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ORIGINAL ARTICLE

Enhanced protease production by *Aspergillus candidus* strain MKA05 using response surface methodology

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Abstract
Protease enzymes are one of the industry's most important enzymes, with a wide range of applications in different sectors, notably detergent manufacture, tanneries, bakeries, breweries, meat tenderization, and protein hydrolysis. The present study represents a first-time approach to filamentous fungi isolation in soil samples from the Bejaia area, situated in northeastern Algeria. The effects of the incubation duration, starting pH, moisture content, culture temperature, and inoculum size on the generation of protease by *Aspergillus candidus* strain MKA05 were optimized using the one-factor-at-a-time (OFAT) method. The test was carried out using wheat bran as a substrate in solid-state fermentation. Moreover, the Box-Behnken design (BBD) experiments were used to explore these variables by means of response surface methodology (RSM). Analytic variance analysis (ANOVA) was performed, and a component-based mathematical equation was derived to express protease production. Both OFAT and RSM optimizations showed a significant enhancement in protease yield, which increased 4.6-fold, with a maximum activity of 3076.92 ± 4.2 U/mL recorded in comparison to the protease activity of 675.11 ± 3.8 U/mL recorded in the standard condition. Substantial R^2 and p-value regression equations were used to examine the impact of individual activity, interaction, and square terms on the generation of proteases. The RSM approach reported that after 8 days, maximum protease production was achieved under optimal conditions: pH 9 using wheat bran as substrate at 24 °C with 53% moisture content.

Keywords Waste biomass conversion · Filamentous fungi isolation · Fermentation · Optimization · Protease production

1 Introduction

Proteases currently have global enzyme market dominance, contributing around 65% of total industrial enzyme sales [1–3]. These biocatalysts represent a specialist class of enzymes mainly involved in protein hydrolysis [4]. Their various industrial applications range across different sectors, including laundry detergent formulations, leather processing, and peptide synthesis[5, 6]. Additionally, they find use in a range of other industries such as food, pharmaceuticals, and cosmetics [7], brewing, paper [8], photography, silk production, baking, textile manufacturing, dairy processing, bioremediation [9], and bioprocessing [10].

Proteases produced by microorganisms are more popular in manufacturing applications than enzymes isolated from plants and animals. Because of their rapid growth and the limited space required to grow them on an industrial scale, microbes are the preferred source of these enzymes [11, 12]. Fungal protease drew the interest of experts in environmental biotechnology because the fungi are able to grow on low-cost substrates and produce vast amounts of enzymes in the growth medium. Fungal protease production has advantages over bacterial protease, particularly as the mycelium can be easily separated from the enzymatic extract by filtration [11, 13, 14]. The fungal sources that produce these hydrolytic enzymes include the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Mucor*, *Humicola*, *Thermoascus*, and *Thermomyces*. Particular emphasis has been placed on the microbial proteases of *Aspergillus* species, which are known to secrete high levels of enzymes in their growth environment [13].

Proteases have been produced in submerged (SmF) and solid-state fermentations (SSFs), and each technique

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