**Research Topics and Seminar (501)**
**Dr. Hassan Al-Haddad**

Presentation And Discussion Of The Paper:

# Expression Of Naphthalene Oxidation Genes In Escherichia Coli Results In The Biosynthesis Of Indigo

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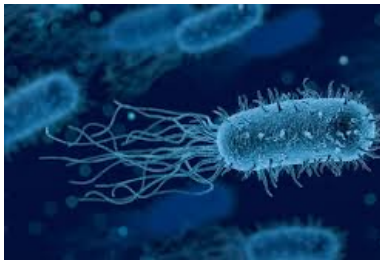
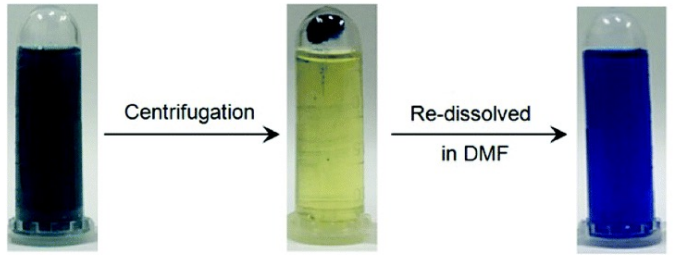
# OUTLINE

- History of indigo
- The paper
- Aim of the study
- Methods
- Results
- Discussion
- Limitations for microbial synthesis of indigo

# HISTORY OF INDIGO DYE

- One of the oldest dyes used for textile dyeing
- **Sources:**
  - Plant extract from the genus *indigofera*
  - Chemical synthesis
  - Microbial synthesis
- **Uses:**
  - Used in ancient history to produce blue colour
  - Dying cotton, wool and silk
  - Production of blue jeans





# THE PAPER

## Expression of Naphthalene Oxidation Genes in *Escherichia coli* Results in the Biosynthesis of Indigo

Abstract. *A fragment of plasmid NAH7 from* Pseudomonas putida *PpG7 has been cloned and expressed in* Escherichia coli *HB101. Growth of the recombinant* Escherichia coli *in nutrient medium results in the formation of indigo. The production of this dye is increased in the presence of tryptophan or indole. Several bacteria that oxidize aromatic hydrocarbons to* cis*-dihydrodiols also oxidize indole to indigo. The results suggest that indigo formation is due to the combined activities of tryptophanase and naphthalene dioxygenase.*

Author(s): Burt D. Ensley, Barry J. Ratzkin, Timothy D. Osslund, Mary J. Simon, Lawrence P. Wackett and David T. Gibson

