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Research Article

CELLULOLYTIC ACTIVITY OF *TRICHODERMA VIRIDE* WITH REGARD TO SELECTED LIGNOCELLULOSIC WASTE MATERIALS

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ABSTRACT
The aim of the study was to assess the cellulolytic activity of a strain of *Trichoderma viride* in the presence of three lignocellulosic substrates, i.e. wheat, barley, and maize straw, in different temperatures (25 °C, 30 °C and 35 °C). Research related to the biosynthesis of enzymes was conducted using the deep method, whereas enzyme activity was assessed on solid media with added carboxymethyl cellulose (CMC). The assessment was based on the activity index (AI) determined for each object of research. The obtained results indicate that *T. viride* produces cellulolytic enzymes, and that their level of activity depends on the type of material introduced into the culture as a lignocellulosic substrate as well as on the temperature. The highest value of AI was found in objects with added maize straw. The optimal temperature for the biosynthesis of cellulolytic enzymes equalled 30 °C.

Keywords: cellulolytic activity, *Trichoderma viride*, lignocellulosic waste materials, bioenergy.

INTRODUCTION

The world's supply of fossil fuels is gradually depleting, whereas the global need for energy increases continuously. The principle of sustainable development dictates that, in order to provide a better future for subsequent generations, non-renewable fuels should be used rationally, and, at the same time, the obtainability of energy from alternative sources should be improved.

Poland has a considerable potential in terms of renewable energy sources, especially wind and biomass energy [Krawiec 2010]. The latter, when subjected to thermochemical or biochemical conversion in the presence of microorganisms, can provide electricity, heat, and biofuels (Figure 1). Importantly, energy can be obtained not only from biomass as a product, but communal, agricultural, forestry, and industrial waste as well.

The main constituent of plant biomass is lignocellulose, which is made up of three types of polymers: cellulose, hemicellulose, and lignin [Perez et al. 2002, Kumar et al. 2009]. Lignocellulosic materials are the most promising feedstock for bioenergy [Perez et al. 2002]. While a range of microorganisms are able to degrade cellulose and hemicellulose by using them as a source of carbon and energy, much fewer microorganisms show the same activity towards lignin [Sanchez 2009]. Among the microorganisms that play an important role in these processes are filamentous fungi, including those from the genus *Trichoderma* [Miettinen-Oinonen and Suominen 2002, Neethu et al. 2012, Rubeena et al. 2013]. Fungi have two types of exogenous enzymatic systems: the hydrolytic system, responsible for degrading polysaccharides, and the lignolytic system, which determines the decomposition of lignins [Sanchez 2009]. Lignocellulosic materials intended for energy production must undergo initial processing with physical, chemical, or biological methods applied independently or in combination, e.g., thermochemical or biochemical methods [Lim et al. 2012]. Another important consideration that may affect the viability of these materials as an energy source is the pos-

