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Biodegradation of cyanide in cassava wastewater using a novel thermodynamically-stable immobilized rhodanese

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ABSTRACT

Extracellular rhodanese obtained from *Aureobasidium pullulans* was employed in both free and immobilized forms for the biodegradation of cyanide present in cassava processing mill effluent (CPME). Crosslinking with glutaraldehyde (at an optimum concentration of 5% v/v) before entrapment in alginate beads resulted in the highest immobilization yield of 94.5% and reduced enzyme leakage of 1.8%. Rhodanese immobilized by cross-linking before entrapment (*cbe*) retained about 46% of its initial activity after eight cycles of catalysis compared to the entrapment in alginate alone (*ea*) which lost more than 79% after the fifth catalytic cycle. A cross-examination of thermodynamic (ΔG_d^\ddagger , ΔS_d^\ddagger , ΔH_d^\ddagger) kinetic (k_d , $t_{1/2}$, D and z – values) parameters at 30–70 °C showed that *cbe* displayed a higher resistance to thermal inactivation when compared to the free enzyme (*fe*) and (*ea*). The efficiency of cyanide biodegradation from the CPME by the *fe*, *ea* and *cbe* were 55, 62, and 74% respectively after 6 h. Biodegradation of cyanide using the *cbe* was monitored using FTIR spectroscopy. Rhodanese immobilized via *cbe* had a higher resistance to thermal denaturation over other enzyme forms. Hence, this makes *cbe* adaptable for large-scale detoxification of cyanide from CPME.

KEYWORDS

Aureobasidium pullulans;
immobilization; kinetics;
rhodanese; thermodynamics


Introduction

Cassava tubers are one of the most important economic crops in Africa as well as the Americas but it is known to contain very hazardous compounds termed cyanogenic glycosides. It is established that a typical cassava tuber (per 1000 g) contains about 75–1000 mg cyanide.^[1] The cyanide amount in a typical cassava processing effluent (e.g., in industries producing tapioca starch) is equivalent to about 200 mg per liter while it is estimated that the typical concentration of the cyanogenic glycosides in cassava wastewater effluents ranges from 10 to 274 mg in 1 L.^[2] These cyanogenic glycosides are released during the tuber processing (washing, peeling, grating, and extraction) which ultimately is then converted to hydrogen cyanides via enzymatic action.^[3] Cassava processing industries then release the hydrogen cyanide-containing waste effluents into the surrounding environment and this causes deleterious effects as cyanides are harmful to all living organisms.^[4]

The toxicity of cyanide occurs via its binding action to cytochrome oxidase leading to abnormalities in respiration.^[2] The downstream effect of prolonged exposure or ingestion of cyanide by humans or aquatic life is death (acute poisoning), nervous system abnormalities or depletion of trace elements, etc. To this end, several approaches have

been designed to remove cyanide from water bodies and this technique falls into two broad categories namely physical/chemical and biological methods.^[5] The current physical/chemical approach mostly involves ozonization, chlorination, etc.^[6] In summary, these chemical techniques are often relatively expensive and it also leads to the release of further toxic reaction by-products into the water-bodies. The biological approach to the treatment of cyanide-contaminated wastewater is regarded as the best approach since it is inexpensive, eco-friendly, highly efficient and no release of toxic by-products is observed.^[5] Hence the use of biological agents such as microorganisms (bacteria/fungi) and enzymes offer an easily adaptable and environmentally friendly approach to cyanide detoxification in these cyanide-contaminated water-bodies. However, the use of microorganisms in the biodegradation of cyanide albeit comes with some known operational problems like the cassava wastewater (*cww*) being low in nitrogen and high in chemical oxygen demand. This causes a situation where the *cww* is not able to support the growth of the microorganism nutritionally. Hence a more viable alternative to cyanide biodegradation is the use of enzymes since they are eco-friendly and they are not affected by the problems associated with utilizing microorganisms for biodegradation of cyanide.^[7]

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