PELT WASTE DEGRADATION USING FUNGI STRAINS

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Microbiological degradation of pelt waste is amongst the permanent concerns of leather processing units. The process may have the purpose of decomposing waste to exploit by-products as biocompost or to obtain proteases through a biotechnological process. These enzymes can be used after purification in various processes that have animal protein as a substrate. They can also be used in raw state for enzymatic hydrolysis.

The paper aims at determining the optimal pH and incubation temperature of Penicillium strains capable of decomposing pelt waste.

Keywords: pelt waste, enzymes, *Penicillium*

1. Introduction

In recent years, there has been an increased interest in the use of biological degradation of leather waste. Leather has a complex composition comprising collagen, keratin, elastin, albumins and globulins [1, 2]. Each of these compounds can be degraded under certain environmental conditions (temperature, humidity, pH, O_2 concentration) under the action of enzyme complexes synthesized by a variety of microorganisms (bacteria and fungi). Leather waste degradation occurs by means of proteolytic enzymes [3, 4].

Recent global legislation on environment protection stipulates the necessity of changes in the leather processing industry [5]. The processes of leather processing and tanning contribute by 80-90% to the total pollution attributed to the industry, by generating toxic gases (such as H_2S), wide variations in pH values (2.8-13), organic and inorganic load (sulphates, chlorides and chromium) and solid waste. The leather industry is warned to opt for cleaner, environmentally friendlier solutions [6].

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Microbiological degradation of pelt waste is amongst the permanent concerns of leather processing units. Microorganisms (fungi and bacteria) play an important role in solving these problems.

Molds or filamentous fungi are eukaryotic spore-forming microorganisms, feeding by absorption, easily adaptable, because they have the ability to form induced enzymes depending on the nature of the substrate they are found, causing degradation [7-9].

The fungi that grow on leather are part of the lipolytic group, since they use fat as their sole carbon source. In addition, this fungi category also synthesizes organic acids that cause colored spots [10-13].

Most degradations found on leather waste are due to the action of fungi, a microorganism group very rich in enzymes. The following strains have been identified: *Penicillium chrysogenum, P. rugulosum, P. brevicompactum, P. luteum, P. decubens, P. aculeatum, P. funiculosum, Aspergillus niger, A. fumigatus, A. ochraceus, A. ventii, A. flavus, A. oryzae, Mucor mucedo, Rhizopus nigricans, Paecylomyces varioti, Scopulariopsis brevicaulis, Verticillium glaucum, Trichoderma* species [14-19].

Their application in leather industry, with the aim of replacing toxic chemicals currently in use, is a relatively new development and is important in terms of biotechnology. The wide variety of proteases and their specificity of action attracted attention of specialists in an attempt to exploit their characteristics in biotechnological applications [20-23].

Microorganisms (bacteria and fungi) are an excellent source of these enzymes and can be used as such for various purposes, including in the leather industry [24-26].

This paper aims to establish the optimal pH and incubation temperature for *Penicillium* strains capable of decomposing pelt waste.

2. Experimental

Samples of ground protein waste were used in the experiments. They are gray-yellowish, with hard, slightly gelatinous, wet consistency.

Materials and methods

Pelt waste was treated with three fungal strains in order to subject it to degradation under controllable laboratory conditions. Experimental variations of parameters were made separately to determine the optimum value of each strain.

Fungal strains belonging to the genus *Penicillium* were used and were given the following codes: 1-1, 1-2, 3.

Fungi culture medium contained 2 g of pelt waste and a nutritional solution (potato-dextrose-agar) specific for this group of microorganisms.

Pelt waste was used as such and the nutritional solution was sterilized.

Experiments were made on media whose pH was adjusted before sterilization at values = 5.0, 7.0 and 9.0, respectively.

The inoculum was represented by a spore suspension containing $1.2x10^4$ units/ml. It was obtained by harvesting spores from cultures obtained on PDA (Potato-Dextrose-Agar) medium after 14 days of incubation at 28°C.

