FOLIA FORESTALIA POLONICA

© PAN Series B, Issue 41, 69-79, 2010

EFFECT OF THE ADDITION OF PESTICIDE ON THE BIODEGRADATION RATE BY MICROFUNGI OF PAPER ARTICLES SUPPLEMENTED WITH CEREAL BRAN AND THEIR BREAKING STRENGTH*

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SYNOPSIS. The article compares rates of biodegradation of paper products with the addition of wheat and rye bran as well as with and without a fungicide (4,5-dichloro--2-octyl-2H-isothiazol-3-one, 3-iodoprop-2-ynyl-N-butylcarbamate). An axenic culture of *Chaetomium globusum* Kunze et Fr. and a mixture of microfungi (with *Aspergillus niger* van Tieghem, *Trichoderma viride* Persoon ex S.F. Gray aggr., *Penicillium funiculosum* Thom) were employed as a biotic agent. The resistance to the colonisation of sample surfaces by the mycelium of the test fungi was adopted as a biodegradation criterion and breaking strength after the mycological test was determined in accordance with the standard: PN-P-50405:1995 (IGT methods). Recycled papers supplemented with 3% wheat bran and with 5% rye bran exhibited the lowest resistance to the development of mycelium on the sample surface and break resistance. Paper products supplemented with 5% wheat bran were resistant to the biodegradation by all test fungi. The addition of 0.125% fungicide to the examined paper products resulted in the complete resistance against test microfungi. Nearly in all examined cases, the breaking strength was higher in the case of samples which contained wheat bran and 0.125% biocide (the difference amounted, on average, to about 7 N in favour of 0.125% concentration).

KEY WORDS: paper test sheets, paper stock, rye bran, wheat bran, microfungi, break resistance

INTRODUCTION

Investigations have been carried out in recent years at the Institute of Chemical Wood Technology (Faculty of Wood Technology, Poznań University of Life Sciences) on the possibilities of application of cereal bran for the production of paper articles from recycled waste papers. The experiments employ by-products from

*The above research project was financed by the Ministry of Science and Higher Education; No. of Research Project: N309 008 31/1223.

the milling industry (rye and wheat bran) used as filling agents for the production, among others, of trays and containers for egg, vegetable and fruits, as well as paper pots for plants (Cofta et al. 2006, Modzelewska 2006).

Cereal bran is no longer used as an additive material during the production of feeds for farm animals because it does not contain any nutrients. Therefore, a problem arose what to do with considerable quantities of this by-product. The quantities of the Dr Cordesmeyer – Sp. z o.o., Gdańskie Młyny i Spichlerze (the Gdańsk mill) alone reach up to 150 tons a day. At the moment, there are two ways in which these wastes can be utilized: either as eco-fuel or as an additive in the production of chipboards. There is, however, another possibility of application of cereal bran, namely as a filling material in the production process of paper products.

Rye and wheat bran

Bran can be described as a by-product obtained during the process of grain milling into flour. It is, by no means, a standardised product of specific chemical composition (Table 1) and texture, and constitutes a mixture of various products of milling of the peripheral parts of the kernel containing smaller or grater admixture of endosperm of various degree of comminution (Gąsiorowski 1994, 2004).

Table 1. Chemical composition of wheat bran and rye bran

Name of compound	Percentage content	
	wheat bran [%]	rye bran [%]
Cellulose by Seifert method	12.70	10.47
Holocellulose according to standard PN 75/50092	43.13	20.80
Tappi lignin	10.40	9.33
Pentosans	24.07	15.27
Substances soluble in hot water	25.84	44.54
Substances soluble in cold water	25.06	26.60
Furfural	12.02	8.82
Mineral substances (ash)	3.67	3.52
Absolute moisture content of raw material = 9 (±2%)		

Having marked the percentage share of particular fractions of mill waste, it has been decided to use cereal bran, with less than 0.4 mm fraction, for manufacturing paper products that undergo determination (Table 2).

