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Ozone Degradation of Lignin; its Impact Upon the Subsequent Biodegradation

Abstract
The possible use of ozone and advanced oxidation processes (AOP) to degrade and eliminate lignin compounds from aqueous solutions, and the determination of the required ozone dose are the primary objectives of this study. The influence of the oxidation methods on the subsequent biological decomposition of the by-products was also investigated.During ozonisation of the alkalilignin aqueous solutions, the polymer is degraded to a degree depending on the ozone dose. Lignin content decreased by about 40 to 96.6% at an ozone dose of 0.1 and 3.6 mgO_3/mgCOD, respectively, accompanied by a drop of COD in the range of 8.8 - 69.6%. An ozone dose of about 1 mgO_3/mgCOD is required to reduce the lignin content by more than 80%; at such a dose, the reduction of COD was about 35%. Lignin proved to be a substance that is practically insusceptible to biodegradation under the test conditions. An increase of the susceptibility to biodegradation of the lignin disintegration products could have been observed at an appropriately high dose of ozone.

Key words: *degradation, lignin, ozone, advanced oxidation process, biodegradation.*

Introduction

The pulp and paper industry has been seen as a large polluter of waters for a long time. The burden of the industry has in the last two decades been largely reduced thanks to improved technology with the result of decreased effluents, and an ever wider use of biotechnology in wastewater treatment. Still, compounds that are highly durable and barely susceptible to biodegradation appear in the effluents of the pulp and paper industry. These are primarily high-molecular lignin compounds (lignin) and low-molecular compounds that are toxic to aqueous organisms: resin acids and chlororganic derivatives, mainly chlorophenols [1 - 6]. Lignin and resin acids are the two groups of compounds that pose the largest problems in classical wastewater treatment [2, 3, 6 - 10]. In the common praxis of effluents' purification in the pulp and paper industry, two processes are employed: mechanical (sedimentation) and biological, mostly aerobic (aerated ponds and activated sludge). The processes can be enhanced with a possible chemical precipitation, which, however, requires the addition of chemicals with often unknown toxicity [1, 8, 10].

The ever stricter environmental protection regulations are a driving force in the pursuit of new methods and an improvement of traditional processes aimed at the limitation of water contaminative substances. An interesting approach to the problem of purifying effluents, containing durable substances that are resistant to biological degradation, is the adoption of a multistage technology. The first step consists of a physical-chemical treatment of the most hazardous effluent streams, while a biological treatment of the joint wastewater streams comprises the final step. Most promising amongst the physical-chemical methods are advanced oxidation processes (AOP), with the use of ozone, ozone and UV irradiation, H_2O_2 and O_3 or all of the agents together [11 - 13].

Lignin is one of the three main chemical constituents of plant matter (cellulose and hemicellulose are the remaining two). Lignin content accounts for between 16 and 31% of the plant mass. In respect of the chemical structure, lignin is a 3-D organic polymer with an aromatic character in which derivatives of phenylpropane are the basic structural elements [14 - 17]. Ether bonds appear in the lignin molecule between the phenylpropane units, and most of the bonds are insusceptible to hydrolysis.

The composition and structure of lignin are diverse depending upon kind of the wood, its habitat, the growing conditions and other factors. This is the reason for the structure of the lignin molecule not being well known or well-defined. In the sulphate pulping process, lignin is separated from the hydrocarbon fraction by the action of strong base (NaOH). Hence, in the effluents from cellulose pulp production, lignin appears in the form of water-soluble sodium salts, which are also called alkalilignins. Lignin content gives a dark brown colour to the effluents, and is resistant to classical biodegradation methods [18 - 20].

Investigations concerning the impact of ozone on lignin decomposition were performed with the following lignin model compounds: coniferyl alcohol, ferulic acid and β-O-4 dimer; these investiga-

Table 1. Characteristics of the lignin preparation and the model lignin solutions.

	Parameter	Unit	Parameter value	
Lignin preparation - Symbol Lign.	Content of dry substance	% wt.	96.6	
	Content of dry organic substance	% wt.	95.0	
Model alkalilignin solutions	**Concentration of model solution**	**g/l**	**1.0**	**0.5**
	COD	mg O_2/l	1950	984
	BOD_5	mg O_2/l	145	110
	Lignin content	mg/l	920	470
	Toxicity	%	70	42

tions showed that compounds readily react with ozone [14, 15, 21 - 23]. It was found that the aromatic structure almost completely disappeared at an adequately high ozone dose.

