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Influence of pH and Alkaline Reserve of Paper on the Growth of some Filamentous Fungi

by M.S. RAKOTONIRAINY, C. HERAUD & B. LAVEDRINE

INTRODUCTION

Artefacts made of cellulose provide a good substrate for the growth of micro-organisms such as filamentous fungi. Some species are very destructive, for example, *Aspergillus* and *Penicillium species*. Machine-made paper of delignified cellulose is a highly vulnerable material. Water content of the substrate is clearly the major factor influencing the growth and metabolic activities of filamentous fungi¹; other parameters that can govern the rate of fungal deterioration include levels of oxygen, temperature, pH or composition of the substrate, etc.

The influence of pH is not clear. It is admitted by several authors that an acidic pH generally stimulates fungal growth^{2, 3, 4}. Other authors assert that whilst the pH of the substrate can greatly influence the formation of mycotoxins or the germination of conidia, it has no marked influence on the growth of some other species^{5, 6, 7}. Research to date has not made it clear whether the alkalinity or acidity of paper can play a role in the development of mould. This has given rise to the question whether deacidification,^{8, 9, 10} which is an effective method for inhibiting paper degradation¹¹ during the ageing process, can affect fungal development.

This work describes experiments carried out in order to answer this question. Different qualities of paper, some of them having being deacidified, were submitted to fungal growths and the development of the moulds was compared.

PREPARATION OF THE SAMPLES

Five different qualities of paper were selected in order to have samples which were neutral, acidic and alkaline (Table 1). Some of them were immersed in aqueous solutions or exposed to an acidic environment in order to modify the pH value and to make the paper acidic or alkaline (Table 2). Another batch was deacidified using CSC Book Saver Spray^{® 12}. The samples were sprayed on one side and then air dried.

Table 1: The tested paper.

Sample	Code	Fibre	Sizing	Filler	Optical brightener
1	linters	cotton linters,			
2	newsprint	softwood chemical pulp	alum rosin		
		mechanical pulp			
3	alkaline copy	softwood chemical pulp			
		softwood and hardwood		calcium	yes
		chemical pulp		carbonate	
4	acidic copy	softwood and hardwood	alum rosin		
		chemical pulp			
5	alkaline copy	softwood and hardwood		calcium	yes
		chemical pulp		carbonate	

Table 2: The treatments.

Acidification		Alkalinization
bath	pollution	bath
acetic acid 10% 5 min	50 ppm NO ₂ at 23°C and 50% RH 5 days	semi-saturated solution of Ca(OH) ₂ 5 min

Table 3: Alkaline reserve and pH of the samples before and after treatments.

Sample	Alkaline reserve (%)				pH			
	untreated	acidic bath	alkaline bath	pollution	untreated	acidic bath	alkaline bath	pollution
1	0.00	0.15	0.15	0.00	6.1	6.7	9.3	3.8
2	0.00	0.04	0.18	0.00	5.8	5.9	7.3	3.6
3	6.78	0.05	6.83	5.23	9.7	6.4	8.6	7.1
4	0.25	0.00	0.10	0.00	6.2	6.1	6.9	4.0
5	9.83	0.11	10.00	7.98	9.3	6.5	9.3	7.1

Table 4: Alkaline reserve and pH of deacidified papers.

Sample	Alkaline reserve (%)			pH		
	untreated	acidic bath	pollution	untreated	acidic bath	pollution
1		7.60	7.40		10.54	10.56
2	6.10	7.60	9.70	10.34	10.47	10.41
3		7.90	10.00		10.63	10.63
4		7.90	9.00		10.48	10.45
5		10.00	9.90		10.57	10.58

Fig.1: Scheme of biological tests

Alkaline reserve and cold extract pH measurements of untreated, treated and deacidified papers were performed according the usual standards ISO/CD 10716 and ISO/DIS 6588 respectively. Alkaline reserves of the papers treated with the CSC Book Saver Spray[®] (based on MgCO_3), were calculated into their calcium carbonate equivalent. The results are given in Tables 3 and 4. The numbers are the average of two measurements.

