single Na^+ channels during the period of pulse application, following pulse onset. The amplitude of the tail current is proportional to the Na^+ current at the time of repolarization and is only seen if repolarization occurs before the inactivation process is complete, and its analysis is particularly used in insecticide research since the tail current characteristics give information about channels in the open state. The tail current in fenvalerate-treated condition is also proportional to the number of open channels, but the decay of the current is much slower. In Figure 3 b it is seen that there is an additional slow component of tail current that does not decay during the time scale shown. This slow component is most likely due to modified open channels which return to the resting state slower by a factor of about 10 as opposed to normal channels. It is also likely that fenvalerate induces some inactivated channels to open spontaneously and become modified, resulting in the appearance of the slow tail current component following fenvalerate addition, as has been suggested earlier for the pyrethroid tetramethrin in squid axons[9]. While both fenvalerate and tetramethrin slow the inactivation and the tail current characteristics, fenvalerate decreases the amplitude of the current elicited during a depolarization pulse, and tetramethrin does not. The difference can partly be explained by the structural differences between fenvalerate and tetramethrin; while fenvalerate is a cyano pyrethroid compound, tetramethrin is a non-cyano pyrethroid.

There is also reason to believe that fenvalerate acts differently on the Na channels of animals in the evolutionary scale. Thus, while fenvalerate decreases the Na current characteristics during depolarization, viz. current amplitude and inactivation time constant of mammalian DRG neurons suggested by our experiments, it only affects the tail current kinetics of amphibian Na channels without affecting the sodium current elicited during the depolarization pulse, demonstrated earlier in myelinated nerve fibres of the clawed frog, *Xenopus laevis*[4]. This difference can partly be explained by inherent subtle differences in the Na channel structures, although it is true that basic structural and functional aspects of voltage-gated ionic channels are strongly conserved over evolution.

The decrease in peak I_{Na} amplitude with fenvalerate addition can be attributed to either closure of a subpopulation of Na^+ channels in the resting state and/or channel modification to decrease single channel conductance. Further, the effects described above could be due to fenvalerate's action on a 'pyrethroid receptor' on the Na^+ channel[3]. These are aspects which are in the process of detailed investigation.

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Characterization of cellulose and hemicellulose degrading *Bacillus* sp. from termite infested soil

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A *Bacillus* strain having a broad spectrum of enzymes responsible for degradation of cellulosic and hemicellulosic components of agricultural wastes was isolated from the termite infested soil. The optimum temperature and pH for growth of the bacterium were 37°C and 7.2 respectively. The isolate was capable of fast growth on soluble products, xylan (μmax = 0.45 h^{-1}) and carboxymethyl cellulose (μmax = 0.20 h^{-1}) rather than on the rice husk (μmax = 0.15 h^{-1}), the insoluble substrate. The organism was characterized morphologically and biochemically. Similarities with *Bacillus licheniformis* are very evident.

TERMITES are prominent members of arid ecosystems. They are considered to be supreme converters of organic matters in soil from the tropics to the desert; they stir and mix and with the aid of bacteria, protozoa and fungi recycle cellulosic materials[1]. The importance of bacterial component of the termite's intestinal microbiota in cellulose digestion has been extensively reviewed[2], however, the role of microorganisms inhibiting termite infested soil is scanty. It is therefore of interest to ascertain the characteristics of bacteria isolated from this specialized ecological niche located in

arid and semi-arid regions and to study their significance in cellulose depolymerization process.

Organisms showing both cellulolytic and xylanlytic properties have been studied extensively because of their ability to degrade both cellulosic and hemicellulosic components of agricultural residues like rice husk, baggasse, etc. These organisms include *Bacillus firmus*, *B. polymyxa* and *B. brevis* which produce both cellulase and xylanase enzyme extracellularly[3]. In this communication, the isolation and characterization of a mesophilic aerobic bacterium harbouring termite mound soil is described together with enzymes involved in breakdown of lignocellulosic substrate-like rice husk.

Soil samples were collected in sterile bottles from live termite mound soil and termite infested tree bark of xerophytic plants growing in arid and semi-arid regions of Northern India. Samples were incubated in an enrichment medium containing the basal medium[4] and eventually the organisms were isolated in pure culture. Endoglucanase, β glucosidase and endo 1,4-β-xylanase activities of the present strain were confirmed by following the screening technique[5].

Negative staining procedures were performed on cells growing in the log phase, prefixed with 3% glutaraldehyde in 0.1 M Na-cacodylate buffer at pH 7.0 for 60 min at room temperature. After washing with distilled water, the cells were stained with 0.5% uranyl acetate.

