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M.P. COUGHLAN

Department of Biochemistry, University College, Galway, Ireland

and

M.T. AMARAL COLLAÇO

DTIA-LNETI, Lisbon, Portugal

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Proceedings of a Workshop on Advances in Biological Treatment of Lignocellulosic Materials, held in Lisbon, Portugal, from 25 to 27 October, 1989, under the auspices of COST (European Cooperation in Scientific and Technical Research)—COST 84-bis, organized with the support of the Commission of the European Communities by Departamento de Tecnologia de Indústrias Alimentares, Laboratório Nacional de Engenharia e Tecnologia Industrial (DTIA-LNETI), Ministerio da Indústria e Energia, Lisbon, Portugal.

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INTRODUCTION TO THE LISBON WORKSHOP

In the late 1960s the European Community launched the idea of facilitating the scientific endeavours of the countries of Europe by promoting a flexible set of arrangements for scientific cooperation. This initiative led to the creation of COST (Cooperation in Scientific and Technical Research) with the participation of the 12 Member Countries, the EFTA Countries, Turkey and Yugoslavia. In 1971 the first 7 COST concerted actions were implemented. This number has now increased to more than 60. COST 84 bis, one such activity, coordinates ongoing multidisciplinary research, within the Community and other contributing countries, on the use of lignocellulose-containing byproducts and other plant residues for animal feeding and industrial purposes. This it does by the holding of regular committee meetings at which appropriate representatives participate; by providing funding for the exchange of personnel between laboratories engaged in relevant research; by assisting the setting up and operation of centres of excellence in specific analytical techniques to which investigators may send/bring samples for analysis; and by the provision of funding for the holding of Workshops at regular intervals.

Several Workshops, dealing with a range of relevant topics, have been held since the foundation of COST 84 bis. The theme of the sixth such Workshop, held in Lisbon (October 25-27, 1989), was "Advances in Biological Treatment of Lignocellulosic Materials." Twenty three papers dealing with a variety of topics within the general theme were presented - and, as in previous Workshops, led, as indeed they should, to vigorous discussion. Each of the papers presented is included in this proceedings as are the summary reports by the relevant chairmen of each session.

Huge amounts of lignocellulosic wastes and residues, of agricultural, forest, industrial and domestic origin are generated annually. Such materials are comprised for the most part of cellulose, hemicellulose and lignin. Clearly, the successful exploitation of the potential of lignocellulosic substances, as sources of animal or human feedstuffs or chemical feedstocks, requires that each of these polymers be utilized to the fullest extent possible. For various reasons, including environmental considerations, biological rather than chemical conversion is the preferred route. This, in turn means that an understanding of the organisms involved, and their relevant enzyme systems, is *sine qua non.* Thus, the theme of the Lisbon Workshop was timely.

The opening lecture of the Workshop reported on the promising results obtained on using carbohydrases to increase the nutritional value of straw as fodder or as a fodder supplement. Session I dealt with solid-state fermentation of straw with white-rot fungi. Emphasis was placed on new developments in composting procedures, the production of

animal feeds and chemical feedstocks, the problems attendant on scale-up of solid-state processes, the possible utility of the soluble lignocellulose produced during fermentation, and on the modelling of the physical process parameters during lignin degradation.

The ultrastructural changes accompanying the biodegradation of plant materials are, as yet, poorly understood. This was essentially the topic of Session II. Papers presented included, the use of various chemical and spectroscopic techniques for studying the effects of chemical and biological treatment on the composition and ultrastructure of lignocelluloses, and procedures for the rapid determination of substrate quality during solid-state fermentation. Session III dealt with the production of enzymes during liquid- and solid-state fermentation of straws and pulps by fungi, the enrichment of seed shells with protein, the use of mixed cultures in bioconversion, the hydrolysis of cellulose in enzyme reactors, and the effects of various parameters on the operational stability of the enzymes involved. Topics discussed in Session IV included, the modelling of rumen processes in vitro and in vivo, the role of anaerobic fungi in the degradation of lignocellulosics, the use of white-rot fungi to increase forage digestibility, and the contribution of rumen fungi and bacteria to the degradation of straw. The last Session dealt specifically with the cultivation of edible fungi on plant residues, the use of white-rot fungi and their enzyme systems in biopulping and biobleaching, the decontamination of polluted air and soils using biofilters based on white-rot fungi growing on straw, and various aspects of the use of such fungi in upgrading lignocellulosic wastes to food, fodder and compost-based products.

