

THE KINETICS OF CHITIN AZURE FOR CHITINASE ACTIVITY ASSAY

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Chitin and its derivatives are widely distributed in the world. They are applied as biomedical materials due to their versatility and biocompatibility. Usually, chito-oligosaccharide from chitin is a better raw material to be made of. However, to assay chitinase activity is difficult, because appropriate substrates are few available. In this work, a substrate, colloidal chitin azure, was developed and the kinetics were determined. First, the optimal reaction parameters, pH, temperature and duration were determined at pH5 as well as 8 at 25 Celsius degree for 30 minutes (Fig. 1-3). The parameters of kinetics, K_m , k_{cat} , V_{max} and specificity constant (k_{cat}/K_m) were determined for the substrates colloid chitin, colloid chitin azure and chitin azure (Table). Though colloid chitin with the lowest K_m value, 20.42 mg/ml, and the highest V_{max} value, 1.25 S-1. But the interference of reducing sugar can not be overcome in the assay with chitin as substrate. The colloid chitin azure was 10^7 more accessible to chitinase than chitin azure. Finally, we also applied the colloid chitin azure to determine the fermentation policy in chitinolytic bacteria.

Table 1. Kinetics parameters of chitinase substrate.

Substrate	K_m (mg/ml)	k_{cat}
Colloid Chitin	20.42	15.00
Colloid Azure	28.28	1.58
Colloid Chitin Azure	25.00	1.44

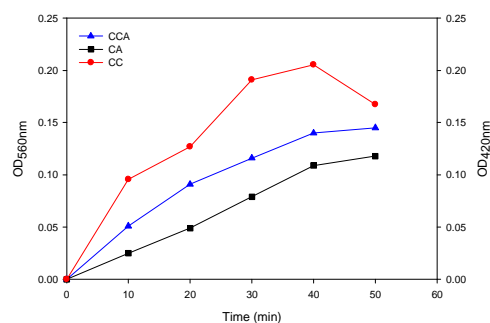


Fig. 1. Kinetics of chitin, chitin azure and colloid chitin azure

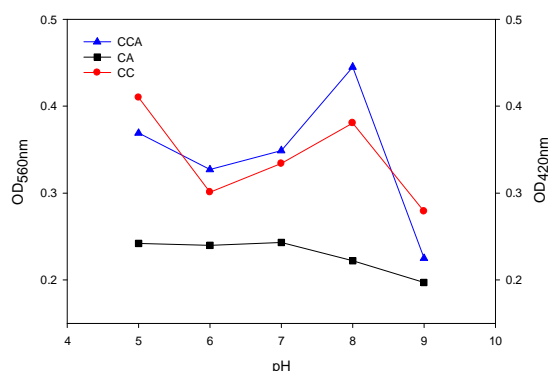


Fig. 2. pH effects of chitin, chitin azure and colloid chitin azure in chitinase activity assay

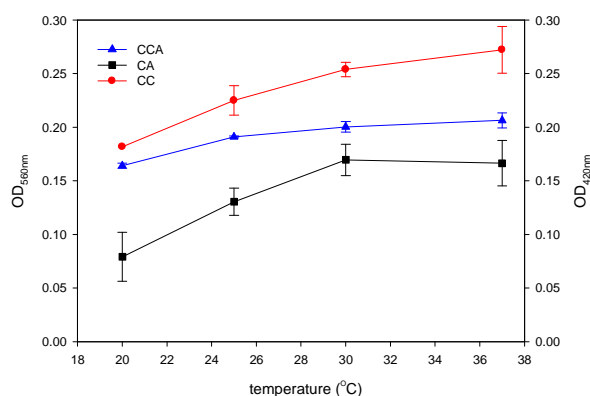


Fig.3. Temperature effect of chitin, chitin azure and colloid chitin azure in chitinase activity assay

REFERENCES

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