Cells grown in 1% CMC were fixed for one hour in 5% (v/v) glutaraldehyde in 0.1 M sodium carcodylate buffer at pH 7.2 at 4°C containing also 0.15% (v/v) Ruthenium red[6], post fixed for 60 min in a solution of 1% w/v O_5O_4 dissolved in the same buffer at room temperature and subsequently post-stained in 1% w/v of uranyl acetate. After dehydration in a graded ethanol series, the cells were embedded in spurr, sectioned and observed under transmission electron microscope.

Biochemical characterization of the strain was done following Bergey's manual[7]. The isolation of DNA and determination of guanine–cytosine content was done by measuring thermal denaturation temperature with a Gilford spectrophotometer model 250[8].

Endoglucanase and β-xylanase activities were determined in the culture supernatant[9]. β-glucosidase and β-xylosidase activities were determined in the intracellular mileu. Harvested cells were washed thrice and suspended in 50 mM PO_4 buffer at pH 7.0. Enzymes were released by ultra sonication (MSE) for 10 min at 18 μ frequency (peak to peak) on ice. The crude lysate was centrifuged at 10,000 g. To the supernatant, 0.03% (w/v) sodium azide was added prior to storage at $-20°$C. The standard reaction mixture for measuring β-glucosidase/β-xylosidase contained 100 μl of suitably diluted intracellular enzyme along with 1 ml of 5 mM p-nitrophenyl β-D-glucopyranoside (PNPG) or p-nitro-

phenyl β-D-xylopyranoside (PNPX), dissolved in 50 mM phosphate buffer (pH 7.0). After incubation at 45°C for 20 min, the reaction was stopped by the addition of 1 ml of 1 M Na_2CO_3. Absorbance was measured at 400 nm. One unit of enzyme activity was defined as the amount of enzyme which produced 1 μmol PNP min^{-1} ml^{-1} from their respective pyranosides.

The isolate was aerobic, gram-positive, rod-shaped sporeforming bacterium with two to three peritrichous flagella. On agar plates, single colonies were 0.5–3.5 mm. in diameter, unpigmented, opaque with hair-like outgrowths attached strongly to agar. The species tend to swarm on solid media. The vegetative cells were 0.9–1.025 μm in length, 0.42–0.53 μm in dia (Figure 1 *a*). The spore was located subterminally, without swelling the sporangium (Figure 1 *b*). The spores showed a more complex ultrastructure to that seen in the vegetative cell sections. The spore protoplast or spore core was surrounded by the germ cell wall, cortex and then the spore coat (Figure 1 *c*). Based on the characteristics of spores, their size, shape and sporangia, this strain could be assigned to the group 1 (ref. 10). The ultrathin sections of the cells grown in cellulose and stained with Ruthenium Red revealed fibrous network around the cell (Figure 1 *d*) which consists of polysaccharide materials. These structures appeared to be related to the clusters of fibrous network observed in *Clostridium thermocellum* around the cell termed as 'cellulosome' which may be of general consequence to the bacterial interaction with and degradation of cellulose[11].

