The Potential Industrial Uses of Forage Grasses Including Miscanthus

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CONTENTS

1. Introduction

This document describes the current state of UK and European research into industrial uses of forage grasses, as well as providing an overview of the components, potential applications and research areas that may provide scope for additional industrial uses. Forage grasses are defined as those grasses that have traditionally been used as fodder or silage for livestock production. In UK and Europe, the rye grasses, *Lolium perenne* (perennial ryegrass) and *Lolium multiflorum* (Italian ryegrass), occupy about 70% of the agricultural areas with *Festuca arundinacea* (tall fescue), *Festuca pratensis* (meadow fescue) and *Dactylis glomerata* (cocksfoot) making up the remainder. This review therefore focuses on these grasses as the traditional forage crops.

A separate section describes current and potential research areas for the giant grass, *Miscanthus*.

2. Current Status of Research

The UK and mainland Europe have reached a position where they no longer seek continued growth in food output from their agricultural resources leading to decline in agricultural industry and rural economies. It is reasonable then to look to satisfy other demands by utilisation of those agricultural resources. The grasslands that have supported rural communities for centuries may again sustain those communities when alternative, industrial uses of the resource are considered and implemented.

In mainland Europe, a number of research and development programmes are already underway to exploit the non-food value of grasslands as sources of new commodity and value-added chemicals and industrial fibres. Indeed, several companies have been formed to commercialise emerging technologies. The following discussion describes current research, development and exploitation activities within Europe and North America.

2.1. EUROPE

2.1.1. Austria

Austria's interest in grasses as potential sources of cellulose has been on-going since at least 1991 when Pirringer reviewed [1] Austria's current and potential production of industrial crops and the land use each accounts for.

In Austria during the last four years a holistic concept for an Austrian Green Biorefinery has been developed [2]. The system is built around grass silage fermentation and the production of lactic acid, other organic acids, proteins, amino acids, carotenoids, other pigments, monosaccharides, and fibres and the resulting applications.

2.1.2. Denmark

In Denmark, a co-operation between South Danish University, Aalborg University, and the local agro-industry in Jutland is developing the concept of integrated usage of green crops such as grass [3]. A development company, Danish Biomass A/S, owned by South Danish University and the local agro-industries has been established and a pilot plant for fermentation of different residues from the involved agro-industries has been built.

The technology for the production of amino acids, developed in co-operation between the universities and agro-industries, is being transferred for industrial commercialisation. All the scientific activities in the area of biotechnological utilisation of biomass and agricultural residues are being brought together within the new Centre for Agro-Industrial Biotechnology at Esbjerg [3].

2.1.3. Finland

In Finland, forage grasses have been investigated as fibre sources for its pulp and paper industry [4]. *Festuca pratensis* and *Festuca arundinacea* were harvested at the seed ripening stage and in the following spring when the plants were totally dry. The amounts of different plant parts (stem, leaf sheaths, leaf blades and panicles) were measured and the composition of ash, silica, iron, manganese, copper, and potassium was measured for each plant fraction. Plant species, plant part and harvesting time affected the mineral composition. The mineral composition was highest in the leaf blades. In each species, stem fractions had the lowest ash, silica, potassium, iron and manganese contents. The potassium concentration was clearly lower in plants harvested in the spring that at the seed ripening stage in the autumn. However, the concentrations of silica, iron, copper and manganese were highest at spring harvesting.

Saijonkari-Pahkala has identified *Festuca arundinacea* as a promising perennial grass as raw material for pulp and paper [5]. It was selected from 24 herbaceous plants, along with reed canary grass, as being most suitable for pulping. Spring harvesting was found to be unsuitable for *Festuca arundinacea*. The fibre content of the raw material increased the later the crop was harvested. Removing leaf blades and using minimum fertiliser application rates increased the fibre content of biomass.

2.1.4. Germany

In Germany in 1998 the Kamm research group started to use different crop juices for lactic acid fermentation [6]. Lactic acid is seen as *the* platform chemical of the future (c.f. ethylene [7]).

Koller has considered [8] the environmental aspects of manufacturing plant based on biomass, especially grass and organic wastes, with respect to resource conservation, emissions reduction, greenhouse effect, ozone depletion potential, water acidification potential, and other pollution consequences. A process is based on the separation of green type versus fibre portions of the substrate by mechanical pressing; the fermentable portion is fermented to protein concentrate (containing 25% lysine) and lactic acid solution. The lactic acid is separated and polymerised to polylactides (which can be used as substitutes for non-biodegradable polymers). The fibre-rich portion is dried (to produce solid fuels and animal feed), fermented to produce biogas fuel, and extracted to recover chlorophyll.

2.1.5. Sweden

Sweden has had an interest in green biomass for food, fodder, fuel, industrial fibre, pharmaceuticals and bio-fertilisers since at least 1988. The processes of wet fractionation for the production of leaf protein concentrate for grasses were described [9].

2.1.6. Switzerland

In Switzerland, the biotechnology company, 2B Biorefineries AG, has developed a technology to produce ethanol, protein concentrate, and technical fibres from forage grasses [10]. 2B Biorefineries has a share capital of Sfr. 1.8 million. Development and growth of 2B Biorefineries have been financed by funds from the founding shareholders and private equity investors (e.g., Zürcher Kantonalbank) and a loan from Credit Suisse. 2B Biorefineries has 12 employees. The first commercial grass plant was established in Schaffhausen in 2001. The technology comprises as main process steps pre-treatment and fractionation of wet forage grass and separation of fibres and protein. No chemicals are added and no solid wastes are produced. The company has plant producing ethanol and biogas (methane). Fibre fractions from grass are marketed as '2B Gratec' to fulfil applications such as loose blow-in insulation, non-wovens, paper and boards.

The 2B Biorefineries grass process can include the extraction of a protein concentrate. This product contains 35–45% raw protein and 8–10% raw fat, depending on the grass quality. The amino acid composition is reported as favourable for all feeding purposes. The product was used in pig and chicken meals and tested in commercial scale operations throughout the last two years. Due to its methionine and carotenoid content, and the fatty acids composition, it is particularly suited for chicken meals. The business of 2B Biorefineries is to license the technology and sell certain key components and engineering to customers.

2.1.7. The Netherlands

In The Netherlands, a sustained green biomass research programme based on forage grasses has led to the opening of a pilot plant in 2002 [11]. Avebe BA operates the pilot plant at Veendam and with other partners has formed a consortium to explore the potential of grass to supply a range of fibre, protein and nutraceutical products. Other members of the consortium are Nom, a private investment group; Meppel, an agricultural co-operative; ABCTA, an animal feed producer; Plant Research International, a plant breeding research organisation; Nedla Co., an alcohol producer; Mommersteeg International, a grasses breeder; and Rabobank, an investment bank. Avebe has identified the following chemicals/materials as being feasible products from grass which are established commodities and already have alternative sources: ethanol, inulin, rubisco, enzymes, lipids/fatty acids, pigments, organic acids, amino acids and potassium fertilisers. Avebe has patent applications under consideration on the refining process that separates the juice/fibre fractions. The protein juice fraction can reportedly replace soya protein. This may be more easily certified as an organic, GMO-free protein source than soya protein.

The project is currently under review by the consortium. Depending on the outcome, a full-scale (50,000 tpa) factory may be commissioned. For this, a new company, Biorefiner bv, will be created. The current pilot plant only processes 3tph of fresh grass. The intended full scale plant would operate at 200 tph and require 10000 hectares per annum of mixed grass sources drawing from a radius of up to 50 km but this would not be started until 2008.

2.1.8. United Kingdom

Forage grass research in the UK is largely carried out by the Institute of Grassland and Environmental Research (IGER), Aberystwyth. The bulk of its work is focused on the improvement of grasses for forage and amenity uses. However, research projects carried out in the Plant, Animal and Microbial Science Department offer opportunities for non-food, industrial uses of grasses. As part of a genomic mapping programme, genetic manipulation techniques are being used to increase the levels of fermentable carbohydrate content [12] within forage as well as to control the levels of phenolic acids within grass cell walls [13]. Enhanced carbohydrate levels and mediated phenolic acid content could provide larger quantities of more easily accessible raw materials as feedstock for the production of fermentation products such as industrial lactic acid.

