BIOTECHNOLOGY

Corn steep liquor lowers the amount of inoculum for biopulping

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ABSTRACT

This research examined the effectiveness of corn steep liquor in reducing the quantity of fungal inoculum during biomechanical pulping , Loblolly pine, Pinus taeda, chips were treated for two weeks with different strains of the white-rot fungus Ceriporiopsis subvermispora on a laboratory scale. On unsupplemented chips, 3 kg/ton of inoculum on a dry weight basis of strain CZ-3 saved 19% electrical energy and improved tear index by 28% compared with the control. With addition pf 0.5% sterilized corn steep liquor on a dry weight basis, inoculum leves as low as 5 g/ton gave equivalent energy savings but did not improve tear index. Two strains saved 28—29% energy and improved tear index by 21—22% when amending 5 g/ton of inoculum of each strain with 0.5% sterilized corn steep liquor. Other augmentation led to 34% savings in energy and 46% improvement in tear index.

Application:

Pulp and paper companiies can obtain a regular supply of corn steep liquor for their use from the nearest location with minimal transportation cost.

BIOPULPING IS THE TREATMENT OF wood chips with lignin-degrading fungi before pulping. It is an experimental process that has undergone extensive research during the past nine years at the USDA Forest Products Laboratory by two biopulping consortia. The main focus was as a pretreatment for mechanical pulp ing. Biopulping reduces electrical energy consumption-a major cost in mechanical pulping—improves paper strength, and lowers effluent toxicity *(1-5)*. Recent studies suggest that fungal pretreatment is also effective on nonwoody plant materials such as kenaf and jute *(6,7)* and for pitch removal *(8)*. It also provides benefits with thermomechanical pulping, chemithermomechanical pulping *(9)*, and sulfite pulping *(8,10)*.

Our work has focused on the white-rot fungus, *Ceriporiopsis subvermispora*, since this fungus is effective on both hardwood (aspen) and softwood (loblolly pine) species *(11-13)*. There is a U.S. patent on the use of *C. subvermispora* in biomechanical pulping *{14)*. Since the fungus does not produce spores, fragmented mycelium has found use as inoculum in previous studies with acceptable results *(8)*. The amount of inoculum used to achieve such results has been high, however. Our goal in the present investigation was therefore to reduce the amount of fungal inoculum with the addition of low cost nutrients. The nutrients stimulate initial fungal growth and establishment in the chips.

We used an inexpensive and readily available nutrient source—corn steep liquor (CSL)—as a fungal nutrient. It comes from the corn wet milling process when the dry corn is soaked (steeped) in a warm dilute sulfurous acid solution. During the process, the grain solubles release and undergo a mild lactic acid fermentation from naturally occurring bacteria. Production occurs throughout the United States and in other countries. Its main uses are as feed supplement for ruminants, a nutrient source for poultry, and a nutrient in fermentation processes. Two preliminary reports discuss the material *(4, 15)*.

EXPERIMENTAL

Wood chips

The experiments used pulp wood sized logs of loblolly pine, *Pinus taeda* L. Freshly cut loblolly pine logs came from the Talladega National Forest in Talladega, AL. Logs underwent debarking and chipping to a nominal size of 16 mm. We thoroughly mixed the freshly made chips, placed them in plastic bags, and immediately froze them to prevent the growth of indigenous microorganisms.

Fungal strains

We used the white-rot fungus, *Ceriporiopsis subvermispora* (Pil) Gilbn. et Ryv., in the present investigation. In our earlier studies, this fungus outperformed others *(12)*. The study used six strains (CZ-3, L-14807 SS-1, SS-3, SS-5, SS-10, and L-105752 SS-4) of the fungus based on their greater lignin degrading ability compared with other strains (unpublished results). They came from the Center for Forest Mycology Research of the USDA Forest Products Laboratory in Madison, WI. We maintained cultures on potato dextrose agar (PDA) (Difco Laboratories, Detroit, MI) slants at 4°C until used. We inoculated PDA plate cultures from these slants and incubated at 27 ± 1°C and 65 ± 5% RH for 10 days.

Inoculum preparation

The culture medium (1-L) contained 24 g potato dextrose broth (Difco Laboratories, Detroit, MI) and 7.27 g yeast extract (Amberex 1003, Universal Foods Corp., Milwaukee, WI). We autoclaved ten 1-L flasks each con-

Composition[1] energy savings	American Maize-Products Co. Hammond, IN	ADM Corn Processing Cedar Rapids, IA	CPC International Inc. Argo IL Batch I	Batch 2	Batch 3
Dry substance, %	42	54	56	51	53
pH	4.0	4.2	4.0	3.9	4.1
Protein, %	45	26	41	41	40
Lactic acid, %	24	2.5	17	16	17
Sugars, %	3	2.5	15	13	12
Energy savings, %[2]	36	30	32	34	33

[1]CSL also contains metal ions, amino acids, vitamins, and other compounds in small quantities (parts per million basis). (Analysis not shown)
[2]Percentage of energy savings calculated on basis of values for untreated control.

