

Some More Thoughts about Indigo Fermentation: Blog Post #7: What I Have Learned from a Biochemist

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It is one thing to put all the ingredients in a “vat” and hope for the best result: a reduced vat that will dye beautiful blues. It is a whole other thing to understand what happens in that vat. I am not a scientist, but the following is some of what I have learned from Dr. Kim Borges, biochemistry professor at Warren Wilson College in Swannanoa, NC. Dr. Borges uses indigo fermentation with her class to help students understand the basic concepts of bacterial fermentation.

She states that “how the indigo reduction happens in the fermentation vat seems to be the big, unresolved question in the microbiology research world.”

The quick reduction vat (made with fructose, iron, etc.) requires very high pH. The glucose (and/or a degradation product of glucose that is made at high pH) appears to donate electrons to the indigo molecules directly.

In the fermentation vats, the concentration of free glucose is much lower. Many different microbes use glucose as a preferred fuel. It is suspected that much of the free glucose will be taken up by community microbes before it reduces indigo, though that is a guess. It is likely that cellulose, starch, arabinoxylans and other complex carbohydrates from wheat bran (and from other plant materials) are converted to simple sugars by some of the microbes in the community. But those microbes will shuttle most of the sugar into their cells for their own use, and so only some of the easily metabolizable sugars like glucose will stay in the fermentation fluid.

It's interesting that most kinds of bacteria can't degrade those complex carbohydrates - only certain microbes specialize in that activity. A functioning sukumo-based vat has *Amphibacillus* and *Alkalibacterium*, which are types of bacteria that degrade wheat bran carbohydrates to produce lactic acid as waste. Other kinds of bacteria seem to be able to use the lactic acid, which helps to keep the pH from falling too much.

I think that one reason we stir the vat daily is to disperse the “pockets” of lactic acid that build up around the wheat bran fermenters and move it to the lactic acid users before the vat spoils. A network of microbes based upon use of hard-to digest complex carbohydrates will develop in a good vat. And somehow some of these microbes transfer electrons from proteins on the outside of their cells to indigo particles (probably carried by anthraquinones and other electron-carrying small molecules). There is still so much to learn!

Regarding the addition of sake (rice wine) or glucose (malt syrup, rice extract, etc.) to the sukumo vat: It doesn't seem like the sake adds enough alcohol to kill bacteria that would de-rail the indigo reduction and spoil the vat. Perhaps those added sugars initially provide easy to metabolize “fuel” that starts the development of the bacterial community. The bacteria that dominate the sukumo vat initially don't seem to be indigo reducers. But they do seem to be good at using up oxygen to create the anaerobic conditions preferred by indigo reducers. Maybe the added sugar helps to cultivate them, and it is worth testing.

As an aside: Rowland Ricketts once told me that he never saw a traditional Japanese dyer use sake in the vat. It is much better to drink it!