As we have said before, the organization of a scientific meeting, the editing and retyping of manuscripts and the preparation of proceedings for publication, cannot be done without the generous assistance of others who are whole-heartedly committed to the project. For this reason we are pleased to acknowledge our gratitude to Dr. Peter Reiniger, Secretary of COST 84, CEC, to Instituto de Promoção Turistica, Lisbon, CFT do LNETI, Editorial do LNETI and all of the staff of DTIA-LNETI, to Sandy Lawson, for her typing excellence, to Rita Richardson, Yvonne Egan and Dorothy Fox who, with unfailing good humour, took care of mountains of FAX messages, and, to our families who saw little of us for several months.

M. Teresa Amaral Collaço and Michael P. Coughlan
Lisbon and Galway

OPENING LECTURE

ENZYME TREATMENT OF CROP RESIDUES

E.R. Ørskov and Y. Nakashima
The Rowett Research Institute,
Bucksburn, Aberdeen AB2 9SB, Scotland

The results of an investigation of the use of crude preparations of enzyme mixtures (see activities below) to preserve and/or upgrade straw is discussed. When moist straws (600 $g.kg^{-1}$) were stored at room temperature with the enzyme mixtures for 30 days the pH was reduced due to fermentation of sugars released by the enzyme action and subsequent production of lactic and volatile fatty acids. If fermentation of the released sugars was prevented by the addition of propionic acid both the solubility of straws and the potential extent of fermentation assessed by *in sacco* incubation was increased. Greater amounts of soluble sugars were released from leaves than from stems of temperate cereal straws. The opposite was found to be the case with rice straw. With combinations of alkali, oxidative and enzyme treatment it is possible to produce high quality feeds from straw.

INTRODUCTION

In this paper the discussion will centre on biological treatment of straw, not by use of micro-organisms, but by use of cell-free enzyme mixtures. Essentially, it is a summary of work carried out during the past 2 years. However, the use of crude enzymes to improve the nutitive value of forages is not a new concept. It has been applied mainly to preserves forages such as silage. In silage, enzymes sometimes have the advantage of increasing the acidity due to the release of sugar from ß-linked polysaccharides that are subsequently fermented to yield lactic and volatile fatty acids (see Henderson *et al.*, 1982 and Bertin *et al.*, 1985). Sometimes, enzyme treatments are also claimed to increase digestibility of silage. In our work we concentrated on the application of enzymes to straw and examination of the degradation characteristics.

The use of enzymes as a possible method of upgrading straw has some advantages. The most important of these is that there are no undesirable chemical end-products. To be successful the preparations used must contain a wide spectrum of enzymes. The commercial preparation (Meicelase) used in these studies contained the activities listed in Table 1.

Table 1. Activities exhibited by Meicelase (from Nakashima *et al.*, 1988).

Substrate	pH of test	Activity (units.g^{-1})
Xylan	5.0	106.3
ß-1,4-galactan	5.0	4.8
Carboxymethylcellulose	5.0	53.9
Avicel	5.0	37.9
Polygalacturonic acid	4.5	5.4
Pectin	4.5	20.9
Starch	4.5	0.4
Arabinan	4.5	2.4
Lichenan	5.0	55.0
Barley mixed link glucan	4.5	3619.5

Table 2. Effects of concentration of polysaccharidase enzymes, moisture contents and particle size on ensiling characteristics of rice straw.

Ensiling concentrations	Final pH	Organic acid (g.kg^{-1} fresh) Lactic	Acetic	Butyric
Cellulase concentrations (g.kg^{-1} DM)				
0	5.21	1.98	3.90	1.24
5	4.87	3.90	2.71	0.57
10	4.82	3.67	3.09	0.21
Significance of linear trend	**	**	*	**
Moisture contents (g/kg)				
500	5.44	2.34	2.06	0.14
600	5.00	3.04	3.33	0.58
700	4.46	4.17	4.31	1.30
Significance of linear trend	**	**	**	**
Particle size (mm)				
20	5.33	1.67	3.38	1.12
5	4.74	4.49	2.56	0.30
2	4.84	3.39	3.75	0.60
Significance of linear trend	**	**	*	**