BioComposites Centre at the University of Wales Bangor has made preliminary investigations of fibre properties and sugar compositions of *Lolium perenne* and *Festuca pratensis*. Results have shown that the cellulosic fibres present are readily processable to form paper products. The fibres may offer a viable alternative to fibres derived from hardwood pulps used in the production of higher value products such as writing paper. Sugar analysis reveals the presence of potentially useful components including D-xylose [14], a precursor of high-value added xylitol, a naturally occurring sweetener.

The industrial exploitation of *Miscanthus* in the UK is led by Bical Ltd. Bical Ltd was established in 1998 as a co-operative of twenty West Country farmers to exploit *Miscanthus* as a non-food crop that offers a wide range of uses, such as poultry and animal litters, thatching, fibre and insulation boards. Currently there are 300 farmers in the consortium. *Miscanthus* is sold as a horse bedding material due to low dust, high absorbency, durability, low odour and biodegradability. Bical Ltd has its own manufacturing facilities using custom-built modular processing plants [15].

Bical Ltd also manufactures and sells biodegradable plant pots made from *Miscanthus* in the UK through Avoncrop Ltd. An expansion in the manufacture of Biopots in the South West is planned, and a feasibility study funded by Government Office Southwest for *Miscanthus* production for composite manufacture in Cornwall is currently underway [16]. The annual market for these pots is estimated to be 20 million per annum in the UK alone. The pots are manufactured from 70% *Miscanthus* blended with natural resins to produce a 100% biodegradable product that can be used as effectively as conventional plant pots.

Bical Ltd is promoting the use of *Miscanthus* for thatching. To date, it has constructed a small-scale demonstration thatch in the south west using *Miscanthus* stems.

Bical Ltd engages in collaborative research with university departments. A research programme has been initiated with the University of Warwick's Manufacturing Group to develop biodegradable plastic car parts using *Miscanthus*. The work has demonstrated its use as a biodegradable structural filler in plastic car parts such as wheel trims. Short lengths of fibres are used to give strength to biodegradable plastics that were previously too weak to be used in many car parts. The plastic car parts developed in this way will not degrade during the life of a vehicle but will biodegrade if they are composted at the end of the vehicle's life. Latest trials show that *Miscanthus* fibres can be blended with Mater-bi starch-based biopolymer (a combination of starch and polycaprolactone) to give a product with good physical properties [17].

Other work in collaboration with the Warwick Manufacturing Group includes using *Miscanthus* as a fibre source in panel boards, paper pulps and as an energy component of compressed fuel briquettes and garden candles. A novel application is as a component of soaps and hand-cleaners in which the dust extracted from the animal bedding serves as an exfoliant/abrasive [17].

A collaboration began in February 2002 with DTI, Cranfield University and Arable Research Centres to produce and develop fully automatic planting and lifting machines to aid in the development of growing *Miscanthus*. The work after its first year has been very successful with crops of *Miscanthus* currently being planted at rates higher than fifteen hectares per day [18].

2.2. NORTH AMERICA

2.2.1. Canada

In Canada, Arbokem, Inc has developed the use of agricultural fibres for newsprint manufacture [19]. Approximately 233 tonnes of newsprint was made using a mixture of old newspapers (68%), thermomechanical pulp (12%) and Agri-Pulp™. The Agri-Pulp™ was made from Oregon ryegrass straw, California rice straw and British Columbia red fescue straw. In the weeks following the paper-making test run, the Agri-Pulp™ newsprint was test printed commercially by the *Los Angeles Times* and other newspapers. The pressroom operators generally found the printing quality and physical strength of the test Agri-Pulp™ newsprint to be somewhat better than those of standard newsprint.

2.2.2. United States

A 'Roadmap for Biomass Technologies in the United States' was published recently [20] in which development and implementation of biorefineries is attributed as an essential contribution to a transition from the petroleum-based economy towards an economy based on renewable resources.

Kashmanian and co-workers in the US have examined [21] the potential quantities and characteristics of leaves and grass clippings as fertilisers. They have further suggested that such by-products are valuable feedstocks for a series of agronomic and non-agronomic purposes to enhance and sustain society.

Vogel and Shearman have described [22] potential new applications for perennial grasses including their use as industrial crops for fuel and paper production.

2.3. CURRENT RESEARCH PROPOSALS IN EUROPE

A Specific Targeted Research Project (STRP) 'New technologies for an integrated, strongly intensified and sustainable production of lactic acid, amino acids and fibres from green biomass' has been submitted into the Framework Programme 6, Priority 3 Nano-technologies and nano-sciences, knowledge-based multifunctional materials and new production processes and devices – 'NMP' [23]. This STRP proposal comprises 10 research and higher education establishments as well as 4 industrial partners. The project is intended to transform traditional forage silage by modern green biotechnology into commodity and higher value-added chemicals and industrial fibres. It will focus on the optimisation of solid-state fermentation, biomass fractionation, enzymatic conversion processes, utilisation of residual liquids, separation technology, product upgrading and chemical conversion, fibre characterisation and technology as well as a sustainability assessment.

The composition of the consortium broadly reflects the state-of-the-art of industrial forage grass research and development within Europe. There are 5 representatives from Austria (Joanneum Research, Graz University of Technology, University of Natural Resources and Applied Life Sciences, Interuniversitaeres Forschungsinstitut fuer Agrarbiotechnologie, and Lactoprot AG), 4 representatives from Germany (Biorefinery GmbH, Biopos e.V., FH-Hannover, and Biopract GmbH), 2 representatives from Denmark (AgroFerm A/S and Danish Research Institute of Food Economics), and one each from Hungary (University of Veszprem), Slovenia (University of Maribor) and the United Kingdom (University of Wales, Bangor).

3. Key Components of Forage Grasses

In the main, the research literature of forage grasses is concerned with their nutritive aspects as fodder grass, hay or silage. From a biochemical perspective the composition of forage grasses is well-described. Thus, a comprehensive inventory of forage grass chemical/material constituents exists in the literature. The components can be conveniently categorised according to their location within the grass, either as a cell wall constituent or as a component within the cell.

3.1. STRUCTURAL, CELL WALL CONSTITUENTS

Cell wall constituents comprise structural polysaccharides (hemicellulose, cellulose), lignin and pectic substances. Generally, there is compositional variation in the amounts and extents of constituents present within cell walls during the growing season.

3.1.1. Hemicellulose, cellulose and lignin

A comparison of the contents of hemicellulose, cellulose, lignin and crude fibre in fresh herbage, hay and silage from meadow grasses has been made [24]. The crude fibre content of grass harvested as fresh herbage was 24.0–35.5% of the dry matter (DM). The crude fibre content increased with delay in harvesting and was higher in hay and silage than in the fresh herbage. The total contents of hemicellulose, cellulose and lignin together were twice that of crude fibre in grasses. During ensiling, the hemicellulose content was decreased by an average of 3–11% and the cellulose remained unchanged while the lignin content was increased by 23% in grasses.

With advancing maturity, the concentrations of cellulose, hemicellulose and lignin, in grasses increase. In general, the digestibility of cellulose decreases during the growing season which is commonly attributed to an increasing lack of accessibility of the polymer to attack by micro-organisms [25]. This variation should be considered when assessing grasses as feedstocks for industrial processes, particularly when a decision to harvest a forage grass specifically for its fibre content is concerned.

Chemical composition and digestibility were studied *in vivo* and *in vitro* [25]. The main finding was that although the grass species studied showed similarity in gross chemical composition, the digestibility varied greatly at comparable stages of maturity. Thus a rapid decrease in digestibility was observed between the two first cuts while only small changes were observed between the two times of harvesting the re-growth. Although the digestibility in this study was studied with reference to ruminants, the variation in this parameter may become significant when considering the use of grass as an industrial fermentation feedstock for, for example, xylitol or lactic acid production.

The more digestible ryegrasses show a two to three-fold increase in (1? 4)-linked D-xylose units without branch points at the *O*-2 and *O*-3 positions, the proportions of those branch points being substantially reduced. The changes were greater in the early cut samples [26].

Similarly, it has been noted that re-growth has a lower nutritional value than the first cut at a comparable stage of growth. *Dactylis glomerata* and *Lolium perenne* were cut 1 to 3 times and analysed chemically. The material from the first cuttings had the highest content of total digestible nutrients, 55.96%. Protein utilisation value was lowest in the third cut grass [27]. Contents of D-galactose and other carbohydrates were much lower in re-growth [28].

Åman and Lindgren studied [29] the change in chemical composition and degradability of six grasses including *Festuca pratensis*, *Festuca arundinacea* and *Dactylis glomerata* which were harvested at two stages of maturity at both the first and second cut respectively. The results are shown in *Tables 1* and *2* (overleaf).

