APPLICATION OF FUNGI AND FUNGAL PRODUCTS IN BIOPULPING
PART II: BIOPULPING OF NON-WOOD FIBRE

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Biopulping is a solid-substrate fermentation (SSF) process where lignocellulosic materials are pretreated with fungi to improve pulping. This paper is the second of two reviews on biopulping where Part I dealt with the biopulping of wood. In this review, we examine the progress in the field of non-wood pulping, because it is evident that the diversity of pulping processes requires development of different SSF processes for optimal results. The global increase in utilisation of non-wood fibre has also led to increased interest in biopulping of these fibres. This review will focus on the biopulping of bagasse, because bagasse is of notable importance to the South African pulp industry.

INTRODUCTION

About 7 % of the world pulp production is from non-wood fibres [1], but non-wood fibre sources are becoming more important in the supply of plant fibre for pulp and paper products [2]. At present 330 mills worldwide, produce pulp from non-wood fibre [3], with two mills in South Africa that utilize bagasse as raw material. Biopulping of bagasse is, therefore, of special significance to the South African pulping industry.

The fungal pretreatment of any lignocellulosic substrate for pulping improvement is now described as biopulping [4], although this term has previously been used only to describe pretreatment wood. Biopulping of wood is usually accomplished by treatment of chips with white-rot fungi that are able to degrade lignin. Effective biopulping of wood has also been achieved with *Ophiostoma piliferum*, a fungus that does not degrade lignin [5, 6]. Improvement of pulping by *O. piliferum* has been ascribed to the reduction of wood extractives to improve penetration of pulping chemicals.

Investigations of non-wood biopulping have focussed only on the modification or reduction of lignin. Biopulping of these fibres, therefore, utilised only white-rot fungi. The biopulping processes are consequently influenced by similar restrictions to those of biopulping of wood and will require special engineering and management solutions [4, 7, 8]. The SSF by fungi will require a certain measure of asepsis during inoculation, as well as controlled temperature, moisture and aeration for growth [4, 8]. The implementation of biopulping processes for the treatment of different types of non-wood fibre will require specific solutions for each process.

BIOPULPING EXPERIENCE

One of the hurdles to overcome in the pulping of bagasse is the seasonal availability of the raw material [9]. This necessitates special storage practices, such as wet bulk storage, to preserve the fibre [10]. However, the long periods of storage (sometimes more than one year) also offer an opportunity to pre-treat bagasse to improve the pulping properties.

Biopulping of bagasse could have a number of advantages. A large quantity of water is used for the preservation of fibre in wet bulk storage of bagasse [9]. Biopulping fungi would also preserve the fibre and could be applied on bagasse with lower moisture content. In a semi-arid country such as South Africa, the saving of water would be a significant advantage. The lignin content of fibre and, consequently, in pulp could be reduced [11]. Fibre discolouration and pith content could be reduced, resulting in improved wet depithing [9]. The reduced lignin and pith content could result in reduced chemical consumption during pulping and in improved pulp quality [12].

Biopulping of bagasse is one of the relatively unexplored fields of biotechnology relating to the pulping industry, although many considerations make such a procedure theoretically feasible. For example, bagasse is stored for long periods [13], allowing enough time to treat raw material with fungi. Capital and other resources, currently used for the preservation of bagasse [13] could be diverted to the biopulping processes. Additionally, the colonization of bagasse by fungi is favoured by the residual sugars and the exposed surface area of the fibres [14].

Biopulping has been applied to several kinds of non-wood fibre, but the literature covers only initial trials where none of the processes were commercialised. A small number of publications deal with fungal pre-treatment of bagasse [15, 16] and do not refer to the soda pulping method that is widely used. One technique combines fungal pre-treatment of bagasse with the so-called Cuba-9 process [16], that is used to produce newsprint with a modified cold soda process prior to mechanical refining. Other publications deal with the fungal treatment of kenaf and jute prior to steam explosion and refining [17], gramineous plants prior to soda pulping [11] and biopulping of wheat straw [18].

The white-rotting fungi are the organisms most frequently used for biopulping of non-wood fibre [11, 15, 16, 17, 18]. These fungi are known to degrade all the wood components, but lignin

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is degraded in varying degrees of selectivity [18]. The selectivity with which lignin is degraded, depends on specific strains of fungi as well as the specific treatment conditions [18, 19]. Cellulase deficient isolates of *Phanerochaete chrysosporium* have been used to enhance selectivity [16], but wild type isolates of *P. chrysosporium* and a hybrid strain of *Pleurotus ostreatus* have also been used [15].

