moscow: Federal Centre of Hygiene and Epidemiology of Rospotrebnadzor; 2008.

of its aqueous extract on the test culture. Red clover was used as a test culture *Trifolium pratense* L.



Figure 1. The photo of hydrolysis lignin
Source: the photo by V.M. Yurk, A.A. Shashkova.

In addition, the calculation of the waste hazard class was carried out according to the Criteria for classifying wastes into I-V hazard classes by the degree of negative impact on the environment⁴. Elemental analysis of substrates was carried out by X-ray fluorescence spectroscopy (ARL Advant’X 4200, TermoFisher Scientific Inc., USA). To calculate the humus content in the soil, the obtained carbon content was multiplied by the coefficient 1,724 according to [14]. The lignin content of the waste was calculated using the approximate gross formula of the compound $C_{31}H_{34}O_{11}$.

Analysis of nutrient and water-soluble components in the aqueous matter of substrates was conducted according to [14]. The following parameters were determined: dry water extract residue, leachate residue, calcium and magnesium ions, sulphate ion. The pH values of water and salt extractions of hydrolyzed lignin were also measured (pH meter 4100, NPP “Infsepark-Analite”, Russian Federation).

To establish the possibility of using hydrolysis lignin for biological remediation, its phytotoxicity was determined. The test crops selected were oat seeds of *Avena Sativa* L. and clover of *Trifolium pratense* L.

⁴ Order of the Ministry of Natural Resources and Ecology of the Russian Federation dated 04.12.2014 No. 536 “On approval of the Criteria for classification of wastes to I-V hazard classes by the degree of negative impact on the environment”. *KonturNormativ*. Available from: <https://normativ.kontur.ru/document?moduleId=1&documentId=265683&ysclid=mb9lftoh6a87783084> (accessed: 15.10.2024).

Both crops are unfriendly to climatic conditions, react to substrate composition and can also be practically used in the restoration of real waste. Hydrolysis lignin and universal soil substrate for planting were used as substrates for growing test cultures (control experience).

Phyto-toxicity assessment experiments were conducted under different conditions as shown in the Table 1. Cultivation of test cultures was carried out on a substrate without additives (experiments No. 1–4), with the application of 2 ml of fertilizer “Ammofosca” in the substrate (experiments No. 5–8) and also with the application of fertilizer “Ammofosca” and 1 ml of biological agent in the substrate, representing a culture of nitrogen-fixing bacteria (Experiments No. 9–12). Each experiment was conducted in two parallels.

Phytotoxicity was assessed using the following methodology. The dry substrate (fraction less than 1 mm) of a weight of 80 g was placed in a plastic pot and moistened. Added fertilizer or a bio-preparation, and then at a depth of 1.0–1.5 cm seeded test crop seeds. In each pot 10 seeds were seeded. Experimental crops placed in the thermostat air XT 3/70 (manufacturer of JSC “Five oceans”). Constant conditions were maintained during the experiment: temperature $25 \pm 1^\circ\text{C}$, duration of daylight 16 hours, periodic watering as needed. The experiment lasted 14 days.

Table 1. Conditions of experimental plant seeding

Exp.	Substrate	Test object	Fertilizer	Biological agent
1	Soil	<i>Avena Sativa</i> L.	–	–
2	Soil	<i>Trifolium pratense</i> L.	–	–
3	Hydrolysis lignin	<i>Avena Sativa</i> L.	–	–
4	Hydrolysis lignin	<i>Trifolium pratense</i> L.	–	–
5	Soil	<i>Avena Sativa</i> L.	+	–
6	Soil	<i>Trifolium pratense</i> L.	+	–
7	Hydrolysis lignin	<i>Avena Sativa</i> L.	+	–
8	Hydrolysis lignin	<i>Trifolium pratense</i> L.	+	–
9	Soil	<i>Avena Sativa</i> L.	+	+
10	Soil	<i>Trifolium pratense</i> L.	+	+
11	Hydrolysis lignin	<i>Avena Sativa</i> L.	+	+
12	Hydrolysis lignin	<i>Trifolium pratense</i> L.	+	+

Source: compiled by V.M. Yurk, A.A. Shashkova.

Nitrogen-fixing bacteria were secreted from the soil. For this purpose, 10 g of ground soil was added to 100 ml of liquid Burka selective medium and cultivated within 7 days at 30°C in a BIOSAN ES-20 shaker-incubator with 250 rpm. The bacteria were then separated from the yeast by seeding in a solid Burke medium. The resulting colonies of bacteria were added back to the liquid medium. The number of cells per 1 ml of suspension is calculated by cytofluorimetric method as 5.9×10^7 pcs/ml.