I. composition of various sources and batches of CSL and their effect on energy savings during biomechanical pulping of loblolly pine chips

taining 100 mL of medium for 20 min at 121°C and 15 psi, cooled them, and inoculated them with 10 plugs cut with a 9-mm-diam. cork bore from lo-day old PDA plate cultures. Flask incubation was at 27 ± 1°C and 65 ± 5% RH for 10 days without agitation. Next we decanted the spent medium from ten cultures, rinsed the mycelial mats with sterile water, and aseptically blended them in a Waring blender. We added sterile water in sufficient quantity to the blended mycelium to make the mycelial suspension stock. There were different dilutions made from the stock to obtain different amounts of fungus depending on the experiment. Reporting of inoculum levels uses dry weight of fungus per dry weight of wood chips. To determine the dry weight of the fungus, we washed the mycelial mat with water to remove the spent medium and dried it at 65°C to constant weight. Dry weights of strains were 0.97-1.83 g per flask.

KEYWORDS
Biochemical pulping, corn culture media, energy conservation, inoculation, mechanical properties, nutrients, pinus taeda, refiner mechanical pulping, steeping liquors, white rot fungi.

Corn steep liquor

Table I shows the composition of the CSL. It came from three different sources: CPC International Inc., Argo, IL, American Maize-Products Co., Hammond, IN; and ADM Corn Processing, Cedar Rapids, IA. Storage was at 4°C. Three batches of E802 CSL came from CPC International Inc. All experiments used 0.5% of sterilized or unsterilized CSL on a dry weight basis. Autoclaving for 20 min at 121°C and 15 psi provided sterilization where necessary. For the work in this table, the loblolly pine chips were inoculated with 5 g/ton of inoculum on a dry weight basis of strain L-14807 SS-3 of *Ceriporiopsis subvermispora* using two-week incubation. Inoculum augmentation was with 0.5% unsterilized CSL on a dry weight basis.

Chip preparation and bioreactor inoculation

We thoroughly mixed thawed chips and placed them in static-bed bioreactors *(11)* for steaming without pressure for approximately 10 min. We next inoculated the bioreactors at room temperature-each containing 1500 g chips on a dry weight basis—with different amounts of fungus in the presence of CSL. We added CSL to the mycelial suspension before inoculation. Bioreactors containing noninoculated chips served as controls. We adjusted the final water content of the chips to 55% on a wet weight basis with sterile water. The bioreactors were then sealed, shaken vigorously, and incubated at 27 ± 1°C for two weeks. Each bioreactor received a continuous supply of sterile humidified air at the rate of 0.02 volume/volume/min.

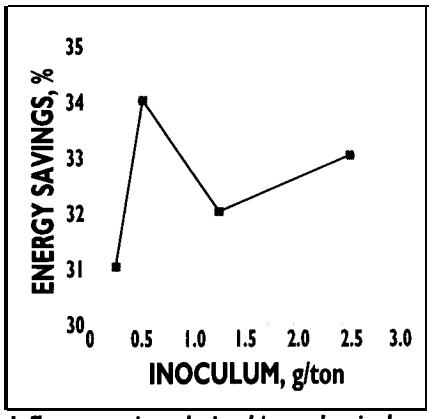
Chip fiberization, pulp refining, and handsheet production

At harvest, the untreated control chips and the fungus-treated chips underwent refining through a single-disc 12-in. atmospheric refiner. Electrical energy consumption during fiberization and subsequent refining was recorded. The untreated control and the fungus-treated chips underwent refining to the same freeness of 100 mL CSF to simplify calculations for energy savings. Details of refining, energy measurements, handsheet preparation, and paper testing are in the literature *(12, 13, 16-18).*

RESULTS

Screening of fungal strains and comparison of sterilized and unsterilized CSL

Table II shows that the 3-kg/ton inoculum of strain CZ-3 without CSL supplementation saved 19% electrical energy and improved tear index by 28% compared with the untreated control. Augmentation of



1. Energy savings during biomechanical pulping of loblolly pine chips for two-week incubation with different inoculum levels, each augmented with 0.5% unsterilized CSL on dry weight basis

Strains	Energy savings[3]	Tear index improvement[3]
Without CSL		
CZ-3[1]	19	28
With sterilized CSL		
CZ-3[2]	18	0
FP-105752 SS-4[2]	22	14
L-14807 SS-1[2]	18	14
L-14807 SS-3[2]	29	21
L-14807 SS-5[2]	28	22
L-14807 SS- 10[2]	22	0
With unsterilized CSL		
L-14807 SS-3[2]	34	46

[1]3 kg/ton of inoculum on a dry weight basis.

