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Full Length Research Paper

Preliminary studies of cellulase production by *Acinetobacter anitratus* and *Branhamella* sp.

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Two Bacteria species, *Acinetobacter anitratus* and *Branhamella* sp., were isolated from the haemolymph of the giant African snail, *Archachatina marginata.* The endocellulase activity of the culture broth was determined during bacterial growth by measuring the release of reducing sugar from carboxymethyl cellulose (CMC). The peak of enzyme production occurred at the late logarithm phase for each bacterial species. The kinetics of CMC and celloboise hydrolysis was found to be the Michaelis-Menten type. The K_M values of CMC substrate were found to be 4.97 and 7.90 mg/ml for *A. anitratus* and *Branhamella* sp., respectively. The K_M values for cellobiose as substrate were 0.32 and 2.50 mM for *A. anitratus* and *Branhamella* sp., respectively. A multiplicity of cellulase complexes in the haemolymph of *A. marginata* is indicated.

Key words: Bacterial cellulase, *Acinetobacter anitratus*, *Branhamella* sp., *Archachatina marginata.*

INTRODUCTION

Cellulases (1,4-β-D-glucan glucanohydrolase, EC 3.2.1.4) are multienzyme complexes, comprising three main components; endo-β-glucanase (EC 3.2.1.4), exo-β-glucanase (EC 3.2.1.9.1) and β-glucosidase (EC 3.2.1.21), which have been shown to act synergistically in the hydrolysis of cellulose (Emert et al., 1974; Ryu and Mandels, 1980). Cellulases are being studied increasingly due to their application in the hydrolysis of cellulose, the most abundant biopolymer and potential source of utilizable sugars, which serve as raw materials in the microbial production for a wide variety of chemicals, food and fuel. Cellulose is obtained either directly or indirectly as forest production or in wastes such as straw, paper waste, municipal solid waste and other industrial wastes.

Cellulose research has been concentrated mostly in fungi but there is increasing interest in cellulose production by bacteria (Crawford, 1986; Li and Gao, 1996). This work was aimed at isolating cellulotic bacteria from the haemolymph of *Archachatina marginata*, and characterizing their extracellular cellulase.

MATERIALS AND METHODS

Growth of bacteria and production of cellulase

All chemicals used were reagent grade, obtained from Eagles scientific, BDH chemical, England, May and Baker and Sigma chemical company USA. The bacteria, *Acinetobacter amitratus* and *Branhamella* sp. used in this study were isolated from the haemolymph of the giant African snail, *Archachatina marginata.* On nutrient agar, pure cultures obtained were identified by conventional test, and maintained on nutrient agar slants.

The two bacteria species were separately grown and tested for production of cellulase in submerged culture in a salt medium, containing 0.01% $MgSO_4$, 0.1% $(NH_4)_2SO_4$, 0.2% KH_2PO_4, 0.7% K_2HPO_4, 0.05% Na citrate, supplemented with 0.1% glucose or carboxymethyl cellulose (CMC) as carbon source. The cultures were grown at 37°C for 24 h. Culture broth was sampled at different time during growth to determine cell density by measurement of absorbance at 660 to 700 nm and enzyme production by carboxymethyl cellulase hydrolysis.

Enzyme assay

Cellulose activity was assayed by the determination of reducing sugar released from carboxymethyl cellulose (CMC). 0.5 ml of culture supernatant fluid was incubated with 0.5 ml 1% CMC in 0.05 M sodium acetate buffer, pH 4.8 at 40°C for 1 h. The reducing sugar product was assayed by the dinitrosalicylic (DNSA) method (Miller, 1959), using glucose as the sugar standard. Controls for carbohydrate produced from substrate and of the enzyme preparation were included. One unit of cellulase was defined as the amount of enzyme which produced 1 µ mole glucose equivalent per minute under the assay conditions.