Oxidation by way of ozonolysis or other AOP methods seems to be the most effective method for purifying effluents of the pulp and paper industry [24 - 26]. Application of the advanced oxidation processes, particularly of ozone, allows a high degradation of lignin to be obtained and the organic substance load in the effluents, such as starch and low molecular compounds, to become lower, although not entirely eliminated [24]. Therefore, the methods can be employed in the first treatment step of the pulp-paper effluents prior to the biological aerobic step [24, 27].

The present knowledge in the domain of lignin degradation is insufficient on account of the variety of lignin compositions and structures depending upon the kind of wood used and the method of pulping employed. This calls for further intensive investigations in that direction.

Aim and scope of the investigation

The aim of the work was to investigate the effect of ozone and other AOP's actions on lignin compounds and to assess the suitability of the methods to the removal of lignin from wastewater.

The work comprised of:

- AOP experiments of the model lignin solutions.
  Variable parameters: ozone dose and UV irradiation – on/off.
- Biodegradation experiments of the model lignin solutions prior to and after ozonisation.
- Chemical analyses and testing of toxicity and biodegradation.

Materials, methods and equipment

Preparing the lignin formulation for the investigations

The used black liquor originated from one of the sulphate pulp mills processing pine wood. The lignin preparation was made by separating the lignin compounds from the liquor, and the separation procedure was based on the authors' earlier experience. A multistage precipitation and re-dissolution of the lignin was employed to get rid of the low molecular compounds including the degraded lignin fragments (so-called hemilignins). Alkalilignin was precipitated in the first step by acidifying the black liquor with 25% H_2SO_4 (analytical purity) to pH = 4. Carbon dioxide, hydrogen sulphide and a certain amount of volatile organic acids were intensively delivered in the course of the acidification. The mixture was left for 24 hours and then the precipitated sediment was decanted, separated by filtration, washed with slightly acidic (pH = 5) water and dried. The obtained preparation was further purified by being dissolved in a diluted NaOH solution and re-precipitated with 25% H_2SO_4 at a pH of about 2. The separated and dried sediment was extracted with ethyl ether to totally remove the resin acids content. The obtained purified preparation contained an average of 3.4% water (96.6% of the dry substance) and 95% of organic matter-lignin (98.3% of the dry substance). The preparation was readily dissolvable in diluted alkalis (0.01 N NaOH solution). A preparation of such purity may be considered a standard of lignin to be used in research and analyses.

Preparation of model alkalilignin solutions

The model solutions for the investigations were prepared by dissolving a weighed amount of the lignin, which was prepared according to section "**Preparing the lignin formulation for the investigations"**, in 100 cm^3 of a 0.1 N NaOH solution. This was diluted with distilled water to 1 l. Solutions with a lignin content of 1000 mg and 500 mg (in 1 l) were used in the investigation.

Characteristics of the lignin preparation and the model solutions are given in ***Table 1***.

Methods and conditions of the experiments

An aqueous solution of the alkalilignin preparation was ozonised at variable ozone doses in some cases using also UV irradiation. The biodegradation of the ozonised solutions was tested with the use of active sludge. The solutions both before and after the experiments were analysed in respect of:

- content of organic substance as COD
- content of substance susceptible to biodegradation as BOD
- lignin content
- toxicity

Experiments in advanced oxidation processes (AOP)

- Ozone dose (mg O_3/mg COD): 0.10 – 3.71,
- Other AOD's: O_3 + UV, O_3+ UV + H_2O_2,
  - UV irradiation: 15 W low pressure lamp,
  - H_2O_2 dose: 2 ml/l of solution,
- Temperature: 20 ± 1 °C,
- pH – initial 9 - 10, after ozonisation 7 - 8.

Biodegradation

On the basis of standard PN-EN ISO 9888:2005 ("Estimation of total biodegradation in aqueous medium; Statistical test – Zahn-Welles method"), a procedure was performed to test the biodegradation susceptibility of the model solutions. An active sludge taken from a regular functioning communal wastewater treatment plant with a slight addition of industrial effluents constituted the inoculum. The amount of the active sludge placed in the chambers of the analyser was adjusted according to the COD values of the tested solutions at the proportion of 1 g of active sludge/1000 mg COD. The testing time was 7 - 10 days.