[2]5 g/ton of inoculum on a dry weight basis of each strain. Inoculum of each strain augmented with 0.5% CSL on a dry weight basis.

[3]Percentage of energy savings or tear index improvement calculated on basis of values for untreated control. To reach 100 mL CSF, control took 1447 W·h/kg dry weight of chips, while strain L14807 SS-3 with unsterilized CSL took only 955 W·h/kg dry weight of chips.

II. Energy savings and improvement in tear index with and without CSL amendment during biomechanical pulping of loblolly pine chips with different strains of **Ceriporiopsis subvermispora** *using two-week incubation*

strain CZ-3 inoculum at 5 g/ton with only 0.5% sterilized CSL led to 18% savings in electrical energy. It did not give improved tear index as Table II shows. Five other strains each with 5 g/ton of inoculum and 0.5% sterilized CSL saved 18–29% electrical energy and improved tear index by 14–22% compared to the control. Strain L-14807 SS-10 was an exception. Table II shows that it did not improve tear index.

Data in Table II show that supplementation of strain L-14807 SS-3 inoculum at 5 g/ton with 0.5% unsterilized CSL saved 34% electrical energy and improved tear index by 46% compared with the control. Levels of L-14807 SS-3 inoculum at 0.25, 0.5, 1.25, and 2.5 g/ton with 0.5% unsterilized CSL showed comparable energy savings of 31-34% as **Fig. 1** shows. This experiment did not determine strength properties. There were similar results with aspen chips treated with L-14807 SS-3 strain of *C. subvermispora* (data not shown). Controls with and without sterilized or unsterilized CSL showed comparable results. Neither strain CZ-3 nor strain L-14807 SS-3 without CSL supplement showed significant energy savings or strength improvements (data not shown).

Effect of different sources and batches of unsterilized CSL

Table I shows that the composition of CSL varies from one source to another and from one batch to another. Comparable results occurred with all sources and batches, however. There were 30-36% savings in electrical energy when supplementing 5 g/ton inoculum of strain L-14807-SS-3 with 0.5% unsterilized CSL in each case. We did not determine strength properties in these experiments.

DISCUSSION

In our previous studies, we established the technical feasibility of biopulping *(3).* The focus of our current research was to scale up the biopulping process so it is economically feasible. One major cost foreseen during scaleup of the biopulping process comes from inoculum production. We therefore reduced the amount of *C. subvermispora* inoculum as low as 0.25 g/ton with CSL amendment. This reduces the cost for inoculum production. Three biotechnology companies based on literature have given us the cost estimates for producing 0.25 g/ton dry weight of fungus. These cost estimates appear to be well within an economical range. CSL is relatively inexpensive-currently US$ 55/ton of semi-solid liquid.

In the present investigation, we have also identified a strain of *C. subvermispora,* L-14807 SS-3, that gives superior results to those obtained with the previous strain, CZ-3 *(13).* During solid-substrate fermentation, reproducibility of results is important. The superior strain identified in this study has routinely given us very reproducible results.

Our data also indicate that unsterilized CSL gave better results than those obtained with sterilized CSL. This could perhaps be due to denaturation of some proteins of CSL

from autoclaving that might be important for fungal growth and subsequent biopulping. Unsterilized CSL even from different sources and batches does not appear to affect the biopulping performance of the fungus. It therefore is possible to avoid the associated costs to make the process more economically attractive. Incubating unsterilized CSL on PDA plates at 27-32°C for 3-4 days reveals the presence of some microorganisms (unpublished data). This does not seem to interfere with the biopulping performance of the fungus. This suggests that *Ceriporiopsis* is aggressive and overcomes any microorganisms present in CSL.

CSL production occurs throughout the United States. Despite variation from sources or batches, its composition does not affect the biopulping efficacy of the fungus. Pulp and paper companies can therefore obtain a regular supply of CSL from the nearest location with minimal transportation cost. We do not know the component of CSL responsible for the beneficial effect. Other nonchemically defined additives, including molasses and yeast extract, have shown promise in biopulping. They have not been as effective as CSL (unpublished data). **TJ**

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