Incubation of media with pelt waste inoculated with *Penicillium* strains for each pH variant was performed at 20, 28 and 35°C.

Culture fluids were harvested from each of three strains of *Penicillium* at 7, 14 and 21 days to determine the proteolytic activity. Also, daily observations were made on the development of three strains of Penicillium in order to establish a possible correlation between the degree of development, proteolytic activity and weight loss of the substrate (pelt waste subject to degradation).

Proteolytic activity was determined by the Kunitz method. Enzyme activity was tested under the following conditions: substrate represented by 1% casein was incubated with culture fluid at 35°C for 30 minutes. Proteolytic activity was expressed in mg of casein/mL.

3. Results and discussion

Proteolytic activity in the medium with pelt waste was specific for each of the three fungal strains tested and for each strain depending on the growth conditions: pH and temperature of incubation.

Fungal strain *Penicillium* sp. 1-1, grown at pH = 5, synthesized proteases with significant activity (1.5 mg casein/mL) after 21 days of incubation at 35°C (Fig. 1).

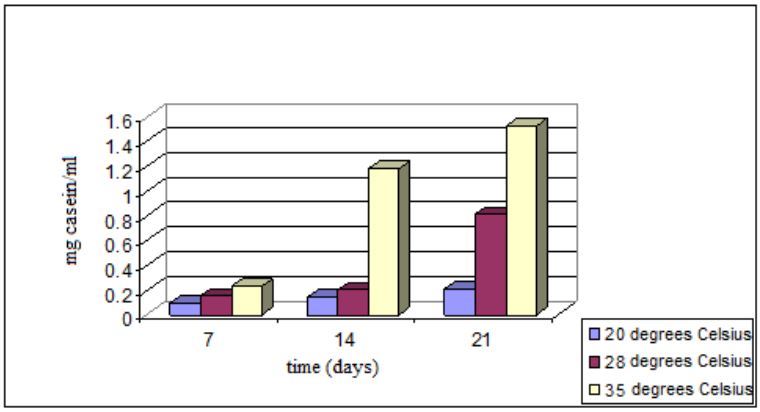

Fig. 1. Dynamics of proteolytic activity in the culture liquid of 1-1 fungal strain at pH 5 and temperature of 20, 28 and 35°C

Activity of 1.2 mg casein/mL was highlighted at pH = 7 and 9 by incubation at 35°C after 14 days and 21 days (Figs. 2, 3).

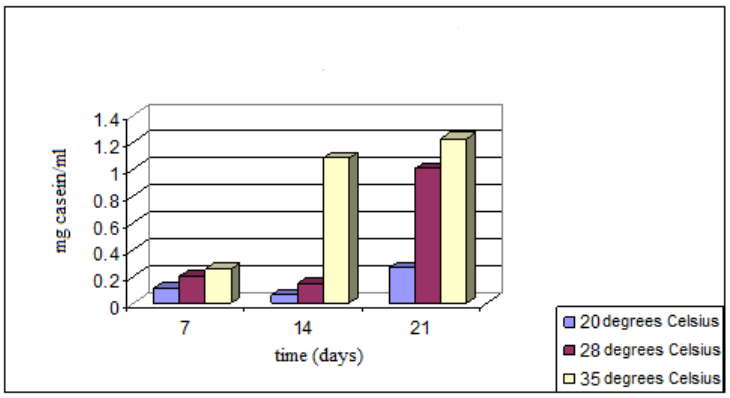

Fig. 2. Dynamics of proteolytic activity in the culture liquid of 1-1 fungal strain at pH 7 and temperature of 20, 28 and 35°C

