CELLULOSE DIGESTION IN *HETEROTMES INDICOLA*, WASMANN AND *COPTOTERMES HIEMI*, WASMANN

by

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ABSTRACT

High activities of cellulase and cellobiase have been found in the gut extracts of the worker caste of two species of *Heterotermes indicola*, Wasmann and *Coptotermes heimi*, Wasmann. The properties of the two enzymes from *H. Indicola* have been investigated. It has been found that the soldier caste of these termites is capable of splitting cellobiose while incapable of breaking down cellulose into simpler sugars.

Introduction

The presence of both cellulase and cellobiase was shown by Trager[1] in the guts of the 'lower' termites *Reticulitermes flavipes* and *Termopsis augusticollis*. The origin, nature and properties of the cellulose digesting enzymes present in the digestive tract of the 'higher' termite *Termes* (*cyclotermes*) *obesus* Rambur were reported earlier from these laboratories[2]. In the present investigation, the nature and properties of the cellulolytic enzymes present in the intestinal tract of *Heterotermes indicola* Wasmann and *Coptotermes heimi* Wasmann are described.

Materials and methods

Insects—The insects of the two species, *H. indicola* and *C. heimi* were collected locally and also at Gonda (Uttar Pradesh). They were found to harbour an abundance of symbiotic flagellates in their hind gut. The experimental studies described in this paper were mostly carried out on the worker caste of *H. indicola*. In the case of *C. heimi* the investigations were confined to the determination of optimum *pH* for maximum cellulase and cellobiase activities. Cellulase and cellobiase activities in the soldier caste of both the species were also determined.

Preparation of gut extract—The method of preparation of the gut extract was the same as described earlier[2]. However, in view of the higher cellulolytic activity found in the case of these species the total number of guts used was 25 instead of 200 used for the higher termite.

Estimation of cellulase and cellobiase activities—The methods used for the estimation of cellulase and cellobiase activities were the same as described earlier[2]. However, in view of the difference in the optimum *pH* observed for these cellulolytic enzymes (see Fig. 3) phosphate buffer of *pH* 4·8 instead of *pH* 4·0 was used in the tests.

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Micro-estimation of end products—The reducing sugars which are the end products of the enzymatic hydrolysis of the cellulose and cellobiose substrates were estimated by the method of Hagedorn and Jensen[3].

Results

Presence of cellulase and cellobiase in the gut extract—To two tubes containing the gut extract were added separately cellulose suspension and phosphate buffer of pH 5·5 and cellobiose solution in the same buffer solution. The reducing groups were estimated initially and after 24 hours from the increase in the reducing sugars, the per cent hydrolysis of cellulose and cellobiose was calculated. The degrees of hydrolysis obtained with cellulose and cellobiose were 11 and 77 per cent respectively.

Optimum pH—The gut extracts of the workers of *H. indicola* and *C. heimi* were separately incubated with cellulose and cellobiose using McIlvaine's Universal phosphate buffers whose pH varied from 2 to 8. The optimum pH as evidenced by the maximum degree of hydrolysis was determined for both the species. The results obtained are represented in Figs. 1, 2 and 3. In order to