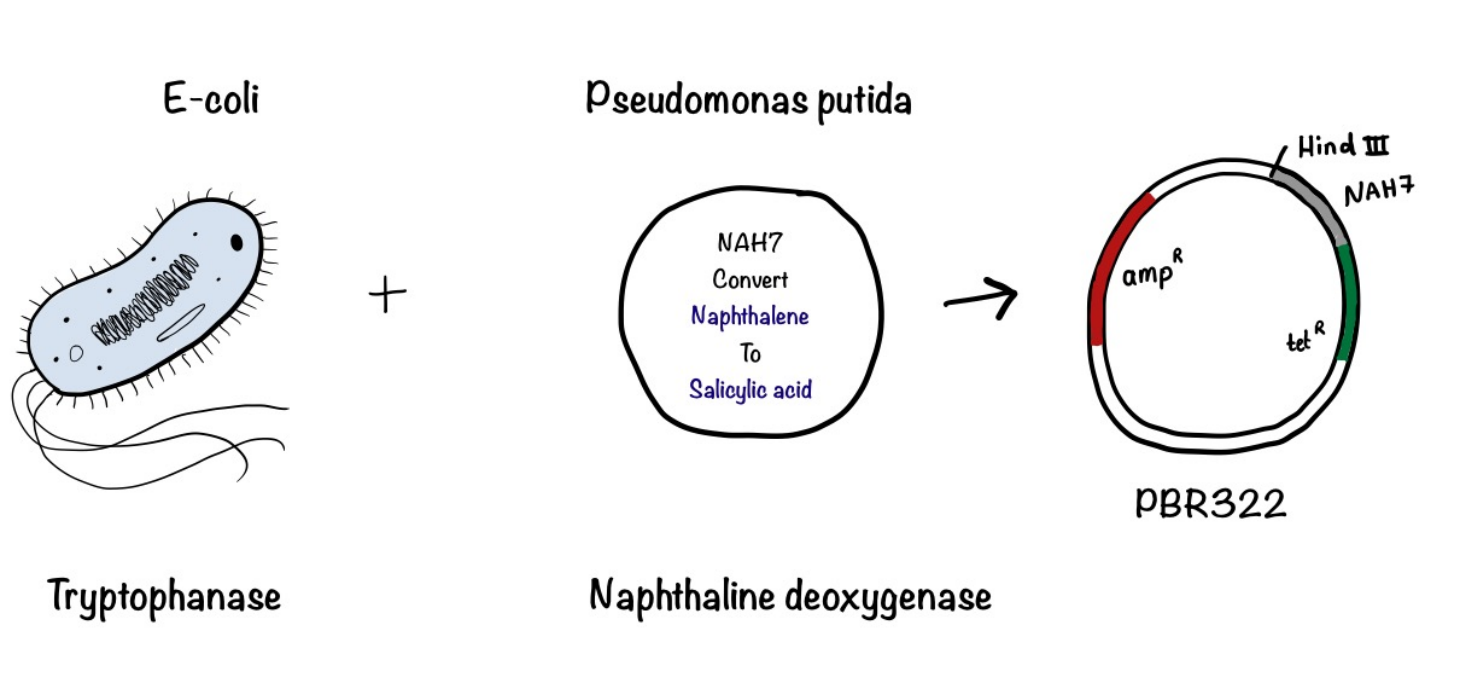
Source: Science, New Series, Vol. 222, No. 4620 (Oct. 14, 1983), pp. 167-169 Published by: American Association for the Advancement of Science

# AIM OF THE STUDY

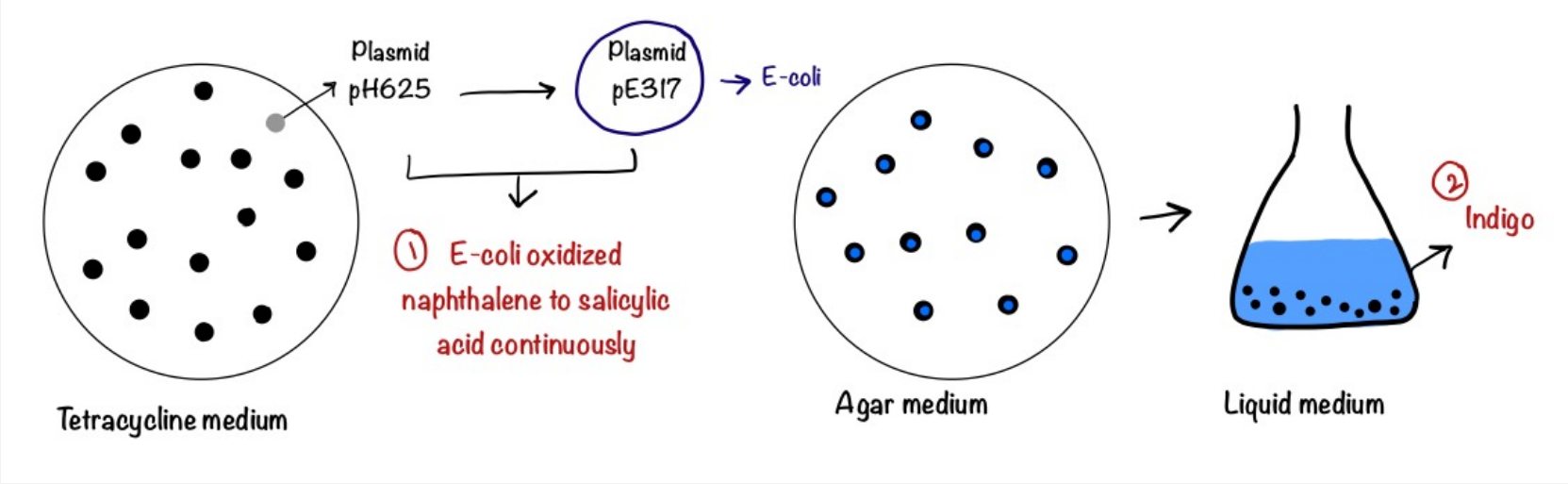
- Produce naphthoquinone and salicylic acid from microorganisms
- Verify that indigo formation is a property of bacterial dioxygenase enzymes