Cellulase activities were measured at different concentrations of substrate, using cellobiose or CMC at concentration range of 1.0 to 10 mg/ml. The Michaelis-Menten constant, K_M, for each substrate was determined from the Lineweaver-Burk plot.

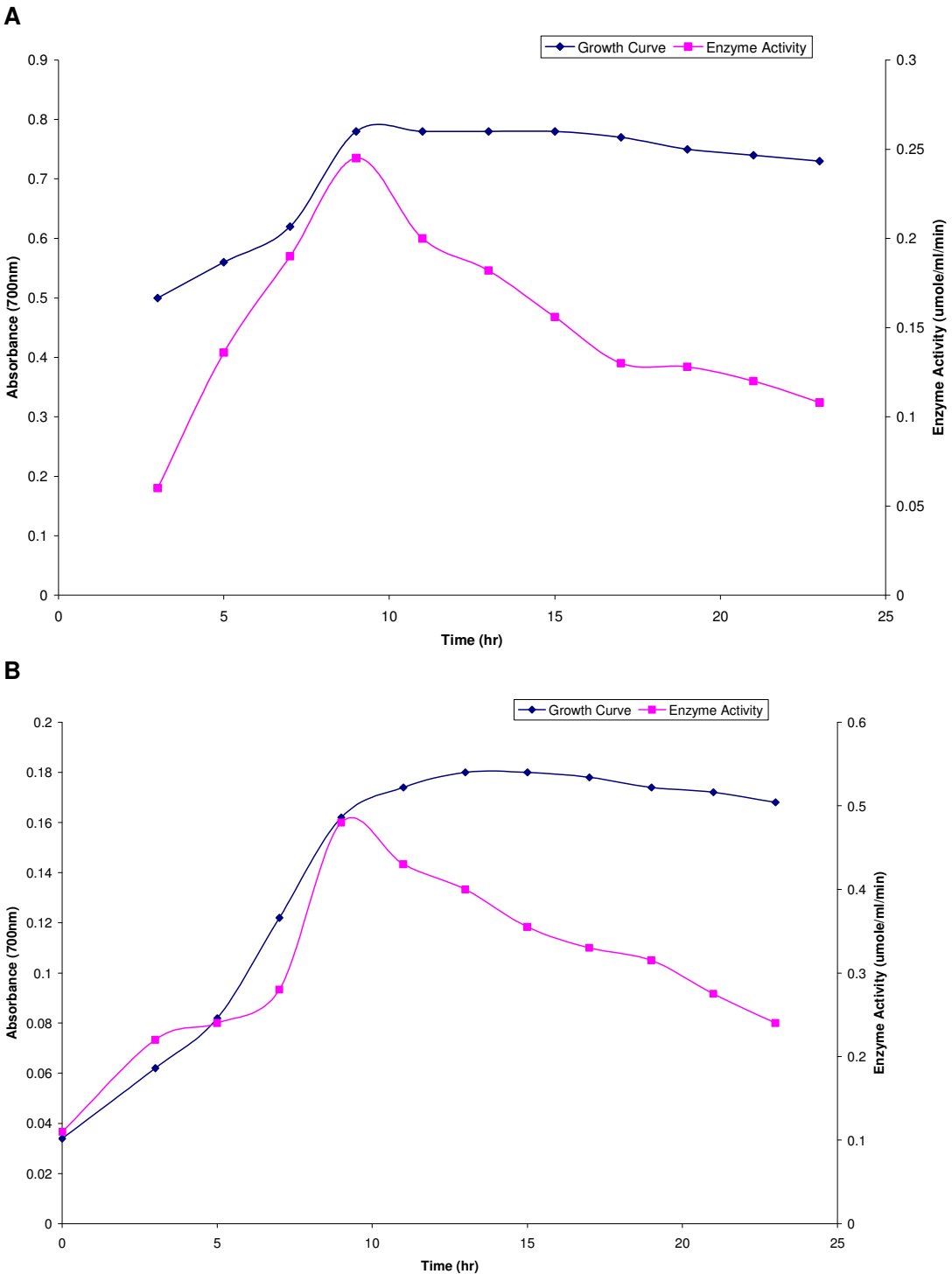

Figure 1. Batch Culture of *Acinetobacter anitratus* and cellulase production. A. Culture in salt medium plus glucose as sole carbon source. B. Culture in salt medium plus CMC as sole carbon source.