Experimental equipment

The AOP experiments were performed in a 1.5 l photo-reactor equipped with ozone detectors. A scheme of the experimental apparatus is shown in ***Figure 1***. After the AOP, the model solutions were biodegraded in laboratory equipment with

controlled aeration. The air inlet was arranged in the bottom of the five cylindrical chambers providing sufficient aeration in the entire volume of the chamber and mixing of the tested solutions with the active sludge suspension.

Analytical equipment, reagents and materials

- Spectrophotometer: CADAS 200 and thermostats LT 100 (Dr Lange GmbH),
- Microprocessor oxygen meter: Oxi 325 with oxygen sensor CellOx 325 (WTW Co.),
- LUMIStox 300 with incubation block (Dr Lange GmbH),
- Filtration apparatus (Whatman Co.),
- Analytical scale type WA-32 (Mera – Wag Co.),
- Cuvette tests LCK 384, 380, 114, 314, 614 (HACH LANGE GmbH),
- Sodium tungstate, sodium molybdate, ortophosphoric acid, hydrochloric acid, sodium-potassium tartrate, sodium carbonate, sodium hydroxide, potassium dihydrophosphate anhydrous, di-potassium hydrophosphate anhydrous, di-sodium hydrophosphate 2-hydrate, disodium hydrophosphate 2-hydrate, ammonium chloride, magnesium sulphate 7-hydrate, calcium chloride anhydrous, and iron chloride 6-hydrate,
- Certified lyophilic bacteria strain *Vibrio fisheri* NRBL-B-11177.

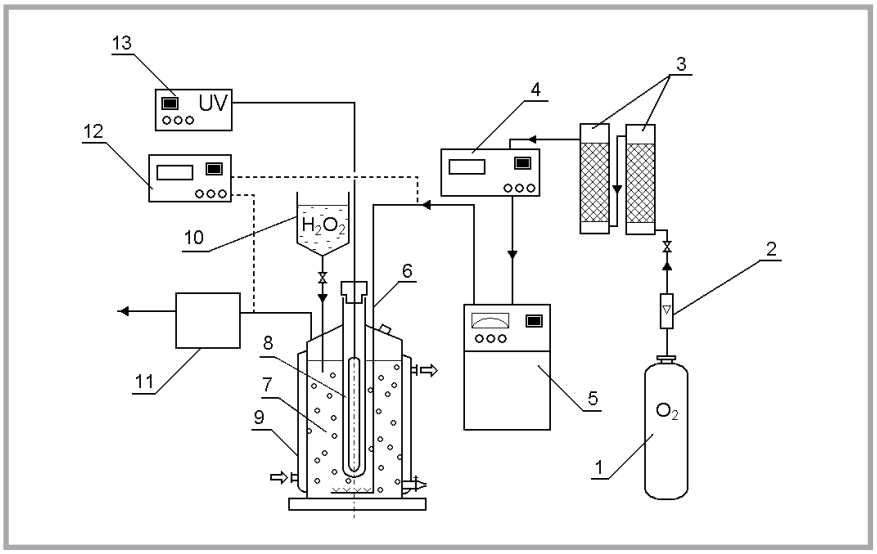

Figure 1. *Schematic diagram of the experimental apparatus set-up: 1 - steel cylinder with oxygen, 2 - rotameters, 3 - drying columns filled with $CaCl_2$ and P_2O_5, 4 – gas flow rate meter, 5 – ozonator, 6 – porous plate, 7 – glass reactor vessel, 8 – quartz tube with UV lamp, 9 – thermostating jacket, 10 – hydrogen peroxide tank, 11 – system of ozone neutralization, 12 – ozone concentration meter to measure ozone content in oxygen at the reactor inlet and outlet, 13 – UV lamp feeder.*

Analytical methods

Lignin content analysis by spectrophotometric methods

A spectrophotometric method for the estimation of lignin content was prepared on the basis of standard PN-76/C-04623.00 ("Testing of lignin and tannin content"). In this method, the reaction proceeding between hydroxyl groups and components of the tungsten-molybdenum-phosphorous reagent was exploited, yielding products with an intensive blue colour. Intensity of the colour was measured by means of a spectrophotometer at a wavelength of λ = 700 nm. The analytical curve was prepared using a standard sulphate lignin solution – a preparation made of spent kraft pulping liquors as described in section "**Preparing the lignin formulation for the investigations**".