Different fungal species have been used to treat non-wood fibres other than bagasse. An isolate of *C. subvermispora* has been used for biopulping of kenaf and jute [17] and *Pleurotus ostreatus* for wheat straw [18]. *Phlebia radiata, Phanerochaete chrysosporium, Pleurotus ostreatus, Panus tigrinus, Phlebia tremellosa* and *C. subvermispora* were used to treat reed canary grass and tall fescue prior to soda pulping [11]. *Lenzites betulina* [12], *Pleorotus ostreatus* and *Phanerochaete chrysosporium* [15, 16] have been used for biopulping of sugarcane bagasse.

The most successful method for the production of inoculum of white-rot fungi for biopulping is to use a pre-inoculum of homogenized mycelium [12]. A pre-inoculum was also used by Johnsrud et al. [16] and inoculum was then produced in a fermenter. By using this method, it was possible to harvest inoculum after eight days. Although special media have been used [16], molasses can provide a convenient nutrient source, as it can be obtained from nearby sugar mills and is inexpensive [12].

In small-scale experiments dried, depithed bagasse (60 % to 70 % fibre) was soaked in water, after which moisture was adjusted to various levels between 50 % and 80 % [16]. The moist bagasse was then sterilized by autoclaving, inoculated and then incubated at 28 °C or 39 °C for 10 to 20 days. Screening experiments with bagasse were done with as little as 2 g of bagasse in 100-ml flasks, but bench scale treatments were done in 1-L flasks or 10 L cylinders [16]. In these bench scale experiments, reactors were flushed with oxygen and relative humidity controlled. Polyethylene bags filled with 1 kg sterilized bagasse at 70 % to 75 % moisture have been used in SSF for the production of biopulp and for the production of enriched animal feed [15]. Degradation was enhanced by oxygenation compared to aeration, especially when it was applied intermittently [16]. Optimal conditions were approximately 65 % moisture and 95 % relative humidity [16].

Moisture of the substrate is one of the key factors influencing SSF. It is important that water is available only as thin films on the bagasse surface to increase the surface-to-volume ratio for oxygen and carbon dioxide transfer [8, 20]. On the other hand, low moisture increases the risk of fire [13] and limits fungal growth [8]. Temperature is one of the most important physical parameters and also the most difficult to control [21]. Maintaining temperature as close as possible to the optimal biopulping temperature for the selected fungus decreases treatment time and may give the fungus the competitive edge over contaminants [8].

Incubation time plays an important role in the degradation of lignocellulosic materials. After biopulping of wheat straw, lignin content, 1 % caustic solubility, cellulose and holocellulose were determined [18]. This analysis showed that most of the lignin was degraded in the latter part of the treatment time. After 20 days of incubation of bagasse under optimal conditions, 2,5 % weight loss and 8,7 % lignin loss were achieved and no discolouration was apparent [16]. Delgado et al. [15] showed 9,6 % lignin decrease after 11 days of treatment with a hybrid *P. ostreatus* and 14,7 % decrease after 35 days. The hybrid *P. ostreatus* was able to modify bagasse more selectively than a cellulase deficient strain of *P. chrysosporium*.

Some researchers hypothesize that reduction of lignin can lead to savings in consumption of pulping chemicals [18, 22]. Optimization of parameters for the kraft pulping of fungal treated wood has shown this not to be true [23]. The consumption of chemicals by fungal mycelium has been investigated by Johnsrud *et al.* [16], who showed that the increase in NaOH consumption was due to the dissolution of mycelium. This point is open to debate, as increased chemical consumption was previously ascribed to the lower-molecular weight products of decay [24]. The effect of fungal treatment on pith content is unknown and might even reduce chemical consumption by degrading pith that generally causes increased chemical consumption [12, 25].

Fungal treatment used in combination with the Cuba-9 process increased the beatability of bagasse pulp and produced hand sheets with strength properties similar to those obtained from reference pulp [16]. On kenaf and jute, the fungal treatment improved strength properties [17]. Soda pulping of fungal treated reed canary grass produced fine paper (furnish composition of 40 % grass, 40 % softwood and 20 % talc) with better printing and strength properties [11].

CONCLUSIONS

The increase in the demand for non-wood fibre [1] will focus research on the development of biopulping processes specifically for non-woody substrates. Apparently, white-rot fungi with selective lignin degrading potential will be the most suitable organisms for application in these processes. The diversity of raw materials that are used for pulping has resulted in different handling and storage practices as well as pulping processes. Specific biopulping processes will consequently be designed to provide optimal benefit to existing operations, but some adaptations to the current procedures to accommodate biopulping will also be necessary.

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