The following paper is a report from a consecutive stage of investigations on the application of cereal bran for the production of selected paper articles. The objectives were realised by setting up two basic aims of the research. Firstly, self-breakage of the obtained paper products was determined, in order to investigate the effect of the addition of different proportions of cereal bran used as fillers, as well as added fungicide on the strength properties of these articles. The application of waste paper in production of paper products is associated with the utilization of waste raw materials (Przybysz et al. 2001, Raczyńska 2003). The same

Table 2. Percentage proportion of individual fractions

Fraction size [mm]	Wheat bran [%]	Rye bran [%]
Over 2.5	0.31	0.52
Over 1	10.91	7.93
Over 0.5	25.81	23.89
Over 0.4	62.97	67.66

is true about attempts at the utilization of cereal bran as fillers. Not only do we utilize wastes, but in addition, we reduce production costs. That is why the second important objective, of the performed investigations, was to determine the impact of the pesticide, contained in the laboratory paper sheets, on the degradation which is caused by microfungi. Sensitivity to fungal attack is particularly important in the case of containers intended for contact with food. The above-mentioned objective was realised by determining the degree of sample resistance to colonization by microfungi (COFTA et AL. 2006).

MATERIAL AND METHODS

The experiments were conducted on small sheets of paper of 200 g/m^2 grammage manufactured in laboratory conditions with the assistance of a Rapid-Köthen apparatus (MODRZEJEWSKI et AL. 1985). The composition of the paper stock was as follows: waste paper, water, starch, aluminium sulphate, fillers (rye and wheat bran) and fungicides. The small paper sheets were manufactured either without or with 3% or 5% content of the experimental filler (rye or wheat bran; Table 4). Simultaneously, small paper sheets containing 0.25% and 0.125% fungicides (4,5-dichloro-2-octyl-2H-isothiazol-3-one, 3-iodoprop-2-ynyl-N-butylcarbamate mixture) were also prepared.

The manufactured paper sheets and investigations of their resistance to biodegradation were carried out in accordance of the methodology eleaborated by COFTA et AL. (2006).

Samples (24 of each type) measuring 15 mm × 95 mm were cut out from each of the prepared paper series. At the same time, control samples of identical dimensions were prepared from No. 3 Whatman blotting paper and subjected to sterilisation to eliminate possibilities of infection.

Next, samples of experimental paper and blotting paper were placed in sterile conditions on the earlier prepared and sterilised Petri dishes filled with the agar substrate and infected with the aid of the aqueous solution of spores of the test fungi. In order to obtain commensurable results, we used microorganisms causing the greatest material contamination and frequently isolated, for example, from library collections and warehouses of paper products. The employed fungi included: *Chaetomium globusom* Kunze et Fr. as well as the mixture of *Aspergillus niger* van Tieghem, *Penicillium funiculosum* Thom, *Trichoderma viride* Persoon ex S.F. Gray aggr. fungi. The Petri dishes were placed in a thermostat maintaining con-

stant temperature of 28°C and air humidity of 95%. The investigations for the first series lasted 8 weeks, for the second series – 4 weeks and the remaining series were stored in the thermostat for the period of 3 weeks. Readings of the degree of colonisation of the samples according to a 5-point scale (Table 3) were performed regularly on day: 4, 7, 10, 14 and 21 of the experiment.

Table 3. Scale used for the evaluation of the sensitivity of experimental samples to fungal infestation

Index	Degree of sample colonization
3	no sign of mycelium growth on sample, there is a zone of inhibition on the medium between the sample and mycelium
2	no sign of mycelium growth on sample, there is no zone of inhibition on the medium between the sample and mycelium
1	less than 1/3 of the sample surface colonized by the test fungus mycelium
0	more than 1/3 of the sample surface colonized by the test fungus mycelium
−1	surface of the examined sample colonized more intensively than the surface of the control sample

The breaking strength of samples with waste paper supplemented with different proportions of bran and biocide, as well as those without fillers was determined in accordance with the PN-P-50405:1995 standard. In addition, this examination also allowed assessing the degree of degradation of the examined paper samples and evaluating values of their break resistance.

