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Conversion of free cyanide to formic acid by a cascade reaction catalyzed by cyanide hydratase and formamidase

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PURPOSE OF THE ABSTRACT

Free cyanide consists of HCN and CN ions in pH-dependent ratios. It is usually found in varying concentrations in wastewaters from gold and silver mining, metal electroplating, jewelry manufacturing, chemical synthesis, coal coking, etc. Today, the treatment of these wastewaters is mainly based on physicochemical principles (precipitation with metals, oxidation to cyanate), followed by a microbial stage.

Enzymatic methods are gaining ground, but they are not yet technologically mature. These methods mainly use cyanide dihydratases (CynDs; EC 3.5.5.1), which hydrolyze HCN to formic acid and ammonia, or cyanide hydratases (CynHs; EC 4.2.1.66), which hydrate HCN to formamide. CynHs generally have higher activities and a higher resistance to alkaline pH than CynDs [1].

Nevertheless, the CynH product, formamide, although much less toxic than free cyanide, still poses a significant health risk. Therefore, it is tempting to combine CynH with amidase to convert formamide into formic acid. In this way, the same product is obtained as with CynD, while taking advantage of the CynH´s properties. Previously, a proof-of-concept was demonstrated using immobilized whole cells of wild-type producing strains with low CynH and amidase activities [2].

To increase the efficiency of this type of cascade reaction, we used a different type of amidase, namely formamidase (AmiF; EC 3.5.1.49) with excellent activities for formamide. An AmiF from Bacillus cereus [3] and a CynH from the fungus Exidia glandulosa [4] were produced in Escherichia coli and purified. Both enzymes were functional at pH 9.0 with specific activities on the order of hundreds U/mg protein, although AmiF had a lower pH optimum. The performance of the cascade reaction was optimized by timing the addition of AmiF or changing the pH after the first step. The cascade was performed in batch and continuous modes, the latter with enzymes immobilized on metal affinity resins. The long shelf life of both enzymes at 4 °C is an additional advantage.

The same principle could be used for analytical purposes. In this case, NADH was produced in a coupled reaction catalyzed by commercial formate dehydrogenase (EC 1.17.1.9) (Figure 1) and its concentration was used to calculate the concentration of free cyanide. The method is currently being optimized with respect to the CynH/AmiF/FDH ratio, cyanide concentration, and reaction conditions.

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FIGURES

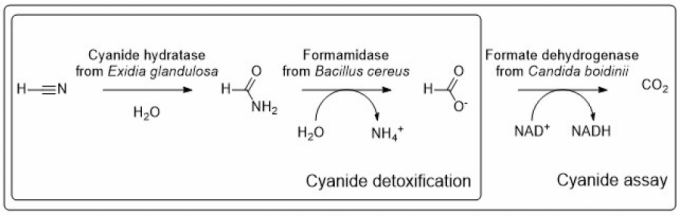

FIGURE 1

Figure 1

Artificial enzyme cascades for the detoxification and determination of free cyanide.

FIGURE 2

KEYWORDS

Cyanide hydratase | Formamidase | Formate dehydrogenase | Cascade reaction

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