

INFLUENCE OF PEAT AND PEAT SUBSTANCES ON THE METABOLISM OF FUNGI

E. KÜSTER

Department of Industrial Microbiology, University College, Dublin, Ireland

There are two main reasons for considering peat and its microbiology at this Symposium. First of all bogland is wide-spread in Ireland, 20 per cent of the country being covered with it. Ireland is lacking in great deposits of coal and minerals, so that peat attains particular economic importance as a naturally occurring raw material for several purposes. It is mainly used as fuel in power stations and briquette factories. Another important product is peat moss which is used as a soil conditioner and as livestock bedding in agriculture and horticulture; it is also exported in huge quantities. Peat as composted shows a very good effect on plants.

Secondly, the microbiology of peat is an under-developed field for the microbiologist, but rich in prospects. Peat is not as sterile a material as is generally assumed. There is some evidence that in peat, micro-organisms are not only in a dormant state, but are also actively living. Many microbial processes of decomposition and transformation of peat substances take place in the natural habitat. This means that peat substances must be used as nutrients to a certain extent. Now, the question arises which components of peat are utilizable and how do they influence the microbial growth.

When we want to work with and to use peat we have first of all to know its chemical composition and biological properties as exactly as possible. Peat is a very heterogenous material varying according to its formation and botanical origin. In general, peat may be defined as a material which derives from dead organic material, chiefly from plants, after an incomplete and anaerobic decomposition. The carbon content increases with progressive carbonization, that of oxygen decreases, hydrogen and nitrogen remain more or less constant. The availability of carbon substances for microbial development decreases with increasing carbonization in the order humic acids, peat, lignite, coal, anthracite¹. The proportion of utilizable carbon compounds depends on the grade of decomposition. Proteins, mono- and disaccharides, and also cellulose are quickly decomposed so that the proportion of lignin and hemicelluloses increases.

In the search for new commercial uses for peat the possibility of microbial fermentations has also been considered. The utilization of peat by micro-organisms to produce substances of high economic value is a promising and worthwhile idea. In Russia for instance, large-scale experiments have been carried out in which peat is used as raw material for the production of alcohol after a chemical and biological pre-treatment². Before starting on this problem of the use of peat as a material for microbial fermentations we have to study and know the general and original microflora of the peat in question.

E. KÜSTER

In accordance with the acid reaction (pH 3·7–4·5) of the peat under examination, the number and proportion of fungi obtained from it was high. As opposed to samples of mineral soils peat samples contain only a few different varieties of organisms. This lack of variety is caused by the acidic conditions, poor aeration, and low content of minerals in peat as habitat for microbes³. *Penicilli* are the dominant fungi in the peat samples (25–40 per cent). The more acidic the peat, the more *Penicilli* occur^{4–6}. The frequent occurrence of *Cladosporium*, *Aleurisma*, *Cephalosporium*, *Paecilomyces* and others is probably due to their strong cellulolytic activity⁷. *Cladosporium*, *Penicillium* and others are also considered as mycorrhizal fungi on typical bog plants such as *Calluna*, *Empetrum* and *Vaccinium*. It has also been observed that *Sphagnum* is able to grow on sterile peat only in the presence of a *Penicillium* species⁸. On examination of a number of peat samples of different quality we found that a relationship exists between quality and microflora (Table 1).

Table 1. Relationship between peat quality and microflora

Quality	Relative total count	Fungi in %	Anaerobic colonies per plate
Excellent	80	20	51
Average	100	55	
Poor	450	90	216

The poorer the quality of peat, the higher are the figures for micro-organisms as obtained by the plate count method. The relationship between the quality of peat and the number of microbes becomes quite clear particularly when comparing the increasing figures for different groups of micro-organisms. The increase in the number of fungi is proportionally higher than that of the bacteria with lower quality of peat. The calculated proportional increase in the number of fungi was 7·5, whereas that of the bacteria was only 4·5. Poor quality is equivalent to low decomposition. This means that poor peat still contains organic matter which can be utilized by micro-organisms. Considerable amounts of hemicelluloses and cellulose have been found in high-moor peat, the less-decomposed layers containing more of these substances than the well-decomposed ones⁹.

