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Pune, India

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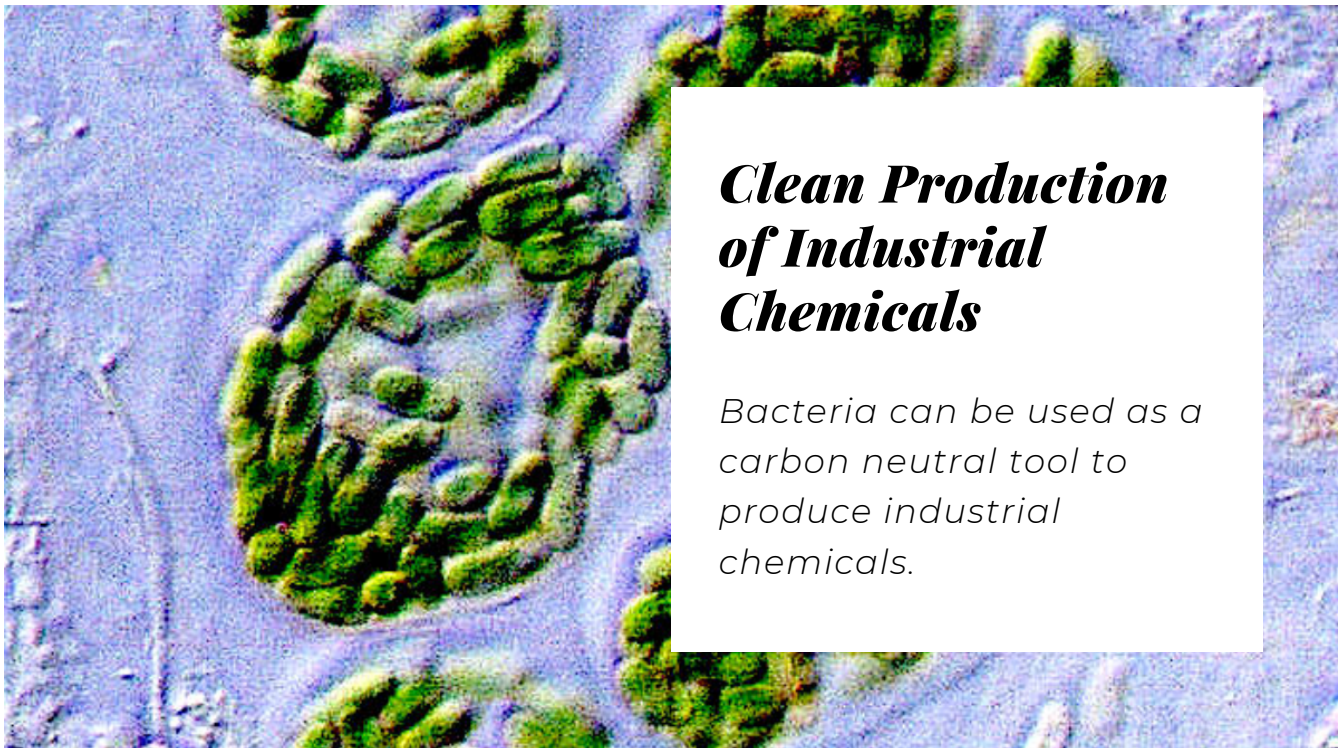


Clean Production of Industrial Chemicals

Bacteria can be used as a carbon neutral tool to produce industrial chemicals.

Research Summary

Want to learn more? Check out Team IISER Pune's web comic, and their synthetic biology youtube series!