The bacillus strain grew with an optimal specific growth rate of 0.45 h^{-1} when grown in xylan followed by 0.20 h^{-1} in CMC and 0.15 h^{-1} when grown in rice husk (Figure 2). The organism could grow in a broad range of temperatures between 30°C and 45°C with optimum at 37°C as determined by their specific growth rate (Figure 3). The pH range for growth varied between pH 6.5 and 8.5 with 7.5 as optimum pH (Figure 4). The organism tolerated 7% sodium chloride in the medium. The isolate could grow anaerobically in a glucose broth. Nitrate reduction and catalase activity could be positively demonstrated. The organism produced acids from glucose, arabinose, D-xylose and D-mannitol but no gas formation took place in any of the tubes. Hydrolysis of casein, gelatin and starch was observed by the organism. The strain utilized citrate and propionate. Degradation of tyrosine did not give a positive result. *Bacillus* strain exhibited a positive result with Voges Proskauer test and pH in VP broth was found to be 6. The guanine-plus cytosine composition of DNA was found to be 47.58 mol%. On the basis of their cellular morphology, biochemical characteristics and substrate utilization, the present *Bacillus* strain showed similarities with *Bacillus licheniformis* except that the present strain showed its optimum growth at
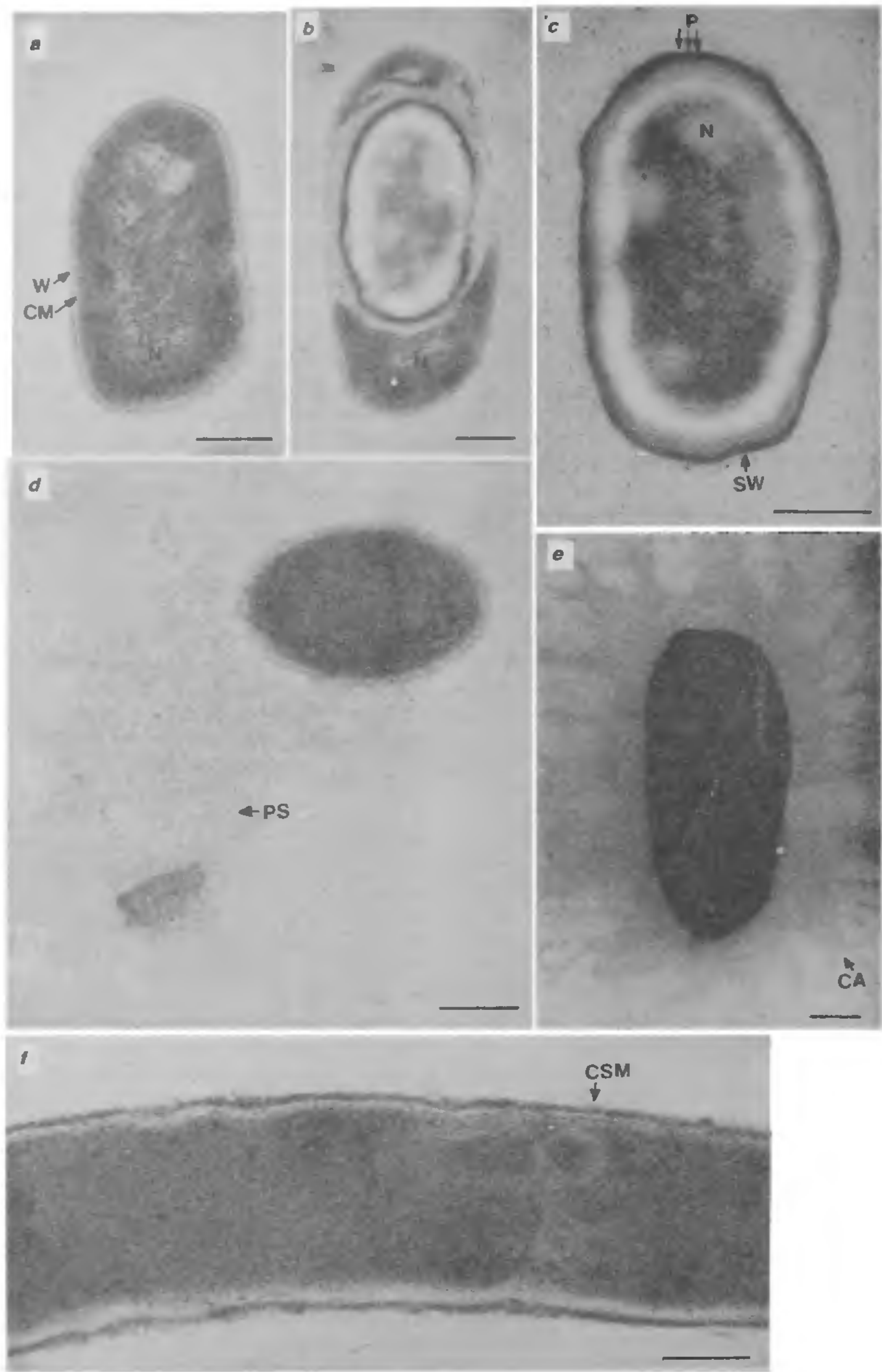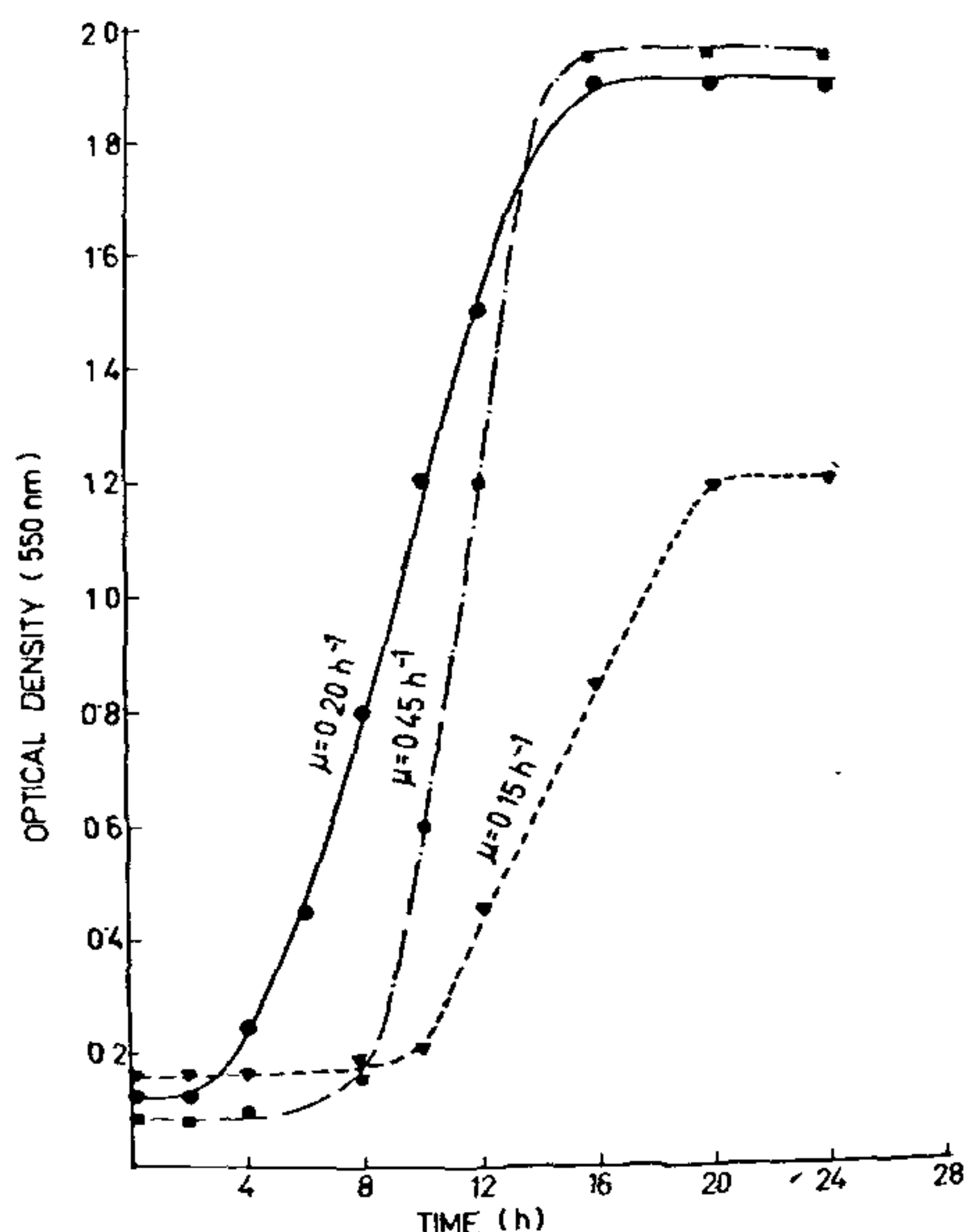
Figure 2. Effect of soluble and insoluble carbon sources (1% w/v) on growth of *Bacillus* .sp. (●) Carboxymethyl cellulose; (■) xylan; both soluble sources. (▼) insoluble lignocellulosic rice husk. Cultures were grown in shake flasks at 200 rpm, pH 7 and temperature 37°C.
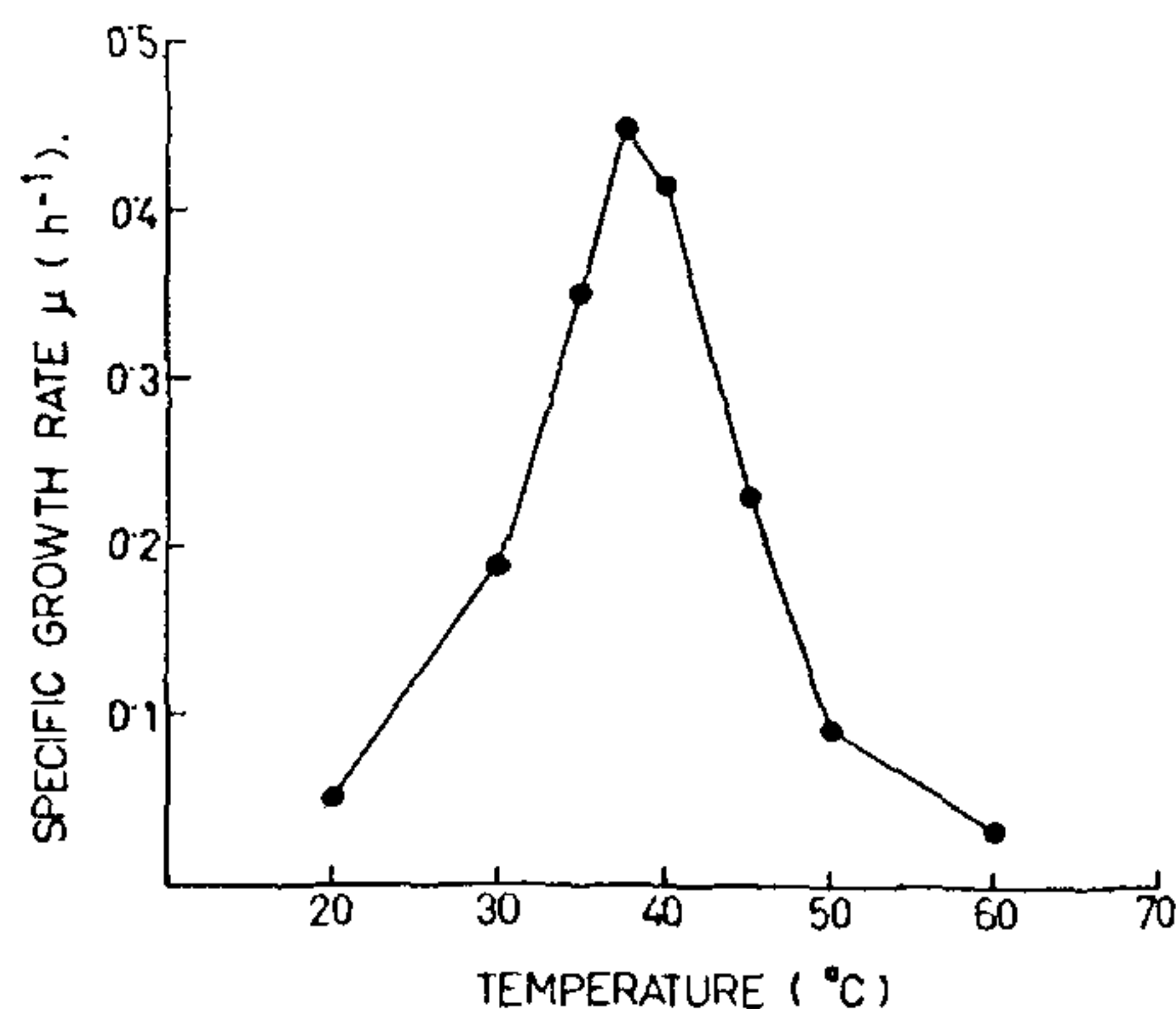