In the course of the carbonization of organic matter, peat formation is one step further on than humic acids. Therefore peat may, to a certain extent, be compared to humic acids with regard to its effect on fungi. A high-moor peat of the *Sphagnum* type for instance contains 25 per cent humic acids¹⁰. It has been demonstrated by several workers^{11, 12} that humic acids are not utilized as a carbon source by fungi such as *Penicilli*, *Paecilomyces*, *Trichoderma* and yeasts, and are used as nitrogen source to a small extent only. The nitrogen compounds of humic acids are slightly hydrolysed by autoclaving and become, probably as amino-acids, a little more available after sterilization. It has been shown that fungi grown on humic acids suffer from nitrogen deficiency and form considerable fat granules in their hyphae. The relationship between nitrogen deficiency and fat production by fungi is well known. Addition of humic acids in increasing

PEAT AND FUNGAL METABOLISM

quantities shows a depressing effect on the growth of fungal colonies but, on the other hand, a stimulating effect on the antibiotic activity of *Penicillium chrysogenum*. It is known that the phase of strongest formation of penicillin coincides with the phase of slow and delayed fungal growth. It has also been shown that fungi grow better on humic acids which are prepared from the same material as that from which the fungal strains were isolated, than on humic acids obtained from other places¹³. For example, the growth of peat fungi is better on peat humic acids than on those of black soil. Small quantities of humic acids stimulate the germination and development of some fungi (*Aspergillus niger*, *Penicillium glaucum*)¹⁴. This effect may be induced, at least partially, by the presence of phenolic compounds in humic acids and probably also in peat. The decolorization of humic acids by growing fungi (*Paecilomyces*, *Polystictus*) seems to be due to their reducing power and is related to the reduction of the carboxyl group of *m*-hydroxybenzoic acid¹⁵. It is suggested that a genuine decomposition of humic acids takes place and that the whole molecule is subject to fungal attack¹⁶. Fungi are also able to utilize phenolic compounds such as aromatic aldehydes (*p*-hydroxybenzaldehyde, syringaldehyde, vanillin)¹⁷ and the corresponding aromatic acids, including *p*-coumaric and ferulic acids^{18, 19}. Even high concentrations of those compounds which are toxic to, or at least depress the growth of bacteria and *Streptomyces*, stimulate fungal development. These results appear to be contrary to observations of an inhibition of spore germination of *Glomerella*²⁰. However, the neutral or positive effect of phenolic compounds on fungi can not be generalized and is limited to specific types of fungi.

Constituents of peat which affect the growth and metabolism of fungi may also consist of quinonoid compounds. These have been found in humic acids and are considered as their precursors and degradation products respectively²¹. There are many studies which demonstrate an antibiotic effect of quinones on fungi and other micro-organisms. On the other hand, using appropriately low concentrations of *p*-benzoquinone²² and naphthoquinone-derivatives²³ an increased respiration of yeast has been shown. This may be due to an uncoupling effect as obtained with 2,4-dinitrophenol²⁴. The change from an inhibiting to a promoting effect on respiration and O₂-uptake also depends on the concentration of glucose present. The lower the glucose concentration the higher is the relative O₂-uptake of yeasts and also of higher plants²⁵.

Now, it is generally accepted that the most easily utilizable substances for micro-organisms are water soluble. For this, cold, warm, and hot water extracts have been prepared from peat. The various water extracts of peat of average quality show different figures for carbohydrates, determined as glucose, with anthrone as reagent²⁶ (Table 2). These water extracts differently prepared, have been used for growth experiments with fungi. Added to a synthetic nutrient solution (Czapek-Dox), they increased the mycelial growth of fungi, the hot water extract showing the highest effect. The weight of mycelia increased in proportion to added quantities of the extracts. This result may not be due to a stimulating effect but is probably caused by an adsorption of precipitated humus-like particles on the fungal hyphae^{11, 27}. In fact, the different water extracts are differently

E. KÜSTER

Table 2. Determination of carbohydrates in aqueous peat extracts

<i>Water extracts</i>	<i>Glucose in %</i>
Cold	0.012
Warm	0.35-0.40
Hot	0.75-1.12
Hot (catalysed)	2.6

coloured and contain increasing quantities of dark brown sediments in the order cold-warm-hot. In respiration studies in the Warburg apparatus, a water extract obtained by autoclaving yielded a higher O₂-uptake by *Cladosporium* than the warm water extract of the same peat sample. A further increase can be attained by autoclaving in presence of catalytic amounts of sulphuric acid. By this so-called wet carbonization a larger amount of fermentable matter becomes available.