* = P<0.01; ** = P<0.001

In the first trials rice straw was used. The effects of moisture, enzyme concentrations and particle size were examined. The results in terms of final pH and concentrations of lactic and volatile fatty acids after 30 days of incubation at 20°C are given in Table 2. The

concentrations of acids were increased by increasing the amount of enzyme used, by increasing the moisture content and by reducing the particle size of the straw. Measurement of degradation characteristics using the nylon bag technique showed that the effects of moisture and particle size were relatively small. The effects of enzyme concentrations are given in Table 3. Solubility as well as the 48 h dry matter loss increased with the addition of enzyme. However, the total digestive potential of the straw, i.e. the asymptote (a + b) from the exponential equation, $p = a + b (1 - e^{-ct})$, where (p) is degradation at time (t) (Ørskov and McDonald, 1979) did not increase. By contrast, the rate constant c was increased. In other words the solubility and the rate of degradation were increased but the extent of degradation was not increased. While intake studies were not carried out on the samples, Ørskov *et al.* (1988) had shown clearly that, as the rate constant increased, food intake increased even if digestibility did not.

Table 3. Effects of the concentration of polysaccharidase enzymes on the solubility, dry matter loss (DML), maximum potential degradability (a + b) and the rate constant (c) of enzyme-treated rice straw in nylon bags in the rumen of sheep using the equation $p = a + b (1 - e^{-ct})$.

	Solubility ($g.kg^{-1}$ DM)	48 h DML ($g.100\ g^{-1}$)	(a + b) ($g.kg^{-1}$ DM)	c (fraction per h)
	----------Mean values----------			
Enzyme concentration ($g.kg^{-1}$ DM)				
0	152	477	624	0.0498
5	196	533	621	0.0677
10	212	565	628	0.0817
Significance of linear trend	**	**	*	**

*, P<0.01; **, P<0.001

Table 4. Effect of cellulase enzyme mixtures on degradation characteristics of stem leaf sheath and leaf blade from barley straw.

Botanical fraction	Enzyme addition ($g.kg^{-1}$)	pH	Solubility ($g.kg^{-1}$)	48-h loss ($g.kg^{-1}$)	Potential ($g.kg^{-1}$)	Rate constant ($fraction.h^{-1}$)
Stem	0	5.6	135	299	403	0.0244
Stem	5	4.7	174	293	383	0.0212
Leaf sheath	0	5.0	185	680	812	0.0347
Leaf sheath	5	4.8	290	677	832	0.0276
Leaf blade	0	5.2	227	775	851	0.0454
Leaf blade	5	4.8	461	789	834	0.0484

Nakashima and Ørskov (1990) also examined the effects of chemical and enzymic pretreatment on different botanical fractions of barley straw. The results for stems, leaf sheath and leaf blade are summarized in Table 4. The degradation with or without enzymes was compared. The pH of the straw silage after 30 d was consistently reduced and solubility, particularly of leaf, was increased. On the other hand, the potential degradability, the 48 h dry matter losses and the rate constants were not changed.

Table 5. Effect of chemical pretreatment followed by enzyme treatment on degradation characteristics of internode of barley straw in polyester bags in the rumen of sheep according to the equation $p = a + b (1 - e^{-ct})$.

Chemical pretreatment	Enzyme addition ($g.kg^{-1}$)	Solubility ($g.kg^{-1}$ DM)	48-h loss ($g.kg^{-1}$ DM)	Potential ($g.kg^{-1}$ DM)	c (fraction per h)
Untreated	0	136	299	403	0.0244
	5	174	293	383	0.0212
NaOH	0	117	283	380	0.0238
	5	179	332	423	0.0238
NaOH + H_2O_2	0	113	380	690	0.0139
	5	195	430	695	0.0145

The effects of treatment of stems (Table 5) and leaves (Table 6) with NaOH, with or without enzyme treatment, are shown above and below, respectively.

The effect of H_2O_2 here is very apparent. The application of enzymes increased solubility particularly for leaves, but, as before, while the potential was increased by chemical treatment it was not consistently increased using enzymes. In fact, in the case of leaves the rate constants were actually decreased.

We examined the actual loss of substrate during treatment with the enzyme preparation and the extent to which the loss could be reduced by inhibiting fermentation of the released sugar. Accordingly propionic acid was used in different concentrations to inhibit fermentation. Concentrations higher than 30 $g.kg^{-1}$ are not included in Table 7