Evaluation of phyto-toxicity of the substrate was carried out according to the following indicators: germination of seeds, dry biomass of plants, length of sprouts and roots, other visually recorded negative changes in different parts of plants.

Results and discussion

The assessment of the possibility of using production wastes is primarily related to the determination of their toxicity and hazard class, which directly affects the design of the waste management procedure and possible options for its recycling. Only non-hazardous and non-toxic wastes that do not contribute to the migration of pollutants through food chains are suitable for biological reclamation.

Phyto-testing of aqueous extraction from hydrolysis lignin according to the methodological recommendations⁵ showed the following results. In the sample under analysis 19 sprouts from 25 planted seeds grew, and in the control (distilled water) 16 of the 25. The mean root size in the experimental sample ($L_{ES} = 31$ mm) is comparable to the mean root length in the control sample ($L_{CS} = 30$ mm), therefore there is no adverse effect of the withdrawal on the plant. In addition, the effect of braking EB calculated from experience is 3.3%, i.e. less than 20% and according to the methodological recommendations,⁶ phytotoxic action has not been proven. Figure 2 shows the results of the test for water withdrawal.

The measurement of the hydrogen indicator of the aqueous and salt extractions from hydrolysis lignin showed the following results: $pH_{aq.} = 6.67$, $pH_{KCl} = 6.34$. The data obtained indicate a weak or neutral retreat environment favourable to the cultivation of various wild-type crops.

The component composition of hydrolysis lignin and the control substrate – universal soil, are given in Table 2. The table shows data only for the main components of the substrates.

As can be seen from the test results, the main component of the waste is lignin – 58.97 wt. %, which is a polymer of plant origin, a component of wood. The mineral fraction of the waste is represented mainly by silicon oxide (14.06 wt. %). The presence of the mineral fraction is a favourable factor, as it contributes to the improvement of physical and chemical properties of soils, regulates toxicity and acidity of media, increases the stability of organic compounds. The studied hydrolysis lignin also contains nutrients necessary for plant growth and development – P, Mg, Na, K, Ca, Mn, S, the total concentration of heavy metals (V, Pb, As, Zn, etc.) is less than 0.15 wt. %.

⁵ Rusakov NV, Kryatov IA, Pirtakhia NV, Tonkopii NI, Kartseva NY, Starodubov AG. *Justification of the hazard class of production and consumption waste on phytotoxicity: methodological recommendations, MR 2.1. 7.2297-07*. Moscow: Federal Centre of Hygiene and Epidemiology of Rospotrebnadzor; 2008.

⁶ Ibid.



Figure 2. The result of phytotest analysis of an aqueous extract from hydrolysis lignin with *Trifolium pratense* L.

Source: the photo by V.M. Yurk, A.A. Shashkova.

Table 2. The content of the main components in hydrolysis lignin and soil

Hydrolysis lignin		Soil	
Component	Concentration, wt. %	Component	Concentration, wt. %
Organic part			
Lignin	58,97 ± 0,66	Humus	52,29 ± 0,22
Mineral part			
SiO ₂	14,06 ± 0,19	SiO ₂	1,42 ± 0,07
CaO	6,54 ± 0,14	CaO	10,08 ± 0,18
Al ₂ O ₃	4,37 ± 0,1	Al ₂ O ₃	0,39 ± 0,02
SO ₃	4,36 ± 0,11	SO ₃	0,35 ± 0,02
Fe ₂ O ₃	2,94 ± 0,09	Fe ₂ O ₃	0,51 ± 0,03
MgO	0,84 ± 0,04	MgO	0,24 ± 0,01
Nutrients			
SO ₃	4,36 ± 0,11	SO ₃	0,35 ± 0,02
P ₂ O ₅	1,79 ± 0,07	P ₂ O ₅	0,47 ± 0,02
Na ₂ O	0,48 ± 0,02	Na ₂ O	0,05 ± 0,003
K ₂ O	0,39 ± 0,02	K ₂ O	0,72 ± 0,04
N	0,20 ± 0,08	N	0,47 ± 0,08
Heavy metal			
Mn	0,059 ± 0,003	Mn	0,018 ± 0,001
Zn	0,022 ± 0,0011	Zn	0,002 ± 0,001
Cu	0,020 ± 0,001	Cu	–
Cr	0,014 ± 0,0007	Cr	0,023 ± 0,001
V	0,0080 ± 0,0004	V	0,001 ± 0,0004
Pb	0,0073 ± 0,0016	Pb	–
As	0,0044 ± 0,0002	As	–
Ni	0,0030 ± 0,0004	Ni	–

Source: compiled by V.M. Yurk, A.A. Shashkova.