# METHODS

- NAH7 DNA cloned in *E-coli*:

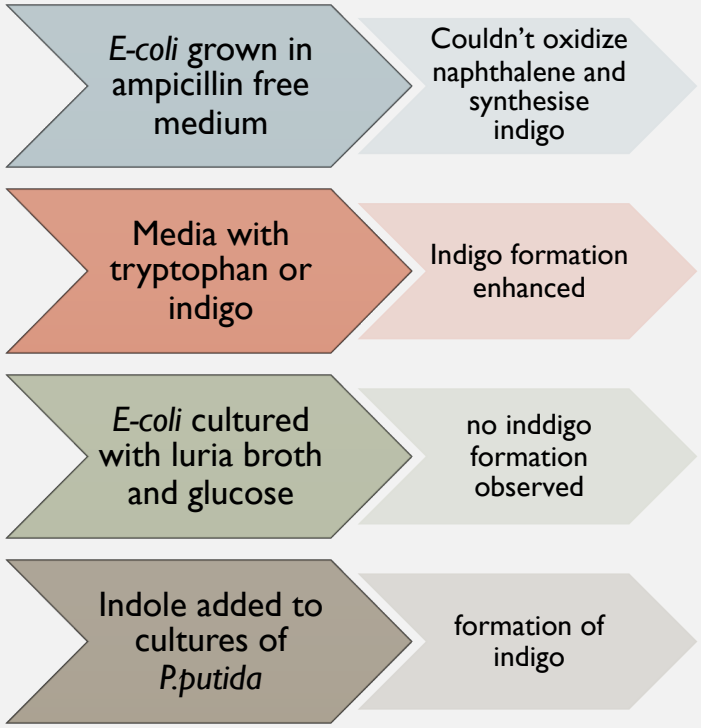


# RESULTS


Plasmid
pH625
Plasmid
pE317
E-coli
① E-coli oxidized
naphthalene to salicylic
acid continuously
Tetracycline medium
Agar medium
② Indigo
Liquid medium

