Using bacteria to dissolve indigo for dyeing

Sir: I've heard that dyeing blue jeans causes a lot of chemical pollution. How could this be lowered?

Image: by Louise Cornelissen, via Pexels.com

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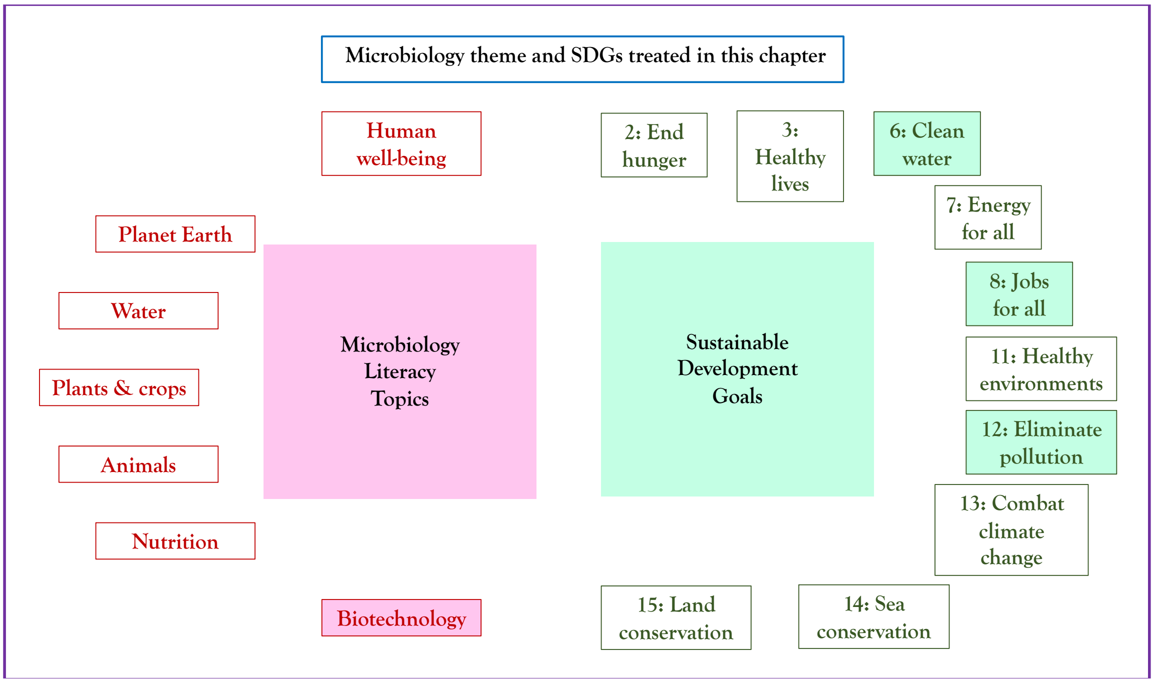
Storyline

Indigo has been one of humankind's favourite dyes since prehistoric times, and remains today one of the most important textile dyes, largely due to the enduring popularity of denim jeans. Indigo is insoluble in water, and to be used as a dye it has to be chemically reduced to a soluble, colourless chemical form, which is called indigo white. For the past hundred years the dyeing industry has relied on the reducing power of alkaline sodium dithionite, (also known as sodium hydrosulphite) but this results in large quantities of sulphur waste to be disposed of. Before the introduction of chemical methods, indigo was dissolved in a fermentation vat, where anaerobic bacteria reduced the indigo. This method is still used on a small scale commercially in India. Harnessing the power of bacteria to dissolve indigo industry-wide would help lessen the environmental impact of producing blue jeans.

The Microbiology and Societal Context

The microbiology: fermentative metabolism of clostridia; generation of reducing potential (redox potential); transition of the bacterial population from aerobes to anaerobes in the fermentation vat; providing optimum conditions of substrate and pH for bacterial growth; optimising conditions for indigo reduction in a pre-scientific age. *Sustainability issues*: environmental pollution, maintaining ancient skills and traditions in local employment.



