**Abstract**

Indigo is a natural dye that is widely used in the textile industry worldwide. However, the synthesis of indigo using toxic compounds such as aniline, formaldehyde, and hydrogen cyanide leads to environmental pollution and poses a threat to workers' health. To overcome these challenges, bio-indigo or indigo biosynthesis has been proposed to replace indigo synthesized from aniline. Among the various biosynthesis methods, Flavin-containing Monooxygenases (FMOs) have been found to achieve the highest yield (1700 mg/L) of bio-indigo. However, the commercialization of indigo biosynthesis still faces several challenges. This review explores the history of indigo biosynthesis by FMOs and identifies the expensive substrate L-tryptophan, unsuitable chassis Escherichia coli, and relatively low yield and high cost compared with the chemical method as the main reasons. Additionally, this paper summarizes the strategies to improve the yield and applications of indigo synthesized by FMOs.

**Keywords:** Flavin-containing Monooxygenase, Indigo

**Introduction**

**1. The Research History of Indigo Biosynthesis by FMOs**

The earliest record of indigo biosynthesis arises from the study of naphthalene dioxygenases (Ensley et al., 1983). In 1989, the pBS959 plasmid was constructed based on the naphthalene dioxygenase gene and pUC19 plasmid, which realized the synthesis of indigo in recombinant E. coli (Boronin et al., 1989). In 1993, a recombinant E. coli

196 capable of producing indigo from glucose was also developed (Murdock et al., 1993).

197 However, the yield was only 135 mg/L. The indigo synthesis pathways of other

198 aromatic hydrocarbon-degrading bacteria were also identified. In 1997, two

**2. The Substrates of Indigo Biosynthesis by FMOs**

**3. The Difference between Synthetic Technologies**