Chemical oxygen demand (COD) was estimated according to standard ISO

Table 2. *Conditions of the ozonization experiments of model of lignin solutions and results.*

Symbol of sample and No of experiment	Lignin and other AOP concentr. at start., g/l	Time of ozonization, h	Ozone dose, $mgO_3/mgCOD$	COD, mgO_2/dm^3		BZT, mgO_2/dm^3		Lignin content, mg/l		BOD/COD	
				Prior to experiment	After experiment	Prior to experiment	After experiment	Prior to experiment	After experiment	Prior to experiment	After experiment
Lign. 3	1	1	0.31	1926	1547	145	140	875	317	0.07	0.09
Lign. 4	1	3	0.93	1926	1220	145	156	875	140	0.07	0.13
Lign. 5	0.5	3	3.02	993	423	110	119	460	22.5	0.11	0.28
Lign. 6	1	3	1.82	1974	1050	145	194	922	87.5	0.07	0.18
Lign. 7	0.5+UV	3	3.71	970	320	110	130	440	16	0.11	0.41
Lign. 8	0.5+UV+H_2O_2	3	3.71	970	308	110	136	440	19	0.11	0.44
Lign. 9	0.5	1	0.20	984	865	n.o.	n.o.	480	250	-	-
Lign. 10	0.5	1	0.50	984	760	n.o.	n.o.	480	116	-	-
Lign. 11	0.5	1	1.02	984	630	n.o.	n.o.	480	74	-	-
Lign. 12	0.5	1.5	1.52	984	538	n.o.	n.o.	480	59	-	-
Lign. 13	0.5	1	0.10	993	906	n.o.	n.o.	431	270	-	-
Lign. 14	0.5	3	3.02	993	445	n.o.	n.o.	431	48.5	-	-
Lign. 15	0.5	2	2.01	993	530	n.o.	n.o.	431	57.5	-	-
Lign. 16	0.5	2.5	2.52	993	520	n.o.	n.o.	431	55	-	-
Lign. 17	1.1	2	0.88	2270	1572	145	110	960	250	0.06	0.07
Lign. 18	0.65	1	0.76	1309	969	110	130	480	85	0.08	0.13
Lign. 19	0.5+UV	1	1.02	992	715	110	98	500	125	0.11	0.14
Lign. 20	0.5+UV	1	0.50	992	773	110	88	500	180	0.11	0.11
Lign. 21	0.5+UV	1	0.10	992	929	110	75	500	370	0.11	0.08
Lign. 22	0.5	3	3.63	992	302	110	132	500	17	0.11	0.44

Table 3. Dependence COD reduction and lignin content on the conditions of advanced oxidation processes (ozone dose, time, UV)

Symbol of sample and No of experiment	Time of experiment, h	Conditions of oxidation		Reduction of COD,%	Reduction of lignin, %
		Ozone dose, mgO_3/mgCOD	UV		
Lign. 13	1	0.10	–	8.8	41.3
Lign. 21	1	0.10	+	6.4	26.0
Lign. 9	1	0.20	–	12.1	47.9
Lign. 3	1	0.31	–	19.7	63.8
Lign. 10	1	0.50	–	22.8	75.8
Lign. 20	1	0.50	+	22.1	64.0
Lign. 18	1	0.76	–	26.0	82.3
Lign. 17	2	0.88	–	30.7	74.0
Lign. 4	3	0.93	–	36.7	84.0
Lign. 11	1	1.02	–	36.0	84.6
Lign. 19	1	1.02	+	27.9	75.0
Lign. 12	1.5	1.52	–	45.3	87.7
Lign. 6	3	1.82	–	46.8	90.5
Lign. 15	2	2.01	–	46.6	87.5
Lign. 16	2.5	2.52	–	47.6	88.0
Lign. 5	3	3.02	–	57.4	95.1
Lign. 14	3	3.02	–	55.1	89.5
Lign. 22	3	3.63	–	69.6	96.6
Lign. 7	3	3.71	+	67.0	96.4
Lign. 8	3	3.71	+ H_2O_2	68.2	95.7

Table 4. Toxicity of lignin solutions -test results (with the use of luminescent bacteria); (TU – Toxicity Unit) = 100/EC50.