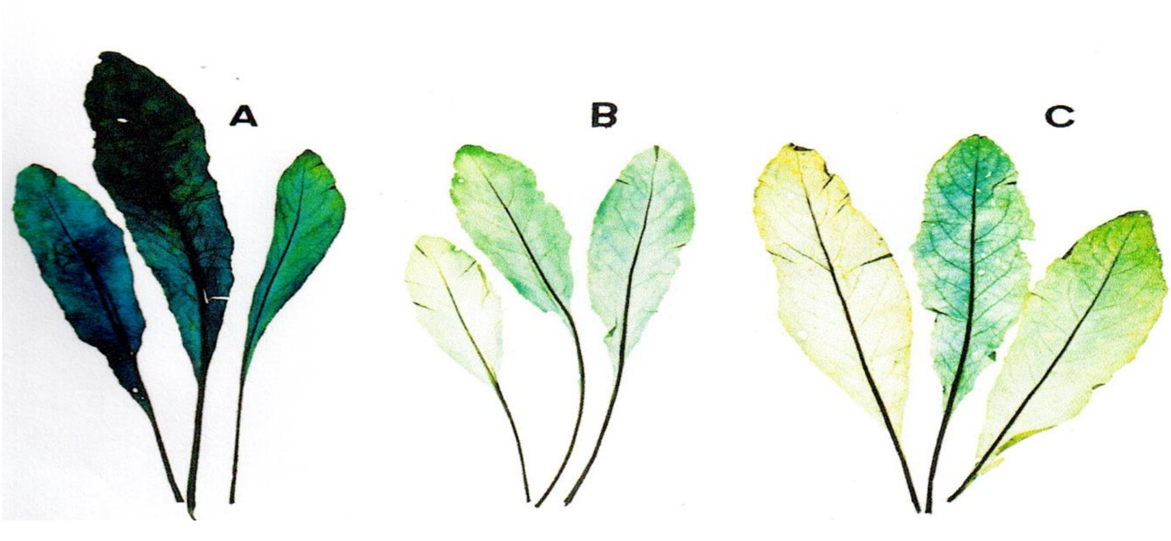
Using bacteria to dissolve indigo for dyeing: the Microbiology

1. ***Indigo: its uses and traditional sources***. For thousands of years indigo has been used to dye textiles of all kinds. Before the advent of the chemical industry in the 19th century, when all dyes were naturally sourced, indigo was the only blue dye. It was also resilient and light fast, compared to other natural dyes, meaning it would not fade even after exposure for long periods to strong light. Now that the chemical industry has given us many alternative synthetic blue dyes, indigo remains

indispensable as a dye for blue jeans because of a unique property: as the jeans are worn and washed the indigo wears away in a particular way so that the jeans acquire a characteristic look that depends on the shape and activity of the wearer. Jeans can be given a "distressed" look even when new by the industrial process of stone washing. Although only a few grams of indigo go into each pair of jeans, such is the scale of jeans production worldwide, some 20,000 tonnes of indigo are used in dyeing every year.

Image: by Brianna Swank, via Pexels.com

Traditionally indigo was obtained from plants that were grown as crops, the leaves harvested and processed so that chemical precursors of indigo in the leaves were converted to indigo. In temperate climates woad (*Isatis tinctoria*) was grown; in the tropics the indigo plant (*Indigofera* spp).



Visualisation of indigo in woad leaves. All the leaves were treated with ammonia, then extracted with acetone and methanol. The leaves in B and C are controls which have been (B) boiled and (C) left to dry in order to destroy the indigo precursor in the woad leaves. From Kokubun et al 1998 Phytochemistry 49, 79-87

In Europe indigo was dissolved for dyeing in a bacterial fermentation called the woad vat. At the end of the 19th century, indigo synthesised by the chemical industry started to replace natural indigo, as it was cheaper and more pure. A result of this was that the British and Dutch colonies in Bengal (now Bangladesh) and the Dutch East Indies (now Indonesia) became less valuable to the colonial powers.–At the same time a new process of dissolving the indigo was found using sodium dithionite as reductant to dissolve the indigo for dyeing.

The amount of dithionite used by the denim dyeing industry is roughly equivalent to the amount of indigo used, the dithionite breaking down into simpler sulfur compounds in the process with the result that about 20,000 tonnes of sulfur waste have to be disposed of every year.

2. ***The traditional fermentation vat.*** The traditional fermentation vat is based on the catalytic properties of bacteria, with the whole process driven by organic substrates that can be waste products themselves of other industries, such as bran from brewing.

