International Journal of
Biological Engineering and Agriculture

ISSN: 2833-5376
Volume 2 | No 12 | Dec -2023

Study of Cellulase Activity of Termites on Cellulose Degradation and their Inactivation with Sodium Fluoride

Kamoljon Nazarov [1], Mirzakhid Rakhimov [2], Askarali Soatov [3], Sirojiddin Sodikov [4]

[1] Candidate of Biological Sciences Associate Professor Head of the Department "Biotechnology", Tashkent State Technical University Republic of Uzbekistan, Tashkent

[2] Doctoral student of the Department of Biotechnology, Tashkent State Technical University Republic of Uzbekistan, Tashken

[3] Doctoral student of the Department of Biotechnology, Tashkent State Technical University Republic of Uzbekistan, Tashken

[4] Student of the direction of pharmaceutical biotechnology Tashkent Pharmaceutical Institute Faculty of Industrial Pharmacy Republic of Uzbekistan, Tashken

Abstract: Currently, very urgent problems are the determination of the distribution of the termite population, as well as physiological and biochemical processes associated with digestive secretions and the activity of symbiont enzymes, the identification of new types of microorganisms-termite parasites, the improvement of control methods based on the creation of poisonous baits based on pathogens-fungi and microorganisms.Since termites have a specialized cellulose digestion system. Various cellulases are involved in the degradation of cellulose in termites and their symbionts. An important area of research is the study of cellulase activity of termites. The purpose of the research is to study the cellulatic enzyme activity of termites. At the same time, the activity of enzymes from different parts of the body of termites was studied. The obtained data show that the obtained suspensions from different places of the termite body after filtration hydrolyze cellulose in the form of filter paper more than suspensions without filtration, this also proves that having different substances in the substrate negatively affects the biodegradation of cellulose samples (in the form of filter paper).

Keywords: Termite, cellulase, cellulose, endo-α-1,4-glucanases, cellobiohydrolases, β-glucosidases.

Introduction. Termites cause serious damage to wooden structures of cultural and historical monuments, strategic facilities, hydraulic structures, settlements and administrative buildings. One family of termites of 25 thousand individuals, living in 100 cm3 of volume, consumes an average of up to 50 thousand cm3 of various types of cellulose per year. At the same time, all this leads to a global carbon cycle and an increase in the concentration of greenhouse gas carbon dioxide in the atmosphere. All this is carried out due to the digestive secretions of termites and symbiont enzymes, as well as due to the activity of biochemical processes.

The hidden lifestyle of termites, strong protection from environmental factors, the functional specialization of castes in the termite mound and the ability to restore their population in a short time make it difficult to use means to combat them. Based on this, the definition of the population ecology of termites and the relationship with vertebrates, invertebrates and microorganisms, as well as the development of modern biological control methods that control the number of termites, are of urgent

importance.

It should be noted that there is insufficient scientific research to protect settlements and other structures from the harm of termites, the reasons for the relocation and spread of termites from natural conditions to urbanized ecosystems. Currently, very urgent problems are the determination of the distribution of the termite population, as well as physiological and biochemical processes associated with digestive secretions and enzyme activity of symbionts, the identification of new types of microorganisms-termite parasites, the improvement of control methods based on the creation of poisonous baits based on pathogens-fungi and microorganisms.

Termites have a specialized cellulose digestion system[1]. Various celluloses are involved in the degradation of cellulose in termites and their symbionts. An important area of research is the study of the cellulase activity of termites. The three main types of cellulases are endo-α-1,4-glucanases, cellobiohydrolases and β-glucosidases, and cellulose degradation requires the synergistic action of three types of glycoside hydrolases. The models and characters of cellulases in termites and their symbionts have been widely described [2], and the cellulase activity of cellulases and their distribution in the digestive system were different in different species of termites. Recently, the distribution of different cellulase activity in each segment of the intestine of termites has been mainly studied, and it has been found that the expression of endogenous cellulase genes has moved from the salivary glands of lower termites to the midgut of higher termites.

Recent studies show that the distribution of cellulase activity in termites is related to their evolutionary levels [4,5].

Materials and methods. Termites. Representatives of termites of the genus *Anacanthotermes turkestanicus*, *Anacanthotermes ahngerianus* are found in Uzbekistan. Our research object is termites of the genus *Anacanthotermes turkestanicus*. Termites were brought from different regions of Uzbekistan.

