The Massachusetts Toxics Use Reduction Institute
University of Massachusetts Lowell

Natural, "Green" Dyes for the Textile Industry

TOXICS USE REDUCTION INSTITUTE
UNIVERSITY RESEARCH IN SUSTAINABLE TECHNOLOGIES PROGRAM

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Natural, "Green" Dyes for the Textile Industry

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The Toxics Use Reduction Institute is a multi-disciplinary research, education, and policy center established by the Massachusetts Toxics Use Reduction Act of 1989. The Institute sponsors and conducts research, organizes education and training programs, and provides technical support to promote the reduction in the use of toxic chemicals or the generation of toxic chemical byproducts in industry and commerce. Further information can be obtained by writing the Toxics Use Reduction Institute, University of Massachusetts Lowell, One University Avenue, Lowell, Massachusetts 01854.

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University Research in Sustainable Technologies

The University Research in Sustainable Technologies program is a joint project of the Toxics Use Reduction Institute (TURI) and the Center for Environmentally Appropriate Materials (CEAM) at the University of Massachusetts Lowell, with support from the Commonwealth's Strategic Envirotechnology Partnership (STEP).

The program taps the research capabilities of the University of Massachusetts to advance the investigation, development and evaluation of sustainable technologies that are environmentally, occupationally and economically sound. The program provides research funding to UMass faculty from all campuses, annually, on a competitive basis and encourages faculty/industry partnerships and cross-campus collaboration. Industry partners provide guidance, propose applications for new technologies and, in some cases, evaluate and/or adopt processes and technologies resulting from research.

Introduction

The textile industry produces and uses approximately 1.3 million tonnes of dyes, pigments and dye precursors, valued at around $23 billion, almost all of which is manufactured synthetically. However, synthetic dyes have some limitations, primarily, (i) their production process requires hazardous chemicals, creating worker safety concerns, (ii) they may generate hazardous wastes, and (iii) these dyes are not environment friendly. This research explores methods where natural dyes are produced from plant tissue and fungal species.

Until the second half of the nineteenth century, all dyes used in textiles were naturally derived. However, with the synthesis of mauveine by Perkin in 1856, the synthetic dye industry has grown at a vigorous rate and all but totally eradicated the use of natural dyes. The large number of synthetic dyes in use today bears witness to the creativity and innovation of textile chemists in successfully satisfying the dyer's demands for simple, reproducible application processes, and the consumer's demand for quality products at a reasonable price. Thus, even though the availability of natural dyes has been known for centuries, the reasons synthetic dyes have been so popular are:

- They are simple to produce in large quantities,
- They can be manufactured at a reasonable price ($10 – 100/kg),
- They can provide the variety of colors that are demanded by today's consumers,
- They provide high color-fastness (*i.e.,* the dye is very strongly bound to the fabric and does not detach after repeated washing cycles).

However, manufacturing of synthetic dyes suffers from the following limitations:

1. Environmentally Unfriendly: The production of synthetic dyes requires strong acids, alkalis, solvents, high temperatures, and heavy metal catalysts. For example, production of a dye designated as Color Index Mordant Blue 23 states, "Treat 4,8-diamino-1,3,5,7-tetrahydroxy-2,6-anthraquinonedisulfonic acid with boiling alkali or dilute acid and convert to the sodium salt" or "Treat 1,5-dinitro-anthraquinone with fuming sulfuric acid in the presence of sulfur sufficient to produce S_2O_3 at 130^0C, hydrolyze with water, and convert to the sodium salt".
2. Increase in Cost of Feedstock or Energy: Petroleum is the starting material for all synthetic dyes and thus the price of dyes is sensitive to the price of petroleum. Also, since synthesis is energy intensive (uses super-heated steam, boiling acids, etc.), the process is sensitive to energy prices and also generates greenhouse gases.
3. Hazardous Waste Generation: Since synthetic production of dyes needs very toxic and hazardous chemicals, it also generates a hazardous waste, the disposal of which is a major environmental and economic challenge. Moreover, some facilities that produced dyes in the past are now "Superfund" sites due to intentional dumping or accidental spills of toxic and hazardous wastes.
4. Increasing Transportation Costs: Since dyes are hazardous materials and are produced at central facilities, transportation of dyes from manufacturing plants to textile dyeing and printing facilities is a major cost item and a logistic challenge.
5. Toxic and Allergic Reactions: There are occupational safety issues involved since production processes use the toxic and hazardous materials and conditions described above.