Symbol of solution and No of sample	Ozone dose, mgO_2/mgChZT	Toxicity results EC50 % of sample		Index TU	Classfication of solution toxicity
		15 min	30 min	30 min	
Preparat. lignin 0.5 g/l	0	44.6	42.3	2.36	moderately toxic
Preparat. lignin 1.0 g/l	0	21.9	18.4	5.43	highly toxic
Lign. 3 (1.0 g/l)	0.31	25.2	25.4	3.94	toxic
Lign. 17 (1.1 g/l)	0.88	21.3	16.6	6.08	highly toxic
Lign. 4 (1.0 g/l)	0.93	18.6	16.3	6.13	highly toxic
Lign. 6 (1.0 g/l)	1.82	24.8	18.8	5.32	highly toxic
Lign. 16 (0.5 g/l)	2.52	47.2	41.5	2.41	moderately toxic
Lign. 5 (0.5 g/l)	3.02	64.3	51.5	1.94	moderately toxic

15705:2002 (ST-COD), using the method of tight reagent glasses.

Biochemical oxygen demand (BOD) was estimated according to the procedure described in standard PN-EN 1899-1:2002.

Toxicity of the model solutions was examined by means of the Lumistox 300 apparatus using the lyophilised luminescent bacteria *Vibrio fischeri* NRBL B-11177 according to the manual and standard PN-EN ISO 11348-3:2002.

Results and discussion

Conditions of the lignin oxidation experiments and the obtained results are presented in ***Table 2*** (see page 193) and ***Tables 3 - 5*** & ***Figures 2 - 6***.

Ozonolysis – effects of ozone oxidation

In ***Table 3*** and ***Figures 2*** & ***3***, the dependence of the reduction of COD and lignin content on the ozone dose is shown. The relationship is in accordance with expectations originating from data from the literature – the reduction of the lignin solutions' COD is a rising function of the ozone dose. Furthermore, it appears that a fairly fast decomposition of lignin proceeds under the action of ozone – 80% of the initial amount at an ozone dose of 1 mgO_3/mgCOD. Compounds formed as a result of lignin decomposition are further degraded to CO_2 and H_2O after a higher dose has been supplied (***Figure 2***). The maximum reduction close to 70% of organic matter (as COD) was attained with the ozone dose of 3.63 mgO_3/mgChZT (trial 22). In that experiment, the maximal reduction of the content of non-degraded lignin reached 3.4% of the initial amount.

The high lignin degradation degree in trials 5 and 22 caused an increase of the biodegradation coefficient BZT_5/ChZT.

Other oxidizing agents (AOP)

UV irradiation at a constant ozone dose causes a certain stabilisation of the lignin structure, which is reflected by the degradation rate decreasing. The effect is more pronounced at low doses of ozone (low degradation degree). This can be seen by comparing the results of the experiments (***Table 3***):