Obtaining a crude enzyme. To obtain enzyme extracts, workers or soldiers were washed with a pre-cooled 0.09% normal saline solution. 1 sample - fifteen sets of heads (including salivary glands), 2 sample – thoracic and abdominal parts of the body of a termite, 3 sample - the whole body of termites. All samples were collected in test tubes and homogenized in 500 ml of 0.1 M sodium acetate buffer (SAB) (pH 5.6) on ice. The test tubes were centrifuged at 12,000 rpm for 15 minutes at a temperature of 4 ° C, and the supernatant was brought to a volume of 500 by adding 0.1 M SUB and used as an enzyme extract. The same volume of 0.1 M SUB was used as a control.

Analysis of cellulase activity. In two test tubes, 50 mg of the colored substrate was suspended in 4 ml of 0.1 M acetate buffer (pH = 4.5) at 40^{o}C, stirring on a magnetic stirrer for 5 minutes. Then 0.1 ml of a solution containing 2-6 mg of the enzyme preparation was added to one tube, while continuing to mix. 0.1 ml of the specified enzyme preparation solution was also added to another tube, and the contents were quickly passed through a filter (control). After 20 minutes. The reaction mixture in the first tube was passed through a filter and the optical density of the filtrate was determined at a wavelength of 490 nm for OC-31 and 375 nm for NOS-OX against the control sample.

The optical density of the hydrolysates was measured using a Spekol-II spectrophotometer (GDR). Cellulase activity was determined by the results of three parallel measurements according to the formula:

$$\text{A (standard units/g)} = \frac{D \cdot 1000}{M};$$

where D is the optical density of the hydrolysate, M is the mass of the enzyme preparation taken for analysis.

The conventional unit of activity was the activity of such an amount of enzyme, which forms 1 unit of optical density at 500, 490, 410 or 375 nm (depending on the type of dye) in 20 minutes.

Results and discussion. Termites play an important role in the degradation of cellulose materials in

nature, and special attention is paid to the activity and expression of termite cellulases [2]. Effective digestion of cellulose in termites requires both endogenous and intestinal microbial cellulases. The present study showed that the thoracic and abdominal parts are the main site of cellulose digestion in termites, which is consistent with the report.

We conducted a comparative determination of the activity of crude enzymes from three parts of the termite body in relation to filter paper, which was determined by the formation of glucose and reducing sugars by the Shomody–Nelson method [6]. The amount of enzyme was taken as a unit of activity, which leads to the formation of 1 μm of BC in 1 min from the corresponding substrate. Table 1 and Figure 1 show the results of these experiments, from which it can be seen that the most active was the samples of the thoraco-abdominal part of the body of termites.

Table 1. Total cellulase activity of different parts of the body in the tested termite workers.

№	Body parts of termites	After 1 houra	48 hours	72 hours
1	A complete body of termites	7,4	3,9	3,2
2	Head of the termite	4,7	4,2	4,2
3	Thoracic and abdominal parts of the termite	10,5	10	9,5

To select the optimal hydrolysis parameters, hydrolysis experiments were carried out at pH 5.0 of the medium and temperature 55-60 ^{O}C. The initial rate of enzymatic hydrolysis significantly depends on the degree of adsorption of enzymes on the substrate. In some cases, the initial reaction rate can be increased by adding surfactants (surfactants), which increase the wettability of cellulose, and consequently its bioavailability for enzymes. We conducted a study of the initial rate of the enzymatic reaction for glucose yield when adding various concentrations of surfactants of Triton X-100. Most effectively increases the initial rate of enzymatic hydrolysis by 0.5% Triton X-100. Higher concentrations of Triton X-100 have no effect.