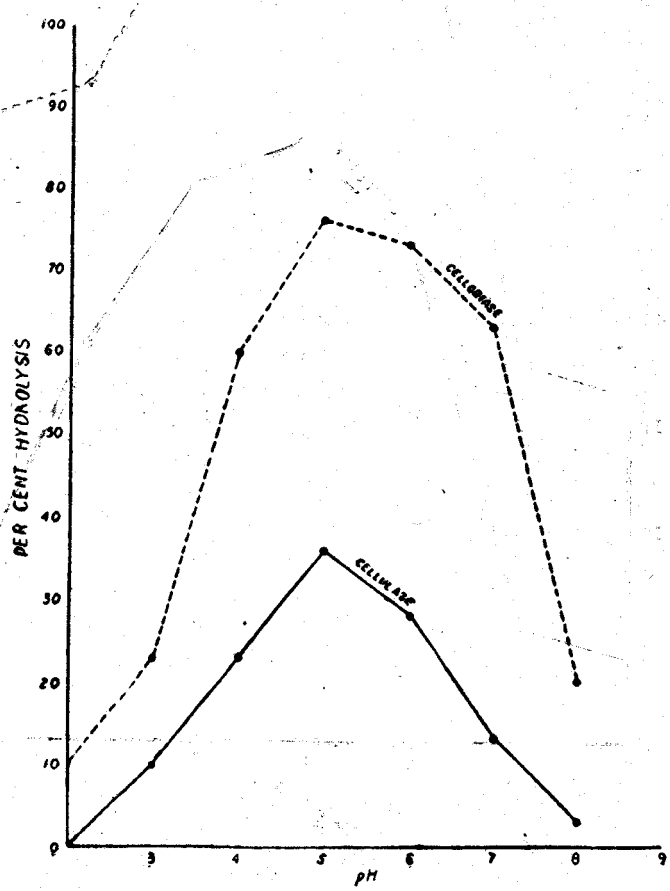


FIG 1

obtain a more accurate value for *H. indicola* the enzymatic activity over a *pH* range of 4·0 to 6·0 was determined. The results obtained are represented in Fig. 3. Cellobiase has a sharp optimum *pH* at 4·8 while cellulase has an optimum range 4·8 to 5·8.

