Three-Step Enzymatic Cascade for Cyanide Detection and Quantification

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Enzymatic hydrolysis of HCN can proceed via two distinct routes: directly through the action of cyanide dihydratase (CynD) [1], or through a two-step cascade involving cyanide hydratase (CynH) followed by formamidase (AmiF) [2]. While both mechanisms have been considered for cyanide detoxification, only the former has been explored for analysis (biosensors). In this study, we explore the latter route for the detection and quantification of free cyanide, highlighting the superior enzymatic activity and alkaline pH tolerance, which benefits the stability of cyanide solutions.

The quest for selective and specific methods for the determination of free cyanide is ongoing, with enzymatic strategies being relatively unexplored. We introduce an enzymatic cascade that converts HCN into HCOOH, the concentration of which is determined photometrically. This process is catalyzed firstly by a CynH from *Exidia glandulosa* [3] and subsequently by an AmiF from *Bacillus cereus* [4]. A third enzyme, formate dehydrogenase (FDH), catalyzes the final step. The production of NADH is monitored at 340 nm, or, following the addition of a soluble tetrazolium salt and 1-methoxy-5-methylphenazinium methosulfate, at 460 nm [5].

The proposed enzymatic cascade offers several advantages, including selectivity, operation under mild conditions, and the absence of hazardous reagents. It efficiently detoxifies cyanide within the reaction sequence. Both CynH and AmiF exhibit high expression levels in *Escherichia coli*. The cascade requires minimal enzyme quantities, and enzyme stability is maintained for extended periods. This assay is compatible with 96-well plate formats, facilitating high-throughput analysis. Comparative assessments with traditional colorimetric assays demonstrate the efficacy and practical benefits of this enzymatic approach for cyanide assay.

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