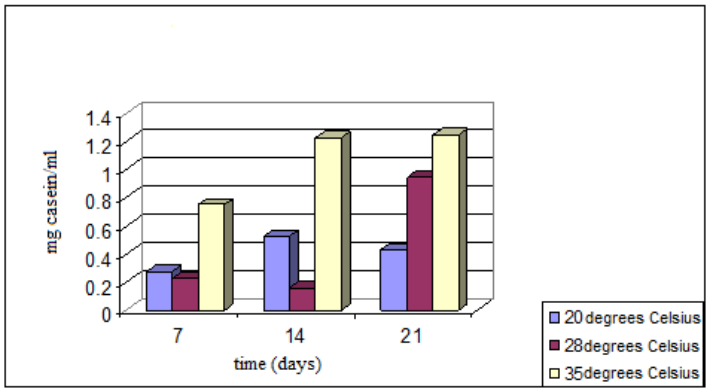

Fig. 3. Dynamics of proteolytic activity in the culture liquid of 1-1 fungal strain at pH 9 and temperature of 20, 28 and 35°C

The morphological appearance of fungal culture *Penicillium* 1-1 shows a great increase by incubation at 20°C, good at 28°C and poor at 35°C. It is noticed that there is no correlation between the degree of culture development and proteolytic activity. The experimental variant with the highest proteolytic activity was also confirmed by weight loss of pelt waste subjected to hydrolysis (0.84g - Table 1).

Table 1

Pelt waste degradation by fungal treatment

Strain code	pH	Temperature (°C)	Weight loss (g)
1-1	5	20	0.20
1-2	5	20	0.18

3	5	20	0.20
1-1	7	20	0.21
1-2	7	20	0.20
3	7	20	0.18
1-1	9	20	0.25
1-2	9	20	0.24
3	9	20	0.30
1-1	5	28	0.31
1-2	5	28	0.20
3	5	28	0.83
1-1	7	28	0.40
1-2	7	28	0.22
3	7	28	0.12
1-1	9	28	0.53
1-2	9	28	0.26
3	9	28	0.25
1-1	5	35	0.84
1-2	5	35	1.05
3	5	35	0.29
1-1	7	35	0.70
1-2	7	35	0.58
3	7	35	0.65
1-1	9	35	0.73
1-2	9	35	0.42
3	9	35	0.30

Penicillium 1-2 fungal strain synthesized proteases in significant amounts (2.9 mg casein/mL) after incubation at 35°C (Fig. 4).

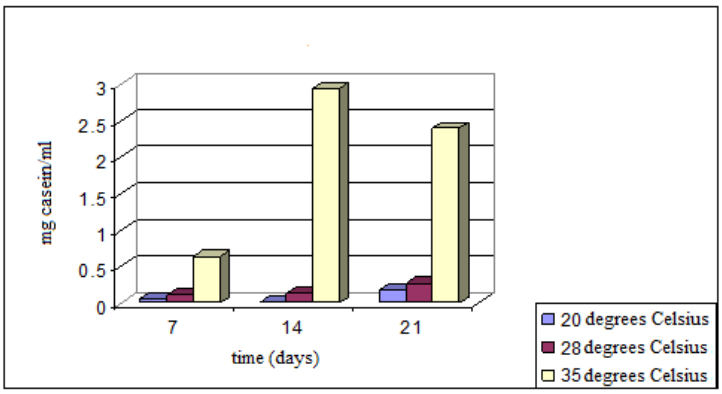

Fig. 4. Dynamics of proteolytic activity in the culture liquid of 1-2 fungal strain at pH 5 and temperature of 20, 28 and 35°C

The optimum biosynthesis pH for this strain has a value of 5, and the time of culture incubation is 14 days. Slightly reduced activities were highlighted at pH = 7 and 9 also, after culture incubation for 14 days (Figs. 5, 6).