# RESULTS

- To Confirm That Naphthalene Deoxygenase Cause Indigo Synthesis



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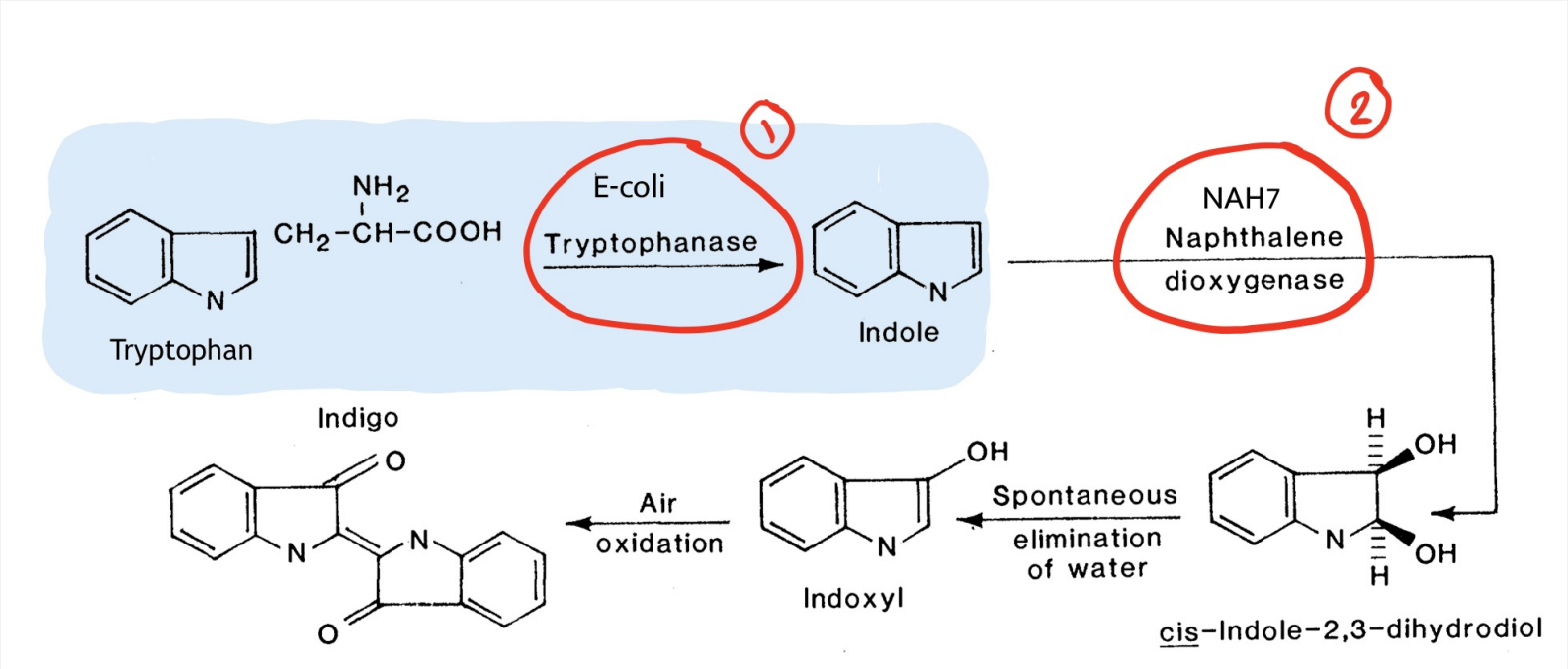
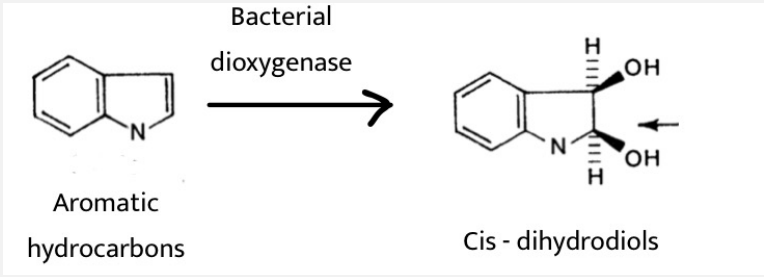



