Improved Bioproduction of Perillic Acid

Benefits of Using In Situ Product Removal

Biotechnological formation of industrially interesting compounds often has limited productivity. Furthermore, the diluted product stream leads to high downstream processing costs. In contrast, the implementation of in situ product removal (ISPR) can provide technical solutions for processes limited by inhibitory products [1]. Here, we discuss the benefits of using ISPR to improve the bioproduction of perillic acid, a promising bioactive agent of industrial interest.

Perillic Acid: Occurrence, Relevance, Production Options

For centuries, plant terpenes and their oxygenated derivatives have been used in medicine for their wide biological activity [2]. Perillic acid, present in Perilla frutescens [3], has been shown to exert potent anticancer and antimicrobial activity [4, 5]. Therefore, this almost odorless monoterpenoic acid is a promising natural bioactive agent of interest to the food, cosmetics and pharmaceutical industries. Nevertheless, the low concentration of the naturally occurring perillic acid makes its extraction economically not viable. Furthermore, its chemical synthesis is an environmentally problematic production process [6]. In contrast, biocatalytic synthesis using a microbial system turns out to be an efficient and ecological method to produce perillic acid already in gram scale [7]. However, improvement of the biocatalytic process remains necessary to meet the volumetric productivities required in the industrial context.

Improved Synthesis by P. putida

The solvent tolerant P. putida DSM 12264 is capable of oxidizing R-(+)-limonene, a cheap and abundantly available by-product of the citrus processing industry [8], into R-(+)-perillic acid as sole and stable product. The biotransformation of limonene by P. putida DSM 12264 yields up to 11 g/L perillic acid after about six days of fed-batch cultivation under non-limiting conditions [7].
Although *P. putida* DSM 12264 withstands extraordinarily high substrate concentrations, accumulation of perillic acid inhibits growth and limonene bioconversion, resulting in limited productivity. To increase productivity, a continuous removal of perillic acid at a rate that alleviates product inhibition effects is necessary.

This approach, generally known as in situ product removal (ISPR), offers additional important benefits such as reducing the total number of downstream processing steps [9,10]. Since limonene and perillic acid are structurally and chemically closely related, the choice of the most appropriate ISPR method will principally depend on its selectivity for the target compound. The exploitation of the carboxy group to selectively adsorb perillic acid on anion exchange resin is the most promising ISPR method to be adapted to the fed-batch bioprocess. Various anion exchange resins were thus tested for perillic acid recovery under biotransformation conditions. These investigations showed that Amberlite IRA 410 Cl was suitable to selectively adsorb perillic acid without affecting cell vitality. Subsequently, this ISPR technique via adsorption was implemented to a fed-batch bioreactor by coupling a fluidized bed of the IRA 410 Cl resin by an external loop (fig. 1). Perillic acid removal by continuous recirculation of the unfiltered broth through the ISPR unit led to a total product concentration of 31 g/L after about seven days of cultivation (fig. 2).

**Quantifying the Benefits of ISPR**

To assess the potential benefits and the impact of ISPR methodologies on a biocatalytic process a quantitative comparison between the conventional and the integrated system is required [10]. The quantified benefits of ISPR for the biocatalytic production of perillic acid are summarized in table 1. The implementation of our ISPR method led to an obvious increase in total perillic acid concentration as well as an increased productivity. These effects reduced
processing time and also simplified downstream processing owing to the treatment of undiluted product solutions. The increased yields reflect a more effective use of biocatalyst and substrate, which can be translated in reduced medium and chemical costs.

**Downstream Processing Evaluation**

Due to a high selectivity for the target product, this anion exchange-based ISPR method also led to a straightforward downstream processing. The perillic acid adsorbed to the resin was completely eluted by 1M HCl/ethanol (40:60 v/v) - thereby regenerating the resin for a successive reuse - and subsequently precipitated by ethanolic distillation. After drying, more than 98% of the eluted perillic acid was recovered as 93% pure white crystals. In contrast, downstream processing of perillic acid produced via a conventional fed-batch bioprocess was significantly more complicated due to a stable emulsified fermentation broth which impaired cell separation. Here, additional processing steps needed, such as centrifugation and sterile filtration of the biomass, led to a 90% pure product with more than 18% loss.

**Conclusion**

This work demonstrates the benefits of using in situ product removal to significantly improve production of perillic acid by *P. putida* DSM 12264. In addition, the selectivity of the ISPR technique used allowed for a considerably simplified downstream processing which resulted in a highly pure product with negligible loss. Productivity and total product concentration obtained with this anion exchange-based ISPR bioprocess represent, to the best of our knowledge, the highest values reported for microbial monoterpene oxyfunctionalizations up to now, making it a promising option for industrial application.

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**References**

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