A traditional fermentation indigo vat used by The Colours of Nature a commercial company based in Auroville, South India. The Colours of Nature revived the ancient traditions related to natural indigo fermentation dyeing after a long search led the founder, Jesus Ciriza Larraona, to a small village and a family that had handed the knowledge of the indigo fermentation vats down for generations. Colours of Nature has used the same water in their vats since 1993 and no water is ever wasted. Jeans dyed in their 200 litre fermentation vats are sold all around the world. The bacteria responsible for indigo reduction in these vats have not yet been identified

But how does the fermentation vat work? From the 15th century onwards recipes and "operating instructions" were printed for the woad vat:

"To start a vat of 5 pounds of woad indigo, add 15 buckets of water, 7 pounds of potash, 1.5 pounds of madder, and 4 handfuls of bran, and heat it all to boiling and leave it for an eighth of an hour...". Recipe for a medieval woad dye vat (Translated from: Un Manuale di Tintoria del Quattrocento, an Italian 15th century dyeing manual)

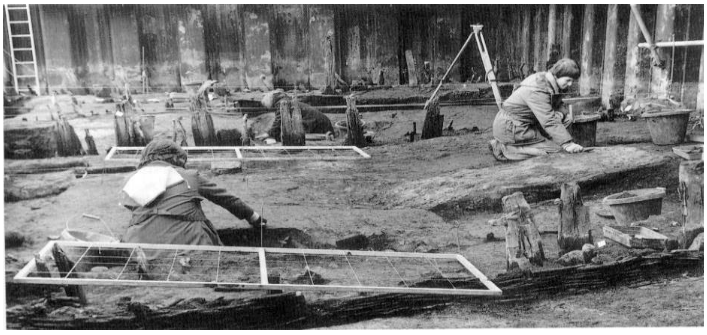
If you set up one of these vats, as experimental archaeologists have done, you can reproduce the whole medieval process: A piece of undyed cloth dipped into the vat becomes infiltrated with the dissolved colourless indigo white. When the cloth is brought out into the air, the indigo white which has penetrated the cloth fibres becomes oxidised by the oxygen of the air back to indigo and turns blue. The cloth can be rinsed of the vat bacteria and other bits and pieces from the vat, but remains permanently dyed blue with indigo which has infiltrated the fibres in the cloth.

$$\text{Indigo (insoluble)} \xrightleftharpoons[+O_2]{-O_2\text{, dithionite or bacterial reduction}} \text{indigo white (soluble)}$$

To the operators of the woad vat in a pre-scientific age this process must have seemed like magic - or alchemy. But they knew exactly how to manipulate conditions in the vat to maximise its effectiveness. The basic ingredient was the processed woad leaves which contained the indigo. To these they added bran, which provided a slow release food for the bacteria in the form of complex carbohydrates. They also added wood ash (potash) which served as an alkali, as it contained potassium carbonate. The fermentation vat was roughly the size of a large beer barrel, and was heated to about 50C by a fire. The periodic carefully judged addition of wood ash and bran allowed the vat to go on reducing indigo for months. How was the indigo reduced?

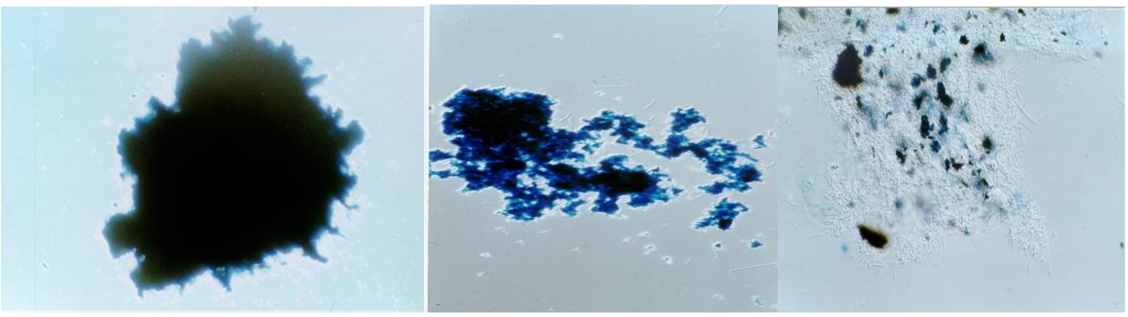
A medieval woad vat 1482. The dyers are stirring the vat with long poles. The complication in this scene is that having previously dyed a cloth blue, the dyers are now dyeing a cloth red. This was probably against the regulations governing their trade, and they are being spied upon by the three people in the left background. (From: Dyeing wool cloth, from "Des Proprietez des Choses" by Bartholomaeus Anglicus British Library Royal MS 15.E.iii, folio 269).

