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Research review paper

Fungal treated lignocellulosic biomass as ruminant feed ingredient: A review

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ABSTRACT

In ruminant nutrition, there is an increasing interest for ingredients that do not compete with human nutrition. Ruminants are specialists in digesting carbohydrates in plant cell walls; therefore lignocellulosic biomass has potential in ruminant nutrition. The presence of lignin in biomass, however, limits the effective utilization of cellulose and hemicellulose. Currently, most often chemical and/or physical treatments are used to degrade lignin. White rot fungi are selective lignin degraders and can be a potential alternative to current methods which involve potentially toxic chemicals and expensive equipment. This review provides an overview of research conducted to date on fungal pretreatment of lignocellulosic biomass for ruminant feeds. White rot fungi colonize lignocellulosic biomass, and during colonization produce enzymes, radicals and other small compounds to breakdown lignin. The mechanisms on how these fungi degrade lignin are not fully understood, but fungal strain, the origin of lignocellulose and culture conditions have a major effect on the process. *Ceriporiopsis subvermispora* and *Pleurotus eryngii* are the most effective fungi to improve the nutritional value of biomass for ruminant nutrition. However, conclusions on the effectiveness of fungal delignification are difficult to draw due to a lack of standardized culture conditions and information on fungal strains used. Methods of analysis between studies are not uniform for both chemical analysis and in vitro degradation measurements. In vivo studies are limited in number and mostly describing digestibility after mushroom production, when the fungus has degraded cellulose to derive energy for fruit body development.

Optimization of fungal pretreatment is required to shorten the process of delignification and make it more selective for lignin. In this respect, future research should focus on optimization of culture conditions and gene expression to obtain a better understanding of the mechanisms involved and allow the development of superior fungal strains to degrade lignin in biomass.

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Introduction

The human population is increasing and consumption patterns are changing towards animal based products such as meat and milk, increasing the demand for animal feed (Boland et al., 2013). To ensure food security, future feed ingredients should not compete with use in human nutrition. Lignocellulosic biomass, consisting of plant cell walls containing lignin, cellulose and hemicellulose, is one of the most abundant waste streams in the world. Cellulose and hemicellulose are polysaccharides which, when converted to sugars, can be used as an energy source. The presence of lignin in biomass forms a major obstacle for the effective utilization of cellulose and hemicellulose by rumen microbes and presently, chemical and/or physical treatments are used to degrade lignin (Chaturvedi and Verma, 2013). There is an increasing interest in alternative treatments to the use of chemicals or expensive physical treatments such as the utilization of white rot fungi to degrade lignin in biomass as indicated by the growing number of scientific papers on this subject. Although white rot fungi have a large potential in selectively removing lignin in biomass, this method requires further optimization to be competitive to conventional treatments such as urea or other alkali treatments. This review intends to provide an overview of research conducted to date using white rot fungi and also intends to illustrate that it is difficult to draw general conclusions from data in the literature on how to improve this type of biological pretreatment. This is in part due to the large variation in methods used or lack in standardization of methods and, sometimes, to the absence of important details such as strain identity. Although the same principle of lignin removal counts for biofuel production, this review focuses on ruminant nutrition. This overview identifies the main steps in the fungal pretreatment that need to be standardized and further optimized to make this method a competitive alternative for more conventional approaches.