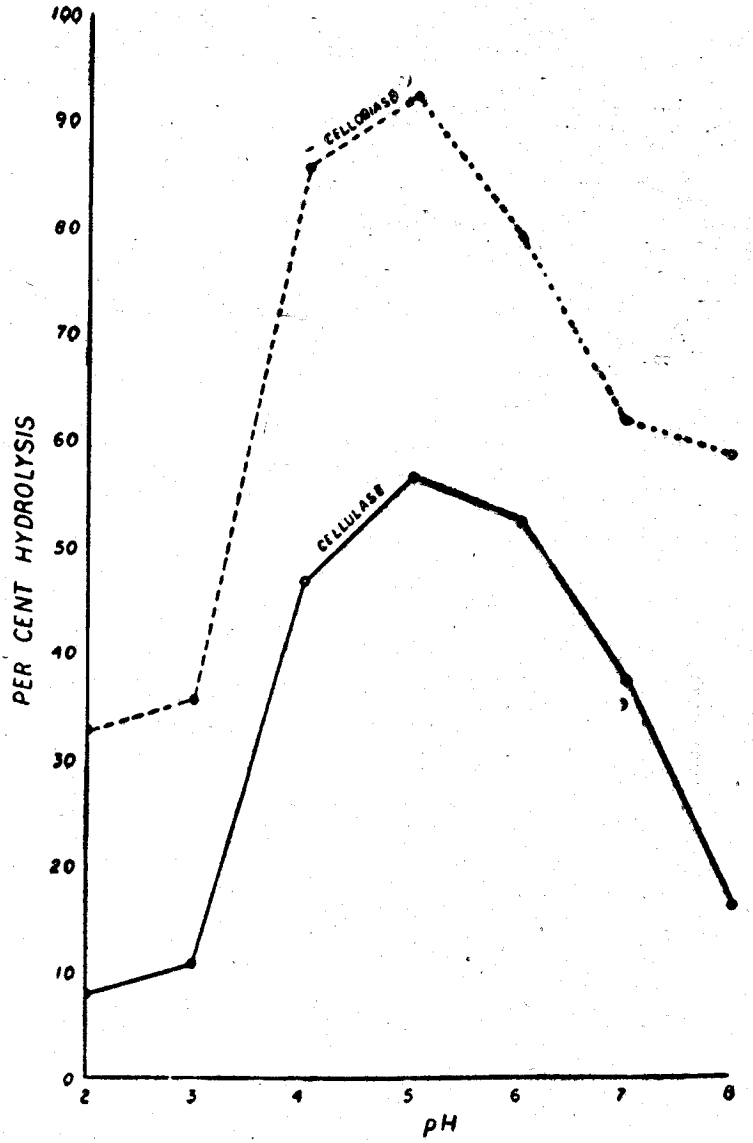


FIG 2

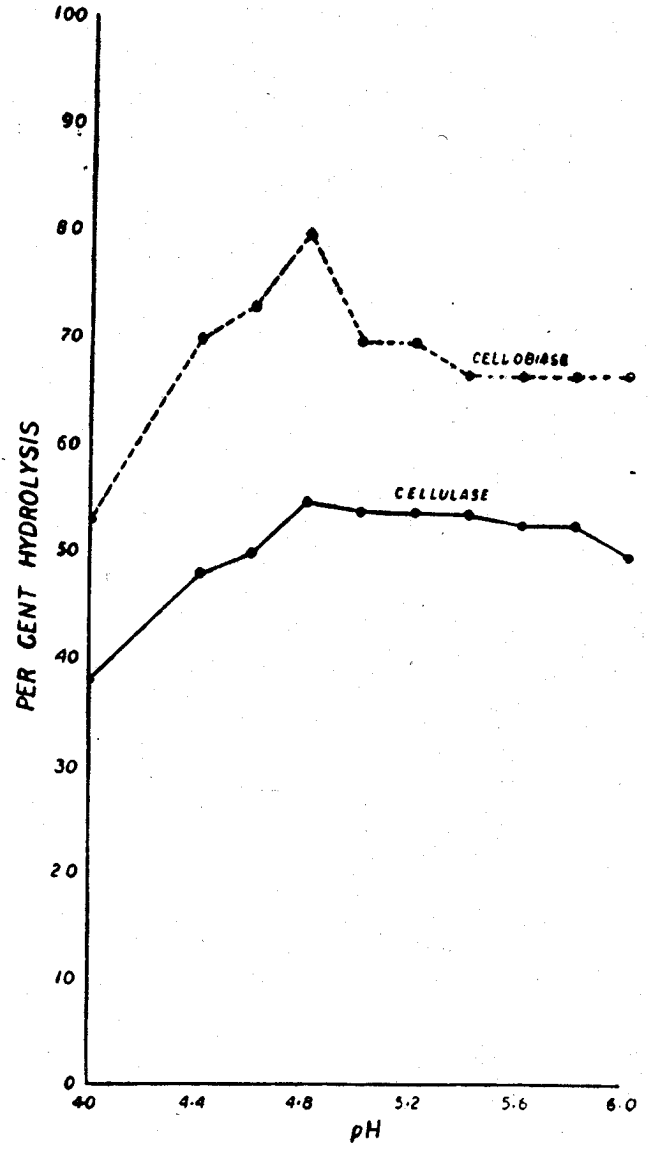

100
90
80
70
60
50
40
30
20
10
0
PER CENT HYDROLYSIS
CELLOBIASE
CELLULASE
4·0
4·4
4·8
5·2
5·6
6·0
pH

FIG. 3

Optimum temperature—Test tubes containing gut extract of the workers of *H. indicola* and the cellulose suspension or cellobiose solution with phosphate buffers of appropriate optimum *pH* were incubated for 24 hours at different temperature levels ranging from 5° to 60° C and the respective enzyme activities determined. The results are represented in Fig. 4. Maximum cellulase activity was obtained in the range 32°—39°C.