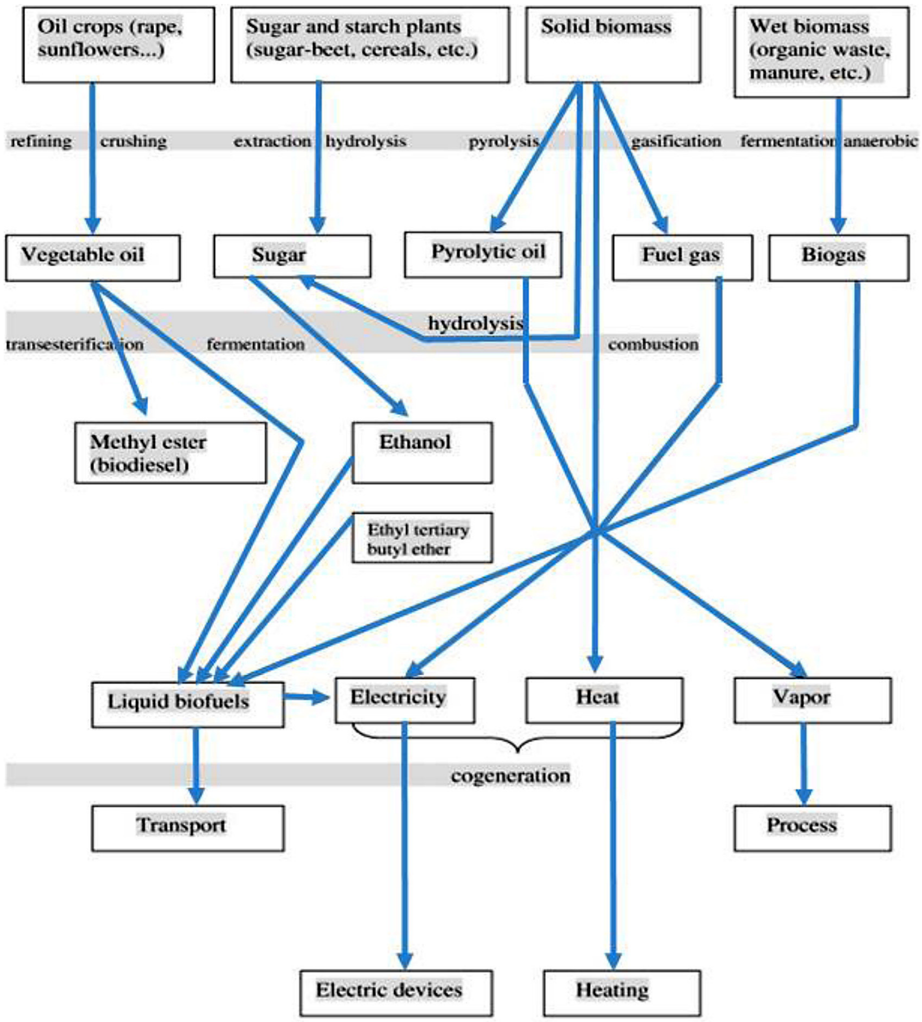


Figure 1. Biomass energy conversion [based on Sanchez 2009]

sibility to provide optimal environmental conditions, adjusted appropriately to the needs of the microorganisms, including the basic ones such as pH and temperature [Tholudur et al. 1999, Bhattacharya et al. 2014].

The aim of this study was to assess the capability of *Trichoderma viride* to biosynthesise cellulolytic enzymes with regard to selected lignocellulosic substrates in different temperatures.

MATERIALS AND METHODS

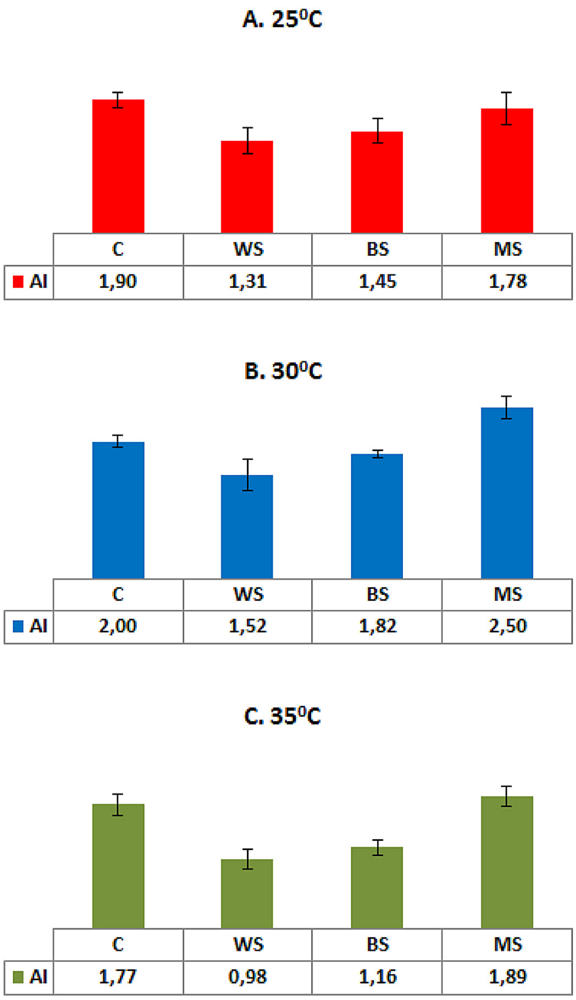
The researched material constituted a strain from the collection of the Department of Microbiology and Environmental Biotechnology. The production of cellulolytic enzymes was assessed in 250 cm^3 Erlenmeyer flasks containing 100 cm^3 of liquid modified medium composed of ($g \cdot L^{-1}$): urea 0.3; $(NH_4)_2SO_4$ 1.4; KH_2PO_4 2.0; $CaCl_2$ 0.3; $MgSO_4$ 0.3; yeast extract 0.25; peptone 0.75 g [Mandels 1975]. Added to the flasks were lignocellulosic substrates weighting 10 g (respectively wheat straw – WS, barley straw – BS or maize straw – MS), that had been broken down and then ground into fragments with a maximum size of 1–3 mm in order to increase bioavailability. Also prepared were flasks with a control medium (C) that contained crystalline cellulose instead of lignocellulosic materials.

