



Dilute nitric-acid pretreatment of oat hulls for ethanol production



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ABSTRACT

Oat hulls are a promising feedstock annually renewable at an industrial scale for the production of second generation ethanol. Chemical pretreatment of oat hulls with a 4% (w/w) nitric acid solution at atmospheric pressure was performed in pilot production to give a pulp in which hydrolyzables account for 79.9%. Acid-insoluble lignin totaling 12.5% in the pulp does not deteriorate the efficiency of enzymatic hydrolysis using an enzyme cocktail of commercial BrewZyme BGX and CelloLux-A. At an initial solid loading of 33.3 g/L, a hydrolyzate was obtained with a reducing sugar yield of 93.0% of the overall hydrolyzables. The high yield is assured only by removal of residual, water-soluble, nitrated lignin by water washing. Alcoholic fermentation was run using *Saccharomyces cerevisiae* Y-1693. Simultaneous saccharification and fermentation with delayed inoculation (dSSF) at an initial pulp loading of 90 g/L was found to improve the ethanol yield by 1.2 times compared to separate hydrolysis and fermentation (SHF), i.e. 0.428 versus 0.343 g/g cellulose, respectively. The ethanol samples obtained from oat hulls contain low percentages of esters and methanol.

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1. Introduction

Ethanol production from cellulosic biomass is one of the most demanded trends in modern biotechnology and is widely discussed both at the governmental level [1] and in the global scientific community [2–19]. Despite multiple micro- and full reviews on problems of the ethanol production from cellulosic biomass and detailed description of research results for individual process stages, of high interest are publications dealing with the engineering aspects of bioprocess research.

The engineering implementation of chemical and biotechnological solutions under development is of prime importance. The key criteria for the ethanol technology to be industrialized include selection of feedstocks [2–7] and their effective pretreatments [3,5–13], commercial availability of enzymes in use [14,15], stability and productivity of microorganism strains in the production environment [10,11,16], optimum process conditions, equipment types and process monitoring system [4,11,14,17–19].

Lignocellulosic biomass is a priority source owing to its infinite volumes, worldwide availability, and universal utilization for converting the same not only into ethanol but also into various bio-based products. Of great interest as cellulosic sources are agri-

cultural residues having no food value—like straw, gramineous plant hulls, citrus skin, sugarcane bagasse, etc. These raw materials are projected to prevail in the near future [2–4]. Special emphasis should be given to residues of glumaceous crops, for example, oat hulls. This is a unique kind of an annually renewable feedstock that is globally spread and has a great technological potential. Firstly, oat hulls account for 28 % of the grain weight and are accumulated at an industrial scale at grain-processing plants. Secondly, this is a morphologically homogeneous raw material naturally calibrated by size and thickness, and requires no milling when processed. Thirdly, this is an annual cellulosic raw material whose chemical composition predetermines the expediency of its processing through the biorefining route. Oat hulls are composed mainly of cellulose (35–45 %), hemicelluloses (32–35 %), acid-insoluble lignin (17–20 %), and are notable for high ash content (4.5–5.2 %). The oat hull ash is low-melting; hence the application of oat hulls as pellet fuel is hindered. It should be noted that, owing to the high content of hemicelluloses, oat hulls were previously considered as a hemicellulosic feedstock and a source of furfural and xylite [20].

There is data on the utilization of oat hulls for ethanol production. For instance, oat hulls are subjected to chemical hydrolysis and then transformed into ethanol in a yield of 0.05 g/g oat hulls [21]. Enzymatic treatment is more preferable, and Lawford et al. [10] reported industrial production of ethanol from oat hulls in Canada, where oat hulls pretreated with sulfuric acid at 200–250 °C are submitted to enzymatic treatment; in addition, a pilot plant

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with a capacity of 50 ton/day was constructed. We have previously investigated enzymatic hydrolysis of pulps derived from oat hulls pretreated by the alkaline delignification method with subsequent nitric acid treatment [22] and by the hydrothermobaric method [23].

Enzymatic hydrolysis of native cellulosic biomass is known to be difficult, for the plant matrix is composed of the three polymers—cellulose, hemicelluloses, and lignin—which all together form a composite material that is more tough and resistant to physical and chemical actions than the components individually. In order that enzyme complexes are able to cleave the individual components, the composite matrix must be broken down; for this purpose, the native raw material should be subjected to physical, physicochemical, or chemical pretreatment [2–13].

From among various pretreatment technologies that have been considered over the past two decades, the main chemical techniques under development are dilute acid pretreatment, hydrothermal pretreatment (liquid hot-water treatment and uncatalyzed steam explosion), and alkaline pretreatment (two types of delignification using sodium or calcium hydroxide and ammonia). Hydrochloric, phosphoric, acetic, formic, or peracetic acid is employed as the acid reagent, but sulfuric acid is in most common use because of its obvious cheapness and relative effectiveness [2–13]. The acid hydrolyzes hemicelluloses and breaks down the lignin structure to increase accessibility of cellulose to enzymes in pretreated biomass. However, the main drawback of sulfuric acid for treating a raw material at temperatures over 120 °C is the partial conversion of the hydrolyzed xylan into furfural that inhibits further biochemical transformation [14]. The reviews on methods for treating lignocellulosic biomass to produce bioethanol [2,3,5–7] lack information about application of nitric acid as a pretreatment chemical.