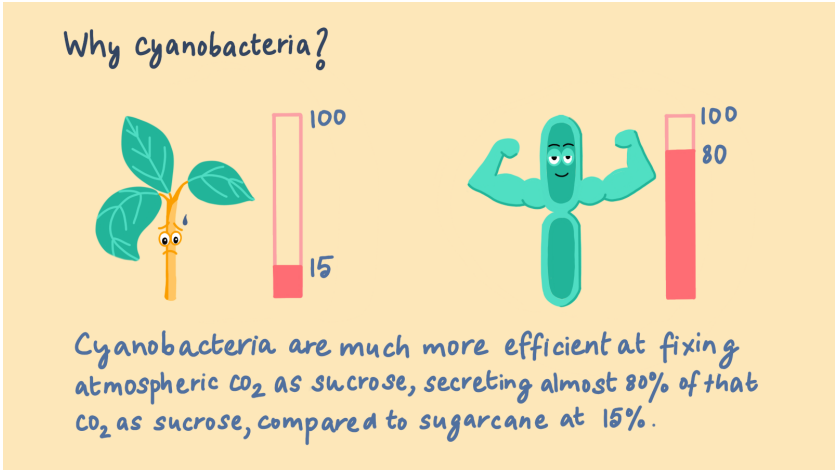
Team IISER Pune is working on the sustainable manufacturing of industrially important chemicals using a co-culture of photosynthetic cyanobacteria and E. coli. A co-culture is created by growing two microorganisms together on the same media. The cyanobacteria is photosynthetic, so it takes in atmospheric carbon dioxide and uses it to produce sucrose, which it then secretes out. The E. coli takes in the sucrose and uses it to fuel its natural processes, though it can be engineered make a specific chemical instead.

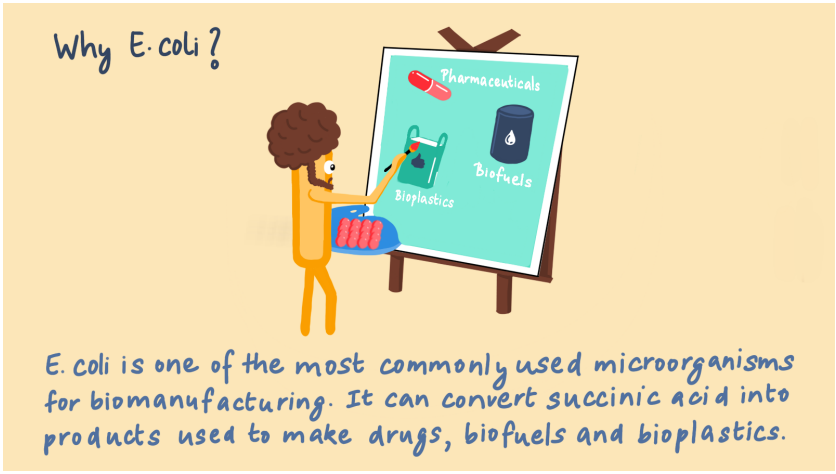
The conventional method for industrial chemical synthesis relies on fossil fuel-based feedstocks, creating problems for the environment. IISER Pune's project aims to replace the conventional method of petrochemical-based synthesis of feedstock that is both polluting and unsustainable, with a carbon-neutral method of synthesis. The project tackles carbon emissions at two levels, first by taking in carbon dioxide from the atmosphere, and second by preventing the emissions that would be generated during conventional feedstock-based synthesis.

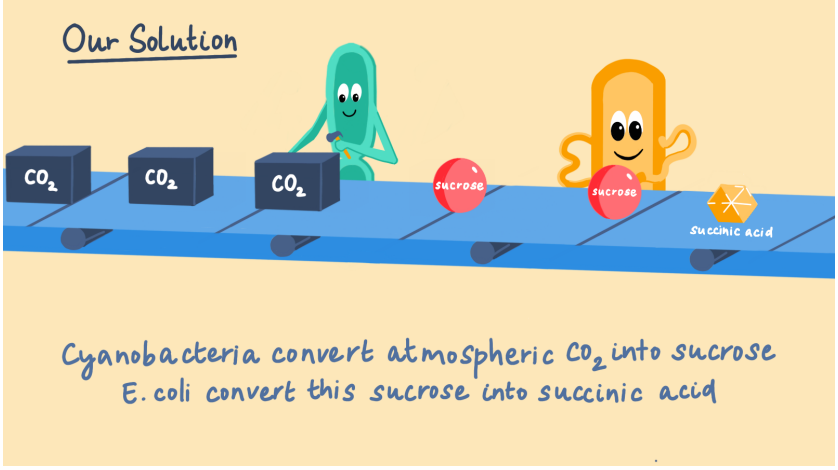
Key Techniques: Wet Lab

Cyanobacteria

The strain of cyanobacteria used will naturally accumulate sucrose when exposed to salt stress, in order to maintain their osmotic pressure. IISER Pune aims to engineer this strain to secrete the sucrose it accumulates, by having it express a sucrose transporter protein. The cyanobacteria is modified through CRISPR Cas12a, to facilitate a genomic integration of a non-native gene which encodes the transporter.







