The Organic Ties between Biology and Textile Chemistry: Fungi, Enzymes and Green Chemistry

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Introduction

Our Scientific Heritage

Science is the curiosity-driven quest to understand the natural world and to explain how we fit into its environment. We know it as the interwoven body of observations, experiment and theory that is used to explain and even predict our how universe has come to be, and our place is in it. For most of recorded human history, what passed as sciences were empirical observations with some attempts made to provide non-supernatural explanations: Thales, Leucippus, Democritus, Aristotle and others from the classical period of Greece all made their attempts, and had dominant influences on accepted explanations of the natural world. Scientific methods as we follow them (as opposed to engineering) date from the Middle Ages with worthies such as Ibn al–Hayam and Roger Bacon being prominent. Most historians say that modern science dates to the scientific revolution in 16th and 17th century Europe with works by Vesalius, Copernicus and Newton totally changing the way we looked at both our human bodies and the universe that we occupy. This was the time of Galileo, Kepler, Leibniz, Pascal, Bacon, Brown, Descartes and Hooke. In a way, it is odd that science seemed to come into its own first in physics, then in chemistry, and lastly in biology. This may simply be the result of our wariness about delving into questions about our essential human nature – this certainly continues to this day. Darwin was aware of the reluctance to explain human origins through science: he delayed publishing his Origin of Species for two decades at least partially because he knew how his very pious wife would react to his ideas. With the increasing specialization of knowledge, the inevitable gap between sciences has grown; the time when a scientist could be broadly and deeply knowledgeable even within a narrow discipline has long passed. Few are Jeffersonian these days, even in their interests, much less their expertise.

Chemistry Emerges

The forerunner of chemistry was alchemy which, though fundamentally flawed, did set the stage for moving chemistry into a science with its experimentation and results-based approach. Chemistry started to emerge from alchemy with the work by Robert Boyle and his “The Sceptical Chymist” in 1661. Antoine Lavoisier created our modern concept of chemistry with his law of the conservation of mass and the careful measurements that it requires. Losing him to the guillotine in 1794 was a blow from which French chemistry did not quickly recover. The atomic theory of John Dalton in the early 1800’s provided the theoretical foundation for chemistry, while in 1869, Mendeleev’s breakthrough with his periodic table and its predictive success provided the clear link between chemistry and physics.
**Biology Moves From Peas To Deoxyribonucleic Acid**

While Darwin was creating a revolution in thinking about origins of species, an obscure Austrian monk named Gregor Mendel was quietly examining the inheritance of certain traits in the 29,000 pea plants he cultivated. His work did not gain fame until after his death, but he is credited with being the originator of the science of genetics. In April, 1953, a one-page report in the journal *Nature* contained this sentence "This structure has novel features which are of consider-able biological interest", which as many have said might be “one of science's most famous understatements”. This statement appeared in the paper where James Watson and Francis Crick presented the structure of deoxyribonucleic acid (DNA) double helix, the molecule that carries genetic code and allows transfer from one generation to the next. Later Watson, Crick and Maurice Wilkins shared the Nobel Prize. Rosemary Franklin, whose x-ray diagrams were crucial to the solving of the DNA structure puzzle, had already died but perhaps would have been included. It is no exaggeration to say that the discovery of the structure of DNA and the implications of that structure changed not only biology, but the way chemists looked at it. DNA is, after all, a polymer and the chemical nature of our existence had become more explicit.

**Textile Chemistry Moves From Art To Science**

Textile chemistry’s change to a science rather than an art or craft can be traced to the accidental discovery of mauveine by the 19-year old William Perkin. Perkin was trying to make quinine, the natural anti-malarial derived from cinchona bark. Perkin’s genius was to recognize that his product might have commercial value. His dye became the rage for a short length of time, and others quickly realized that he had opened up a completely new area for chemistry to be applied to commerce. Within a few decades, the British had lost their initial lead and the Germans had come to dominate the dye industry. Germany used their increasing expertise in organic synthesis to move into (and dominate) drug manufacturing, thus creating a direct link between synthetic chemistry and the human body.

