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Zygomycetes and cellulose residuals: hydrolysis, cultivation and applications

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Zygomycetes and cellulose residuals: hydrolysis, cultivation and applications

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Abstract

Zygomycetes is a class of fungi living worldwide as saprobes, as part of mycorrhizae, and as parasites. Humans have used some zygomycetes for centuries in the production of traditional foods, e.g. Indonesian tempe. In the present thesis, the experimental focus was on two zygomycetes strains, *Mucor indicus* CCUG 22424 and *Rhizopus sp.* IT.

One of the distinguishing features of *M. indicus* is its dimorphism. The different cell forms were influenced by the culturing conditions. After inoculation, when the initial spore concentration was high (6-8×10^6 spores/ml), yeast-like growth dominated under anaerobic conditions. With a smaller inoculum, yielding 1-2×10^5 spores/ml, and access to oxygen, filamentous forms dominated. Only negligible differences in ethanol yield (390-420 mg/g hexoses), productivity (3-5 g/l/h), and inhibitor tolerance were observed. Differential expressions of probably four genes were observed between the yeast-like and filamentous growth forms.

Lignocelluloses are a suitable substrate for cultivating zygomycetes, as they occur in abundance, particularly since zygomycetes, unlike *Saccharomyces cerevisiae,* can utilise pentoses. Lignocelluloses require pretreatment to achieve efficient hydrolysis of the cellulose. N-methylmorpholine-N-oxide (NMMO) was tested for pretreatment of spruce and birch. Reducing wood chip size and/or prolonged pretreatment, promoted hydrolysis yield. Best yields were achieved from <2 mm chips and 5 h pretreatment. The hydrolysate was used for fermentation with *M. indicus*, resulting in 195 and 175 mg ethanol/g wood, and 103 and 86 mg fungal biomass/g wood, from spruce and birch respectively.

Orange peel is another potential substrate. However, the hydrolysate contained 0.6 % (v/v) D-limonene, ten times higher than the concentration inhibiting *S. cerevisiae*. *M. indicus* was more resistant and successfully fermented the hydrolysate, producing 400 mg ethanol/g hexoses and 75 mg fungal biomass/g sugars. Both *M. indicus* and *Rhizopus* sp. grew in 1.0 % and 2.0 % D-limonene, although the latter was unable to grow in the hydrolysate.

A third substrate was also used, spent sulphite liquor (SSL), which is a by-product from sulphite paper pulp mills. The SSL was diluted to 50 % and used for airlift cultivations of *Rhizopus* sp. In 1.0 vvm aeration, up to 340 mg biomass/g sugars was produced. Prolonged cultivations generally decreased the protein (from 500 to 300 mg/g) and lipid (from 70 to 20 mg/g) contents. In contrast, the cell wall fraction, measured as alkali-insoluble material (AIM), increased (160-280 mg/g), as did the glucosamine (GlcN) content (220-320 mg GlcN/g AIM). The produced fungal biomass could serve as animal feed, e.g. for fish.

Keywords: Zygomycetes, fungi, lignocelluloses, ethanol, fish feed, animal feed, dimorphism, airlift, pretreatment