BIOLOGICAL TEST

Biological tests on the all paper samples (untreated, acidified, alkalized) were carried out using the following eight fungal strains from our mycological collection: *Ulocladium spp.*, *Aspergillus penicilloides*, *Aureobasidium pullulans*, *Eurotium herbariorum*, *Fusarium solani*, *Penicillium brevicompactum*, *Stachybotrys atra* and *Trichoderma viride*.

They were used as a suspension of spores adjusted to 1.10^6 spores per mL. Spores were harvested from a pure culture on malt-agar plates and mixed with deionised water. A microdrop of Tween 80* was added to the suspension in order to prevent spores aggregation. The number of spores was counted using the Malassez cell**. The concentration was adjusted if necessary. Then, for a given strain, each sample of paper (4 x 4 cm), placed on an agar medium in a Petri dish, was inoculated with 9 equidistant spots of 5 µL of a suspension (Fig. 1). Experiments were repeated twice for each strain. After an incubation period of 21 days at 26°C, the behaviour of the different strains was estimated by the growth level. We

* A polymer of sorbic acid, used in microbiology to reduce the agglutination of spores.

** An instrument used in medicine and microbiology to count, e.g., blood corpuscles.

considered 5 levels: 0: no growth; 1: development of mycelial filament; 2: beginning of the sporulation; 3: good sporulation; 4: maximum of sporulation.

RESULTS

Type of paper, charges and fillers did not influence the development of the tested strains (Table 5). Growth depended only on the species. Our results showed a relatively poor growth activity of *E. herbariorum* and *F. solani* on the different papers, while the growth of *A. pullulans* and *A. penicilloides* was very high. Moreover, no general change was observed after the different treatments had been applied to the papers. This means that the pH and the reserve alkaline had no significant effect on fungal growth. For three strains (*P. brevicompactum*, *F. solani*, *E. herbariorum*), growth appeared to be slightly better on polluted papers. On deacidified papers, the complete inhibition of *A. pullulans*, *E. herbariorum* and *T. viride* was observed. For the other strains, growth levels were unchanged or decreased slowly.

Statistical analysis

To analyse the possible interaction between the several parameters (acidity, alkalinity, types of paper, fungal growth level), results were treated with Principal Component Analysis (PCA) using SPAD version 4.5 software for Windows^{13, 14, 15}. PCA is a multivariate procedure, which rotates the initial data in a multidimensional space, so that maximum variability is projected onto the axes. PCA correlation circles were established. To carry out the analysis, we retained the first two factorial axes. For paper which had not been deacidified, the axis F1 and F2 describe 50.34% and 26.46% respectively of the variability. This can account for 76.80% of the initial variability of the data. For deacidified papers, values are 46.97% and 26.71% respectively, i.e. 73.68% of the total inertia. The interpretation of the circles takes into account the relative positions of the variables. The closer the variables on the circle, the more they are correlated. To read the circle, it is necessary to look at the angle between each pair of variables. The reading of the circle can be summarized in following way:

- acute angle: high positive correlation. For a given individual, the two variables are close and move in the same direction;
- approximate right angle (near 90°): low correlation between the two variables;
- obtuse angle: high negative correlation. The two variables move in opposite directions.

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Table 5: Growth levels of the tested strains on the different untreated, treated and deacidified papers after 21 days of incubation. The numbers indicate the growth intensity: 0: no growth; 1: development of mycelial filament; 2: beginning of the sporulation; 3: good sporulation; 4: maximum of sporulation.