The content of the organic part represented by humus in the control substrate is slightly lower than in the waste. It should be noted that plant residues are present in the control soil.

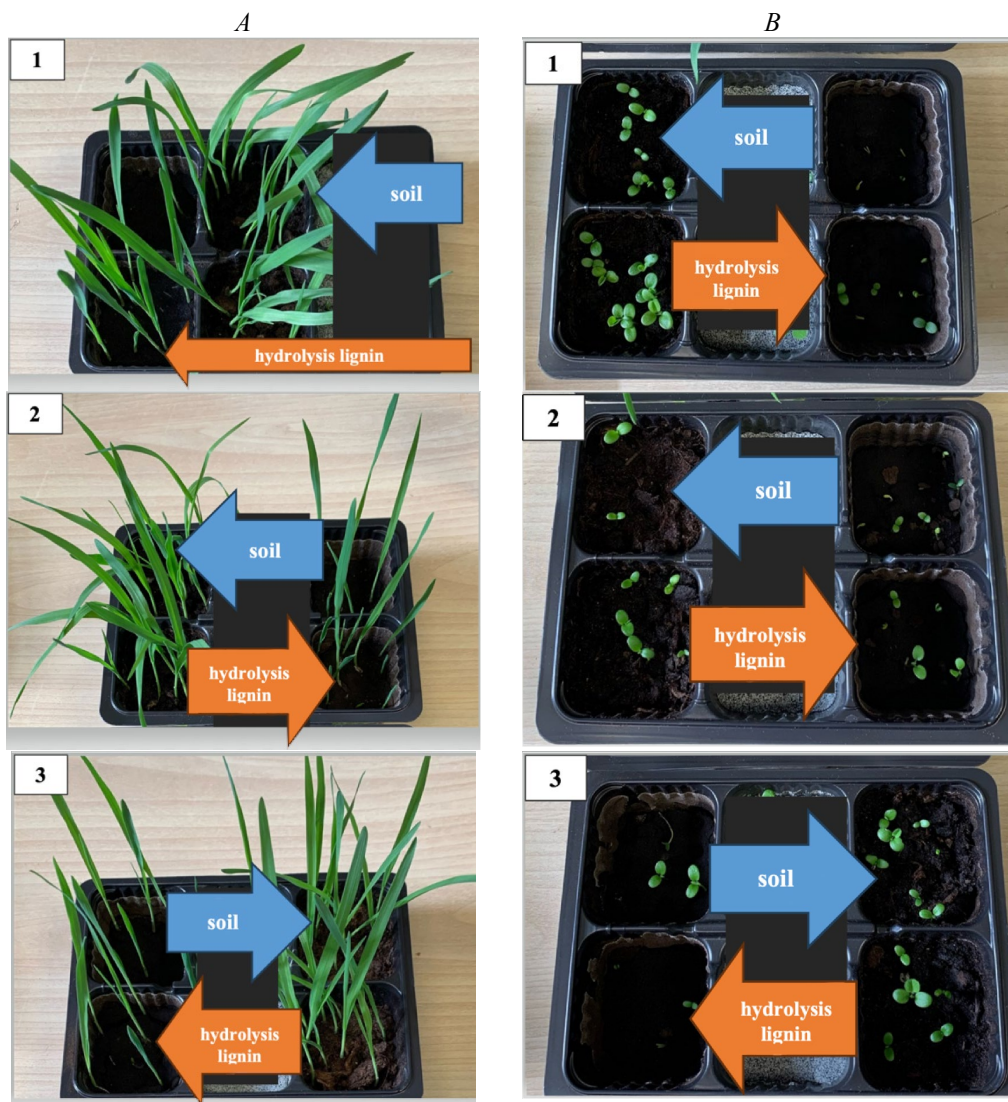


Figure 3. Results of phytotesting of hydrolysis lignin:

A – test object is oats (*Avena Sativa* L.); *B* – test object is red clover (*Trifolium pratense* L.);
 1 – cultivation on a substrate without additives; 2 – with the addition of fertilizer “Ammophoska”;
 3 – with the addition of fertilizer “Ammophoska” and with a bioagent *Azotobacter*

Source: the photo by V.M. Yurk, A.A. Shashkova.