Indigo is synthesized by two enzymes in *E-coli*

# RESULTS

Indigo formation by different bacterial strains with different hydrocarbons




| Organism | Relevant phenotype | Relevant genotype of plasmid | Reference or source | *cis*-Diol* formation | Indigo formation |
|---|---|---|---|---|---|
| *P. putida* PpG7 | $Nah^+$ | Wild-type (NAH7) | (*18*) | + | + |
| *P. putida* NCIB 9816 | $Nah^+$ | Wild-type (pTX1)† | (*3*) | + | + |
| 9816-11 | $Nah^-$ | nddA‡ (pTX1) | CAM | + | + |
| 9816-C2 | $Nah^-$ | | CAM | − | − |
| *P. putida* Np | $Nah^+$ | Wild-type | (*10*) | + | + |
| *P. putida* | $Tol^+$ | Wild-type | (*19*) | + | + |
| 39/D | $Tol^-$ | *tod*D | (*20*) | + | + |
| F106 | $Tol^-$ | *tod*C | CAM | − | − |
| F102 | $Tol^-$ | *tod*A *tod*E | CAM | − | − |
| F26a | $Tol^-$ | *tod*A *tod*B | CAM | − | − |
| *P. putida* BG | $Xyl^+$ | Wild-type (TOL) | CAM | − | − |
| *Pseudomonas* sp. | $Cre^+$ | Wild-type | (*21*) | − | − |
| *Beijerinckia* sp. | $Bp^+$ | Wild-type | (*22*) | + | + |
| B836 | $Bp^-$ | bddA‡ | (*22*) | + | + |

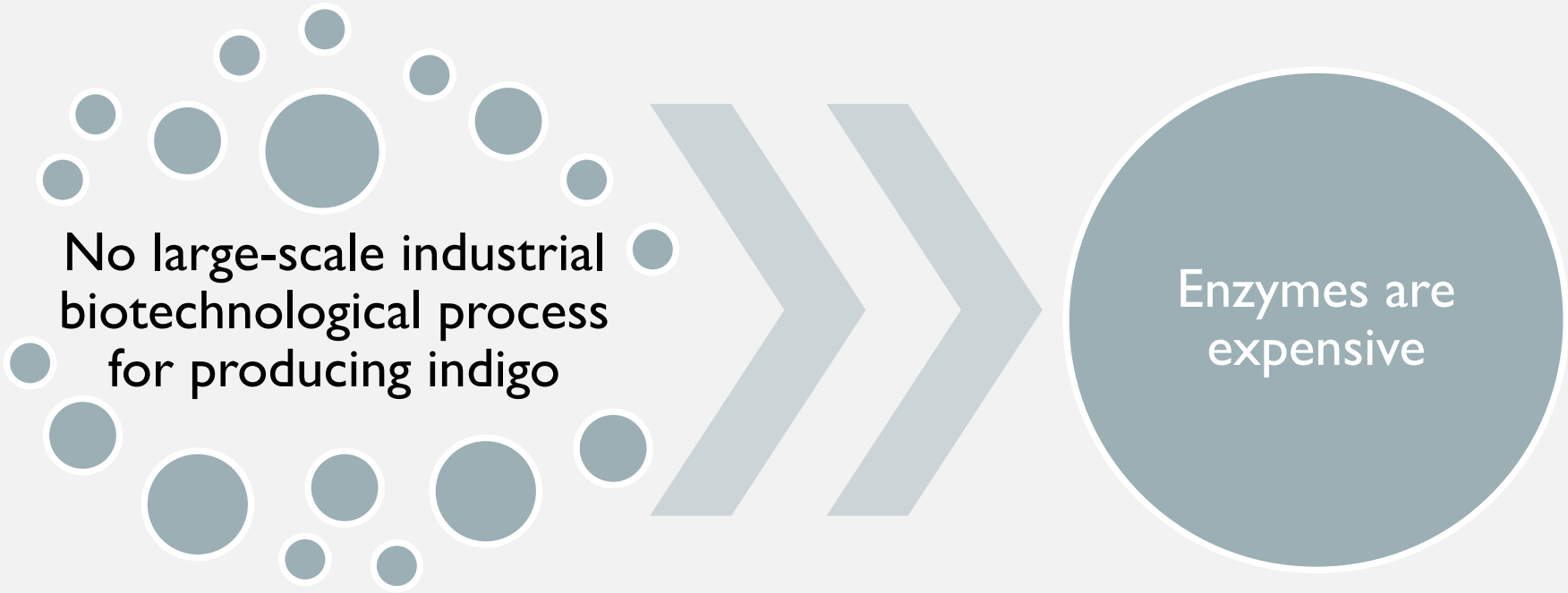
*Initial oxidation products derived from aromatic hydrocarbons are *cis*-dihydrodiols. †This strain of *P. putida* contains a single plasmid. The relation of this plasmid to plasmids in different strains of NCIB 9816 (*23, 24*) is unknown. ‡Strains lack active naphthalene diol dehydrogenase (nddA) or biphenyl diol dehydrogenase (bddA).