The dramatic moment came in 1897 when Felix Hoffman created acetylsalicylic acid, named aspirin by his company Bayer. This was only the first of many synthetic drugs coming from the increasing expertise in synthetic organic chemistry.

On the fiber side of textile chemistry, Count Hilare de Chardonnet is usually given credit for inventing “artificial silk”, displaying it at the 1889 Paris World Fair. Though rayon and acetate fibers came soon from this beginning, the recognition of macromolecules (polymers) as reality, and the creation of truly synthetic fibers, would have to wait until the 1920’s and 30’s, with the seminal work of Staudinger and Carothers, the former considered the father of polymer science and the latter inventing nylon 6,6.

For centuries, there have been connections between textile chemistry and biology, but as an art or craft rather than as science. In one sense, enzymes and textiles probably have a very long history. There are at least two examples that go back millenia. One would be the retting of flax by laying the harvested plants on the ground and allowing microbes to free the fibers from the stem by the actions of the pectinases and xylanases that the microbes exude. More recently, Dan Akin at the USDA Russell Research Center in Athens, Georgia, spearheaded efforts to use commercially produced pectinases in a controlled process to produce flax fiber with better and more consistent fiber quality.

Another example, but initially a negative one, is the rotting of cellulose fibers by the actions of fungi that produce cellulases. The fungi are ubiquitous in nature and cotton fabrics left in warm, wet environments will quickly “rot” if not protected with a finish that poisons the fungi and prevents them...
from secreting cellulases. Of course, the positive side is that *Trichoderma reesei* was discovered to be the problem with tents rotting in the south Pacific during World War II, and that knowledge subsequently led to the development of cellulase production that is used in textile and garment finishing, laundry detergents, and in the conversion of biomass into ethanol.

**My Interest In Biological Ties To Textile Chemistry**

My courses in science, engineering and textiles at Auburn and ITT, and then in the chemistry program at Clemson, made me aware of the connections between disciplines, and whetted my appetite for broader understandings of what it meant to be seeking an education. In one of my polymer classes at Clemson, I wrote about the “protein machine” of Robert Merrifield, and the description of the discovery of the structure of DNA in “The Double Helix” by James Watson. Both of these men were Nobel Prize winners and though neither thought of themselves as polymer chemists, they in fact were just that. Merrifield was constructing proteins and Watson was elucidating the structure of the greatest polymer of them all, deoxyribonucleic acid, our ultimate “Morse Code” of organic existence.

Watson’s book had a profound influence on me. I had not studied biology beyond my sophomore class in high school and heard only vaguely about DNA and RNA. By the middle of my graduate work at Clemson, I had accumulated a fair knowledge of polymer science, thanks to Howard Clark, Bob Barker, and especially John Lundberg. Much of what I learned in polymer and fiber science related to the polymers of life. It was evident to me even then, the late 60’s, that biologists were going to have to learn much more about chemistry as they pushed into the chemical origins of life, and chemists were going to be increasingly involved with biology as biomedical research became ascendant through the funding of the National Institutes of Health (NIH) and the National Science Foundation (NSF).

**Encounters With Biology At Georgia.**

After many fruitful years at Auburn University, I moved in January of 1994 to the University of Georgia to become the head of the department of textiles, merchandising and interiors. Georgia had a strong textile chemistry group built by Terry Perenich. I was fortunate to arrive at UGA when the state of Georgia had put into effect a competitive grant program that encouraged the universities and industry to work together on applied research projects. One real bonus from that program was the opportunity to get to know better and to work closely with Fred Cook at Georgia Tech. In the next few years we were able to put together a strong research and technology transfer group in our department that included three future Olney Award winners, Nolan Etters, Warren Perkins and George Baughman, as well as other strong scientists such as Charles Yang, Patti Annis, Karen Leonas, Helen Epps.