It is interesting to note that nutrient biogenic components – N, P, K – are higher in the waste than in the soil. Also, in the waste there is an increased sulphur content compared to the control soil, which is a consequence of the use of sulphuric acid in the process of hydrolysis of timber.

According to the results of the conducted research, it can be concluded that the composition of hydrolysis lignin does not contain toxic components, and the content of nutrients is not less than in the control soil. Calculation of the hazard class in accordance with the Order of the Ministry of Natural Resources and Ecology of the Russian Federation dated 04.12.2014 No. 536⁷ showed that the waste can be classified as the 5th class if confirmed by experimental method. Thus, the considered hydrolysis lignin can be used as a substrate for biological recultivation.

The appearance of plants after phytotoxicity evaluation experiments is shown in Figure 3. As can be seen, no negative changes were recorded in the samples of test plants 14 days after the start of the experiment. Both test crops in all experimental conditions do not show any damage to leaves or shoots, which confirms the absence of toxic bioavailable components in the composition of the hydrolysis lignin studied.

The results of measurements of test parameters of experimental crops sown on hydrolysis lignin and control soil are given in Table 3.

Table 3. Measurement results of test parameters of experimental crops on hydrolysis lignin and control soil

Test culture	Without additives		With fertilizer		With a biological agent	
	Hydrolysis lignin	Soil	Hydrolysis lignin	Soil	Hydrolysis lignin	Soil
1	2	3	4	5	6	7
Percentage of germination of test object seeds, %						
<i>Avena Sativa</i> L.	55	100	85	95	55	100
<i>Trifolium pratense</i> L.	60	75	65	45	30	60
Dry biomass, g						
<i>Avena Sativa</i> L.	0.185	0.375	0.225	0.420	0.155	0.410
<i>Trifolium pratense</i> L.	0.010	0.023	0.034	0.010	0.002	0.015
Average length of test object plantlets, cm						
<i>Avena Sativa</i> L.	2.3	4.7	2.8	3.7	1.6	4.5
<i>Trifolium pratense</i> L.	1.5	3.1	1.4	0.8	0.7	1.8
Average root length of test objects, cm						
<i>Avena Sativa</i> L.	10.5	9.6	12.4	10.7	10.5	11.8
<i>Trifolium pratense</i> L.	2.6	2.4	2.2	1.3	1.0	1.5

Source: compiled by V.M. Yurk, A.A. Shashkova.

⁷ Order of the Ministry of Natural Resources and Ecology of the Russian Federation dated 04.12.2014 No. 536 “On approval of the Criteria for classification of wastes to I-V hazard classes by the degree of negative impact on the environment”. *KonturNormativ*. Available from: <https://normativ.kontur.ru/document?moduleId=1&documentId=265683&ysclid=mb9lftoh6a87783084> (accessed: 15.10.2024).

For all experiments, a lower percentage of germination of test crops on hydrolysis lignin was observed compared to the control soil. This can also be observed in Figure 3. The highest value of biomass was also recorded when the test plants were grown on the test soil.

Summarising the results of determination of all the studied indices, it can be concluded that oats grow best on substrates (hydrolysis lignin and soil) to which fertilizer was additionally applied. Red clover grows better on substrates without additives. This can be explained by the peculiarities of the test crops and their different needs for nutrient components at different periods of growth and development.

It should be noted that hydrolysis lignin contains a sufficient amount of nutrients but does not give a greater biomass growth compared to control samples. Probably, these elements are in forms inaccessible for plants, so the fertiliser application has a positive effect on the growth of test-cultures.

The average length of seedlings and roots is practically unaffected by the addition of fertiliser. At the same time, additional application of the biopreparation reduces the value of these indicators in both test-cultures grown on hydrolysis lignin. The samples grown on potting soil show less susceptibility to the presence of bacteria. As shown in the results, the application of the biopreparation, which is bacteria of the genus *Azotobacter*, was ineffective in the case of plants germinated on hydrolysis lignin. The possible reason for this is the abundant multiplication and feeding of microorganisms with mineral elements, which become insufficient to meet the needs of plants.

In general, the values of germination and plant development indicators can be considered satisfactory, which indicates the promising use of hydrolysis lignin for biological reclamation.