	Sample	not deacidified				deacidified		
		un- treated	acidic bath	pollution	alkaline bath	un- treated	acidic bath	pollution
<i>Stachybotrys atra</i>	1	2	4	3	3		4	4
	2	4	4	4	0	4	4	4
	3	4	3	4	3		4	4
	4	2	4	2	4		4	4
	5	4	4	4	4		4	4
<i>Aureobasidium pullulans</i>	1	4	4	4	4		0	0
	2	4	4	4	4	0	0	2
	3	4	4	4	2		0	0
	4	4	4	4	4		0	0
	5	4	4	1	4		0	0
<i>Eurotium herbariorum</i>	1	0	1	0	1		0	0
	2	1	1	4	1	0	0	0
	3	0	1	1	1		0	0
	4	1	1	4	0		0	0
	5	1	2	0	1		0	0
<i>Trichoderma viride</i>	1	2	4	2	2		0	0
	2	2	4	4	3	0	0	0
	3	2	3	2	3		0	0
	4	3	3	4	2		0	0
	5	2	4	2	2		0	0
<i>Penicillium brevicompactum</i>	1	1	1	3	1		2	4
	2	2	2	4	3	3	2	4
	3	2	2	3	2		2	2
	4	3	3	4	3		1	4
	5	2	3	4	2		2	3
<i>Aspergillus penicilloides</i>	1	4	4	4	4		2	3
	2	4	4	4	4	3	3	4
	3	4	4	4	4		3	3
	4	4	4	4	4		2	4
	5	4	4	4	4		3	4
<i>Fusarium solani</i>	1	1	1	1	1		1	2
	2	1	1	4	1	1	1	3
	3	1	1	2	1		1	1
	4	1	1	4	1		1	3
	5	1	1	4	1		2	3
<i>Ulocladium spp.</i>	1	3	3	3	3		2	4
	2	4	3	4	3	4	4	4
	3	3	3	4	3		2	2
	4	3	4	4	4		4	4
	5	3	3	4	4		4	4

Fig. 2: PCA correlation circles. 1: pH; 2: alkaline reserve; 3: type of paper; 4: fungal growth.

The length of a variable (distance of the arrow extremity from the centre of the circle) is an indication of how well the coupled variable is approximated and how much it contributes to the analysis. When the variables are close to the centre, it means that some information is carried out on other axes and that any interpretation might be hazardous. So, it is necessary to look at the correlation circle on the other axes.

Fig. 2 presents the PCA correlation circles when papers are both deacidified and not deacidified. The different variables are close to the circumference of the circle, which means that they are well represented. The two correlation circles are very similar. It seems that deacidification of papers does not change the relationship between the growth and the others parameters. Along F1 we find the variables “pH”, “alkaline reserve” and “type of paper”. These three parameters appear very close to each other and are significantly positively correlated. The variable “growth” is along the F2 axis and had no correlation with pH, alkaline reserve and the type of paper.

CONCLUSION

This experiment showed that, with the papers and tested species, the alkaline reserve, the pH and the type of paper does not influence fungal growth. Nevertheless, some deacidification treatment such as Book Saver Spray® may influence growth depending on the strain. Growth of *A. pullulans*, *E. herbariorum* and *T. viride* were inhibited. For the others strains, we observed little or no effect. The inhibit-

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ing effect observed may be due to the nature of the solvent (n-propanol) used as the propellant of the deacidifying solution and not to the deacidifying compound itself. Finally, pollution (NO₂) did not influence a paper's susceptibility to fungal damage. However, we noted sometimes that the rate of deterioration might be faster in the case of some polluted paper. This may be due more to the physical condition of the cellulose fibres than to the pH itself. If deacidification treatment with the CSC Book Saver Spray[®] is an efficient method of reducing the acidity of paper and to provide an alkaline reserve, our result shows clearly that it can not be used to prevent fungal growth on paper.

SUMMARIES

Influence of pH and alkaline reserve of paper on the growth of some filamentous fungi

The effect of different parameters on the growth of some microfungi strains was evaluated. pH, alkaline reserve and the type of paper have no significant influence on the fungal growth. Actually, the observed differences depended only on the intrinsic characteristics of each mould species.

Influence du pH et de la réserve alcaline du papier sur la croissance de quelques champignons filamenteux

Les effets de différents paramètres sur la croissance de quelques souches de champignons microscopiques ont été évalués. Le pH, la réserve alcaline ainsi que le type de papier n'ont aucune influence significative sur la croissance fongique. Les différences observées dépendent uniquement des caractéristiques intrinsèques de chaque espèce.

Der Einfluß von pH und alkalischer Reserve auf das Wachstum einiger Schimmelpilze

Es wurde der Einfluß einiger Papierparameter auf das Wachstum von Schimmelpilzen untersucht. pH, alkalische Reserve und Papierart haben keinen signifikanten Einfluß. Gewisse Unterschiede, die beobachtet wurden, sind ausschließlich auf die unterschiedlichen Schimmelpilze zurückzuführen.

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