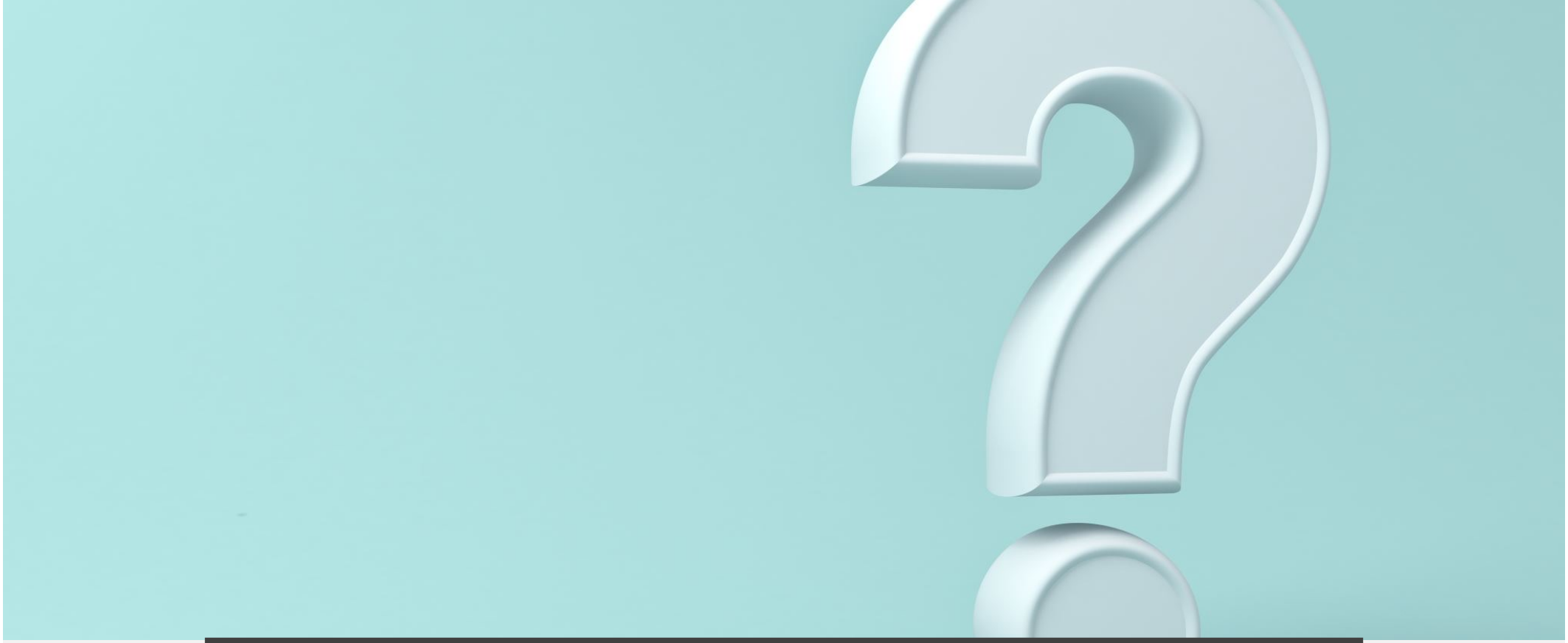
# DISCUSSION

- Provide a sustainable alternative for chemical synthesis of indigo dye from microorganisms

Pollution produced from denim factories using chemically  synthesised indigo

# LIMITATIONS OF MICROBIAL SYNTHESIS OF INDIGO





# THANK YOU FOR LISTENING