Table 1. Composition of grasses harvested at early first cut and late first cut (% of DM of unextracted material, sugar residues given as anhydrosugars).

	Dactylis glomerata	*Festuca pratensis*	*Festuca arundinacea*
Early first cut			
80% Ethanol Extract	31.5	29.2	28.2
Crude Protein	16.6	14.2	14.3
Polysaccharides	37.5	38.8	40.5
Rhamnose	0.1	0.1	0.1
Arabinose	2.6	2.6	2.8
Xylose	11.2	10.6	13.1
Mannose	0.2	0.1	0.2
Galactose	1.4	1.0	0.9
Glucose	19.1	21.3	20.0
Uronic acids	2.9	3.1	3.4
Glu/Xyl+Ara	1.4	1.6	1.3
Klason lignin	7.7	10.3	9.1
Ash	10.0	10.2	10.0
NDF	51.4	55.4	55.4
ADF	28.5	31.4	30.8
Permanganate lignin	4.2	5.2	4.8
Residue of organic matter			
in vitro	12.1	15.8	16.5
in vivo	25.8	22.8	27.1
Leaf per cent	48.0	41.0	42.0
Late first cut			
80% Ethanol Extract	28.1	24.9	26.6
Crude Protein	10.6	9.8	9.8
Polysaccharides	44.2	48.3	44.9
Rhamnose	0.1	0.1	0.1
Arabinose	2.8	3.1	2.5
Xylose	12.1	15.5	14.4
Mannose	0.2	0.2	0.2
Galactose	0.8	0.9	0.9
Glucose	25.3	25.4	22.9
Uronic acids	3.0	3.1	4.0
Glu/Xyl+Ara	1.7	1.4	1.4
Klason lignin	13.2	13.8	15.0
Ash	8.6	8.5	8.5
NDF	57.9	62.3	62.4
ADF	32.6	35.6	34.3
Permanganate lignin	6.0	6.2	5.7
Residue of organic matter			
in vitro	21.1	24.6	28.9
in vivo	31.8	32.2	35.7
Leaf per cent	17.0	34.0	25.0

Table 2. Composition of grasses harvested at early second cut and late second cut (% of DM of unextracted material, sugar residues given as anhydrosugars).

	Dactylis glomerata	*Festuca pratensis*	*Festuca arundinacea*
Early second cut			
80% Ethanol Extract	22.2	22.7	25.6
Crude Protein	9.9	10.0	10.0
Polysaccharides	47.2	44.8	44.1
Rhamnose	0.1	0.2	0.2
Arabinose	3.2	3.2	3.1
Xylose	11.9	10.2	12.5
Mannose	0.2	0.2	0.2
Galactose	1.2	1.5	1.1
Glucose	26.3	26.2	24.0
Uronic acids	4.2	3.2	3.1
Glu/Xyl+Ara	1.7	2.0	1.5
Klason lignin	13.1	13.8	13.8
Ash	9.7	11.5	10.9
NDF	65.1	60.5	61.2
ADF	40.0	37.0	34.0
Permanganate lignin	8.3	5.4	5.0
Residue of organic matter			
in vitro	20.5	19.4	18.4
in vivo	30.9	28.4	29.2
Leaf per cent	55.0	73.0	77.0
Late second cut			
80% Ethanol Extract	24.3	22.0	24.8
Crude Protein	9.0	8.7	9.7
Polysaccharides	45.8	46.9	43.3
Rhamnose	0.3	0.2	0.1
Arabinose	2.9	3.2	2.9
Xylose	10.5	11.8	11.2
Mannose	0.2	0.4	0.2
Galactose	1.2	1.7	1.3
Glucose	26.7	26.4	23.4
Uronic acids	4.0	3.3	4.2
Glu/Xyl+Ara	2.0	1.8	1.7
Klason lignin	16.0	19.0	12.9
Ash	9.8	10.6	11.5
NDF	63.5	62.3	60.6
ADF	41.2	39.2	34.7
Permanganate lignin	8.6	7.4	5.7
Residue of organic matter			
in vitro	19.1	19.9	16.8
in vivo	32.9	29.6	30.1
Leaf per cent	68.0	81.0	77.0

Composition studies have also been driven by recognition of the influence of covalent bonding between the cell wall polymers on the utilisation of the cell wall as a nutrient source.

Morrison investigated [25] the variation in hemicellulose and lignin composition of grasses over the growing season. It is known that these two cell components are covalently linked and it is believed that the lignin has a strong influence on the digestibility of the hemicellulose moiety. In this study, ten varieties of temperate grasses were studied by harvesting at five stages of maturity, taking only a first cut. The lignin and hemicellulose contents were measured with the hemicellulose being further fractionated into linear and branched hemicellulose by iodine treatment. The hemicelluloses were analysed for the neutral sugars L-arabinose, D-xylose, D-galactose and D-glucose. The results are shown in *Table 3*.

Table 3. Hemicellulose concentrations (g per kg DM) in leaf and stem tissue of forage grasses.

	Leaf					Stem				
Cut no.	1	2	3	4	5	1	2	3	4	5
Lolium perenne S24	83	114		162		101	133		244	
Lolium perenne Reveille	79	104		140		97	136		211	
Lolium perenne S23	76	120	167	183	211	99	137	204	228	291
Lolium perenne Barpastra	78	111	153	180	199	89	136	183	194	272
Festuca pratensis	113	159		202		147	177		270	
Festuca arundinacea S170	124	172		194		162	201		193	

Lignin-carbohydrate complexes from *Lolium perenne* contained high proportions of D-glucose residues (ca 50%). Leaf tissue complexes had the highest D-glucose content, while stem and leaf sheath were very similar. The other neutral sugar residues present in these complexes were mainly L-arabinose and D-xylose. The polysaccharide components of the lignin-hemicellulose complexes contained mainly D-xylose (63–77%) and L-arabinose (19–28%) [30].

Forage grass lignin was more extensively solubilised by acid detergent than forage legume lignin. Forage plant lignins were characterised by guaiacyl-syringyl lignin with *p*-hydroxyphenylpropane units. The number of ferulic acid cross-linkages in the cell wall matrices of forage grasses increased with plant maturation [31].

Two classes of phenolic-carbohydrate complexes were purified from the water-soluble products obtained from the digestion of *Lolium perenne* cell walls with a cellulose preparation [32]. They contained D-glucose, D-xylose, L-arabinose, D-galactose and D-mannose in the ratios 3.6:10:6.3:1.4:2.3 and 5.3:10:3.0:1.1:2.1, respectively. The complexes were based on (1? 4)-β-D-xylan chains to which were attached residues of L-arabinofuranose and D-galactopyranose. Mixed linkage (1? 3), (1? 4)-β-D-glucan chains also appeared to be integral components of these complexes.

The principle outcomes of these studies are as follows:

- The quantities of hemicellulose increased with increasing maturity with the increase being higher in stem tissue compared to leaf tissue. For example, the hemicellulose content of *Lolium perenne* leaf tissue increased from 7.6% to 21.1% of dry matter over the study period. The D-xylose content of the linear hemicellulose increased concomitantly from 69% to 85%.
- The hemicellulose content of stem tissue increased from 9.9% to 29.1% with the linear hemicellulose increasing from 74% to 91%.
- The linearity of hemicelluloses tended to increase with crop age.

- Higher lignin contents were associated with hemicellulose of a higher linear: branched ratio.
- Hemicellulose also showed higher D-xylose:L-arabinose ratios.

In summary:

- Time of harvesting has a significant effect on the sugar composition.
- Hemicellulose sugars increase during the growth season
- Stem tissue contains greater amounts of hemicellulose (xylans) than leaf tissue.
- Digestibility of grasses decreases with age, which may affect yields of fermentation-derived products such as xylitol and lactic acid. Although overall at later maturity, the absolute quantities of potentially fermentable sugars (e.g., D-xylose, present as hemicellulose) are greater, their accessibility to fermentation media may be reduced.

3.1.2. Pectic substances

Extraction of mesophyll cell walls from leaves of *Lolium perenne* afforded 25 mg of a uronic acid polymer per gram of material [33]. The polymer was identified as a 1,4-linked homogalacturonan essentially free from neutral sugar residues, with a low degree of acetylation (3.6%) and methyl esterification (3.3%). Thus, the pectin was similar to the pectins of dicotyledons but the amounts found were substantially lower than in the majority of dicotyledonous plants. On that basis, there appears to be little scope for industrial end-uses of forage grass pectins.