Escheria coli

E. coli will simultaneously be engineered to express the necessary transporters and enzymes needed to take in sucrose and break it into glucose and fructose. This will be done by transforming the *E. coli* with a plasmid containing the necessary genes. *E. coli* can further be modified to convert the sucrose into industrially relevant metabolites, which may differ depending on the desired molecular end product.

Objective

IISER Pune aims to identify and define the function of the genes, promoters, and terminators that they use. They also aim to develop a better understanding of the co-culture method between cyanobacteria and *E. coli*. In order to do this, experiments will be done to make measurements of the growth rates of both microorganisms, in the presence of each other and in isolation. The sucrose production rate in the cyanobacteria, the sucrose consumption rate of the *E. coli*, and the yield of metabolites produced by the *E. coli* will also be measured.

With this technology, industrial chemicals can be produced without emitting massive amounts of greenhouse gases!

Key Techniques: Dry Lab

Metabolic Models

The dry lab team is working with genome scale metabolic models of the bacteria present in the team's co-culture, to try and gain insights into how they can improve the manufacturing process. In these metabolic models, the bacteria are defined by the set of chemical reactions that occur inside their cells. The interactions between a bacterial species and its environment is modelled by the rate of exchange of the various metabolites that move across the cell membrane, and how that affects the chemical reactions that are key to the species' survival.

These models are used for:

1. Optimal strain design. This involves exploring what modifications (such as gene deletions or over expressions) can improve the yield of sucrose in the cyanobacteria, and the industrially important metabolite in the *E. coli* strain.
2. Determining how changing certain parameters would affect the yield of the end product from the co culture. For example, the bacteria can be grown at either aerobic conditions (with oxygen in the environment) or at anaerobic conditions (without oxygen). This would affect the stability of the co culture and how much end product is produced. The models can help to determine what level of oxygen would result in the best yield. Similarly, changing parameters such as the sucrose production rate of the cyanobacteria, or the carbon dioxide concentration might affect the final yield.
3. Building a dynamic model for the co culture. This will help to determine how each species will grow over time and how much of the end product will be formed, given initial conditions of sucrose and cell biomass for each species. This will enable an understanding of how parameters such as the initial ratio of biomass of the two species will affect the system's target chemical production levels.

Cyanobacteria can be grown in carbon-rich waste, and different strains of *E. coli* can be produced to manufacture different chemicals.

Value of this Research

The climate is changing. Carbon dioxide levels in the atmosphere continue to rise unchecked. The chemical and petrochemical industries alone are responsible for 18% of all industrial carbon dioxide emissions. Because of this, it is important to develop an alternative and sustainable method of chemical synthesis, one that no longer relies on fossil fuels, and that doesn't produce any greenhouse gas emissions.

Biomass produced from photosynthetic organisms is a promising alternative to fossil fuels, as a carbon source for chemical synthesis. However, generating plant biomass can be resource intensive. It requires large amounts of land, water, and fertilizer, and can lead to further environmental degradation. This method could also end up competing for resources with food production.

Cyanobacteria on the other hand are far more efficient at photosynthesis and sucrose production compared to plants. They have been shown to secrete over 80% of their absorbed carbon as sucrose, in comparison to the 15% secreted by plants such as sugarcane. They also require just a fraction of the resources that plants do, not to mention they can be cultivated using industrial emissions, polluted land, and waste water for food.

From the IISER Pune Team to You

"Don’t stop asking questions, look for science in everything. Don’t accept what’s given to you at its face value, keep trying to learn more. Be curious and be creative. Get to know your peers by working with them and learning from them, science is not a one person job. Most importantly, don’t let anyone discourage you from pursuing science."

~ Team IISER Pune

The above note was written in answer to the question: *What advice would you give to a high school student who is interested in pursuing the sciences?*