At Auburn, my collaborations had been primarily with engineering and chemistry, but a change occurred after I arrived at Georgia. The University of Georgia is especially strong in the biological and environmental sciences with many internationally recognized scientists. As I met with various research leaders on campus and at other government research centers located near the campus, I became aware of possible ways to combine my environmental interests with research projects that might benefit the Georgia textile industry. Many opportunities occur by happenstance and my visit to the USDA Russell Research lab near campus was like that. I went there to investigate their nuclear magnetic resonance spectroscopy (NMR) facilities, but that visit turned out to be a window to opportunities to combine chemistry with biology in ways I would have never envisioned during much of my career. While there, I was introduced to Dan Akin, a research microbiologist; he and I subsequently enjoyed many years of collaboration. He also was my pathway to discussions with the late Karl-Erik Eriksson, a distinguished biochemist at UGA. Dan and Karl-Erik reinforced interests I had for some time in looking at enzymatic treatments of cotton, and I collaborated with both in my research. Karl-Erik was responsible for stimulating my interest in examining white rot fungi and their potential for decolorizing waste dye effluent. Later on, in my attempt to help out the small town of Washington, Georgia, with a problem
they were having with whole effluent toxicity, I became acquainted with two scientists at the US Geological Survey based on campus, Peter Lasier and Parley Winger. My collaborations with them centered around investigations of the use of a small aquatic organism named *Cereodaphnia dubia* (sometimes incorrectly called a water flea), and its use in determining whole effluent toxicity.

**Biology and Textile Chemistry: Enzymes and Bioscouring**

As was indicated earlier, the application of enzymes to flax has been done through retting of flax fibers for centuries, though not in any deliberate scientific sense. The use of amylases to remove starch sizing from cotton fabrics dates to as early as 1917, with bacterial amylase derived from *Bacillus subtilis* used for desizing by Boidin and Effront(1). Widespread commercial use came much later but was common by the 1950’s. Cellulases were the next enzyme to be widely used on cotton. The original uses were for replacement of pumice stones in “stone washing”, biopolishing of fabrics and incorporation into detergents to remove fuzz and thereby reduce scattering of light and “brightening” the fabrics (2). Cellulases can be used for these same purposes on other cellulosic fibers such as linen, ramie and hemp.

The scouring process is part of the cotton yarn and fabric preparation sequence that includes desizing and bleaching. The scouring and bleaching processes have three major objectives: a) they remove a thin outer layer of waxes and complex carbohydrates, thus making the fabrics water absorbent; b) they both break up and decolorize cotton seed coat fragments (sometimes called motes); and c) they remove the natural cream color of the cotton and create the white base that is used for dyeing. Although using amylases for desizing has been in use for decades, using enzymes for scouring cotton fiber is a relatively new idea. The German literature of the early 1990’s alluded to the possibility in three separate papers (3,4,5). Shortly thereafter, research papers on cellulases (6-9) on cellulases suggested that other enzymatic treatments to cotton fibers might be feasible.

From 1996 to 2000, a series of papers appeared addressing the possibility of successful scouring of cotton fabrics with enzymes (10-25). Although several enzymes, including cellulases, proteases and lipases, were examined, pectinases proved to be the most effective. Their laboratory work was not fully published at the time, but Novo Nordisk (now Novozymes) reported in 1996 (11) on an alkaline pectinase that was particularly effective in creating water absorbability in cotton fabrics, and they subsequently announced its commercial availability in 1999 (22,23). This product had two major advantages over the enzymes that had been examined previously. The pectate lyase, marketed as “BioPrep 3000L”, had very high specific activity and its maximum effectiveness at a pH range of 8-10, which made its use very compatible with subsequent hydrogen peroxide bleaching conditions. In 2002, Novozymes was awarded the EPA Presidential Green Chemistry Award for developing “a cost-effective, environmentally compatible preparation process”.

At the same AATCC conference as the Novozymes paper by Krebs-Lange (11), Li (10) from our laboratory reported his first results using enzymes to accomplish the objective of creating water absorbent cotton fibers. His paper concluded that, out of a number of enzymes examined, pectinases (enzymes that break down pectins) could be successfully used to scour cotton, thus allowing the elimination of the use of sodium hydroxide in this stage of wet processing. Our lab and others were convinced that this process would have the advantages of providing a process for scouring that would lower the effluent pollutant load, be much less environmentally damaging, and save significant amounts of energy. The yarn, fabrics or garments produced from processes like these can accurately be marketed as being “green” or “eco-friendly”, a cachet that is increasingly sought by knowledgeable consumers. One result of that 1996 conference was a personally as well as professionally rewarding collaboration with Brian Condon and Jim Liu of Novozymes in trying to deal with the intractable seed coat fragment problem.