- Lign. 13 and Lign. 21
- Lign. 10 and Lign. 20
- Lign. 11 and Lign. 19

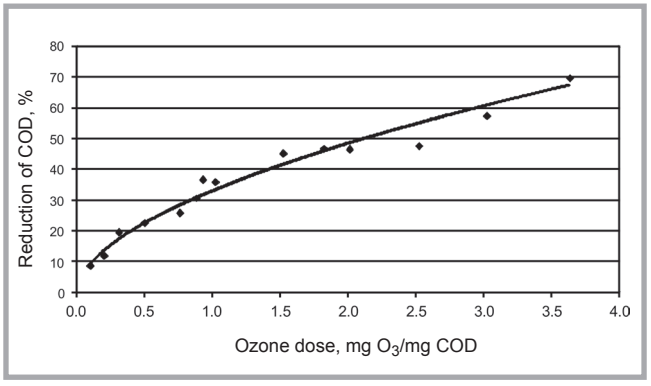

Figure 2. *Dependence of COD reduction on lignin solutions on ozone dose.*

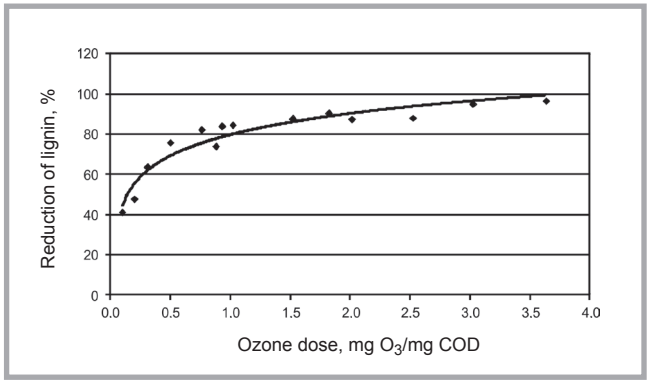

Figure 3. *Dependence of lignin reduction on ozone dose.*

The effect of UV irradiation and H_2O_2 action on COD and lignin reduction was not as distinct in trials 7, 8 and 22, probably on account of the prevailing impact of high ozone dose. Still, the highest reduction occurred in trial No. 22 without UV irradiation. The biodegradation coefficient BOD_5/COD was distinctly increased in all three of the trials (7, 8 and 22).

Toxicity

Lignin solutions with concentration of 1 g/l are highly toxic, and at 0.5 g/l are moderately toxic, against the luminescence bacteria *Vibrio fisheri* according to the proposed classification [28, 29].

The impact of ozonisation upon the toxicity of solutions containing lignin degradation products is not unequivocal. This calls for further in-depth investigations with the use of various methods and test organisms. Thanks to the results obtained, the lignin degradation products may be regarded as equally or even more toxic than the starting lignin (***Table 4***). However, the results indicate that the dependence of toxicity on the ozone dose arrives at a maximum after which a decrease of the toxicity proceeds at an adequately high ozone dose (high lignin decomposition degree); for example higher than 1.8 mgO_3/mgCOD.

Biodegradation

The results of the biodegradation tests of the lignin solutions prior to and after the ozonisation are presented in ***Figures 4 - 6***. The course of the tests indicates that both lignin and its degradation products are compounds that are not susceptible to microbial degradation at low ozone doses. A temporary limitation of the COD values seen in some of the tests (experiments 17 and 18 – ***Figure 5***) is an effect of a transitional retention on the surface of the active sludge flocs, and flotation, along with the formed foam.

There were no signs of intoxication of the active sludge during the biodegradation tests of the lignin solutions, nor any cell lysis, which would manifest itself by a turbidity of the fluid beyond the sediment. Also the COD did not rise above the initial value.

In the trials with high doses of ozone (7, 8 and 22 - ***Figure 6***), a distinct improvement of the biodegradation occurred. However, the lignin decomposition products cannot be deemed easily biodegradable as the reduction in COD after 8 days was below 50%.

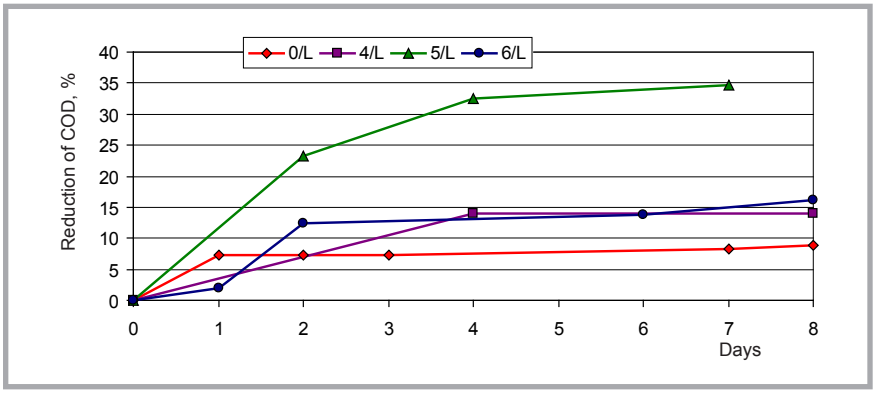

Figure 4. *Graph of biodegradation of model lignin solutions before and after ozonisation (experiments No 4, 5 and 6).*