3.2. CELL CONTENTS

The cell contents of forage grasses contain sugars, fructans, amino acids, proteins, silica, alkanes, starches, minerals, nucleic acids, lipids and alkaloids. Protein and sugars comprise the greatest components. The non-structural carbohydrate concentration of leaves and stems is highest in winter for *Lolium perenne* at 13% of dry matter. Seasonal variations in elemental concentration are small [34]. *Festuca pratensis* and *Dactylis glomerata* are characterised by high cell wall contents.

3.2.1. Sugars

D-Glucose, D-fructose, D-sucrose and fructans are the main non-structural carbohydrates in *Lolium perenne* tissues [35]. The D-glucose, D-fructose, D-sucrose, as well as D-xylose, D-mannitol, D-sorbitol, glycerol, and D-maltose contents of *Dactylis glomerata*, *Lolium perenne* and *Festuca pratensis* cut three times at different dates without interim harvesting have been recorded [36]. The results are reported (*Table* 4) overleaf. Significant findings were:

- *Lolium perenne* contained the most water-soluble carbohydrate (27%) in the early season, compared to *Festuca pratensis* (13.8%).
- This figure decreased steadily through the growing season to 7.8% in early September although this rose to 16% at the end of the month.
- *Lolium perenne* also contained the most xylose (0.26%) in mid-season.
- In *Lolium perenne*, glucose levels peaked in early August and again in late September. Although xylose levels similarly peaked in early August, there was no corresponding peak in late September. Similar trends were observed in *Festuca pratensis*.

Table 4. Changes in water soluble carbohydrate contents and mono- and disaccharide contents (% on dry matter basis) of *Lolium perenne* and *Festuca pratensis*

	Monosaccharides				**Sugar Alcohol**		**Disaccharides**			
Species	Glc	Fru	Xyl	Mann	Sorb	Glyc	Sucr	Malt	Mono+Disacch	WSC
Lolium perenne										
June 6	3.11	5.43	0.00	0.05	0.05	0.24	0.15	0.10	9.12	27.0
June 21	3.82	4.62	0.05	0.00	0.00	0.24	0.14	0.14	9.02	16.1
July 6	2.89	3.92	0.08	0.04	0.04	0.20	0.12	0.16	7.45	18.1
Aug 6	4.36	6.25	0.26	0.13	0.00	0.46	0.26	0.26	11.98	9.2
Aug 21	2.43	5.10	0.23	0.08	0.00	0.32	0.00	0.16	8.32	9.8
Sep 3	1.46	1.87	0.11	0.00	0.00	0.19	0.11	0.08	3.83	7.8
Sep 30	5.00	7.47	0.14	0.07	0.00	0.40	0.27	0.20	13.53	16.0
Festuca pratensis										
June 6	2.38	4.91	0.00	0.00	0.00	0.24	1.42	0.10	9.04	13.8
June 21	2.93	3.48	0.00	0.00	0.00	0.19	0.12	0.12	6.85	9.0
July 6	2.13	3.33	0.07	0.00	0.00	0.18	0.00	0.21	5.95	12.5
Aug 6	2.07	3.30	0.15	0.25	0.00	0.20	0.30	0.40	6.66	4.9
Aug 21	1.74	2.81	0.07	0.07	0.00	0.34	0.00	0.20	5.22	7.6
Sep 3	1.53	0.47	1.80	0.19	0.12	0.19	0.19	0.27	4.78	6.6
Sep 30	4.56	6.56	0.13	0.13	0.06	0.44	0.19	0.19	12.25	10.3

Glc: D-glucose; Fru: D-fructose; Xyl: D-xylose; Mann: D-mannitol; Sorb: D-sorbitol; Glyc: glycerol; Sucr: D-sucrose; Malt: D-maltose; Mono+Disacch: monosaccharides + disaccharides; WSC: water-soluble carbohydrate

In an analogous piece of work, Fales and colleagues [37] reported results for *Festuca arundinacea* but for stems. The stems were extracted with 95% ethanol and water to afford D-glucose, D-fructose, D-sucrose and fructans. The fructan extract was hydrolysed with sulfuric acid and shown to contain D-glucose and D-fructose. A hemicellulose fraction was hydrolysed and found to contain D-xylose, L-arabinose and small amounts of D-glucose.

3.2.2. Fructans

In grasses, fructan reserves are mobilised from vegetative plant parts during seasonal growth, after defoliation during grazing. In expanding leaves, fructans are accumulated in cells of the elongation zone [38].

Fructan structures have been characterised in *Lolium perenne* belonging to essentially three series: inulin series, inulin neoseries and the levan neoseries [39].

Festuca arundinacea contains an inulin and neokestose based series of oligosaccharides [40].

Fructans are an important class of carbohydrate that have considerable biotechnological importance [41]. The first is that they are a potential source of D-fructose, for which there is a growing market in the food industry as a sweetener. The utilisation of fructans has been reviewed by Fuchs [42]. Their main uses are in the food sector. More pertinently, fructans could be chemical feedstocks from which a variety of chemicals can be produced. Hydrolysis to D-fructose and subsequent dehydration leads to hydroxymethyl furfural (see 5.1.2.) which, like lactic acid, is considered to be a key chemical intermediate for chemistry based on renewable raw materials. Similarly, hydrolysis of inulin to D-fructose followed by catalytic hydrogenation yields D-mannitol/ D-sorbitol mixtures from which D-mannitol can be easily crystallised. D-Mannitol, like xylitol, is a valuable, non-cariogenic low-calorie sweetener. Other chemicals that could be derived from fructans include ethanol, other organic solvents and chemicals such as furans [43].

Inulin is the best known sub-class of fructans. Inulin is colourless and odourless, and has a pleasant slightly sweet taste; it is moderately soluble in water and acts as a gel-forming agent at concentration >30%; it is also a foam stabiliser and texturing agent. Its calorific value is 4kJ/g, but it acts as a dietary fibre; it suppresses putrefying bacteria and selectively supports bifidobacteria and lactobacilli in the colon. In food applications, its main functions are to replace fat and sugar, to enrich with dietary fibre, to activate bifidobacteria and lower cariogenicity. It is classified as a foodstuff [44].

3.2.3. Amino acids

The amino acid composition of *Festuca pratensis*, *Dactylis glomerata* and *Lolium perenne* has been studied. There were no significant differences between the species. As the grasses aged, decreases occurred in aspartic acid, glutamic acid, alanine, tyrosine and phenylalanine and increases occurred in threonine, serine and proline. Lysine, histidine, arginine, glycine, valine, methionine, isoleucine and leucine did not change with plant age nor did the total amino acid content [45].

From amino acid analysis in 6 crops and the corresponding juice, the amino acid composition of the juice deviated only slightly from that of the crop but the contents of glutamic acid and aspartic acids were somewhat higher and correspondingly the content of other amino acids, in particular arginine, glycine, alanine, tyrosine and phenylalanine somewhat lower [46].

The degradation of protein and amino acids of juice extracted from ryegrass can be reduced by adding hydrochloric acid. For complete preservation, the pH must be less than 3. Heating to 80 ºC also has a preservative effect [47].

3.2.4. Proteins

Leaf protein concentrate is obtained by green crop fractionation. The acceptability of leaf protein concentrate in the human diet has been discussed by McDougall [48].

Lesnitski has also advocated [49] the manufacture of high protein feeds from green mass as a partial replacement of, for example, soybean protein and dried skim milk.

3.2.5. Silica

In comparison with elements commonly associated with the nutrition of higher plants, silicon has received relatively little attention. Biogenic amorphous silica (BAS) is a natural constituent of living matter. In some plants, a portion of BAS exists externally as pointed or irregularly shaped fibres, and these have been implicated as human toxicants [50].

Silica deposits commonly called phytoliths occur in cell walls, cell lumens or in extracellular locations. Silicification occurs in roots and the shoot including leaves, culms and in grasses, most heavily in the inflorescence. Biogenic silica structures provide support and protection [51].

Grasses are heavy accumulators, but considerable variation occurs between and within species. Deposition is heaviest in inflorescence bracts [52].

Silica has been detected in the leaf mesophyll of *Lolium multiflorum* at an estimated concentration of 1–2%. Samples were also subjected to a range of techniques for the removal of organic matter, that confirmed the presence of silica throughout the cell walls [53].