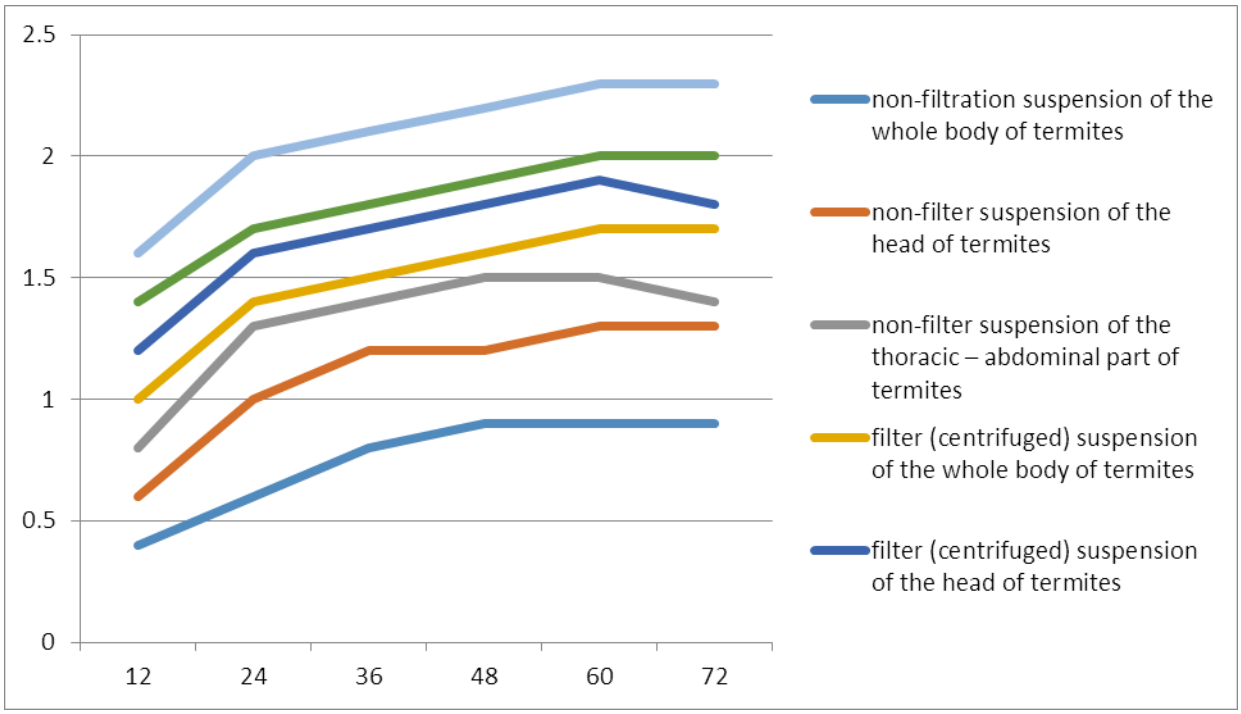

Fig. 1. Glucose yield (g/l) during enzymatic hydrolysis of filter paper using various suspensions obtained from different parts of the body of termites: sample I, sample II, sample III.

In the second stage of our research, it was planned to solve problems that included the preparation of suspensions of the head, thoraco-abdominal and whole body of termites, in this section the biodegradability of filter paper was determined under the action of a suspension obtained from different parts of the body of termites, the optimal ratio was non-filtration suspension 1,2,3; filtration (centrifuged) suspension 4,5,6; and 7 pure enzyme the drug (control).

Subsequently, we studied the effect of inhibitors on the cellulose activity of the termite enzyme. The data obtained indicate that sodium fluoride at a concentration of 10 mg/l inhibits cellulase activity in all samples and, although this effect manifests itself to varying degrees (a decrease in activity from 1.5 to 5 times).