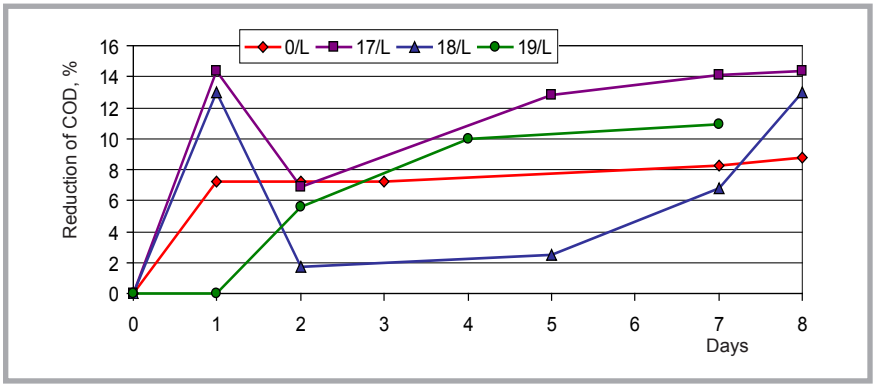

Figure 5. *Graph of biodegradation of model lignin solutions before and after ozonization (experiments No 17, 18 and 19).*

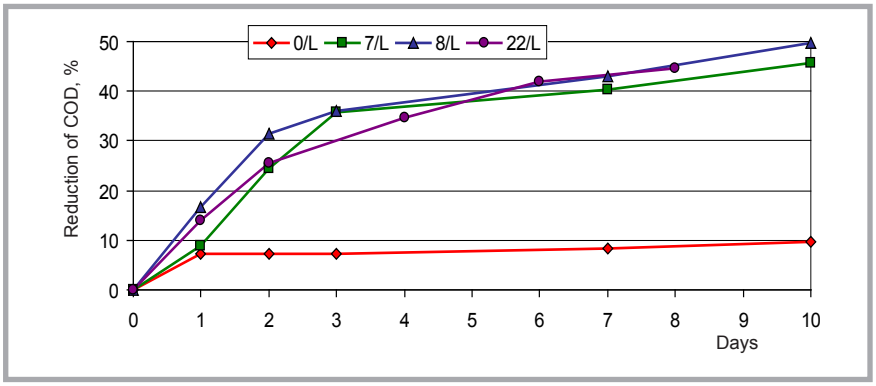

Figure 6. *Graph of biodegradation of model lignin solutions before and after ozonisation (experiments 7, 8 and 22).*

The ultimate effects of the biodegradation testing in dependence on ozone dose in the singular experiments are presented in ***Table 5*** (see page 196).

Summary

Ozonisation of lignin causes the nearly complete degradation and total decomposition of a substantial part of the organic matter, which is manifested by a distinct decrease of COD (70% at 3.6 mg O_3/mg COD of ozone dose). Disappearance of the dark colour of the model lignin solutions is a spectacular effect of the ozonisation.

Lignin proved to be insusceptible to biodegradation under the test conditions. The same conclusion also arises from the low biodegradability coefficient BOD/COD in the range of 0.06 - 0.11. Ozone-induced lignin decomposition products do not manifest improvement of the biodegradability at low ozone doses, while

Table 5. Results of biodegradation testing of model lignin solutions (test time: 8 - 10 days).

Symbol of solution and No of sample	Oxidation conditions		Reduction of COD as a result of biodegradation, %
	Ozone dose	UV	
Starting solution of lignin preparation	0	-	9.7
Lign. 21	0.10	+	8.0
Lign. 20	0.50	+	8.3
Lign. 18	0.76	-	13.0
Lign. 17	0.88	-	14.4
Lign. 4	0.93	-	14.0
Lign. 19	1.02	+	10.9
Lign. 6	1.82	-	16.1
Lign. 5	3.02	-	34.8
Lign. 22	3.63	-	44.6
Lign. 7	3.71	+	45.7
Lign. 8	3.71	UV + H_2O_2	49.7

at high doses (e.g. 3.6 mgO_3/mgCOD) they were more prone to biodegradation.

The use of UV irradiation along with ozone produces a rather negative effect by slowing the process of lignin decomposition down to a certain degree.

The use of ozone in the effluent treatment allows the dark colour and a substantial part of the organic matter originating from lignin to be removed. This is much more favourable since lignin does not undergo a biological decomposition under the conditions and time applied routinely in classical wastewater aerobic treatment plants with active sludge. A primary purification step in the treatment of effluents contaminated with lignin applied prior to the secondary biological treatment process allows the cumulated efficiency of the removal of organic substances to be improved.

Acknowledgments

The study was funded by the Polish Committee for Scientific Research, Grant No. 4 T09B 008 24 and the Polish Ministry of Science and Higher Education

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Received 19.10.2012 Reviewed 12.11.2012