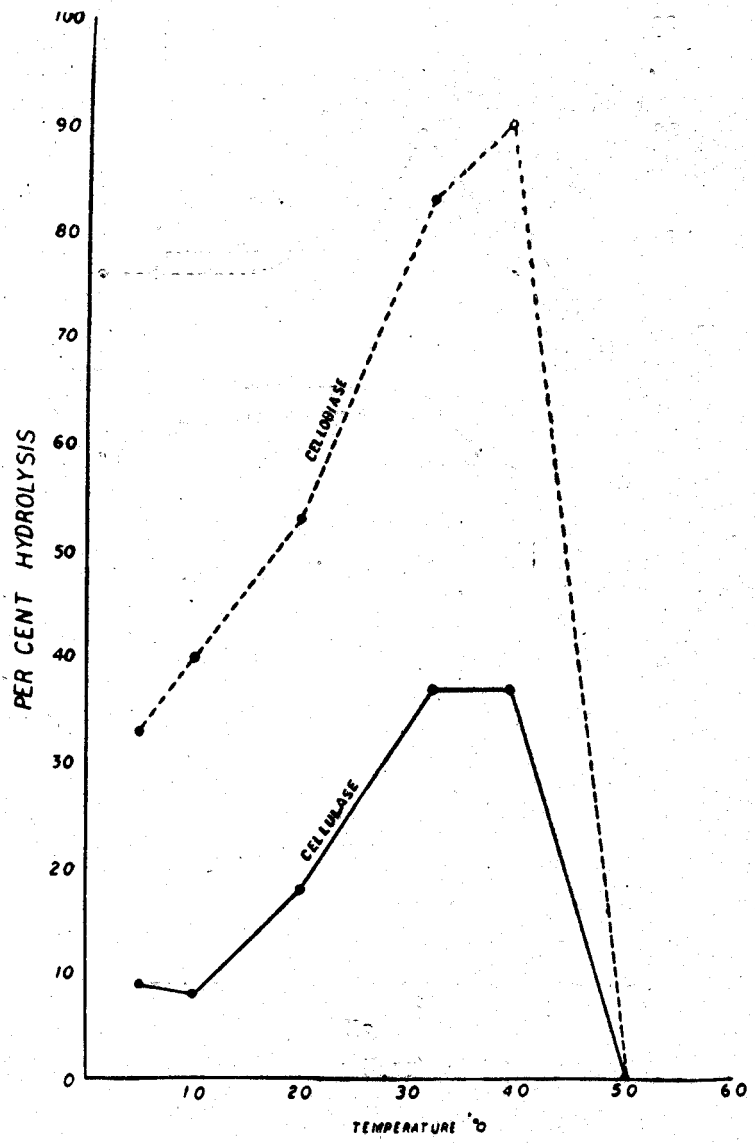


FIG. 4

Cellobiase activity was the highest at 39°C. In either case the enzyme activity was destroyed at 50°C.

Heat stability—The stability towards heat of cellulase and cellobiase was investigated by maintaining the gut extracts of the workers of *H. indicola* for one hour in water baths maintained at different temperatures ranging from 40° C to 60°C and then estimating the cellulase and cellobiase activities. The results are represented in Fig. 5. It will be observed that the cellulase and cellobiase activities are completely destroyed at 60°C and 70°C respectively.

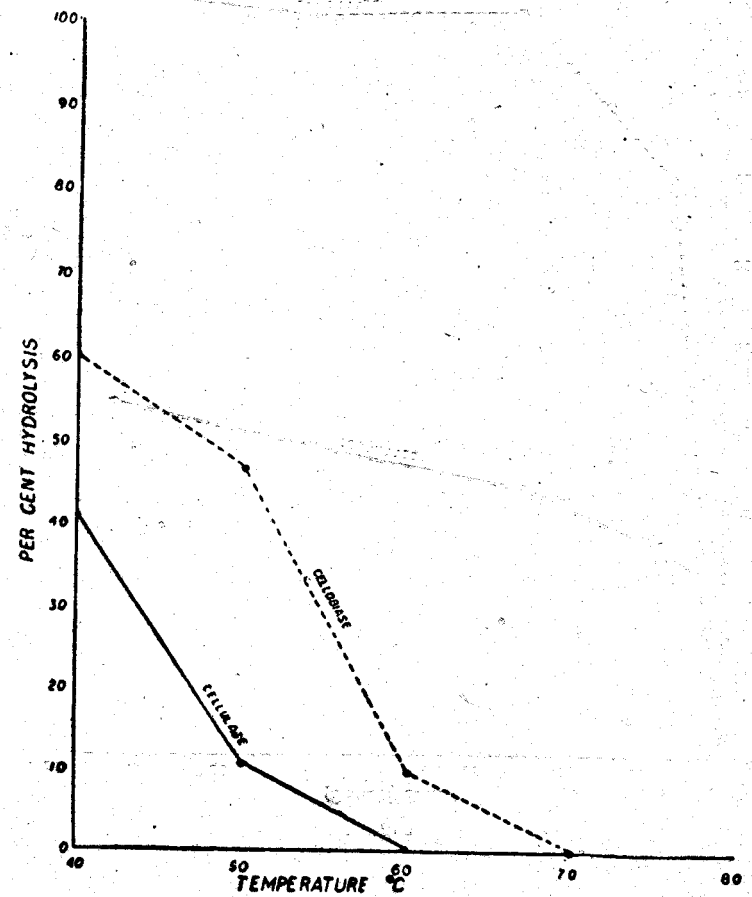


FIG. 5.