RESULTS AND DISCUSSION

A. anitratus and *Branhamella* sp., aerobic and gram-negative bacteria, were isolated and purified from nutrient agar plates inoculated with the haemolymph of *Archachatina marginata.* Identification was carried out by conventional tests and by Cowon and Steel (1978) methods.

Figures 1A and1B show the growth of *A. anitratus* in a basic salt medium with glucose as the sole carbon source and with CMC as the sole carbon source, respectively.

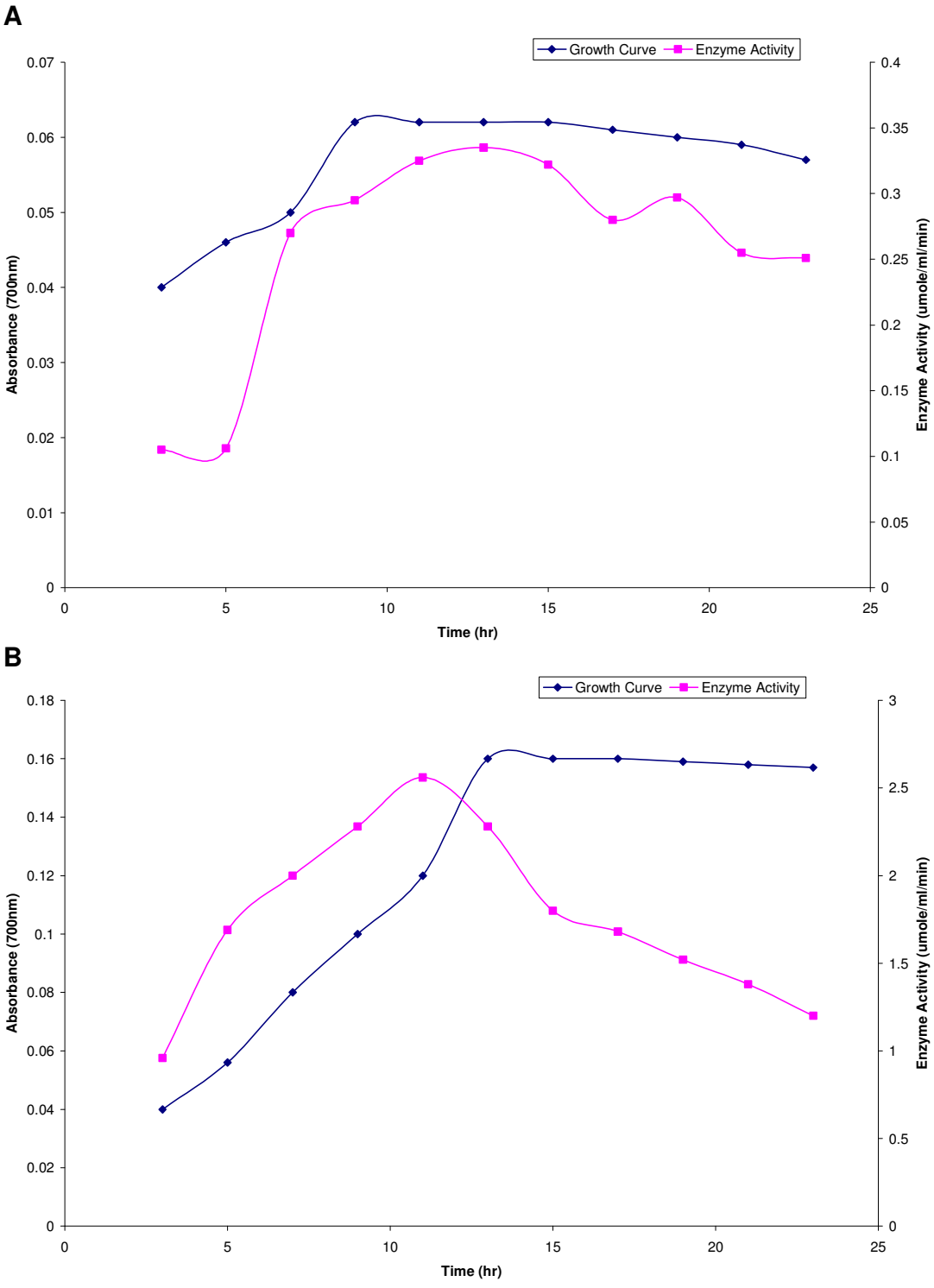

Figure 2. Batch Culture of *Branhamella sp* and cellulase production. A. Culture in salt medium plus glucose as sole carbon source. B. Culture in salt medium plus CMC as sole carbon source.

The cellulase production of each culture system is also shown. Maximum enzyme production was observed at the late logarithm phase, about 9 h after inoculation, when glucose or CMC was used as substrate. Figures 2A and 2B show the growth of *Branhamella* sp. with glucose and CMC, respectively. From the cellulose activity curves shown, the maximum enzyme production was observed at the about the 9th h when CMC was used as substrate,