Thus, if bioengineered natural, 'green' dyes can be produced at a comparable price, the following benefits will be realized:

1. Reduce the use of toxics since starting materials are environmentally benign with associated benefits in terms of waste disposal and occupational safety.
2. Production can be "decentralized" resulting in savings in transportation costs.
3. After extraction of the dye, the biomass can be used for energy generation (e.g., through anaerobic treatment to generate methane, which in turn, can be sued as a fuel) and the growth media can be recycled; thus, there are virtually no wastes generated.
4. Possible beneficial aspects such as higher UV absorption by the fabric (which contains natural dye) can result in reduced incidence of melanoma.

It is clear, however, that if natural dyes are to be considered as an alternative to the synthetic dyes used today, they have to manifest the same characteristics of synthetic dyes as those listed above. Specifically, the major challenges in this field are:

1. To produce natural dyes in the quantities required,
2. To produce natural dyes at a reasonable price,
3. To produce natural dyes that have high color-fastness.

The major avenues of production of "green" dyes are:

- Extraction from plants
- Extraction from arthropods and marine invertebrates (*e.g.,* sea urchins and starfish)
- Extraction from algae (*e.g.,* blue-green algae)
- Production from bacteria and fungi

Secondary Metabolites

Regardless of the source, it is believed that products which may be harnessed as "green" dyes are in essence secondary metabolites produced by the organism. These secondary metabolites are low molecular weight natural products that have a restricted taxonomic distribution, possess no obvious function in cell growth and are synthesized for a finite period by cells that are no longer undergoing balanced growth. However, they have specialized survival functions in nature and are observed to be numerous in organisms occupying densely inhabited environments and are believed to have a prominent role in the coexistence and coevolution of species allowing interaction within a community. Functions of secondary metabolites can be listed as follows:

1. Competitive weapons against other organisms
2. Able to chelate toxic metals present in the environment so that it is not bio-assimilable
3. Form structural and extracellular protective agents
4. Act as agents of organism-host symbiosis

Despite their diversity, secondary metabolites are all produced from a few key intermediates of primary metabolism, which includes all of the anabolic and catabolic processes that are finely balanced to keep the organism alive. Primary and secondary metabolisms are intimately related, with secondary metabolites depending on precursors and energy generated through primary metabolism. For example, the following scheme may be representative of natural anthraquinone compounds:

Primary Metabolite (Lipid) ⟶ ***Low MW Intermediate (Acetyl Coenzyme)*** ⟶ ***Anthraquinone***

Use of Cells for Dye Production

The unique characteristic of the generation of the secondary metabolite (which can be used as dye) presents a scientific and technological challenge; namely, how can one get yields that can be used in a commercial scale when the cell produces these chemicals only under conditions of stress (most probably as a defense mechanism). We will present our approach to this problem in the next section, along with experimental data obtained from research being conducted in one area. Table 1 presents a compilation of natural, "green" dyes that have been researched and published in open literature.

Table 1 – Natural, "Green" Dyes Produced at Bench-Scale Level

Chemical Name	Type of Dye	Produced from	Classification
Indigo	Indigoid	Escherichia coli	Bacteria
Indigo	Indigoid	Nocardia globerula	Bacteria
Saffron	CI Natural Yellow 6	Crocus sativus L	Plant
Chrysophanol	Anthraquinone	*Curvularia lunata*	Fungus
Helminthosporin	Anthraquinone	*Curvularia lunata*	Fungus
Cyonodontin	Anthraquinone	*Curvularia lunata*	Fungus

An effective biotechnology solution to manufacture of these and other dyes or dyestuff intermediates will impart the following benefits:

1. The medium in which these plant cells or fungi or bacteria grow contain no expensive or toxic chemicals
2. The process is carried out at low temperature (around 30° C) compared to the fuel-consuming very high temperatures in the synthetic process
3. The process is typically run at neutral pH as opposed to very high acidic or alkaline conditions in the synthetic process
4. The process is very "environmentally friendly" and "sustainable"

However, the key factors are, (i) high yield of the product, and (ii) high purity.

Plant tissue and cell culture system may circumvent seasonal and geographic restriction of the plants, and as sources of useful secondary products. Plant cell cultures also provide effective systems for exploring plant physiology and plant biochemistry. The value of the technique of plant tissue and cell culture is that cell and tissue systems can be subjected to direct experimental control.