Optimum period of incubation—Using gut extracts of *H. indicola* cellulase and cellobiase activities were estimated initially and after incubating at 37 ± 1°C for 3,6, 24, 48 and 72 hours. In both the cases, most of the hydrolysis took place at the end of 24 hours. Incubation for longer periods did not bring about any appreciable increase in enzymatic activity. The results are represented in Fig. 6.

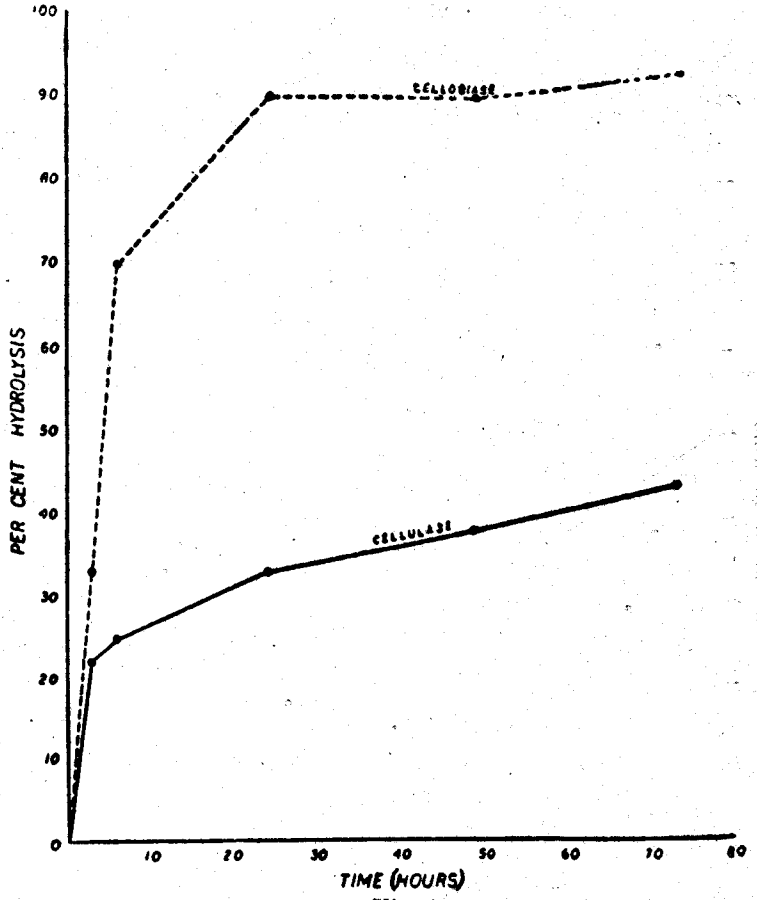


Fig. 6.

Cellulase and cellobiase activities in relation to enzyme conscentration— These experiments were not carried out on purified enzymes. The concentration of the crude enzymes in the gut extract of the workers of *H. indicola* was varied by adjusting the number of guts used for preparing the extract. 1, 5, 10, 25, 75 and 100 guts per ml. were used for the different concentrations of the enzymes. The results are represented in Fig. 7. With both the enzymes the activity increases with the increase on the number of guts.