The results of chemical analysis of water extract from hydrolysis lignin and soil are presented in Table 4. The observed values of the dry residue index allow us to conclude that the waste contains more soluble impurities than the control soil. Moreover, half of these impurities are represented by water-soluble organic matter (which can be determined by the difference between the dry and calcined residue). Despite this, the growth and development of test-cultures is better on the control soil than on the investigated waste of hydrolysis lignin. Probably, some of the mineral components in the composition of the aqueous extract from the solution do not belong to the nutrients required by plants for seed germination and seedling development.

It is possible that when hydrolysis lignin is used for reclamation of territories in the process of its decomposition, overgrowth with herbaceous crops and development of soil biocenosis, the soil solution will be acidified, which will lead to additional leaching of nutrient components. However, this will clearly not be the case at the initial stage of reclamation and other fertilisers that will contain calcium, magnesium and other useful components required by plants can be considered as nutrient additives.

It should also be noted that despite the higher gross sulphur content in the hydrolysis lignin, there is less sulphate in the aqueous extract than in the control soil. The low concentration of sulphate ions can be explained by the fact that the work considered waste from the lignin storage facility, which had already been deposited in the landfill for some time. During this period, some number of water-soluble constituents may have been leached by infiltration of surface runoff. The sulphate ion may form poorly soluble salts with some metals or be part of the hydrolysis products of lignin. Thus, this component will not inhibit plant growth and negatively affect the reclamation process.

Table 4. Results of chemical analysis of aqueous extracts of hydrolysis lignin and control soil

Parameter	Unit of measurement	Hydrolysis lignin	Soil
Dry residue	%	1.395	0.86
Calcined residue	%	0.71	0.59
Calcium ions	mg-eq / 100 g	1.0	0.15
Magnesium ions	mg-eq / 100 g	–	0.03
Sulfate ions	mg-eq / 100 g	0.17	0.25

Source: compiled by V.M. Yurk, A.A. Shashkova.

Conclusions

The results of the conducted studies showed that the hydrolysis lignin under consideration does not contain toxic components and does not have a phytotoxic effect on test-cultures, which indicates the prospect of its use as a substrate for plant cultivation. However, despite the sufficient number of nutrient components such as phosphorus, nitrogen, potassium and sodium, the growth and development of oat and red clover test-cultures is rather slow. This may be due to the presence of these elements in the composition of the waste in a form that is not available to the plants, or to the lack of other important components, such as magnesium, which is necessary during the seed germination stage and seedling growth and development.

The use of this waste is economically feasible for biological recultivation, as it will reduce the area occupied for its disposal and reduce environmental damage. Mixtures of hydrolysis lignin with other, more nutritious substrate can give the fastest result of overgrowth of recultivated areas. In this case, lignin will play the role of a soil structuriser.

Based on the remote location of some timber processing plants, it is not always possible to add nutritious substrates to the reclamation material without damaging the existing ecosystems. Therefore, sowing different types of plant and microorganism cultures capable of forming a sustainable biocenosis on the same substrate is also of practical and scientific interest in the future. The selection of a combination of species can contribute to a faster process of soil formation with the participation of hydrolysis lignin.

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Bio notes:

Victoria M. Yurk, Ph.D. of Chemical Sciences, Associate Professor, Department of Chemical Technology of Fuel and Industrial Ecology, Ural Federal University, 19 Mira St, Ekaterinburg, 620062, Russian Federation. ORCID: 0000-0002-5261-742X; eLIBRARY SPIN-code: 2477-3934. E-mail: v.yurk@yandex.ru

Aleksandra A. Shashkova, Bachelor's Student, Department of Chemical Technology of Fuel and Industrial Ecology, Ural Federal University, 19 Mira St, Ekaterinburg, 620062, Russian Federation. E-mail: aleksa_shashkova2207@mail.ru

Vyacheslav A. Snegirev, Assistant Lecturer, Department of Chemical Technology of Fuel and Industrial Ecology, Ural Federal University, 19 Mira St, Ekaterinburg, 620062, Russian Federation. ORCID: 0000-0002-6348-4271. E-mail: v.a.snegirev@urfu.ru

Natalia A. Tretyakova, Ph.D. of Chemical Sciences, Associate Professor, Department of Chemical Technology of Fuel and Industrial Ecology, Ural Federal University, 19 Mira St, Ekaterinburg, 620062, Russian Federation. ORCID: 0000-0003-3319-270X; eLIBRARY SPIN-code: 3385-5894. E-mail: n-tretyakova@mail.ru.