The work on “bioscouring” continued into the decade of 2001 (26-36). A continuing question as bioscouring was explored by scientists and industry is to what degree do the waxes and pectins need to be removed in order to create the desired level of hydrophilicity in the fabric. A wide-ranging paper by a
research group based in the Netherlands and Belgium addressed this issue (37). A porosimeter developed at the Textile Research Institute (39) was used to study changes in hydrophilicity. The porosimeter had the advantage over the commonly used AATCC Drop Test (40) and various absorption tests in that the results are said to be independent of fabric density and structure. From these measurements they were able to calculate a structural contact angle. The research group used these calculations to benchmark the effects of enzymatic treatments against standard alkaline scouring. From this work they concluded that wax and pectin removal were the essentials for scouring, and that in the enzymatic processes the removal of the outermost layer of wax was foremost. Other reports from this group introduced a cutinase-pectinase mixture, combined with added mechanical action, that allowed them to reduce the scouring temperature from 50 degrees C with pectinase to 30 degrees C with the mixture (38).

**Biology and Textile Chemistry: Seed Coat Fragments**

In order to create a total enzymatic preparation sequence for cotton fibers, an enzymatic process for removal of seed coat fragments (SCFs) with enzymes is needed. The seed coat fragments are created, for the most part, during the ginning process. Since the fragments remain with fibers attached, these get incorporated into yarns and then into fabrics, appearing as small dark spots in the greige fabric. In our lab, Kim and others analyzed the chemical and physical nature of the SCFs and applied a series of enzymatic treatments to the seed coat fragments, as well as analyzing the materials removed (41-45). Figures 2 and 3 show an optical microscopic cross section (43) and an analysis of the layers of the fragment (46). The complex nature of the chemical and physical structures of cotton seed coat fragments ensure that finding an enzymatic route to removing and/or decolorizing these contaminants will require a complementary approach, perhaps with two of more enzymes with pretreatment to increase accessibility. Dhandapani has pursued that latter approach (47). Csiszar and colleagues have attempted to use enzymatic methods to remove seed coat fragments. In their work, they utilized cellulases to clip fibers from the seed coat fragments. This detached the fibers entrapped in the yarns and made it easier for the SCFs to fall off. Another effect, and one we are pursuing further, is that the cellulases can attack the cellulosic part of the SCF structure and thereby open up the fragments to further chemical attack (48,49). Later, research by the same group showed that chelation by EDTA in a mixture of hemicellulase and xylanase enzymes hydrolyzed the SCFs much faster than the cotton fabric itself. The removal of calcium also sped up the removal of colored impurities in the alkaline process (50,51). The part of the seed coat fragments that gives them their dark color is the lignin, a complex polyphenolic set of compounds. Investigations in the UGA labs and others are focused on the degradation and removal of these lignins by xylanase or laccases (52). A unique approach was the use of solid state fermentation to apply a mixture of hydrolytic and oxidative enzymes (53). The seed coat fragments were used as a carbon source for the production of the enzymes, which were then applied to seed coat fragments in fabrics. In other research, the conclusion was a combination of cellulases and pectinases, followed by hydrogen peroxide, was needed (54). At present, despite advances, a practical enzymatic process for decolorizing seed coat fragments in woven fabric has not been achieved. Present research in the UGA laboratory is focused on improving accessibility for enzymes into the seed coat fragment structure.

**Biology and Textile Chemistry: White Rot Fungi**

Azo dyes, which account for approximately one-half of all known dyes, are commonly used as coloring agents in the food, pharmaceutical, and textile
industries. As a result, they are the most common synthetic colorants released into the environment. Because they are highly colored, azo dyes are readily visible in effluent water and can be the focus of significant environmental complaints centered around that visibility. These compounds are also of concern because some of the dyes, dye precursors or their biotransformation products, such as aromatic amines, have been shown to be carcinogenic (57).