In *Lolium perenne*, only the epidermal cell walls of the leaf edges and the trichomes contained silica [54].

The *Lolium perenne* variety Fortis which shows some resistance to stem borer and had many silica bodies between the veins of the leaf sheath [55].

The silica content of each of 4 cuts of 3 *Dactylis glomerata*, 4 *Festuca pratensis*, 5 *Lolium perenne*, 5 *Lolium multiflorum* are reported by Puffe and colleagues [56]. Silica content is lower in legumes than in grasses.

3.2.6. Alkanes

The total *n*-alkane (C27–C35) contents of *Dactylis glomerata*, *Lolium multiflorum* and *Lolium perenne* were found to be 143, 681 and 531 mg/kg dry matter in the species, respectively. In all cases, C29 and C31 had the highest concentration [57]. These levels do not appear to be high enough for commercial exploitation.

3.2.7. Starch

The starch content of forage grasses is low: a maximum of 3% of starch is accumulated in field grown grasses. Cocksfoot contains more starch than *Lolium perenne* or *Lolium multiflorum* [58]. This low level rules out the industrial use of forage grass starches.

3.2.8. Minerals

The dry matter of extracted juice of *Lolium perenne* has a high mineral content [46] that may be exploitable as plant fertiliser (see 5.8).

3.2.9. Alkaloids

Perloline, perlolidine and loline are alkaloids of the *Lolium* spp. The toxicity of lolium species is due to a symbiotic fungal infection of plants [59]. The alkaloids are effective antifeedants and may offer utility in the agrochemical industry but their low quantities may make extraction non-viable (see 5.4).

3.2.10. Antifreeze protein

A plant antifreeze protein from *Lolium perenne* has been reported [60]. Present in organisms enduring freezing environments, antifreeze proteins have the ability to inhibit damaging ice crystal growth. The macromolecular antifreeze protein present in *Lolium perenne* has superior ice recrystallisation inhibition activity when compared with fish and insects antifreeze proteins [61].

4. Processing

Green crop fractionation was considered in 1980 as a means of providing edible protein for man [48]. Fractionation separates the crop into pressed matter which, with the de-proteinised juice, can be used to feed ruminants, and leaf protein concentrate that can be give directly to man [48].

The machinery for fractionating green herbage has been described in 1987 [62]. Fibre fractions and juice fractions are typically separated. The process of obtaining and storing the fractionation products of green herbage involved cutting and chopping of the plant material, pressing out the juice, purifying and conserving the juice, isolating and coagulating the protein, drying of the material left after pressing and of the juice itself and then separating the coagulate from the grass whey into containers for storage.

Further developments have occurred since then and the pilot plant developed by Avebe [11] has a patent pending on the refining process. It is not entirely clear but it is likely that the process consists of hammer-milling and extraction of the sap with a screw press. A prototype fractionation implement has been developed by IMAG-DLO and an industrial partner (Avebe?). The further processing of the press sap and the press cake is discussed [63].

Grass juice and crude protein have been separated and concentrated with a self-cleaning disc-type centrifugal machine [64]. The crude protein content of separated and concentrated juice increased with rotor speed, discharge cycle time, feed flow rate, original crude protein content and temperature.

4.1. PROCESSING FOR FIBRE

Non-wood fibres have been used to manufacture all kinds of papers, including printing, writing and packaging. Interest in non-wood fibres has increased recently, but implementation is still lagging because of the lack of economically feasible, environmentally acceptable technology compatible with such feedstocks. Such feedstocks are expected to play an important role in improving the sustainability of the pulp and paper industry [65] thus permitting a more rational utilisation of forest resources. Non-wood fibre pulps can be used effectively in combination with recycled papers, improving many of their attributes and permitting an overall cost reduction because of a decrease in the use of starch. Collecting non-wood fibres and using them for pulp and paper production could provide an alternative, non-food use for the land in those countries that have agricultural surpluses [66].

4.1.1. Semi-chemical pulping

A process that addresses the problems of pulping and liquor recovery has been described [66] as well as its application to the production of semi-chemical pulps. The process starts with atmospheric alkali cooking in a continuous digester. The semi-chemical pulp obtained is washed, refined and screened, and sent to the paper mill for corrugated paper manufacture. From the black liquor obtained in the pulping process, lignin is initially recovered by precipitation followed by a post-treatment to improve filterability. Most of the silica remains with the filtrate and the resulting lignin cake is high in purity and contains less than 1% silica and less than 3.5% sugars. Lignin sales increase overall mill revenues and make possible a reduction in minimum plant scale required for economic operation. The filtrate after lignin recovery can be processed in a biological treatment plant. Alternatively, oxygen-based wet oxidation of the filtrate can be used to generate energy and green liquor. From the latter, a precipitate that typically accounts for 70–90% of the silica in the wet-oxidation feed can be filtered, effectively purging silica from the cycle. The filtered green liquor can be causticised to generate white liquor for re-use in pulping.

4.1.2. Steam explosion

Steam explosion of ryegrass straw has been reported in a patent application to yield separate portions of usable straw pulp and a usable aqueous by-product comprising lignins and hemicellulose. The pulp was blended with Kraft pulp and old corrugated containers to make linerboard [67].

4.1.3. Mechanical pulping

A recent Chinese patent describes the production of non-polluting grass pulp and a method for reclaiming its by-product [68]. Grass is processed into refined grass chip and refined grass residue, the refined grass chips are treated by soaking with water, softening, washing and pulping to produce high quality grass pulp, and the refined grass residue is mixed with an additive containing functional preparation (organic selenium, organic calcium) and carrier (refined grass powder) to produce a high-quality fibre feed.

4.2. PROCESSING FOR CHEMICALS

Products based on renewable raw materials are predicted to become an important substitute for petroleum-based goods within the next two decades. The chemical components of forage grasses can potentially offer a broad range of chemical starting materials and higher value-added intermediates to offset demand for petroleum-based materials. The key to effective utilisation of these components is their effective extraction and/or conversion in a pure enough form and in an environmentally benign way. Products obtained by fermentation or direct extraction are discussed below.

4.2.1. Fermentation

Today, only a few chemicals are produced from renewable resources by fermentation. In Europe, the biotechnological production of lactic acid, acetic acid and ethanol are the only processes that are currently applied in technical scales and which can compete with petrochemical manufacturing routes. While the fermentation routes are limited by metabolic pathways of microorganisms, a wide range of chemicals can be produced. Most natural compounds are degraded by some type of microbe and even many man-made compounds can be attacked by bacteria. In environments devoid of oxygen (or other suitable inorganic electron accepter), this degradation involves fermentation. Fermentation is brought about under controlled conditions by inoculation of a substrate with an appropriate yeast or bacterium. These organisms act upon the substrate to microbiologically convert it to the desired product.

4.2.1.1. Lactic acid

The effects of various fermentable carbohydrates on silage fermentation in *Dactylis glomerata*, *Lolium perenne*, *Lolium multiflorum* and *Festuca pratensis* were as follows: D-fructose and D-glucose monomers and oligomers were converted almost exclusively to lactic acid whereas D-galactose and L-arabinose were metabolised to ethanol and acetic acid [28]. Indeed it is well-known that the common forage grasses yield lactic acid by fermentation as a primary product of the ensiling process.

Lactic acid represents a chemical with a small world market volume of 54500–59000 tonnes per year. While the market for traditional applications of lactic acid is estimated to be growing by 3–5% annually, new products based on lactic acid may increase the world market share significantly. Lactic acid is produced on an industrial scale mainly by fermentation. After heating to approximately 70 ºC, the broth is acidified with sulfuric acid to pH 1.8. The precipitated salts and biomass are removed by filtration and the resulting liquid is treated with activated charcoal to remove any colourants. The clarified lactic acid solution is then ion exchanged and concentrated to 80%. Good purification can also be obtained by liquid-liquid extraction where lactic acid is extracted into an organic solvent and then back-extracted into water, or by calcium salt formation and a re-release of the acid (for pharmaceutical grade material) [69].

4.2.1.2. Lysine

Green juice from *Lolium multiflorum* has been shown by Andersen and Kiel [69] to be well-suited as a medium for lysine fermentation. After pre-treatment with lactic acid bacteria, it is possible to obtain high enough contents of bio-available essential amino acids methionine and lysine that can be stored for months.

The world market for lysine comprises some 250,000 tonnes per year and is currently growing at 8–10% per year. Different strains of *Corynebacterium* can be used in lysine fermentation.