The media were inoculated by introducing discs with a diameter of 2 mm cut from a seven-day-old mycelium of *T. viride* (the material was cultivated on the PDA medium at 30 °C). Each culture was incubated for seven days at 25, 30 and 35 °C. In order to determine the activity of the produced enzymes, 0.1 cm^3 of culture liquid was taken from flasks with the appropriate objects of research and transferred centrally onto a medium solidified on Petri dishes (composition as stated above) with added carboxymethyl cellulose (1% w/v). The experiment was performed three times. After seven days of incubation at 30 °C, the dishes were flooded with a 1% aqueous solution of Congo red for 15 minutes and bleached using 1M NaCl (20 min). Next, the diameter of the clear zone around the colony was measured (in mm). AI (activity index) was determined based on these measurements by comparing the size of the clear zone to the size of the colony. The results were subjected to statistical analysis using the Statistica ver. 10.0 computer program by StatSoft Poland.

RESULTS AND DISCUSSION

The annual yields of lignocellulosic biomass in the world is about 220 billion tons [Ren et al. 2009]. Lignocellulosic substrates are renewable, available in large quantities [Bisaria and Ghose 1981] and undergo bioconversion. As many authors indicate, strains belonging to the genus *Trichoderma* [Khokhar et al. 2012, Neethu et al. 2012, Sartori et al. 2015], may play an important role in these processes, as also confirmed by the presented research. It was found that for the tested strain of *Trichoderma viride*, the presence of lignocellulosic materials in the growth medium benefitted the production of cellulolytic enzymes (Figure 2.A–C), which is significant taking into account that the materials are waste that can be used to produce biofuels. Also noted were differences in the activity of the strain depending on the applied lignocellulosic substrate. All waste materials contained substances essential for fungal growth and activity, i.e. the source of carbon or nitrogen [Kancelista et al. 2013], but the highest AI was observed in the presence of maize straw.

The average content of cellulose, hemicellulose, and lignin in materials such as those used in this study may amount to, respectively, 29–35%, 26–32%, and 16–21% for wheat straw, 31–34%, 24–29%, and 14–15% for barley straw [Sanchez 2009], and approx. 49%, 29%, and 7.5% for maize straw [Song et al. 2014]. Maeda et al. [2011] indicate that cellulolytic enzymes may bind with lignin, causing their effectiveness to drop considerably. Maize straw contains markedly less lignin that the other substrates, which means that the potential level of the reduction of enzymatic activity may be lower in this case.



C – control, WS – wheat straw, BS- barley straw, MS – maize straw

Figure 2. The activity index (AI) in the presence of various lignocellulosic substrates

It is not only the type of substrate, but the environmental conditions as well that have a significant effect on the amount of the produced cellulolytic enzymes [Herculano et al. 2011]. The conducted experiments indicated that the optimal temperature is 30 °C. Similar results were obtained by Bhattacharya et al. [2014], who assessed the relationship between the effectiveness of cellulase production by *T. viride* and such parameters as pH, incubation time, or temperature. In the presented research, an increase in temperature led to an increase in the activity of the strain with regard to maize straw. The observed values of AI were higher than in the other objects of research, including the control. No similar relationship was observed with regard to the other lignocellulosic substrates, in which an increase in temperature over 30 °C reduced the activity of the strain, which is also confirmed by other authors [Malik et al. 2010].

CONCLUSIONS

The influence of two factors, different lignocellulosic waste materials and temperature, were examined in the current work. The results showed that the lignocellulosic waste substrates introduced into growth media induced the biosynthesis of cellulolytic enzymes by the strain of *Trichoderma viride*. Cellulolytic activity varied depending on the type of substrate (maize straw>barley straw>wheat straw). The optimal temperature for the biosynthesis of extracellular cellulolytic enzymes is 30 °C, regardless of the type of the lignocellulosic substrate.

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