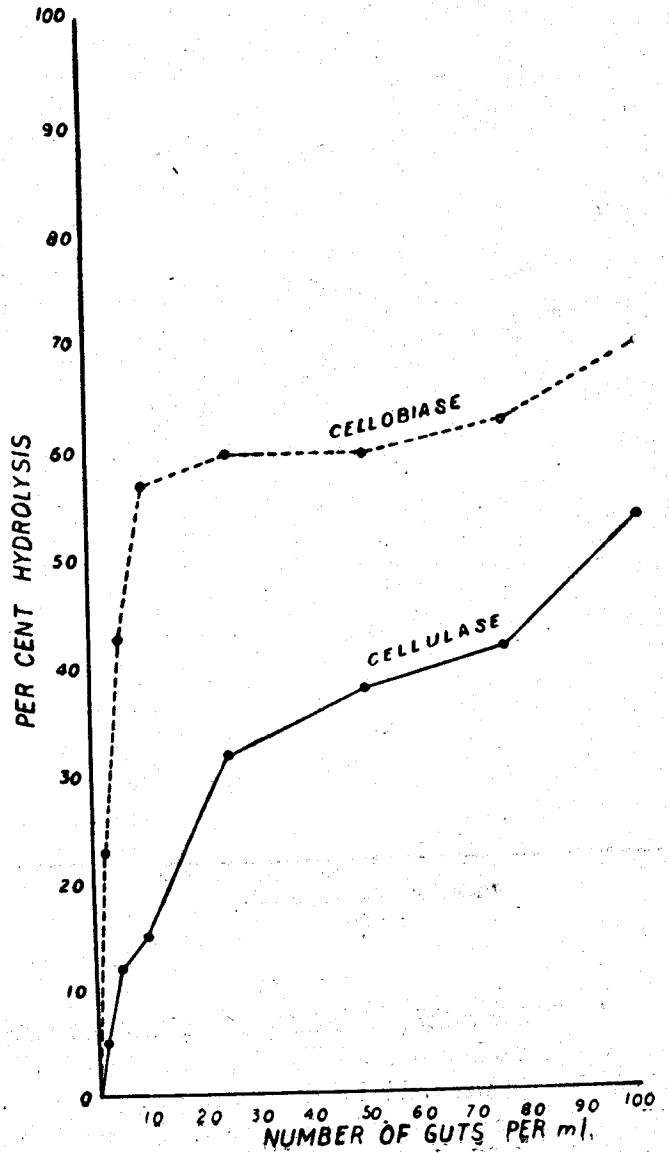


Fig. 7

Effects of storage on the enzymatic activity—The gut extract of the workers of *H. indicola* containing cellulase and cellobiase were stored at 10°C and the activities of the two enzymes measured initially and at intervals of 1, 2, 3 and 4 weeks. There was a gradual fall in cellulase activity upto the third week after which it remained more or less the same. Within the limits of experimental error, the cellobiase activity remained constant during the 4 weeks of storage. The results are shown in Fig. 8.

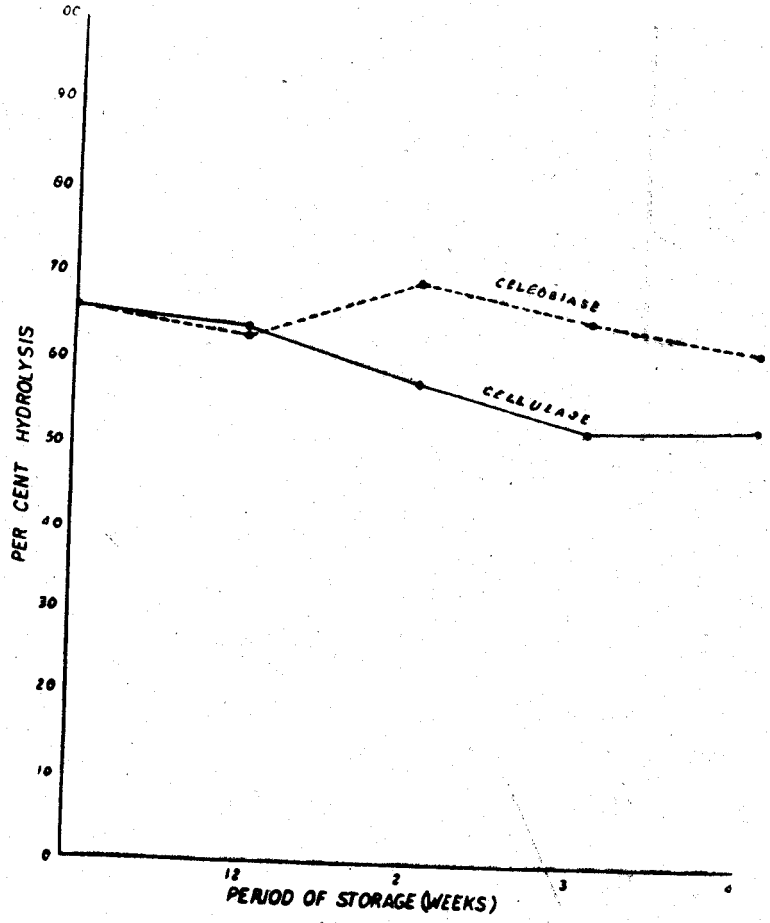
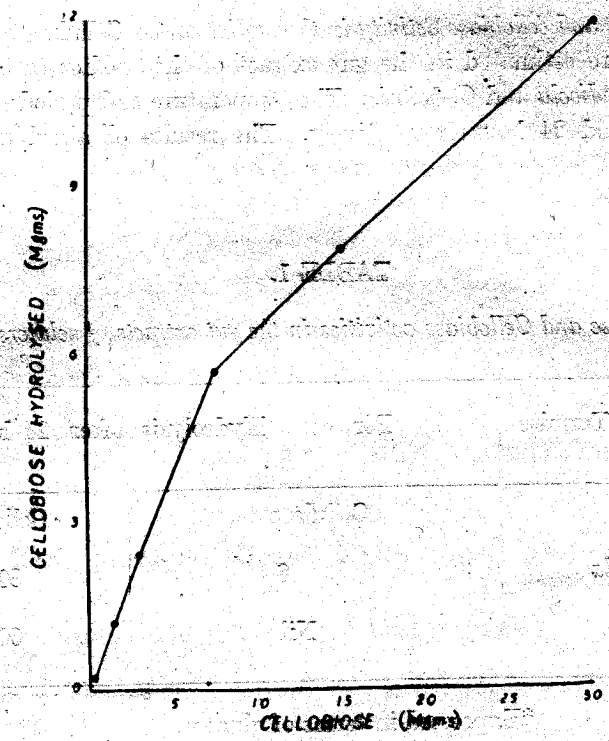


