ABSTRACT

Kinetics and Applications of Free
and Immobilized Linamarase

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This thesis deals with the kinetics of free linamarase and this enzyme immobilized onto an activated 2-fluoro-1-methylpyridinium Fractogel support as well as an epoxide (a co-polymer of glycidylmethacrylate and ethylene glycoldimethacrylate) support. The study aims to characterise both free and immobilized linamarase in terms of their kinetic parameters. It also focusses on the application of the immobilized linamarase for the quantitation of cyanide in cassava.

The data obtained for the free enzyme showed a K_m of 0.2 mM; a V_{max} of 1.71×10^{-6} mol min^{-1} and a temperature optimum of 55°C. The kinetic data obtained from the flow kinetics of the immobilized linamarase showed that the enzyme immobilized on FMP activated Fractogel and epoxide supports have $K_{m(app)}$ values of 0.32mM and 0.26mM, C'_{max} values of 0.34×10^{-4} and 1.35×10^{-4} mol min^{-1}, pH optimum of 7.0 and temperature optima of 50°C and 45°C respectively. The systems also showed excellent storage stability, retaining 69% and 96% of their activity after 211 days and 82 days respectively for the Fractogel and epoxide supports when stored in phosphate buffer (pH 7.0) at 4°C.

In the process of developing a method for quantifying cyanide in cassava samples, a rapid and sensitive flow injection method was developed for the

fluorometric determination of trace amounts of cyanide. It is based on the reaction of o-phthalaldehyde (OPA) or 2,3 naphthalene dialdehyde (NDA) and taurine with cyanide ions. Calibration curves are found to be linear in the range 10^{-7} to 10^{-5} and 10^{-8} to 10^{-5}M respectively for OPA and NDA when the FIA manifold is operated under optimized conditions. Within day precisions of 1.97% and 2.1% and detection limits of 1.8×10^{-7}M and 5.4×10^{-8}M are achieved for OPA and NDA respectively. When the method is applied to river water, linear calibration curves with a detection limit of 2.8×10^{-7}M is obtained. Up to 48 samples can be analyzed per hour.

Excellent recovery yields (99 - 102%) and within day CV's (<3.3%) are achieved.

A linamarase bioreactor is incorporated into the FIA manifold using the o-phthalaldehyde reagent to determine releasable cyanide levels in cassava parenchyma and cortex tissues. Calibration curves are found to be linear in the range 10^{-5} to 10^{-4}M. Up to 13 samples can be analyzed per hour. Recovery yields varied between 95 - 99% and within day CV between 0.66 - 4.38%.

A linamarase electrode is also described for the quantitation of free and releasable cyanide in cassava extracts. Recovery yields with this biosensor varied between 95% - 99%. Both the within day and day to day CV's were less than 6.4%. Up to 5 cassava samples can be analysed per hour. There is only a 20% decrease in biosensor activity over a period of 150 days.