3. ***The microbiology of traditional indigo reduction***. We now know that the agent in the traditional European woad vat responsible for indigo reduction was a bacterium, called *Clostridium isatidis*. This anaerobe was isolated by microbiologists from a medieval woad vat set up in a laboratory according to the medieval recipe. There are two reasons we believe that this bacterium was responsible for reducing the indigo. First, a pure culture of the organism grown on a chemically defined medium reduced and thereby dissolved indigo, when oxygen was removed. Secondly, *C. isatidis* was grown from spores found in the sediment of a woad vat that archaeologists had found in a dig in York dated to the Viking Period (about 1,000 CE). The spores were viable – after about a thousand years in the ground!



Archaeologists at the dig of Viking York. It was from this dig that a sample of the sediment of a woad vat was found, from which spores of the indigo-reducing bacterium Clostridium isatidis were germinated.

Initially the woad vat contains oxygen from the air, but aerobic respiring bacteria rapidly consume this oxygen and when it is depleted they stop growing, the resulting anaerobic conditions favour the growth of fermenting bacteria, such as C. *isatidis* and provide the conditions for indigo reduction.



Sequence of light microscope images showing the dissolution of blue indigo by the bacterium Clostridium isatidis. The multiplying bacteria show as white rods clustering around the gradually disappearing indigo particle, which is reduced to the colourless, soluble indigo white. (Unpublished, P. John)

The fermenting bacteria produce gases and acids as by-products of the breakdown of the carbohydrates they consume, so the vat fizzes when the fermenting bacteria are active and the pH declines. In the case of the woad vat, the gases that come off are hydrogen and carbon dioxide. If the acids were allowed to accumulate and were not neutralized, the bacteria would stop growing, as the vat pH would sink too low. The medieval operators were aware that they had to keep the vat "sweet" by adding wood ash for it to continue to operate. We now know that the wood ash contains an alkali in the form of potassium carbonate. We also now know that the reduction of indigo to indigo white is favoured by an alkaline pH so the dyers had to get the pH just right; sufficiently alkaline for indigo to be reduced, but not too alkaline in which case the bacteria would not have been able to flourish. They accomplished all this without a pH meter or litmus paper, but relied on the traditional skills handed down by the masters of their craft and by practice.

There is evidence that the woad vat was used in prehistoric times, with one of the first written observations being that of Julius Caesar in the mid 1st century BC who observed that: "All the Britons dye their bodies with woad (*vitrum*), which produces a blue colour, and this gives them a more terrifying appearance in battle". Other classical writers confirmed this, including Pliny in the 1st century AD: "In Gaul there is a plant like plantain, called *glastum*, with it the wives of the Britons, and their daughters-in-law, stain all the body, and at certain religious ceremonies march along naked, with a colour resembling that of the Ethiopians". These later Iron Age Britons could have used indigo pigment as a powder mixed with egg, beef fat or milk to make tattoos or to paint their bodies. Alternatively, they may have immersed themselves in a woad vat, dyeing their skin as one would dye a piece of cloth. (There is an amusing song that celebrates this body art, The National Anthem of the Ancient Britons:

https://www.monologues.co.uk/Parodies/Ancient_Britons.htm. Here is a selection of different people singing the anthem to suit different audiences: https://www.youtube.com/watch?v=em4F-DXsM-Q, https://www.youtube.com/watch?v=dAs1-4ZM2Ms, https://www.youtube.com/watch?v=FwlLA4KF-No).