Fig. 8

Cellulase and cellobiase activity in relation to substrate concentration—To the gut extract of the workers of *H. indicola* varying amounts of substrate (cellulose and cellobiose) were added and cellulase and cellobiase activities estimated. The results are represented in Fig. 9 and Fig. 10 for cellulase and cellobiase respectively. With the increase in concentration of the substrate the quantity hydrolysed also increased.


CELLOBIOSE HYDROLYSED (Mgms)
12
9
6
3
0
5
10
15
20
25
30
CELLOBIOSE (Mgms)

Figs. 9

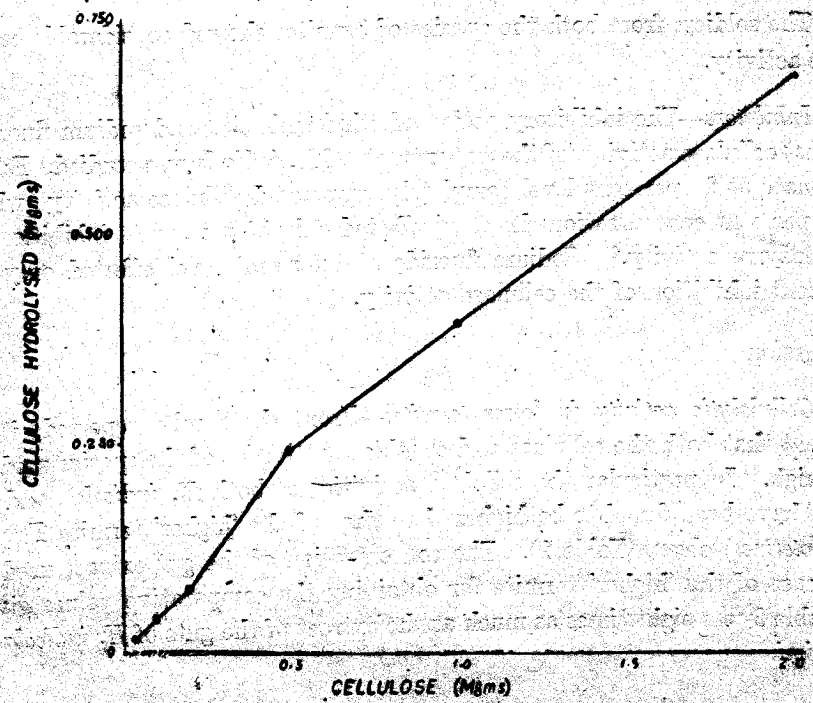

CELLULOSE HYDROLYSED (Mgms)
0.750
0.500
0.250
0
0.5
1.0
1.5
2.0
CELLULOSE (Mgms)