Prior to placing the samples in the grips of the breaking machine, the papers were taken away from Petri dishes, cleaned and dried. The series of samples without fillers were removed from the thermostat after the period of 8 weeks, the series with the addition of 3% rye bran – after the period of 4 weeks and the remaining samples were taken out 3 weeks after sample infection. The series of samples which remained in the thermostat longest were the most difficult to recover as they broke easily and were difficult to remove in one piece. Samples with the addition of the fungicide were not colonized by the test mycelium which facilitated their removal and the cleaning process. The paper articles which were manufactured using waste paper were then subjected to drying. The series without fillers as well as with the addition of 3% and 5% rye bran were dried for the period of 7 days, while the remaining samples – for the period of 10 days. The relative moisture content of the examined articles prior to the determination of their breaking strength amounted to 7 ±1%.

RESULTS

Fungicide investigations should be conducted employing microorganisms which are specific for a given product which is to be protected. Furthermore, in order to

obtain practicable results, it is essential to carry out investigations which cause the greatest damage of a given material.

It is evident from laboratory practice that it is advisable to work with microorganisms characterised by different sensitivity to a given fungicide and which do not exhibit significant deviations when experiments are repeated. That is why we decided to use *Ch. globusom* which develops very well on pure cellulose and is frequently isolated in different libraries (Zyska 1997, 2000). On the 7th day of the performed experiment, the applied *Ch. globusom* mycelium colonised almost the entire surface of samples prepared from the waste paper stock. This showed that paper sheets manufactured from waste paper stock without any addition of fungicide undergo biodegradation. Earlier investigations revealed that the addition of cereal bran to paper stock increased even more the susceptibility of such material to degradation by *Ch. globusom* (Cofta et al. 2006). The observed influence of cereal additives can be attributed to considerable quantities of nutrients used by microorganisms which colonise paper materials (Perkowski et al. 2008). This hypothesis was confirmed partially by the presented investigations.

However, paper sheets manufactured in the laboratory from waste paper stock supplemented with 5% wheat bran were totally resistant to the development of the *Ch. globusom* mycelium after 7 days of the mycological test and after 3 weeks, the samples were only slightly colonised by the test microfungus. This was probably caused by synergism between substances inhibiting the growth of microorganism found in the waste paper stock (printing paint, fillers etc.) used to manufacture the experimental samples and compounds present in the cereal bran.

Due to considerable sensitivity to the attack of different microorganisms present in cereal bran, paper articles manufactured with their participation may contain various mycotoxins. In our climatic conditions, cereals are frequently infected by *Fusarium* spp. (Chełkowski et al. 2001) which is a well known species capable of manufacturing large quantities of mycotoxins which may inhibit the growth of other microorganisms (Bottalico 1998).

Therefore it would be necessary to examine what kinds of compounds are found in the wheat bran employed in the studies, as well as in the waste paper stock used to manufacture laboratory paper sheets. This should explain the obtained result.

The research results obtained for the treatments without the addition of fungicides and differing with regard to the applied biotic factors differ for the mixture of microfungi and for the *Ch. globusom*. The only exception is the test in which the samples were produced from the waste paper stock and 3% addition of wheat bran. The obtained results indicate a very high sensitivity to the biodegradation both by the mixture of *A. niger*, *T. viride*, *P. funiculosum* as well as by the axenic culture of the *Ch. globusom* fungus (colonization index – 0). Differences occurred in the biological activity of the fungal mixture and the *Ch. globusom* fungus for the remaining test options. On the fourth day of the test, *A. niger*, *T. viride*, *P. funiculosum* microfungi obtained comparable colonization indices which means that they colonized the sample surface with a similar rate. However, during the consecutive days of the experiment, samples were colonized more readily by the *Ch. globusom* fungus in comparison with the mixture of test microfungi (Fig. 1).