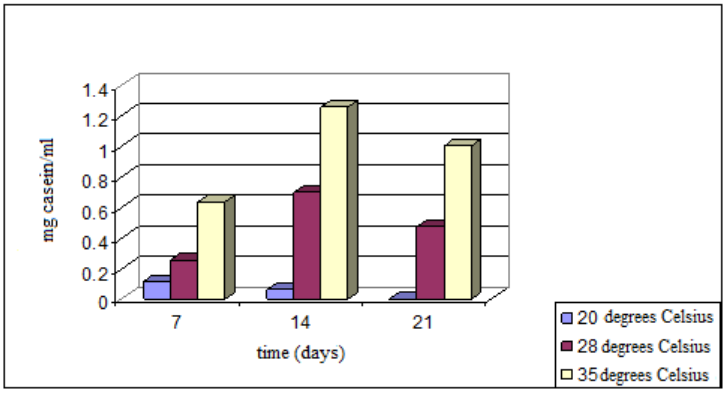

Fig. 5. Dynamics of proteolytic activity in the culture liquid of 1-2 fungal strain at pH 7 and temperature of 20, 28 and 35°C

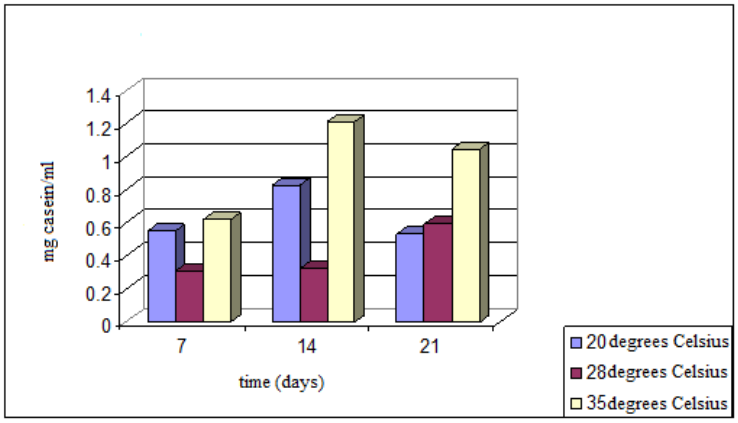

Fig. 6. Dynamics of proteolytic activity in the culture liquid of 1-2 fungal strain at pH 9 and temperature of 20, 28 and 35°C

Penicillium 1-2 fungal culture shows a good development by incubation at 28°C on the medium with pH = 7 and 9 and a reduced growth on medium with pH = 9.5 and incubated at 28 and 35°C. It is noticed that there is no correlation between the degree of culture development and proteolytic activity.

The experimental variant with the highest proteolytic activity was also confirmed by weight loss of pelt waste subjected to hydrolysis (1.05g, Table 1).

Penicillium 3 fungal strain synthesizes proteases with maximum activity (1.5 mg casein/ml) in the culture medium with pH = 5, after 21 days of incubation at 28°C (Fig. 7).

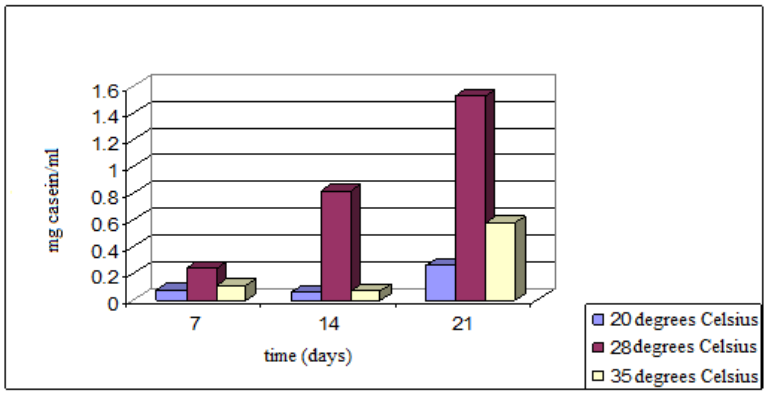

Fig. 7. Dynamics of proteolytic activity in the culture liquid of 3 fungal strain at pH 5 and temperature of 20, 28 and 35°C

Slightly lower proteolytic activity of this strain (1.1 mg casein/ml) was revealed on culture medium with pH = 7, after incubation for 14 days at 35°C

(Fig. 8). The lowest proteolytical activities were obtained in the nutritional medium of pH = 9 (Fig. 9).