4.2.1.3. Xylitol

The production of xylitol comprises two stages: solubilisation of xylans from the plant biomass followed by conversion to the sugar alcohol. Chemical reductions are difficult to perform economically on the industrial scales required but biological conversion is essentially simple and a very low-energy process.

Xylans are easily extracted from grasses by acid-catalysed steam treatment [70]. Conditions are typically 10 min exposure at 170 °C to 197 °C in the presence of Lewis acids or calcium chloride to liberate 6% of the water-soluble xylans.

The processes for xylitol production by fermentation have been reviewed [71]. Chemical reduction is effected by hydrogenation using nickel-alumina and production rates of 75kg/h have been achieved. The biological processes are usually based on fermentation by *Candida* species of which many are known to be industrially useful.

Recovery of the xylitol is the rate- and cost- limiting step of the whole process and may take a week to complete. However, a recent report suggests that recovery by crystallisation is more feasible than previously thought [72].

The composition of industrial raw material media for conversion of D-xylose into xylitol by yeast fermentation has been optimised by Plackett-Bumman design and response surface analysis [73]. A conversion rate of 80.4% has been obtained in an optimised medium that is prepared from inexpensive industrial raw materials.

Mixed cultures have been used for the production of xylitol and ethanol from a mixture of cellulosic D-glucose and hemicellulosic D-xylose and for the production of xylitol from D-xylose in a hemicellulosic sugars' mixture [74]. For ethanol production, the process was studied in continuous aerated conditions. For xylitol production, the utilisation of *Lactobacillus reuteri* in association with the xylitol-producing yeast, *Candida guilliermondii* permits to reduce the accumulation of arabinitol from L-arabinose and to produce xylitol. Similar fermentative parameters were obtained from wheat straw hemicellulosic hydrolysate [74].

Xylitol may be recovered from fermented hemicellulosic hydrolysates by a crystallisation methodology. The procedure consists of dilute solution evaporation up to super-saturation, super-saturated solution cooling, separation of crystals by centrifugation, and final filtration [72].

D-Xylose may be isolated and used as the starting substrate in which case the hydrogenation of D-xylose to xylitol in a trickle bed reactor at 120 ºC, 4.0 MPa hydrogen pressure has been studied [75].

Hydrogenation over a Raney nickel catalyst in the range 40–70 bar and at temperatures of 80–140 ºC afforded xylitol as the main hydrogenation product but small amounts of xylulose and arabinitol were detected as by-products. A process simulator, using the kinetic and mass transfer effects, was developed to predict the behaviour of industrial reactors [76].

A European Project 'Development of xylo-oligosaccharides and xylitol for use in pharmaceutical and food applications' [77] has goals to (1) optimise selective processes for lignocellulosic wastes fractionation; (2) purify different xylo-oligosaccharides as intermediates and (3) optimise the fermentation of xylo-oligosaccharide for xylitol production. Raw materials studied include corn cobs, wheat bran, breweries' spent grains and *Eucalyptus* wood biomass.

4.2.2. Direct extraction

4.2.2.1. Fructans

High fructan concentration grasses may be suitable for processing and extraction in the same way as chicory roots as an industrial source of D-fructose [78].

4.2.2.2. Silica

A process has been described for the manufacture of high purity amorphous silica from bio-genic materials [79]. Rice hulls are given as the example. The hulls are finely divided, screened, subjected to surfactant wash, rinsed and soaked in water to accelerate and enhance penetration of an oxidising solution. The oxidising solution removes organic compounds, and volatile impurities are removed by heated oxidation to leave silica. The remaining silica may be rinsed with water, acid solution or other solution to remove even trace impurities. At the end of the process, a fine white amorphous silica of extreme purity is produced.

5. Products and Target End-Uses

5.1. CHEMICALS/SOLVENTS

5.1.1. Acetone, butanol, ethanol

An acetone-butanol-ethanol (ABE) blend may serve as an excellent car fuel, which can be easily mixed not only with petrol but also with diesel. The fermentative production of ABE used to be the second largest industrial fermentation after ethanol production. In 1945, 60% of the butanol demand of the United States was met by fermentation. Worldwide, no ABE plants have operated since 1981.

However, an American study has re-evaluated [80] the economics of production of acetone, butanol and ethanol from corn and grasses, following the advent of a new hyper-butanol-producing strain of *Clostridium beijerinckii*. Assumptions such as by-product credit for gases and complete conversion of liquors to fermentation by-products have been taken into consideration. Assumptions were also made regarding the costs of starting raw materials. Worst case scenarios were outlined and outcomes appear in almost all cases to be feasible.

The production of ethanol from *Lolium multiflorum* has been studied in Ireland [81]. Production is a practicable proposition at yields of about 1000 litres per hectare per year. It was concluded at the time of the work (1990) that even at greatly increased energy prices, exploitation of the process appeared to depend on the value of the residual grass in terms of its suitability for silage making or use as an animal feed (hay).

5.1.2. Hydroxymethyl furfural

Hydroxymethyl furfural is a furan derivative that can be synthesised in different ways from sugars such as D-fructose, D-glucose, D-sucrose and high-fructose syrups. Hydroxymethyl furfural is considered as an alternative to petrochemicals because it can be transformed into a variety of intermediate chemicals which have a possibility for industrial applications [82]. Many such applications include the field of polymers, pharmaceuticals, insecticides and opto-electronic materials.

5.1.3. Ethyl Lactate

Ethyl lactate works in a range of micro-electronic and chemical applications and offers the benefit of extreme purity. The solvent is useful as:

- a photo resist carrier solvent
- edge-bead remover
- clean-up solvent for semiconductor manufacture
- low-cost, environmentally friendly solvent

Ethyl lactate is non-toxic and biodegradable. The U.S. Food and Drug Administration has approved its use in food products. It is suitable for a wide range of industrial and consumer uses and could replace up to 80% of conventional chemical solvents. However, the cost of producing it has been too high to compete with lower-priced chemical solvents. Ethyl lactate sells for US$3.50 to US$4.50 per kg, compared with about US$2.00 to $3.75 per kg for conventional chemical solvents.

Ethyl lactate is produced by esterification of lactic acid with ethanol and sulfuric acid as a catalyst.

5.2. HUMAN NUTRITION

5.2.1. Vegetable protein

Increased production of plant protein may be required to support the production of protein-rich foods that can replace meat in the human diet to reduce the strain that intensive animal husbandry poses to the environment. Eight crops were appraised in Europe as sources of sustainable protein-rich foods [83]. Peas, lucerne and grasses were the most promising with grasses estimated to yield 2500 kg of protein per hectare.

5.2.2. Health drinks

Gaynor and Hickey have reported [84] a new nutritional powder composition consisting of a blend of grass juice powders that is readily soluble in a fluid for ingestion by humans. When digested, the mixture provides users with an energy boost and associated feelings of well-being when the mixture is taken as part of a regular regimen to supplement normal nutritional intakes.

5.2.3. Cholesterol mediation

Polysaccharide-lignin complexes from fodder grasses have been demonstrated to be active sorbents of cholic acid, a metabolite of cholesterol [85]. Equations were derived for calculating the sorption of cholic acid by the grass material. The equations can be used to construct dietary fibre having the desired properties, for example for cholesterol metabolism in man and animals.

5.2.4. Food preservatives

A recent Japanese patent describes [86] the preparation of food preservatives from grass fibre powder. The grass fibre powder is contained within a moisture-permeable, water-impermeable microporous biodegradable polymer film and in combination act to preserve food.

5.2.5. Xylitol

Xylitol, currently produced in the main from Finnish birch, commanded a £100M market in 2000 and demand in Europe is predicted to grow at 4.2% (average annual growth rate, AAGR) up to 2005 [87]. Consumption data for 2000–2005 are shown in *Table 5*. In global terms, Western Europe accounts for the greatest consumption of xylitol.

Table 5. Xylitol consumption by geographic area through 2005 [87]

Area	2000	2005	AAGR% 2000-2005
Western Europe	13	16	4.2
U.S.	10	13	5.4
Japan	7	8	2.7
Asia (except Japan)	3	4	5.9
Rest of the world	1	2	14.9
Total	34	43	4.8

Xylitol prices with current ranges of US$4 to US$5 per kg are fairly high although the price has decreased substantially over the last ten years [88]. This was a consequence, ultimately, of the economies of scale of production. The raw material for production is hemicellulose, which is abundantly available, with the main industrial application being paper production where the nature of the industry tends to drive the price down. Some D-xylose is used in pet food preparations as a filler and digesting aid. Quantities used for xylitol production are relatively small and therefore a strong impact on the price of xylitol is not seen. As the application of xylitol is now standard in chewing gum preparations with only marginal substitution, higher prices of xylitol would not have a substantial impact on its use. The overall trend for the price of xylitol is down 2 to 3 percent per year (Table 6).