Table 4. Mean values of resistance of papers to the colonization with tested microfungi (*Ch. globusom* and mixture *A. niger*, *T. viride*, *P. funiculosum*)

Types of the examined paper		4 d		7 d		10 d		14 d		21 d	
Contents of addition in waste paper	Acronym	Ch*	M**	Ch	M	Ch	M	Ch	M	Ch	M
–	A	1.85	1.75	0.25	1.62	0	1.62	0	1.5	0	1.37
Rye bran 3%	B	2.75	2.37	1	2.37	0	2.37	0	2.37	0	2.37
Rye bran 5%	C	0.12	2.25	0	2.25	0	1.87	0	1.75	0	1.75
Wheat bran 3%	D	0	0.12	0	0	0	0	0	0	0	0
Wheat bran 5%	E	3	3	3	2.87	2.12	2.75	1.5	2.75	1.5	2.75
Fungicide 0.125%	F	3	3	3	3	3	3	3	3	3	3
Fungicide 0.25%	G	3	3	3	3	3	3	3	3	3	3
Fungicide 0.125% + rye bran 3%	H	3	3	3	3	3	3	3	3	3	3
Fungicide 0.125% + rye bran 5%	I	3	3	3	3	3	3	3	3	3	3
Fungicide 0.125% + wheat bran 3%	J	3	3	3	3	3	3	3	3	3	3
Fungicide 0.125% + wheat bran 5%	K	3	3	3	3	3	3	3	3	3	3
Fungicide 0.25% + rye bran 3%	L	3	3	3	3	3	3	3	3	3	3
Fungicide 0.25% + rye bran 5%	M	3	3	3	3	3	3	3	3	3	3
Fungicide 0.25% + wheat bran 3%	N	3	3	3	3	3	3	3	3	3	3
Fungicide 0.25% + wheat bran 5%	O	3	3	3	3	3	3	3	3	3	3

Ch* – *Ch. globusom*.
M** – mixture (*A. niger*, *T. viride*, *P. funiculosum*).
d – day.

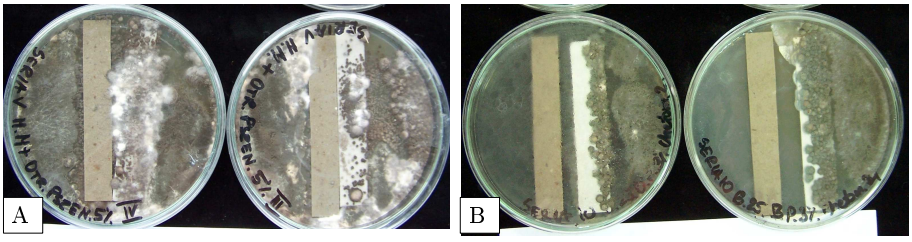

Fig. 1. Examples of photographs showing different levels of colonization of samples 21 days after infection. A – test fungus *Ch. globusom*, series 5 (with the addition of filler – 5% wheat bran), B – series 10 (with the addition of 0.125% biocide and filler – 3% wheat bran)

Additional investigations were also conducted with the aim of elucidating whether fungicide supplementation caused any changes in the rate with which sample surfaces were colonized by test fungi. Table 1 presents the diversity of options together with the obtained results. It turned out that the treatment in which the paper articles were manufactured from waste paper supplemented with 3% wheat bran and 0.125% fungicide was the most interesting. The obtained results corroborated a very high effectiveness of the applied fungicide (Table 4). Both in this treatment, as well as in the remaining ones, the obtained colonization indices amounted to 3. This indicates that it is necessary to carry out more mycological tests reducing the fungicide concentration. This will ensure reduction in the content of chemical substances affecting natural environment of these paper products were to be applied commercially.

After the mycological test, the degradation rate of the examined paper articles was assessed by determining their breaking strength. Table 5 presents mean values of the obtained results on breaking strength.