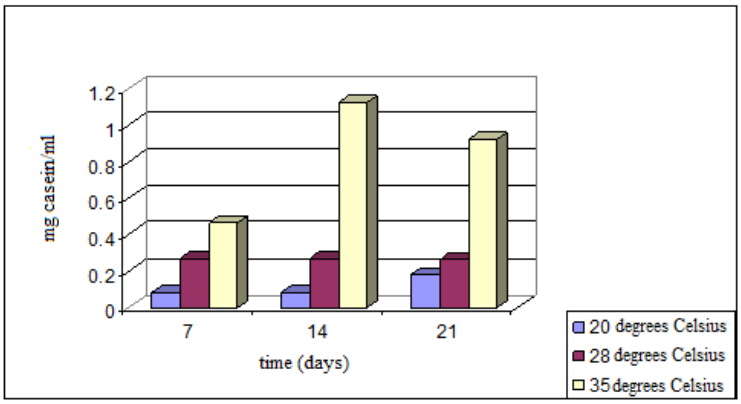

Fig. 8. Dynamics of proteolytic activity in the culture liquid of 3 fungal strain at pH 7 and temperature of 20, 28 and 35°C

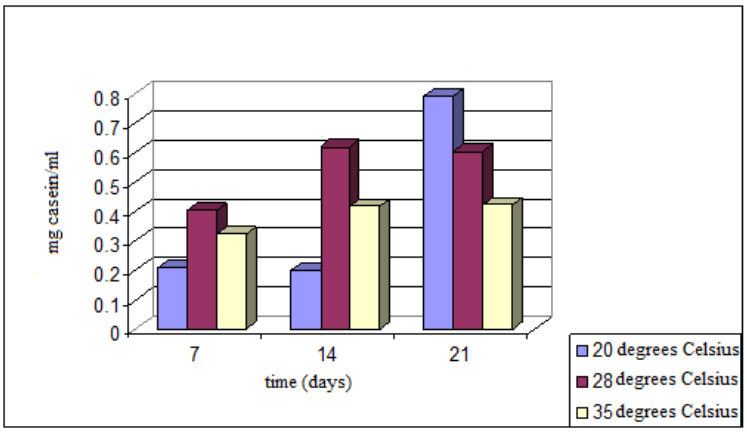

Fig. 9. Dynamics of proteolytic activity in the culture liquid of 3 fungal strain at pH 9 and temperature of 20, 28 and 35°C

Penicillium 3 fungal strain has a very good development on the medium with pH = 5-9 and incubation at 20°C, a good development on the medium with pH = 5-7 and incubation at 28°C. This strain has a reduced development by incubation at 35°C regardless of the pH value. It is noticed that there is no correlation between the degree of culture development and proteolytic activity. The experimental variant with the highest proteolytic activity was also confirmed by the weight loss of pelt waste subjected to hydrolysis (0.83g - Table 1) .

4. Conclusions

Three selected fungal strains have been tested to synthesize proteases with significant hydrolytic activity on pelt waste. Proteolytic activity of tested strains is different depending on the pH of the nutritional medium, temperature and incubation time. Protease synthesized by tested bacteria strains intensified over the incubation period; all experimental variants showing the maximum of enzymatic activity occur at 14 days and depends also on pH.

All tested strains have a very good development by incubation at 28°C. Two of fungal strans grow well on pH = 7, while the third strain grows well at all pH values. Biosynthesis activity of the proteases is not directly correlated with the biomass synthesized by the tested strains.

Results obtained in this study showed the ability of some strains of the genus *Penicillium* to synthesize large amounts of proteolytical enzymes and to degrade leather waste, as well as the possibility of using these microorganisms as a source of proteases in various biotechnological processes.

Acknowledgements

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