The use of nitric acid for pulping has been known since the XIX century. Apart from the breaking of chemical bonds between cellulose, hemicelluloses, and lignin, the dilute nitric acid solution at a boiling temperature first causes hydrolysis of hemicelluloses to form soluble xylose and its soluble precursors, and then cellulose is partially hydrolyzed and the sugars are transformed into simple organic acids (formic, acetic, etc.) and carbon dioxide; moreover, oxidative nitration of lignin occurs [24]. In the Republic of Belarus, a dilute nitric-acid delignification was studied with winter rye and a possibility was shown of obtaining radioactively pure cellulose and nitrolignin from the radionuclide-contaminated feedstock [25]. There is a report on the application of nitric acid for chemical hydrolysis of loblolly pine [26].

The feasibility of using nitric acid for pretreatment of cellulosic raw materials for ethanol biosynthesis is not obvious because oxidative nitration products of lignin may act as inhibitors during enzymatic hydrolysis (inhibition of the complex of cellulases, β -galactosidases and cellobiases) or during alcoholic fermentation (inhibition of the enzyme complex of yeast to inhibit biocatalysis via the Embden-Meyerhof-Parnas pathway and subsequent pyruvate decarboxylation to form acetaldehyde followed by reduction of acetaldehyde to ethanol).

The aim of the present work was to examine the dilute nitric-acid pretreatment of oat hulls at atmospheric pressure under pilot production conditions and investigate enzymatic hydrolysis of the resultant pulp and ethanol biosynthesis.

2. Materials and methods

2.1. Microorganisms and inoculum preparation

The experiments were performed with the *Saccharomyces cerevisiae* Y-1693 strain (Russian National Collection of Industrial

Microorganisms, Moscow). This strain was isolated from a fermenter of the Kotlas Pulp and Paper Mill (Koryazhma city, Russia) and was further used for ethanol production on sulphite liquors. The strain has a feature of being resistant to harmful impurities in hydrolyzates. A sterilized malt extract was employed as the medium for inoculum preparation. Into an Erlenmeyer flask (250 mL) containing the medium (100 mL) was inoculated a loopful of cells grown on a malt-extract agar slant, and the strain was then incubated at 28 °C for 48 h. The resultant culture was used as the inoculum. The inoculum was introduced in a ratio of 10% to the medium volume, the cell concentration of the inoculum being 120 ± 10 million CFU/mL.

2.2. Feedstock

Oat hulls were obtained from a local grain-processing factory, “Biysk Grain-Elevator” (Biysk, Russia), and had the following chemical composition (on oven-dry basis, w/w): 36.7 ± 0.2 % cellulose, 34.8 ± 0.2 % hemicelluloses, 19.4 ± 0.1 % acid-insoluble lignin, and 5.5 ± 0.1 % ash content.

2.3. Nitric-acid pretreatment

Oat hulls were pretreated in one stage with 4% (w/w) dilute nitric acid in a solid-to-liquid ratio of 1:20 (w/v) under atmospheric pressure at 94–96 °C for 4 h. Pretreatment was run at a pilot production site using a 250-L standard reactor which was loaded at 80% of the rated reactor volume; the equipment and piping manifold are made of stainless steel.

2.4. Post-pulping

The pretreated oat hulls were squeezed from the nitric-acid liquor on a filter *in vacuo* and washed with water by two ways: (i) until the wash waters have a neutral pH but are orange-yellow color (pulp 1) and (ii) until the wash waters have a neutral pH but are colorless (pulp 2). The resultant pulps had similar appearance and, in both cases, were like a loose, homogeneous material, in which case the oat hull lemmas became soft and flexible. The weight of each sample was 4.0 ± 0.1 kg on oven-dry basis. The pulps were identical in chemical composition. Both substrates were then used for enzymatic hydrolysis in the wet state (78.0 ± 0.3 % moisture).

2.5. Separation of inhibitor from pulp 1

Pulp 1 was additionally washed with water in the digester in a solid-to-liquid ratio of 1:20 (w/v) under agitation at 100 rpm for 30 min at 60 °C. The resultant wash waters were orange-yellow color and had neutral pH. The wash waters were collected, evaporated *in vacuo*, and then dried *in vacuo*. The yield of the dried product (hereinafter “inhibitor”) was 0.019 ± 0.001 % (w/w) of the pulp weight.

Afterwards, pulp 1 was subjected to a control washing. The wash waters had a neutral pH and were colorless, that is, pulp 1 was turned into pulp 2.

2.6. Nitrated lignin

Nitrated lignin was isolated by treating alkaline lignin with 4% (w/w) nitric acid at 94–96 °C for 3 h. The alkaline lignin was separated from the spent liquor after oat hulls had been treated with 4% (w/w) sodium hydroxide under atmospheric pressure at 94–96 °C for 4 h, in a solid-to-liquid ratio of 1:20 (w/v). The conditions of lignin separation from the spent liquor were as follows: neutralization with hydrochloric acid for lignin coagulation, heating to

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