These water extracts of peat are at least partially comparable with fulvic acids, the water-soluble part of humic acids. It has been shown that fulvic acids containing lower-molecular compounds of different kinds have a greater effect on the growth and metabolism of micro-organisms and higher plants than the true humic acids.

Drain water from a bog was also used in respiration experiments. It may be defined as an aqueous extract of peat obtained under natural conditions. Bog water in a concentrated form showed a stimulating effect ranging between that of hot- and cold-water extracts. The effect increased when using bog water purified with charcoal. It means that organic substances with an inhibiting effect have been removed by the charcoal treatment. This was confirmed by an antibiotic test in which the washings of the charcoal showed the strongest antibiotic effect (Table 3).

Table 3. Antibiotic activity of ether extracts of bog water

<i>Test organisms</i>	<i>Ether extracts of</i>		
	<i>Raw</i>	<i>Purified</i> (inhibition zones in mm)	<i>Washings</i>
<i>E. coli</i>	1	2	1.3
<i>Bac. subtilis</i>	2	3.5	6.5
<i>Cladosporium</i>	1.5	5	8

In Warburg experiments with purified hot-water extracts of various peat samples it was demonstrated that the respiration was higher on the purified solution during the first 2-3 days. After this the O₂-uptake decreased below that of the non-purified extract. This means that the better growth and respiration is due to the removal of inhibiting substances. But, utilizable carbohydrates have also been removed by the purifying treatment so that the O₂-uptake rapidly decreases after 3 days.

It is well known that treatment with ether, toluene, or benzene-alcohol removes waxes and other incrusting substances from peat so that the utilizable carbohydrates become more available for micro-organisms. For this, water extracts have been prepared from peat after ether- and benzene-alcohol treatment respectively. The respiration on differently treated peat,

PEAT AND FUNGAL METABOLISM

as well as on its water extracts, was determined. In general, the water extracts of ether extracted samples showed a greater effect on respiration and metabolism than those of untreated peat.

The respiration on benzene-alcohol extracted peat was low on the 3rd day, reached a very high maximum at the 5th day and decreased after that, but was distinctly higher at this point than that of the other two samples (*Table 4*).

Table 4. O₂-uptake on differently treated peat

<i>Peat sample</i>	3	4	ml O ₂ /h 5	6	7 days
Untreated	15	9	7	7	5
Ether-extracted	14	10	6	5	4
Benzene-a.coloh extracted	4	22	61	25	25

This particular benzene-alcohol effect appeared with all the peat samples except well-decomposed peat of good quality. This means that the highly decomposed peat seems to contain no carbohydrates which become more available by this treatment. Only a benzene-alcohol mixture induces this peculiar effect but neither an ether-alcohol mixture, nor alcohol or benzene alone. The content of reducing sugars after acid hydrolysis of benzene-alcohol treated peat is 2-4 times higher than that of ether-treated peat. The afore-mentioned purification effect on hot-water extracts also occurred with those which were prepared from benzene-extracted material. The delayed respiration, called the benzene-alcohol effect, is eliminated by the purification treatment.

Waxes, resins, tannins, higher alcohols *etc.* are soluble in benzene-alcohol. Only a few of these substances are available to fungi, but some may also show an inhibiting effect on fungal growth. For example, a substance with antifungal activity which inhibits the germination of fungal spores²⁸ has been extracted from high-volatile bituminous coal²⁹ and also from peat in smaller quantities³⁰, using organic solvents only. On the basis of mass spectrometry and u.v.-spectra it is suggested that the phenolic material which is responsible for the activity probably consists of diphenanthryl or similar systems. Peat bitumen, soluble in benzene-alcohol, contains a mixture of acids or acidic groups of differing strength, as was recently shown by anion-exchange chromatography³¹. In humic acids also acid carboxyl groups and the weaker acid phenolic hydroxyl groups were differentiated by electrometric titration³².

The benzene-alcohol extract itself as obtained from peat did not show any antibiotic effect even in high concentrations. The test has been carried out with the paper disc method against gram-positive and -negative bacteria as well as a number of fungi. This lack of an apparent inhibiting or promoting effect of the benzene-alcohol extract may be due to its low or negative solubility in water. Water and acetone-water extracts respectively have been prepared from the evaporated benzene-alcohol extract and also tested by the same method. Here also no inhibiting effect was recorded. Both extracts were quite acidic (pH 4.0-4.5). With the acetone-water extract

no acid-induced inhibition was observed. The inhibition zones with the water extract were always smaller than those of the corresponding controls. This could mean that the negative acid effect was eliminated by stimulating factors present in the extracts. Further studies on this effect are in progress as well as attempts to isolate the substances involved and to examine their chemical nature and biological activity.