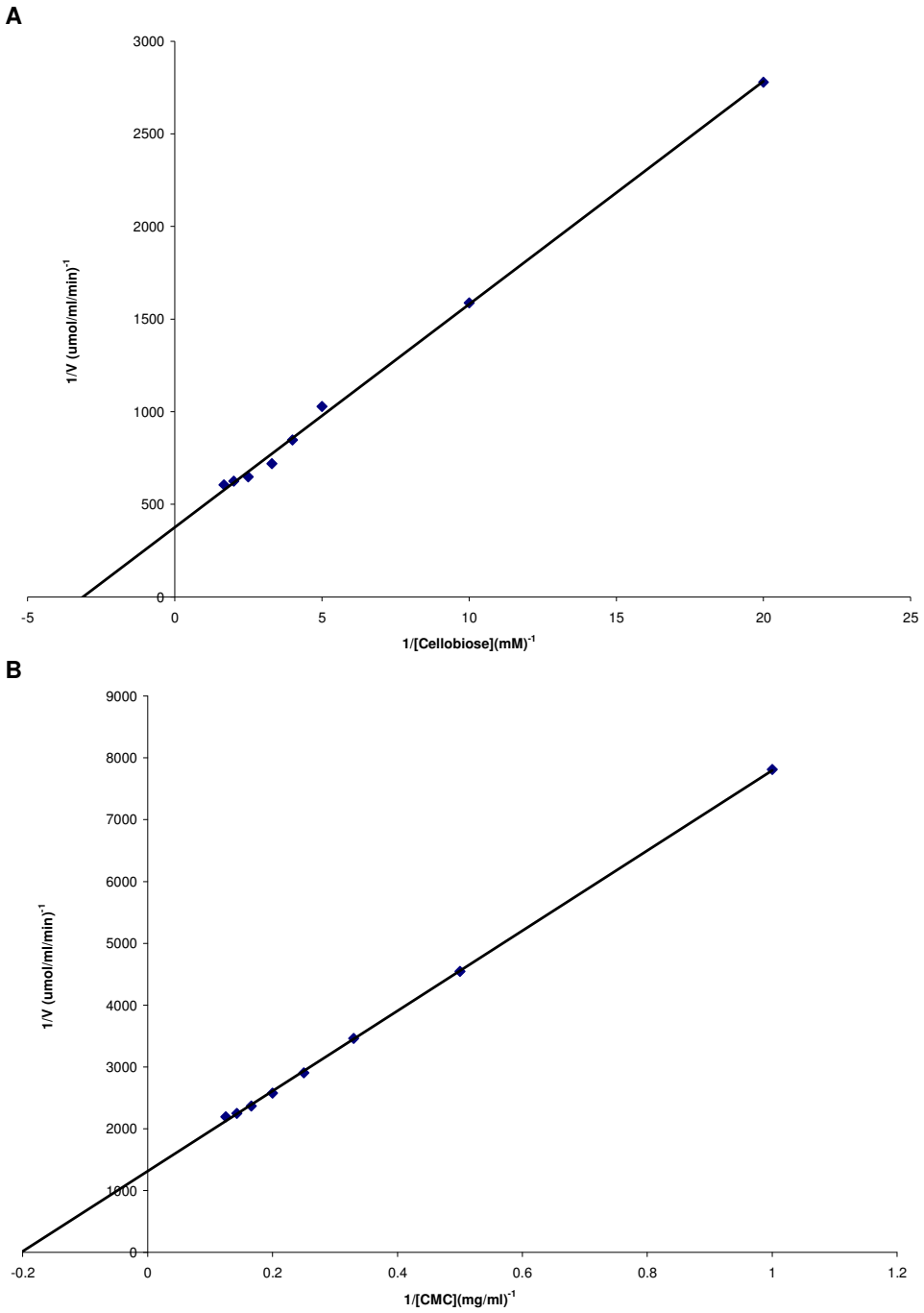

Figure 3. Lineweaver-Burk plots of cellulase activity at different substrate Concentration for *Acinetobacter anitratus*. A. Cellulase activity at different cellobiose concentrations. B. Cellulase activity at different CMC concentrations.

while with glucose the peak of enzyme production was observed at about the 13th h. The maximum enzyme activities of *A. anitratus* culture supernatant were 0.48 and 0.24 U/ml for CMC and glucose, respectively. For *Branhamella s*p., the maximum enzyme activities of the culture supernatant were 2.56 and 0.34 U/ml for CMC and glucose, respectively. A higher production of cellulase when CMC served as substrate may be as a result of induction of the enzyme, since cellulose is know to be a universal inducer of cellulase synthesis. Paul and Varma (1993) had reported the induction of endocellulase by CMC.

The Lineweaver-Burk plot of the extracellular cellulase from *A. anitratus* and *Branhamella* sp. are different as shown in Figures 3 and 4. The Michealis-Menten constant, K_M, for CMC is 4.97 mg/ml for *A. anitratus* and 7.90