Table 5. Break resistance of paper sheets manufactured from waste paper stock without additives and with the addition of fillers and biocides expressed in N – mean values from 10 measurements

Type of the examined paper sample	Type of the examined sample		
	type of the examined control sample	*Ch. globusom*	*A. niger, T. viride, P. funiculosum*
A	37.82	1.592	1.347
B	37.509	0.735	3.858
C	37.766	0.612	6.002
D	72.899	35.782	26.447
E	52.631	42.391	40.155
F	55.272	44.479	36.799
G	46.868	43.348	40.817
H	84.182	65.929	49.563
I	85.877	69.482	53.679
J	89.057	75.680	52.405
K	96.481	70.823	64.851
L	69.163	49.000	44.198
M	91.115	67.522	55.786
N	77.126	66.272	53.165
O	89.290	71.975	55.909

The worst break resistance was determined in samples manufactured without fillers, as well as those with the addition of 3% and 5% rye bran which, in turn, were characterised by exceptional brittleness and fragility. All the samples were strongly twisted and difficult to place in the grips of the breaking apparatus. Samples without the addition of fungicide were far less brittle and deformed which was probably the reason why the obtained values were higher.

As it was mentioned in the Methodology, all laboratory paper sheets were stored in identical conditions, The sample series without fillers was removed from the thermostat after the period of 8 weeks, the series with the addition of 3% rye bran – after 4 weeks and the remaining samples – after 3 weeks from infestation.

The samples which remained in the thermostat the longest were the most difficult to recover, were easily broken or could not be taken out in one piece. Samples supplemented with biocide were not colonised by the test mycelium which became apparent when they were removed from the thermostat and cleaned. Sheet samples manufactured with the addition of cereal bran and biocide turned out to be characterised by the highest break resistance.

Sample series manufactured with the addition of 0.125% and 0.25% biocide and 3% and 5% content of wheat bran turned out to be particularly resistant (Table 5, Fig. 2). In the case of samples filled with wheat bran and biocide, the values were as follows: 3% filler and 0.125% biocide, for the sample colonised by *Ch. globusom* estimates 75.68 N and colonised by fungal mixture estimates 53.2 N; 5% filler and 0.125% biocide for the sample infected with *Ch. globusom* estimates 70.8 N and colonized by a mixture of fungi estimates 64.85 N; 5% filler and 0.25% biocide for the sample infested with *Ch. globusom* estimates 72 N and colonized by a mixture of fungi estimates 55.9 N.

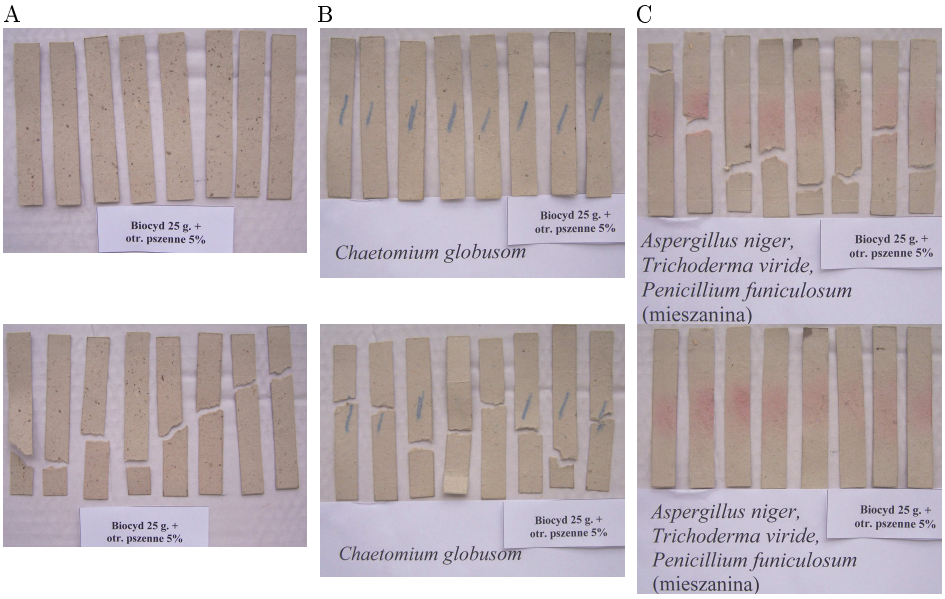

Fig. 2. Photographs showing examples of paper samples before and after determination of the break resistance (series with the addition of 25 g of biocide = 0.125% + 5% wheat bran): A – samples not infected by micro-mycelium (biocide 0.25%, 5% wheat bran), B – samples colonized by *Ch. globusom* micro-mycelium (biocide 0.25%, 5% wheat bran), C – samples colonized by the mixture of microfungi (biocide 0.25%, 5% wheat bran)