Table 6. Price trends for xylitol, through to 2005 [88]

($/kg)			AAGR%	
1995	2000	2005	1995-2000	2000-2005
7.2	4.5	3.8	-9.0	-3.3

The number of producers of xylitol is quite small. Firstly, xylitol is a relatively small market because it is a relatively new product. Xylitol is produced mainly by companies who are dedicated to polyols, such as Cerestar and Roquette. The market share by company [89] is shown in *Table 7*.

Danisco also has some xylitol production facility in place. The multi-purpose plant originally belonged to Cultor (Xyrofin Oy). After the take-over of Cultor by Danisco, this plant was modernised and is mostly active in some sorbitol production. The total capacity is small. There are constant rumours that Cultor/Danisco will stop the production of polyols completely. Occasionally, Chinese traders also offer xylitol. However, it is not clear in which plant such product is manufactured.

Table 7. Xylitol market share by producers, 2000 [89]

Company	Market Share %
Cerestar	30
Roquette	40
Danisco	25
Others	5

Xylitol is now a standard component of chewing gum preparations. The biotechnological production of xylitol from the D-xylose in hemicellulosic hydrolysates of biomass including straws and stems has been carried out in Brazil with the yeast, *Candida guillermondii* [90]. Whilst wheat and rice straws were used as substrates, forage grasses were not studied. It is our expectation that forage grasses would yield D-xylose and hence xylitol in the same fashion.

Further Brazilian work has identified species of grasses related to esparto as sources of xylitol [91]. The grasses included *Aristida pallens*, *A. setifolia* and *A. reparia*. *A. pallens* was the most satisfactory source in terms of quantity and production of xylans high in D-xylose and with only traces of impurities.

5.2.6. Fructans

In the search for substances that can replace a proportion of fat in fat spreads and so contribute to lower fat intake, requirements for an 'ideal' fat have been formulated.

A promising carbohydrate, inulin, a fructan, has been identified as suitable. Inulin can be made from chicory on an industrial scale but it is also present in large quantities in forage grasses. It is freely available and not limited by patents. Furthermore, it can be combined with dietary fibre for manufacturing diet and health foods [92]. Inulin is used in many products as a drug-carrier or conjugate [93].

5.3. BIOREMEDIATION

Silage residues have been used as sources of natural chelates to improve the ecological and economical balance of leaching techniques for the remediation of metal-polluted soils. Silage effluent containing various aliphatic carboxylic acids, sugar acids and amino acids was used to remove about 75% of cadmium and more than 50% of copper and zinc from contaminated soils [94]. The trial supported the conclusion that biomass residues have potential to serve as extractants in remediation techniques.

Porous carbon fibres have been obtained from cut grass by baking in an oven in a roped form for the formation of coiled carbonised fibres. The porous carbon fibres are useful for sound absorbers, adsorbents, purification materials and radio wave absorbers [95].

5.4. ANTIFEEDANTS

Festuca arundinacea and *Lolium perenne* can become infected with fungal endophytes (*Neotyphodium* spp). The symbiosis between plant and fungus leads to the synthesis of alkaloids that have been shown to be either toxic or act as feeding deterrents against insect pests. Alkaloid production/accumulation is enhanced by decreased mowing frequency in *Festuca arundinacea* and *Lolium perenne* [96]. Such alkaloids may have a role as insecticides for agrochemical use [97,98] or in the clinic as a result of their pharmacology [59].

5.5. SILICON PRODUCTS

5.5.1. Silicon carbide

Ryegrass has been proposed as a raw material for the production of polytypically pure β silicon carbide in an economically effective and ecologically compatible procedure. When the particle size of starting raw material is defined then the particle size of the developing silicon carbide is also controlled [99]. Silicon carbide has many industrial applications and is a valuable chemical used for cutting, grinding and polishing applications. Silicon carbide is also used in the electronics industry in hostile environments where its ability to function in high temperature, high radiation conditions overcomes the limitations of conventional silicon-based systems. It is used as a component of blue and violet light-emitting diodes.

5.5.2. Filter aids

Highly purified biogenic silica has an intricate and diatomaceous SiO_2 structure and a high SiO_2 specific volume. These products exhibit extreme brightness and can be used in filtration processes [100]. In one example the adsorbent has been used for the removal of proteins in chillproofing of beer [101].

5.5.3. Zeolites

Artificial zeolites are manufactured by heating a mixture of grass husks and plants with aqueous alkali solutions to elute silicic components. These are mixed with aluminium-enriching agents and treated under heat and pressure. The zeolites show high cation exchange property to be useful as fertilisers [102].

5.6. THERMOPLASTICS

Adhesive films have been produced from grass fibre by the preparation of alkali cellulose and then film-forming. The adhesive film may be used as agricultural mulching film or packing material [103]. Grass fibre has been proposed as a component of a biodegradable protein/starch-based thermoplastic composition. The grass fibres function as reinforcement filler. The composition is processed by conventional methods such as extrusion and injection moulding, into packaging material or articles that are low density and have high compressive strength, tensile strength and good resilience [104].

5.7. PULP AND PAPER

A study of the pulping characteristics and mineral composition of 16 field crops grown in Finland showed that the most suitable species for alkali cooking were the grass and cereal crops, which gave the highest pulp yields and the lowest amounts of rejects. On the basis of the test results, *Festuca arundinacea*, *Festuca pratensis*, reed canary grass and spring barley were selected for further study [105]. Further work selected *Festuca arundinacea* and reed canary grass as worthwhile candidates [5].

5.8. NUTRIENT SUPPLEMENT FOR CROPS

Grass clippings have been investigated to determine how effective they are as a nutrient supplement for cabbages [106]. Even at the same application rate, soil ammonium- and nitrate-nitrogen concentrations and yields were very different during each year of a three-year trial. The authors concluded that to avoid over or under-fertiliser application, then targeted nitrogen supply from grass clippings should be less than necessary to grow cabbage. Additional nitrogen fertiliser can then be applied as necessary.

5.9. EMULSIFIERS

The polysaccharide and protein components of *Festuca* spp cell walls have been transformed into emulsifiers by extraction and treatment with xylan-hydrolysing enzyme preparations. The emulsifiers are useful, for example, for food, cosmetics, pharmaceuticals, industrial chemicals' applications [107].

5.10. LACTIC ACID

Lactic acid is well-established in the food industry as an additive for preservation, flavour, and acidity. New applications for lactic acid include the use of derivatives such as ethyl esters to replace hazardous solvents like chlorinated hydrocarbons in certain industrial applications, Furthermore, lactic acid may be polymerised to biodegradable plastics as demonstrated by Danone Inc. in the form of yoghurt pots. Lactic acid is typically made by micro-organisms that are able to convert or ferment sugars obtained from agricultural crops, such as corn. The lactic acid market for the U.S. is currently about 50,000 tons/yr.

Lactic acid is used to manufacture cheese, confectionery, chewing gum, alcoholic beverages, baked goods, puddings and snacks. It may also have physical or functional effects permitting uses as anti-microbial agents, curing and pickling agents, flavour enhancers, flavouring agents and adjuvants, pH control agents.

Lactic acid and it salts are:

- Good buffers
- Effective humectants
- Natural preservatives
- Safe since they are used in the food and pharmaceutical industry
- Natural constituents of the skin as part of the natural moisturising factor

Lactic acid is a valuable compound in the sense that it is vital to the food industry.

6. Recommendations for Further Areas of Research

6.1. BIOMASS PROCESSING

Plant biomass represents both the dominant foreseeable source of feedstocks for biotechnological processes as well as the only foreseeable sustainable source of organic fuels, chemicals and materials. A variety of forms of biomass, notably many cellulosic feedstocks are potentially available at a large scale and are cost-competitive with low-cost petroleum whether considered on a mass or energy basis, and in terms of price defined on a purchase or net basis for both current and projected mature technologies, and on a transfer basis for mature technology. Thus, the central, and surmountable, impediment to more widespread application of biomass is the general absence of low-cost processing technology.