FIG. 10

Cellulase and cellobiase activity in the soldier caste—Cellulase and cellobiase activities were estimated in the gut extract of the soldier caste of the two species, *H. indicola* and *C. heimi*. The temperature and period of incubation were 33°C and 24 hours respectively. The results obtained are given in Table 1.

TABLE 1.

Cellulase and Cellobiase activities in the gut extracts of soldiers.

Species of Termite	Per cent Hydrolysis after 24 hours*	
	Cellulase	Cellobiase
H. indicola	8	80
C. heimi	Nil	60

*Expressed in terms of glucose.

The soldiers from both the species of termites showed considerable cellobiase activity.

Inhibitors—The inhibitory action of ethyl malonate and sodium fluoride against cellulase activity, of the gut extracts of *H. indicola* was studied. Ethyl malonate at 2 per cent level completely suppressed cellulase activity while at 1 per cent concentration the ester effected only 50 per cent inhibition of the enzyme activity.[4] Sodium fluoride at 4 per cent level effected only 20 per cent inhibition of the cellulase activity.

Discussion

Cellulolytic activity in lower termites—From the results reported, it is evident that both the cellulase and cellobiase activities of the species studied are high. In particular the cellulase activities of both *H. indicola* and *C. heimi* have been found to be higher than that of the 'higher' termite *Termes* (*Cyclotermes*) *obesus* (Table 2). The concentration of the gut extract used in the case of the 'higher' termite for obtaining the comparative values given in Table 2 was eight times as much as the extract of the guts of *H. indicola* or *C. heimi*.

TABLE 2

Comparative activities of Cellulase and Cellobiase in the 'higher' and 'lower' Termites Studied.

Species of Termite	Per cent Hydrolysis after 24 hours*	
	Cellulase	Cellobiase
T. obesus	35	93
H. indicola	37	90
C. heimi	57	93

*Expressed in terms of glucose.

The range of optimum temperature for maximum enzymatic activity for both cellulase and cellobiase as well as their heat stability were similar to those reported earlier for the 'higher' termites[2]. Cellulase and cellobiase activities were completely destroyed at 50°C after 24 hours incubation and at 70°C after one hour incubation.

The optimum pH for both cellulase and cellobiase activities was found to be 4·8. This figure is slightly higher than that reported for the enzymes elaborated in the guts of the 'higher' termite *T. obesus*. The difference in the optimum pH observed is perhaps to be sought in the fact that the agencies which contribute these enzymes in the 'lower' termites investigated are flagellates[5,6] while those contributing these in the 'higher' termite are certain cellulolytic bacteria[2].

Cellulolytic activity in the soldier caste—A high degree of cellobiase activity has been observed in the gut extracts of the soldier caste of both species of lower termites, *H. indicola* and *C. heimi*. Cellulase activity was, however, low in *H. indicola* and absent in C. *heimi* (Table 1.) But in the case of 'higher' termite *T. obesus*, it has been reported[2] that both cellulase and cellobiase activities were completely absent in the soldier caste.

Conclusion

Experiments were carried out to study the digestion of cellulose by two species of 'lower' termites *Heterotermes indicola* Wasmann and *Coptotermes heimi* Wasmann. High activities of cellulase and cellobiase have been found in the gut extracts of the worker caste of both the species. The optimum pH for maximum cellobiase activity has been found to be 4·8 while cellulase has an optimum range 4·8 to 5·8. Maximum cellulase activity was obtained in the temperature range 32—39°C while cellobiase activity was the highest at 39°C. In either case the enzyme activity was destroyed at 50°C. The soldier caste of both the species has been found to possess considerable cellobiase activity. Ethyl malonate at 2 per cent concentration completely inhibited cellulase activity. Sodium fluoride showed poorer inhibition.

Acknowledgement

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