4. ***Traditional indigo fermentation in modern times.*** Even today, jeans can be bought that have been dyed in a fermentation vat. These are made in India (https://www.youtube.com/watch?v=hes05oYzd6c). We don't know which bacteria are responsible for the indigo reduction in these vats, but they do have something in common with the medieval woad vat. In both the medieval woad vat and in the Indian vats, the tradition is to add to the vats a small amount of an ingredient that helped the dyeing. For the woad vat it was madder (see recipe above), a natural dye used to dye cloth red; in India it is seeds of *Cassia tora,* a common weed in the tropics. Both madder and the Cassia seeds contain organic compounds called anthraquinones. In fact it can be shown in the laboratory that a pure synthetic anthraquinone speeds up the process of indigo reduction. Physical chemistry experiments show that the anthraquinone interacts with the crystal structure of insoluble indigo to facilitate its reduction to the soluble indigo white. So again, without any knowledge of chemistry as we understand it today, medieval and contemporary operators of the fermentation indigo vat have learned to optimise the chemistry of the process.

Relevance for Sustainable Development Goals and Grand Challenges

- **Goal 6. Ensure availability and sustainable management of water and sanitation for all** (*assure safe drinking water, improve water quality, reduce pollution, protect water-related ecosystems, improve water and sanitation management).* The bacterial system of indigo reduction described here uses less water and produces less pollution in the form of sulfur waste than the currently employed chemical reduction industrial process, as the fermentation vats once set up can be maintained for long periods of time. However, the scale and speed of modern industrial production means that much research into scale-up will be required.
- **Goal 8. Promote sustained, inclusive and sustainable economic growth, full and productive employment and decent work for all** (*promote economic growth, productivity and innovation, enterprise and employment creation).* The fermentation method of indigo reduction provides local employment to a significant number of people, albeit a specialised niche industry process, like that currently operating in India (https://www.youtube.com/watch?v=UEMcjmyjoOY). To scale-up to a modern industrial process would require considerable research and development on the bacterial system to discover how to control the bacterial process, and also provide the speed of reduction that modern economic conditions demand. However, this would also create employment for skilled personnel and help promote a sustainable future.
- **Goal 12. Ensure sustainable consumption and production patterns** (*achieve sustainable production and use/consumption practices, reduce waste production/pollutant release into the environment, attain zero waste lifecycles, inform people about sustainable development practices).* The bacterial system of indigo reduction offers a sustainable process that uses cheap and easily obtained carbohydrate substrates to generate a sufficiently reducing potential for indigo reduction. In effect the bacterial cells act as dispersed, micrometre-scale electrodes delivering reducing electrons to the indigo particles. However, the safety of the indigo-reducing bacteria would need to be assured, for example some people might show an allergic reaction to the indigo-reducing bacteria that remained on the dyed textile. Nevertheless, if we could discover the chemical/biochemical mechanism the bacteria use to reduce the indigo, then we could apply that in a large-scale electrochemical process of indigo reduction. This would in effect replace chemistry with physics.

Potential Implications for Decisions

The present technology of indigo reduction is well-established and the investments that have already been made in these industrial plants allow for a cheap dyeing process for the mass-market product that are denim jeans. If we were to invest in a clean electrochemical process derived from research into bacterial indigo reduction, then jeans might become more expensive. Are we willing to pay more?

The Evidence Base

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Further reading

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Glossary

Reducing power. The ability of a chemical substance to reduce, that is donate electrons, to another chemical substance. A powerful reducing agent will have a highly negative oxidation/reduction (or redox) potential.

Fermentation. Living organisms obtain metabolic energy (ATP) from oxidation-reduction reactions, transferring electrons from an electron donor to an electron acceptor. In fermentation the final electron acceptor is an organic compound.

Aerobic respiration. Living organisms obtain metabolic energy (ATP) from oxidation-reduction reactions, transferring electrons from an electron donor to an electron acceptor. In aerobic respiration the final electron acceptor is molecular oxygen.

Electrochemical process. A chemical reaction that is driven by the application of an electrical current, or the reverse in which a chemical reaction creates an electrical current. The former process can use electricity to reduce a compound like indigo without recourse to a chemical reductant such as dithionite.

Niche product. A product that targets a specific section of a larger market. Niche products appeal to a particular demand, and are often more expensive than more widely sold products.

Sodium dithionite. A strong reducing agent used industrially to reduce indigo. It is also known as sodium hydrosulphite. In alkaline solution dithionite ($Na_2S_2O_4$) reacts directly with O_2 to form sulphite and sulphate.