Additionally, there are a few major hurdles in the use of forage grasses like their availability at a constant quality throughout the year, the fractionation technology, limitations due to the metabolism of micro-organisms, and the lack of integrated technologies.

Technology and research challenges associated with converting plant biomass into commodity products must be considered relative to overcoming the recalcitrance of cellulosic biomass (converting cellulosic biomass into reactive intermediates) and product diversification (converting reactive intermediates into useful products).

Advances are needed in pre-treatment technology to make cellulosic materials accessible to enzymatic hydrolysis, with increased attention to the fundamental chemistry operative in pre-treatment processes likely to accelerate progress.

Important biotechnological challenges related to the utilisation of cellulosic biomass include developing cellulase enzymes and organisms to produce them, fermentation of D-xylose and other non-glucose sugars, and ‘consolidated bio-processing’ in which cellulase production, cellulose hydrolysis, and fermentation of soluble carbohydrates to desired products occur in a single process step [108].

6.2. ETHANOL PRODUCTION

The main problems in ethanol production are (1) comparatively low yields and concentration of monosaccharides in plant hydrolysates; (2) large amounts of impurities in hydrolysates; (3) incomplete assimilation of substrate compounds during fermentation; and (4) severe contamination of the environment by wastewater. New methods of hydrolysate and wastewater purification and a recycling scheme for water used in ethanol production are required.

6.3. *ARISTIDA PALLENS*

A literature report describes this South American grass as a satisfactory source of xylans high in D-xylose, with only traces of impurities. This grass may then be an ideal raw material for the ultimate production of xylitol. Whilst it grows in Brazil and Paraguay, there is no published data to indicate its ability to grow in Northern Europe. This grass could be considered as an industrial crop for xylitol/fibre production.

7. Miscanthus

Miscanthus is a perennial plant with an estimated productive lifetime of at least 10–15 years, and both the stems and leaves of the crop can be harvested annually. *Miscanthus* is a promising non-food crop, yielding high quality lignocellulosic material for both energy and fibre production. It is characterised by relatively high yields, low moisture content at harvest, high water and nitrogen use efficiencies and an apparently low susceptibility to pests and diseases [109].

Much *Miscanthus* work in Europe has been carried out under the umbrella of the *Miscanthus* Productivity Network [110]. Its main objective was to generate information on the potential of *Miscanthus* as a non-food crop in Europe.

Whilst water-use efficiency of *Miscanthus* is high, it is nevertheless requires irrigation at most sites to achieve its maximum potential yield [111]. The first phase of *Miscanthus* production, planting, is potentially the most capital intensive one. The high investment required for planting arises from the inability to propagate *Miscanthus* by seed in Europe. Thus, highly mechanised, cost-effective methods of plant propagation need to be developed [112]. Bical ltd in the UK is making good progress towards this goal with support from the DTI [18].

7.1. APPLICATIONS

7.1.1. Pulp and paper

In recent years, interest has risen in the use of *Miscanthus* as a suitable raw material for the paper industry [113,114]. At present, the use of fibres from non-woody crops for paper pulp production in Europe is below 1% of total production [115]. Paper pulp from non-woody crops is mainly produced in developing countries and the raw materials which are most widely used are straw, bagasse and bamboo.

It has been established that the production of paper-pulp from cellulose-rich herbaceous crops is possible by several different chemical or thermomechanical processes. *Miscanthus* has been exploited for the production of paper pulp in China. In addition, a number of investigations have been carried out in European countries on the production of paper pulp from *Miscanthus* using both conventional and innovative processes.

Wheat straw and *Miscanthus* chips have been evaluated as raw materials for fibre and pulp production following delignification. Both were fractionated to give high chip yields [116]. The mechanical strengths of *Miscanthus* pulp are very high compared to the respective properties of wheat straw pulp [117].

Steam explosion technology was used by Pignateli and co-workers to obtain valuable paper products by processing *Miscanthus* fibre with high yield and low pollution. The pulps showed significant and positive evolution of the main physical, mechanical and optical properties desired for utilisation of the resulting paper for various applications [118].

7.1.2. Energy Production

Miscanthus can be used as a raw material for energy production. Energy production alternatives that have been examined are co-combustion with coal and combustion in farm heating plants. The alternatives have been discussed [119].

7.1.3. Fermentation products

The chemical composition of *Miscanthus* leaves and stalks have been analysed [120] in a comparative study of green plants harvested in September and dry plants harvested in March. Analysis of the lipophilic and hydrophilic constituents indicated that green plants contained higher amounts of glyceride and other fatty esters than dry plants. The predominant fatty acids were linoleic acid, linolenic acid and palmitic acid. D-Sucrose, D-glucose and D-fructose contents of green plants also exceeded those of dry plants. Whereas the leaves of dry plants were a poor source of constituents due to senescence and leaf-fall during winter, those of green plants were rich in fatty acids and other lipids. The green stalks were particularly rich in soluble sugars. On the basis of the high cellulose, sugar and lignin content, *Miscanthus* has been evaluated as a raw material for the production of fermentable pentose sugar solution. Papatheofanous and co-workers [117] reported that 86% (w/w) of the original pentosans are hydrolysed to a fermentable sugar solution after a two-stage chemical treatment.

7.1.4. Construction/building materials

Miscanthus has been a subject of interest as a source of fibre to be used in building materials. The European Union supported a demonstration project in 1992 which investigated the used of *Miscanthus* for the production of panel boards and building blocks [121]. Harvey and Hutchens [122] reported that *Miscanthus* fibre structure is particularly suitable for the production of medium density fibreboard (MDF). They also found that sample MDF made from *Miscanthus* was comparable with that made from wood chips.

7.1.4.1. Light natural sandwich materials (LNS)

LNS materials are light building materials used for plane and mould structural parts with high form stability at low weight, used for a broad range of applications. LNS can substitute sandwich materials made from plastics or light metals which are regarded as high technology products as well as wood-based materials or insulating materials. The use of *Miscanthus* as the core material for LNS has been demonstrated in Germany [123]. Despite the superior quality of *Miscanthus* stems, there are still problems regarding the stem quality for LNS production.

A recent project on LNS funded by the European Commission (FAIR) co-ordinated by Kai-Uwe Schwarz, DIAS will provided the basis for scaling up the production of high performance LNS from *Miscanthus*.

7.1.4.2. Thatching

Miscanthus has been used as a material for centrutries in Japan. A project entitled Thatching: use of *Miscanthus* was conducted in Denmark during 1995-6, aimed at scaling up production and investigating possible commercialisation of the utilisation of *Miscanthus* for thatching. One hectare of *Miscanthus* was established in the spring of 1995, a preliminary market survey was made, yields were measured, the quality of the straw for thatching was evaluated, preliminary tests on harvest machinery were carried out. Bical Ltd is actively promoting its use in the UK by working with UK thatchers. A large potential market exists for UK grown *Miscanthus* since, at present, up to 40% of material (reed grass) is imported.

7.1.5. Bio-remediation

Miscanthus has been assessed for its ability to grow in west Cornwall on land which was polluted by heavy metals as a result of tin mining. The growth and heavy metal uptake of *Miscanthus* grown on soils and mine waste polluted by copper, zinc, and arsenic were studied over a two year period by CSM associates [124]. The results obtained showed that the copper arsenic and zinc content in above-ground biomass was slightly lower in *Miscanthus* grown on unpolluted soil than on polluted soil. However, *Miscanthus* grown on mine waste did not show enhanced metal uptake.

Miscanthus was demonstrated to grow well in soil amended with sewage sludge, despite high concentrations of phytotoxic metals, and also in lead-contaminated soil [125]. However, as in the cases above metal uptake was such that it could not be considered as a means of bio-remediation in contaminated material. In soil contaminated with artificial spoils, however, *Miscanthus* did show significant concentration of zinc at 500 ppm. For other metals, the metal concentrations proved too phytotoxic for the plants to survive [125].

Further research was recommended to assess the full environmental benefits and risks of growing and using such plants [124].

7.1.6. Other possible uses of Miscanthus

The increasing number of publications, communications, reports and meetings regarding *Miscanthus* in past years has brought about an interest in the investigation and development of commercial end uses for the crop. Some of the uses which have not already been mentioned are the use of *Miscanthus* fibre material in geo-textiles, its use as canes to support ornamental pot plants and the use of *Miscanthus* ash arising from combustion processes as a fertiliser [126].

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