The nonspecific nature of the “White Rot” lignin-degrading systems of fungi is of interest as a way to decolorize textile effluent with minimal impact on the environment. Although their function in nature is to degrade lignin, they have the capability to degrade other types of compounds because they function by secreting a suite of enzymes, but primarily lignin peroxidases, manganese peroxidases and, in some cases, laccases. Ligninolytic fungi are able to degrade numerous aromatic organic pollutants via oxidative mechanisms. The ligninolytic enzymes are extracellularly excreted by the fungi to initiate the oxidation of substrates in the extracellular environment of the fungal cells. All of these enzymes are generally believed to form during secondary metabolism of white rot fungi. Various investigations have shown that white rot fungi can efficiently degrade various organic pollutants other than lignin. For example, a characteristic of lignin peroxidase, which is also shared by non-ligninolytic peroxidases, is its relative unspecificity for aromatic substrates. Examples include polycyclic aromatic hydrocarbons, persistent environmental pollutants such as DDT, alkyl halide insecticides, xenobiotic compounds, and dinitrotoluene.

The similarities of the molecular structures of the above compounds to those of dyes were obvious to the early investigators. This initiated interest in using the fungi for decolorization of dye wastewater. The decolorization of azo dyes using the white rot fungus Phanerochaete chrysosporium was first described by Cripps et al. (55). They showed that three azo dyes, Orange II, Tropaeolin O, and Congo Red, could be decolorized by Phanerochaete chrysosporium. Other white rot fungi, such as B. adusta and T. versicolor, also showed the ability to degrade azo dyes efficiently (56). In the UGA laboratory, Cao screened a number of white rot fungi including P. chrysosporium, P. cinnabarinus, T. versicolor, C. subvermispora, C. stercoreus, P. ostreatus, P. tremellia, P. oxysponum, and P. pini for their capability in degradation of commercially used dyes (59). This and other work showed that Pleurotus ostreatus, had high potential for decolorizing a variety of azo dyes (57,58). Of some interest in considering the viability of using these whole organisms in textile waste water treatment is the research by Cao that showed that starch in the wastewater acts as a very good nutrient for the white rot fungi (63).

There has been extensive examination of white rot fungi in dye decolorization over the last decade, particularly in European laboratories. However, despite the known ability of the fungi to break down dyes and render them colorless, there had been few attempts to comprehensively examine the dye effluent systems after biotreatment by white rot fungi and determine the molecular nature of the process. Decolorization, by itself, demonstrates only the transformation of the chromophoric group of a dye, but does not reveal much about the mode of degradation of the dye molecules. The objective of one of our research programs was to develop methods to identify by of this paper is to review the efforts made at the University of Georgia and other research laboratories in developing decolorization techniques based on either the use of whole microorganisms, or the enzymes produced from these organisms. These efforts were led by Xueheng Zhao, Wang Lu and Yiping Lu in our laboratories (65-73). They were able to develop techniques using thin layer chromatography, high performance liquid chromatography (HPLC) and capillary electrophoresis coupled with electrospray ionization ion trap mass spectrometry to determine degradation products and pathways.

**Biology-Ceriodaphnia Dubia and Aquatic Toxicity**

Ceriodaphnia dubia is a famous (perhaps infamous), almost microscopic in size, water organism used for testing the aquatic (not human) toxicity of water. It is used because of the long history of studies done on it in the upper mid-west. When water-
using operations such as dyeing and finishing plants apply for Georgia Environmental Protection Division permits, a whole effluent toxicity test (WET) must be done to determine the effect of the effluent on aquatic organisms. We were contacted by a municipality and asked if we could help them determine why they were failing these WET tests, but were unable to pinpoint any one particular problem in their effluent water. This led, eventually, to work with several textile dyeing and finishing operations. This work was done examining how alkalinity, water hardness, and various ions in the water might affect the viability of *Ceriodaphnia dubia* (*C. dubia*), apart from the presence of other chemicals in the effluent.

