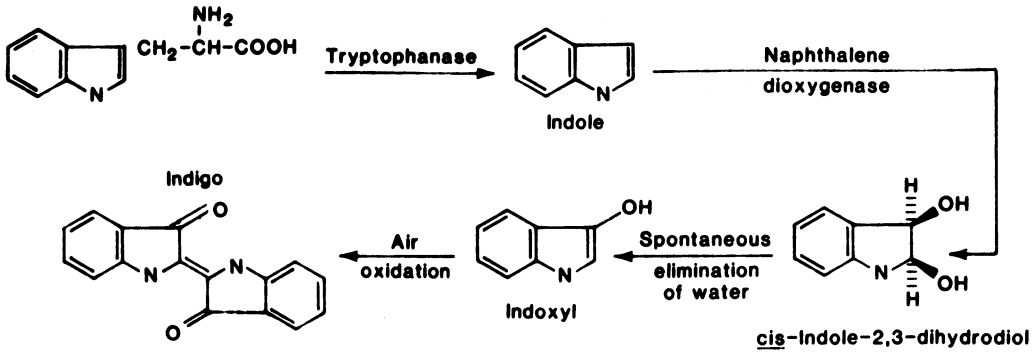

Figure 14.8. Proposed pathway for indigo biosynthesis in a recombinant strain of *E. coli.* Indole is formed from tryptophan by tryptophanase, a natural enzyme in *E. coli.* Naphthalene dioxygenase formed by the expression of the cloned *Pseudomonas* DNA oxidizes indole to indigo; *cis*-2,3-dihydroxy-2,3-dihydroindole and indoxyl have not been isolated. Their inclusion is based on the known activities of aromatic hydrocarbon dioxygenases and established mechanisms for the chemical synthesis of indigo. (With permission, from B. D. Ensley et al., *Science 222:*167, 1983, and American Association for the Advancement of Science.)

- Several pathways can be combined in a single organism by recruiting enzymes from more than one organism.
- Can move a pathway from an organism that grows poorly to one that can be more easily cultured.
- The genetically engineered cell can be proprietary property.

Cells that have engineered pathways face many of the same limitations that cells engineered to produce proteins face. Two issues that perhaps assume greater importance with metabolic engineering are stability and regulatory constraints.

Protein products are of high value and can be made in batch culture. Instability is avoided by inducing overproduction only at the end of the culture cycle. The productive phase is too short for nonproducers to grow to a significant level, and cells are not reused. With metabolically engineered cells, the same strategy is untenable. Lower product values necessitate cell reuse or, at least, extended use. The use of antibiotics as selective agents may be undesirable because of contamination of product or cost. For a culture with a 1.5-h doubling time and a 20-h batch cycle, a continuous system has a 14-fold advantage in productivity over a batch system. Although the levels of protein overproduction are lower in metabolically engineered cells, they can experience as high a level of metabolic burden as "protein producers" because of the diversion of cellular building blocks to nonessential metabolites. Also, if the cells are used to treat hazardous compounds, the genetically engineered cells will face competition from a natural flora.

In addition to the need for extra efforts in engineering design to ensure genetic stability, regulatory approval may be more difficult. If a genetically engineered cell is to be used to treat hazardous wastes, containment of the engineered organism is difficult or impossible. Pump-and-treat scenarios for leachates allow the possibility of control. *In situ* use of such organisms would have to satisfy the constraints for deliberate release.