Figure 3. Effect of temperature on specific growth rate of *Bacillus* sp. ranging from 20°C to 60°C.
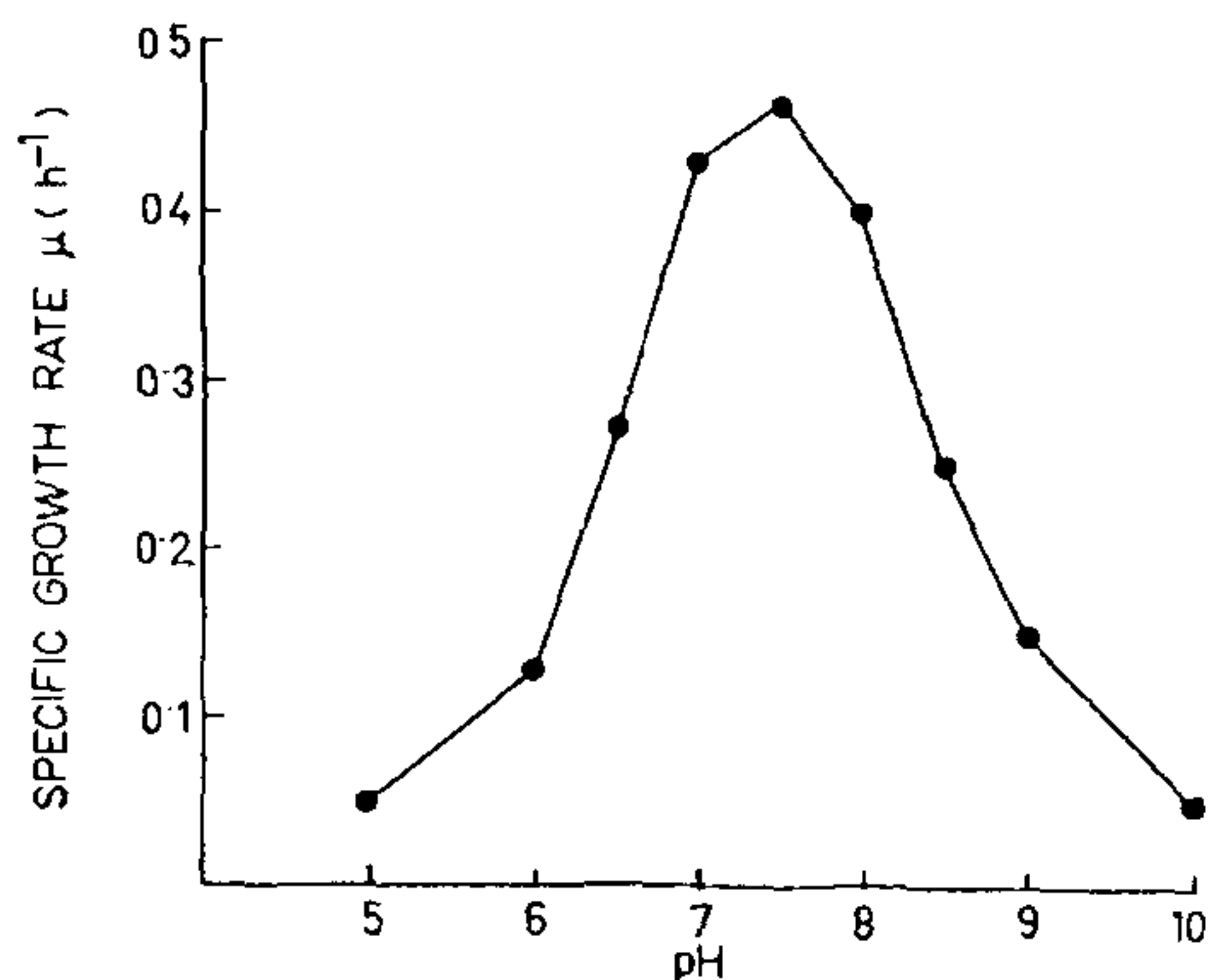