At the identical content of the applied filler, significantly higher values of self-breakage were observed in paper samples with a lower percentage content of the applied fungicide, i.e. 0.125%. In addition, the examined articles colonized by a mixture of microfungi were characterised by poorer break resistance. This tendency was also observed in the case of papers filled with rye bran and biocide (Table 5).

For samples filled with rye bran and biocide, these values were as follows: 3% filler and 0.125% biocide for the sample colonized by *Ch. globusom* estimates 66 N and for that infected by the mixture of fungi estimates 49.6 N; 3% filler and 0.25% biocide for the sample colonized by *Ch. globusom* estimates 49 N and for that infected by the mixture of fungi estimates 44.2 N; 5% filler and 0.125% biocide for the sample colonized by *Ch. globusom* estimates 69.5 N and for that infected by the mixture of fungi estimates 53.7 N; 5% filler and 0.25% biocide for the sample colonized by *Ch. globusom* estimates 67.5 N and for that infected by the mixture of fungi estimates 55.8 N.

Recapitulating this phase of investigations, it should be said that the inclusion of 0.125% fungicide sufficiently protected the examined paper articles against mycological infestation and, at the same time, did not reduce significantly the breaking strength of the examined samples. The performed assays confirmed current presumptions that the application of cereal bran as fillers for the production of containers intended for contact with food articles, makes it necessary to use a biocide (Fornalski and Godlewska 2007). Future investigations will show whether it is possible to reduce biocide concentration from 0.125% to 0.06%.

The comparison of the obtained results for break resistance of the samples of paper articles colonized by micro-mycelium (both *Ch. globusom* and fungal mixture) with the control samples, makes it possible to conclude that the difference in the breaking strength remained, on average, at the level of 10 N.

The addition of the experimental biocide, irrespective of the applied concentration, contributed to the improvement of the break resistance of the papers subjected to the mycological test.

The applied Whatman No. 3 blotting paper used a control sample to determine fungal growth was characterised by low breaking strength ranging from 25-39 N.

CONCLUSIONS

1. All the examined paper products without fungicides were colonized by the test fungi with the exception of the paper supplemented with the addition of 5% wheat bran which was only slightly colonized by *Ch. globusom* fungi as well as by the mixture of *A. niger*, *P. funiculosum* and *T. viride* fungi.
2. The addition of 3% wheat and 3% and 5% rye bran to the paper stock as fillers resulted in the increase of their susceptibility to colonization by test mycelia in comparison with samples which did not contain cereal bran.
3. Paper samples without additives or supplemented with 3% wheat bran and 3% or 5% rye bran were more susceptible to the infection by *Ch. globusom* fungi than by the mixture of *A. niger*, *P. funiculosum* and *T. viride* fungi.
4. The addition to the paper stock of 0.125% and 0.25% concentrations of experimental biocides caused that all the series of the manufactured papers exhibited a very good resistance to the colonization by the test mycelium.

5. The biocides and wheat bran used in the experiments increased the breaking strength of samples in comparison with the series which did not contain the above-mentioned fillers.
6. Experimental paper sheets containing biocides, in comparison with unprotected samples, exhibited almost 100% greater resistance to breaking. The difference between samples containing 0.125% and 0.25% biocides was negligible (on average, about 7 N in favour of the 0.125% concentration).
7. The addition of biocide, irrespective of the applied concentration, improved the breaking strength of papers subjected to the mycological test.
8. The break resistance of paper samples colonized by experimental mycelia (both *Ch. globusom* as well as fungal mixture) in comparison with the control samples was, on average, by about 10 N lower.
9. At identical contents of the applied filler, significantly higher breaking strength values were recorded in the case of paper samples with lower percentage content of the fungicide, i.e. 0.125%.

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Received in January 2009

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