Plant cell walls and lignin

Lignocellulosic biomass can be defined as plant materials with a high content of lignified plant cell walls which contain, besides lignin, also hemicellulose and cellulose. Further, only small amounts of ash, proteins and pectins are present in lignocellulosic biomass (Grabber, 2005). Lignin content and composition differ between species and changes during plant maturation (Grabber, 2005; Susmel and Stefanon, 1993) when more lignified secondary cell walls are developed (Grabber, 2005). Cellulolytic microbes in the rumen can degrade most of the carbohydrates present in plants, and therefore roughage high in plant cell walls can be used as a main feed ingredient for ruminants. Cellulose, and also hemicellulose, in plant cell walls is limitedly accessible to the rumen microbes, because of the direct (covalent) or indirect (ester or ether) linkage to lignin (Ding et al., 2012; Jalc, 2002; Jeffries, 1994; Susmel and Stefanon, 1993; Vanholme et al., 2010). Cellulose is present in either a crystalline or amorphous form with the former being more difficult to degrade by enzymes (Dashtban et al., 2009). Although the term lignin describes a large group of aromatic polymers, in general, lignin consists of 3 building blocks namely *p*-coumaryl alcohol (*p*-hydroxyphenyl propanol), coniferyl alcohol (guaiacyl propanol) and sinapyl alcohol (syringyl propanol) (Jeffries, 1994; Vanholme et al., 2010). Lignin plays an important role in providing strength, protection against pathogens, improving water conduction and preventing degradation of structural polysaccharides by hydrolytic enzymes (Grabber, 2005). Lignin can only be degraded aerobically and is not degraded in the anaerobic condition in the rumen. As such, the amount of dietary lignin is, therefore, negatively correlated to dry matter digestibility (Arora and Sharma, 2009) and as such is used as an indigestibility marker (Van Soest, 1993). The structure of lignin is as important as the type and ratios of its constituent building blocks in terms of the degradability by wood decay fungi. Highly branched lignin is less degradable than more linear lignin (Grabber, 2005) and syringyl-rich lignin, for example, renders poplar more resistant to degradation by wood decay fungi (Skyba et al., 2013).

Plant residues with a high lignin content, although rich in cellulose and hemicellulose, are often regarded as (organic) waste. In the European Union, a total of ~153 M dry tons of agricultural residues are produced originating from, for example, wheat, barley, rye, oats, maize and forests (Diamantidis and Koukios, 2000; Ericsson and Nilsson, 2006; Scarlat et al., 2010). Municipal waste, approximately 10% of the total waste, has a variable composition and can contain amongst others paper and paper products, food and garden waste (Blumenthal, 2011; Eurostat, 2009). Organic waste is now mainly used in biogas and energy production, soil improvement, as bedding in animal husbandry, mushroom production and disposed of in landfills (Blumenthal, 2011; Scarlat et al., 2010). Another source of lignocellulose includes energy crops (e.g. miscanthus) specially bred for biofuels which contain a high carbohydrate content (McKendry, 2002).

Chemical and/or physical methods to degrade lignin

Current methods to increase the accessibility of cellulose and hemicellulose in lignocellulosic biomass include physical, physicochemical and chemical treatments. The effectiveness of these treatments has been reviewed by several authors. Therefore, only a short summary including advantages and disadvantages will be provided here. For more in-depth discussions on this topic, see articles by Agbor et al. (2011), Chaturvedi and Verma (2013), Hendriks and Zeeman (2009), Sarkar et al. (2012) and Sarnklong et al. (2010).

Physical treatments of lignocellulosic biomass to improve digestibility, like grinding, soaking or pelleting, can result in a lower cellulose crystallinity resulting in cellulose being better degradable by enzymes (Agbor et al., 2011; Hendriks and Zeeman, 2009; Sarkar et al., 2012; Sarnklong et al., 2010). These physical treatments have a limited feasibility to be applied on farm, because machines or industrial processes are required (Sarnklong et al., 2010). However, often reduction of particle size is needed for successful processing (Mosier et al., 2005).

Chemical treatments with acids (e.g. sulphuric or nitric acids) or alkalis (e.g. sodium hydroxide or potassium hydroxide) result in a lower cellulose crystallinity and high sugar yields (Agbor et al., 2011; Hendriks and Zeeman, 2009; Sarkar et al., 2012). Although sodium hydroxide is used for pretreatment to improve digestibility of rice straw, it is an expensive method which can cause environmental pollution

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