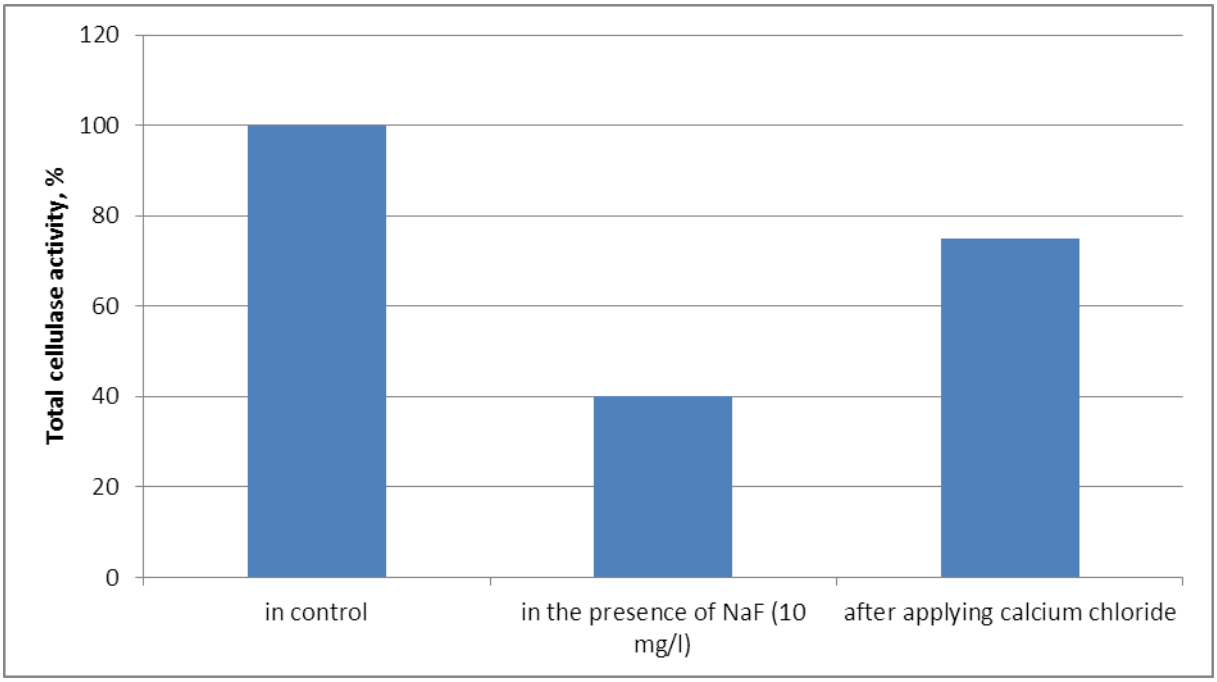

Fig. 2. Reactivation of the enzyme of the abdominal part of the termite with calcium chloride after fluoride-dependent inhibition of cellulase activity

As for the dynamic shift in termites, our results showed that cellulase activity and degrading activity of filter paper were most strongly concentrated in the intestine of the studied termites than in other samples (body parts, samples I and II) of termites, which is confirmed by the studies of EG [7], the dynamic change in cellulase activity is confirmed by the corresponding Fujita report A., T. Miura, T. Matsumoto (2008) and Tokuda G (2009). They hypothesized that the main position of activity is towards the head/forelimb with the evolution of arboreal termites, and evolved arboreal termites such as Rhinotermitidae and Termitidae termites may have higher HD activity in the thoracic part of the termite body.

As for the comparison of cellulase activity in whole termite bodies, it was suggested that the degrading activity of filter paper and the percentage of CBH in complete cellulases increase with an average increase in evolutionary status.

The obtained data show that (Fig. 1.) the obtained suspensions from different places of the termite body after filtration hydrolyze cellulose in the form of filter paper more than suspensions without filtration, this also proves that having different substances in the substrate negatively affects the biodegradation of cellulose samples (in the form of filter paper).

The data obtained makes it possible to conclude that termites in the thoracic and abdominal parts, the cellulase activity of the termite is gradually decreasing. This phenomenon can be explained by the fact that there are various impurities in the composition of the resulting suspension and they can affect the cellulase activity, since it has been repeatedly proven that multinomial factors such as temperature, substrate, state and composition of the substrate can affect the activity of cellulase[3].

It has been shown that sodium fluoride at a concentration of 10 mg/l inhibits cellulose activity in all samples and, although this effect manifests itself to varying degrees (a decrease in activity from 1.5 to 5 times).

The experiment translated in Figure 2 serves as proof that the observed effect is determined precisely by the fluoride anion, during which an equimolar amount of calcium chloride was added to the

reaction medium (enzyme-substrate-buffer) containing sodium fluoride (10 mg/l). After precipitation of calcium fluoride, partial reactivation of the enzyme occurred.

Literature

1. Назаров К.К., Рахимов М.М. Изучение целлюлазной активности термитов по деградации целлюлозы // Universum: химия и биология: электроный научный журнал 2021.11(89).29-32 URL:https://7universum.com/ru/nature/archive/item/12389
2. Correlation of cellulase gene expression and cellulolytic activity throughout the gut of the termite Reticulitermes flavipes. Nakashima et al. 2002; Tokuda et al. 2007; Zhou et al. 2007
3. Watanabe H. & G. Tokuda 2010. Cellulolytic systems in insects. Annual review of entomology. 55: 609-632.
4. Willis J.D., C. Oppert, J.L. Jurat-Fuentes 2010. Methods for discovery and characterization of cellulolytic enzymes from insects. Insect Science 17(3): 184-198.
5. Tokuda G., N. Lo, H. Watanabe 2005. Marked variations in patterns of cellulase activity against crystalline- vs. carboxymethyl-cellulose in the digestive systems of diverse, woodfeeding termites. Physiological Entomology 30(4): 372-380.
6. Tokuda G., Lo N., Watanabe H., Arakawa G., Matsumoto T., Noda H. 2004. Major alteration of the expression site of endogenous cellulases in members of an apical termite lineage. Molecular Ecology 13(10): 3219-3228.
7. Nelson M.I., Kelsey R.G., Shafizaden F. Anhancement enzymatic hydroly-ses by Simultaneus attrition of cellulosed Substrates. // Bio-technol and Bioeng, 1982, vol 24,p 293-294.
8. Lo N., G. Tokuda, H. Watanabe 2011. Evolution and function of endogenous termite cellulases. In: D.E. Bignell et al. (eds.), Biology of Termites: a modern synthesis, pp.51- 67. Springer Science+Business Media, London New York.