*Ceriodaphnia dubia* were cultured in four reconstituted waters with hardness and alkalinity concentrations ranging from soft to the moderately hard water that is required by whole-effluent toxicity (WET) testing methods for culturing test organisms (74-76). The effects of these culture formulations alone and in combination with two levels of Cl$^{-}$, SO$_2^-$, and HCO$_3$ on reproduction of *C. dubia* were evaluated with the standard three-brood test. Reproduction was significantly reduced when test waters had lower hardness than culture waters. However, reproduction was not significantly different when animals cultured in low-hardness waters were exposed to moderately hard waters. The hardness of the culture water did not significantly affect the sensitivity of *C. dubia* to the three anions. Conversely, increased hardness in test waters significantly reduced the toxicities of Cl$^{-}$ and SO$_2^-$, with HCO$_3$ toxicity following the same pattern. Alkalinity exhibited no consistent effect on Cl$^{-}$ and SO$_2^-$ toxicity. The physiological stress of placing animals cultured in moderately hard water into softer test waters might contribute to marginal failures of otherwise nontoxic effluents.

The conclusion of this part of our studies with *C. dubia* was that the standard WET protocol should be revised to allow the culture of *C. dubia* under lower hardness conditions to better represent local surface water chemistries, particularly since most water sources in Piedmont Georgia have very soft water, and it is this lack of calcium that may well be the major cause of WET failures in some locations. In time, these results could put the evaluation of “chronic toxicity” on a more rational scientific and common sense basis and save Georgia companies much time, effort and money often wasted by dealing with “false positives”.

The primary objective of the second major study in this area was follow up with what we had learned about the sensitivities of *C. dubia*, and provide information to the textile industry regarding the interactive effects of ions common in textile wastewaters. The salts and dyeing chemicals in textile effluents often contribute to failures in toxicity tests, but little was known about interactive effects. Effluents from textile dyeing and finishing processes often contain substantial concentrations of chloride, sulfate, bicarbonate and sodium with low concentrations of hardness (calcium and magnesium). Collectively, these ions account for most of the total dissolved solids (TDS) in textile wastewater and are the main sources of toxicity in many textile wastewaters. However, effluents having similar concentrations of TDS often differ from one another in toxicity due to the composition of ions making up the TDS and the presence of process chemicals. Our research focused first on silicate and phosphate toxicity, and the effect of using test animals cultured in water with Piedmont Georgia characteristics, rather than the “standard” animals usually employed.

Three major ionic constituents of textile wastewater, chloride, sulfate and bicarbonate, were examined in some detail. Chronic toxicities of Cl$^{-}$, SO$_2^-$, and HCO$_3$ to *Ceriodaphnia dubia* were evaluated in low- and moderate-hardness waters using a three-brood reproduction test method. Toxicity tests of anion mixtures were used to determine interaction effects and to produce models predicting *C. dubia* reproduction. Effluents diluted with low- and moderate-hardness waters were tested with animals acclimated to low- and moderate-hardness conditions to evaluate the models and to assess the effects of hardness and acclimation. Sulfate was significantly less toxic than Cl$^{-}$ and HCO$_3$ in both types of water. Chloride and HCO$_3$ toxicities were similar in low-hardness water, but HCO$_3$ was the most toxic in moderate-hardness water. Low acute-to-chronic ratios indicate that toxicities of these anions will decrease quickly with dilution. Hardness significantly reduced Cl$^{-}$ and SO$_2^-$ toxicity but had little effect...
on HCO₃. Chloride toxicity decreased with an increase in Na⁺ concentration, and HCO₃-toxicity may have been reduced by the dissolved organic carbon in effluent. Multivariate models using measured anion concentrations in effluents with low to moderate hardness levels provided fairly accurate predictions of reproduction. Determinations of toxicity for several effluents differed significantly depending on the hardness of the dilution water and the hardness of the water used to culture test animals. We believe these results can be used to predict the contribution of elevated anion concentrations to the chronic toxicity of effluents; to identify effluents that are toxic due to contaminants other than Cl⁻, SO₄²⁻, and HCO₃; and to provide a basis for chemical substitutions in manufacturing processes.

**Summary**

Chemistry, textile chemistry and biology may have evolved separately, but today the connections and interactions are encountered by scientists and engineers quite regularly. In our labs, we have been challenged to enter the realms of enzymology, fungi, biochemistry, animal biology, and even plant biology. We have been fortunate to have collaborators in every phase who helped us bridge into areas that were new to us, but totally necessary in order to solve problems in textile chemistry.

**References**