Figure 4. Effect of pH on specific growth rate of *Bacillus* sp. ranging from 5 to 10.

37°C and cannot tolerate temperature beyond 45°C as reported for *B. licheniformis*[7].

Table 1 represents the pattern of enzyme induction with different substrates. High cellulase and hemicellulose activities observed when growing on natural substrates like rice husk compared to the cultures grown on artificial substrates, viz. CMC or xylan, probably result from cooperation between various inducers derived from the components of the lignocellulose complex. These results are in agreement with the earlier report on *Streptomyces* growing on lignocellulose[12]. The results also revealed that the new isolate is an efficient cellulose and hemicellulose degrader thereby can be chosen as a model for evaluating factors governing the synthesis of these enzymes.

The results obtained emphasize the role of this bacterium as a potent cellulose and hemicellulose

Table 1. Enzyme activities induced by different substrates at the onset of stationary phase.

Medium	Endo-glucanase	β-gluco-sidase	β-xylanase	β-xylo-sidase
CMC	0.9 ± 0.07	1.63 ± 0.12	1.2 ± 0.15	1.2 ± 0.04
Xylan	0.32 ± 0.01	0.05 ± 0.01	9.2 ± 0.47	3.3 ± 0.32
Rice husk	1.2 ± 0.13	1.48 ± 0.13	11.5 ± 0.52	4.1 ± 0.46

Enzyme activities were induced with 1% substrate; all enzyme activities are expressed as IU ml; experiments were completed in triplicate; data are mean ± range about the mean

Figure 1. *a–d* and *f*, Ultrathin sections of chemically fixed, spurr embedded cells and *e*, negatively stained cell. All magnification bars ◀ 0.25 μm. CM, capsular material; N, nucleoid; P, periodic layer in the spore wall, PS, polysaccharide material, R, ribosome, SW, spore wall; W, cell wall, CSM, cellulosic material.

degrader in the termite infested soil of semi-arid ecosystem.

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