A detailed paper will be published elsewhere.

SUMMARY

On the basis of the studies reported in the literature and from our preliminary experimental results it may be suggested that peat as an energy source for micro-organisms is used to a limited extent only. Peat also contains substances which are more effective as stimulants than as nutrients for micro-organisms. The addition of peat as a whole or of some of its fractions influences and may probably improve microbial fermentations.

References

- ¹ H. DeBarjac. *Abstr. VI. Int. Congr. Microbiol. Rome* **3**, 115 (1953).
- ² Minnesota Peat Mission, *Report* (1958).
- ³ H. Burgeff. *VIII. Int. Congr. Botany Paris*, Sect. 7 (1954).
- ⁴ M. D. Bogopolski. *Instorf Proc.* No. 10, 114 (1934).
- ⁵ J. J. Moore. *Proc. Roy. Dublin Soc.* **26**, 379 (1954).
- ⁶ T. G. Zimenko. *Mikrobiologiya* **26**, 761 (1957).
- ⁷ J. G. Boswell and J. Sheldon. *New Phytologist* **50**, 172 (1951).
- ⁸ H. Burgeff. *Ber. Deut. Botan. Ges.* **69**, 257 (1956).
- ⁹ S. A. Waksman and K. R. Stevens. *Soil Sci.* **26**, 239 (1928).
- ¹⁰ H. J. Rehm and G. Sommer. *Zentr. Bakteriolog. Parasitenk. Abt. II* **115**, 594 (1962).
- ¹¹ W. Flaig and H. L. Schmidt. *Arch. Mikrobiol.* **27**, 1 (1957).
- ¹² H. Schönwälder. *Arch. Mikrobiol.* **30**, 162 (1958).
- ¹³ E. Küster. *Sonderh. Z. "Landwirtsch. Forsch."* **4**, 80 (1953).
- ¹⁴ E. A. Kudrina. *Rept. Inst. Soil Sci. Dokutscheva* **38**, 185 (1951).
- ¹⁵ H. M. Hurst, A. Burges, and P. Latter. *Phytochemistry* **1**, 227 (1962).
- ¹⁶ P. Latter and A. Burges. *VII. Int. Congr. Soil Sci.* **2**, 643 (1960).
- ¹⁷ M. E. K. Henderson and V. C. Farmer. *J. Gen. Microbiol.* **12**, 37 (1955).
- ¹⁸ M. E. K. Henderson. *J. Gen. Microbiol.* **14**, 684 (1956).
- ¹⁹ D. Knösel. *Z. Pflanzenernähr. Düng. Bodenk.* **80**, 225 (1958); **85**, 58 (1959).
- ²⁰ B. T. Lingappa and J. L. Lockwood. *Phytopathology* **50**, 644 (1960).
- ²¹ W. Flaig. *Suomen Kemistilehti* **33**, 229 (1960).
- ²² W. Flaig and W. DeJong. *Arch. Mikrobiol.* **37**, 355 (1960).
- ²³ J. M. Starkow. *Proc. Acad. Sci. USSR* **104**, 287 (1955).
- ²⁴ W. Flaig and W. DeJong. *Arch. Mikrobiol.* **37**, 369 (1960).
- ²⁵ M. Ruiz Amil and W. Flaig. *Ann. Edafol. Agrobiol. (Madrid)* **19**, 11 (1960).
- ²⁶ R. H. Brink, P. Dubach, and D. L. Lynch. *Soil Sci.* **89**, 157 (1960).
- ²⁷ A. Burges and P. M. Latter in *The Ecology of Soil Fungi*. (Ed. by D. Parkinson and J. S. Waid), p. 239, University Press, Liverpool (1960).
- ²⁸ N. C. Schenck and J. C. Carter. *Science* **119**, 213 (1954).
- ²⁹ A. A. Mills. *Nature* **184**, 1885 (1959).
- ³⁰ M. H. Rogoff and I. Wender. *Nature* **192**, 378 (1961).
- ³¹ J. D. R. Thomas. *Nature* **193**, 975 (1962).
- ³² J. J. VanDijk. *Sci. Proc. Roy. Dublin Soc. Ser. A* **1**, 163 (1960).