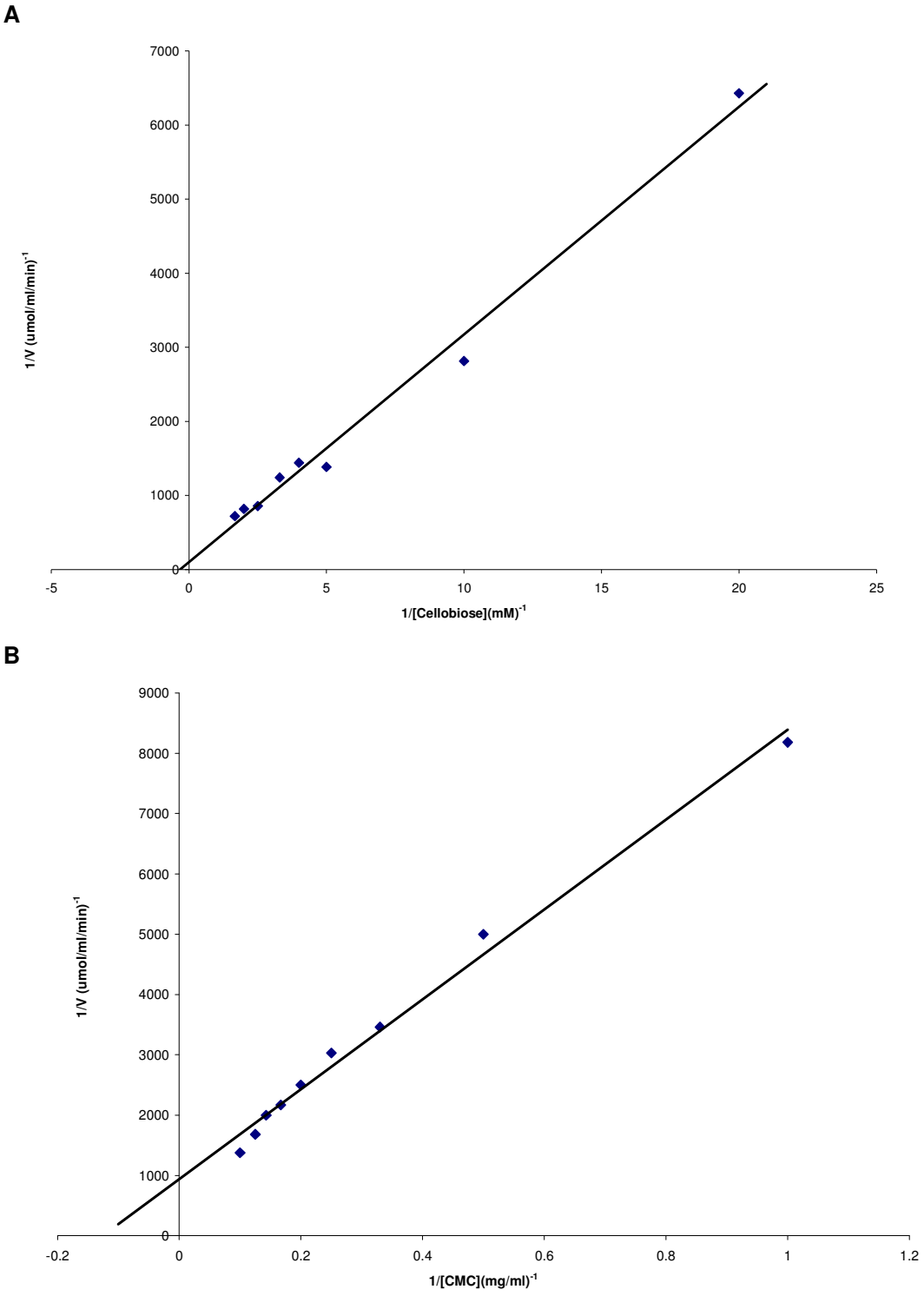

Figure 4. Lineweaver-Burk plots of cellulase activity at different substrate Concentration for *Branhamella sp.* A. Cellulase activity at different cellobiose concentrations. B. Cellulase activity at different CMC concentrations.

mg/ml for the *Branhamella* cellulase, indicating a higher affinity of the *Acinetobacter* enzyme for CMC. Similarly, the K_M values for cellobiose are 0.32 and 2.54 mM for the *A. anitratus* and *Branhamella* sp. Cellulose, respectively, indicating again a higher affinity of the *Acinetobacter* enzyme for cellobiose. The K_M of 4.97 mg/ml for CMC in

the *Acinetobacter* enzyme system is similar to that estimated for two CM-cellulases purified on Sephadex G-200 from snail haemolymph (Ekperigin, unpublished data, 2000). The K_M values for CMC of these partially purified CM-cellulases with molecular weights of 112,202 and 67,000 were estimated to be 4.3 and 2.8 mg/ml, respectively. The enzyme reaction with cellobiose can be ascribed to β-glucosidase component of the cellulase complex.

The cellulase producing bacteria reported in this paper need further investigation and characterization, especially in the area of culture conditions of the production. It will also be desirable to determine if the cellulases are true cellullase that will convert crystalline and amorphous celluloses, found in several cellulosic wastes quantitatively to glucose.

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