TITLE: SCALING FOR BIOREACTOR AND SACCHARIFICATION PROFILE OF CORN STRAW BY ENZYMATIC COCKTAILS PRODUCED BY *MYCOTHERMUS thermophilus* AND *TRICHODERMA reesei* RP698 CULTURES

AUTHORS: CONTATO, A. G.[1]; SANTOS, R. A. C.[2,3]; SCARCELLA, A. S. A.[1]; GRANDIS, A.[4]; BUCKERIDGE, M. S.[4]; SILVA, R. N.[1]; POLIZELI, M. L. T. M.[1,5]

INSTITUTIONS: 1. Faculdade de Medicina de Ribeirão Preto (FMRP), Universidade de São Paulo, Ribeirão Preto, SP, Brazil; 2. Instituto de Biologia, Universidade Estadual de Campinas, Campinas, SP, Brazil; 3. Faculdade de Ciências Farmacêuticas de Ribeirão Preto (FCFRP), Universidade de São Paulo, Ribeirão Preto, SP, Brazil; 4. Departamento de Botânica, Instituto de Biociências (IB), Universidade de São Paulo, São Paulo, SP, Brazil; 5. Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto (FFCLRP), Universidade de São Paulo, Ribeirão Preto, SP, Brazil.

ABSTRACT

The increase in agro-industrial activity has led to the accumulation of large amounts of lignocellulosic biomass. The cost of producing bioethanol from agro-industrial waste (2G-bioethanol) depends on biomass recalcitrance and enzymes involved in saccharification. Therefore, optimizing enzyme production comprises a promising strategy to overcome this limitation. Here, we evaluated the saccharification profile of the corn straw using a fungal enzymatic extract obtained from *Mycothermus thermophilus* and *Trichoderma reesei* RP698 in a co-cultivation system, in media supplemented with the same biomass. The enzyme production was compared using Erlenmeyer Flasks and a stirred tank bioreactor containing 4.5 L of minimum medium supplemented with 1% corn straw, 35 °C, initial pH 6.5, 275 rpm, airflow rate 2 v.v.m., for 120 hours. The hydrolysis occurred at 50 °C, under 200 rpm stirring, for 24 h. After that, the samples were centrifuged (4800 rpm) at 4 °C. Sugar hydrolysate profiles were determined using a High-Performance Liquid Chromatography YL9100 model (Young Lin Instruments) system equipped with the Rezex™ ROA-Organic Acid H+ (8%) 300 × 7.8 mm column and the YL9170 Refractive Index Detector. The analysis was performed at 80 °C, using 0.005 N H2SO4 as the mobile phase at a flow rate of 0.5 mL/min, with the detector cell temperature of 40 °C and a 25 min run time. Maximal xyloglucanase, arabinanase, polygalacturonase, and feruloyl esterase were produced at 24h. However, high levels of endoglucanase, β-glucosidase, xylanase, β-xylosidase, and arabinofuranosidase occurred at 48h, and acetyl xylan esterase and β-galactosidase at 72h. In the final analysis period (120h), several cellulases and hemicellulases still presented satisfactory activities. All enzymes produced in the bioreactor presented increased activities, except xyloglucanase, which reduced from 2.29 to 0.39 U/mL. In addition, we also evaluated the cocktail protein content, and within 24 hours of co-cultivation, protein production was low. However, over the following days, we observed increased protein concentration, reaching a peak at 120 hours with 330 µg protein.mL-1. After saccharification, it was recorded an amount of 1.91 ± 0.046 and 1.51 ± 0.047 of released glucose and xylose, respectively. Thus, scaling up and the co-cultivation of these fungi were shown to be promising for an enzymatic cocktail design.

Keywords: enzymatic cocktail, corn straw, co-cultivation, *Mycothermus thermophilus*, *Trichoderma reesei*

Funding: São Paulo Research Foundation (FAPESP), processes 2018/07522-6; 2014/50884-5 and 2017/25862-6.