Cranberry plants (*Vaccinium macrocarpon*, *Ericaceae*) growing in outside bogs receive different external environmental stresses. The environmental stresses are divided into biotic stresses (attacked by insects, mites and fungi) and abiotic stresses (physical stresses: light, temperature, and wounding; chemical stresses: nutrient elements, water, and chemicals). At the cellular level, there is no report on responses of the cranberry plants to these environmental stresses.

Flavonoids (anthocyanins and flavonols) are secondary products of plants [1]; and have been found to have important therapeutic values against arterial diseases and cancer because of their antioxidant properties [2, 3]. The accumulation of anthocyanins and flavonols in plants is largely influenced by various environmental stresses.

Description of the Cranberry Plant as a Pigment Source

In cranberry fruits, the anthocyanins are cyanidin and peonidin based chromophores, whereas the flavonols are myricetin and quercetin based chromophores (Figure 1).

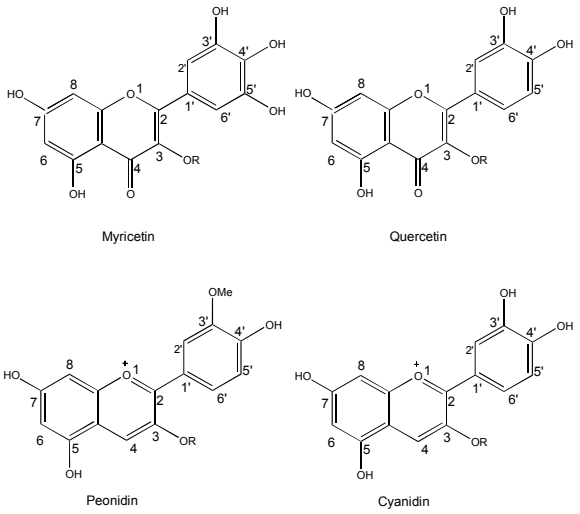

Figure 1: The structural differences between the different types of anthocyanins and flavonols present in cranberry plants. Myricetin and quercetin are flavonols and cyanidin and peonidin are anthocyanins. R represents where the sugar groups are attached to the chromophores.

Cyanidin-3-galactoside, cyanidin-3-arabinoside, peonidin-3-arabinoside, and peonidin-3-galactoside are the four different types of major anthocyanins present in cranberries. The five different flavonols present in cranberries are myricetin-3-arbinoside, myricetin-3-diglactoside, quercetin-3-galactoside, quercetin-3-arabinoside and quercetin-3-rhamnoside [4-7].

Flavonols contribute to the red color displayed by the anthocyanins through intermolecular co-pigmentation [8]. Intermolecular co-pigmentation of anthocyanins with other compounds increases the color intensity and causes the wavelength of maximum absorbance to shift toward higher wavelengths (bathochromic shift). In addition, because flavonols have strong UV absorbance, these pigments play important protective functions against damaging effects of UV radiation [9].

A major advantage with anthocyanins and flavonols for their use as natural dyes is the fact that they have attached sugar groups, which can be removed without loss of their colors. The chemical group freed after removal of sugars could then be used to attach these dyes with cellulose in fibers.

Description of Research

Two kinds of primary species, American Cranberry plant tissue and two fungal species, *Curvularia lunata* and *Curvularia pallescens,* can be used in natural growth media to produce dyes or dye precursors. The technology is simple to implement two steps:

(i) Grow the primary species in the laboratory or in a bioreactor with the right growth medium,

(ii) Harvest the dye or dye precursors from the plant or fungal cells.

We have successfully established cranberry cell culture system from cranberry (*Vaccinium macrocarpon*, *Ericaceae*) stems, leaves and leafstalks by using Gamborg's B5 medium containing 5 mM 1-naphthaleneacetic acid (NAA), 5 μM 2,4-di-chlorophe-noxy acetic acid (2,4-D), and 2.5 mM kinetin at 25^{o}C in the dark.

Production of Pigment from Cranberry Callus

Production of flavonoids in cranberry callus varies under different stresses such as light irradiation (red light of 660 nm and far-red light of 730 nm), temperature-changing (from 25^{o}C to 4^{o}C, or 37^{o}C), and wounding.

Cranberry callus produces anthocyanins only on exposure to light. Production of anthocyanins in cranberry callus was induced under continuous light irradiation. However, because anthocyanin is red color, it was observed that only top layer of the callus, which received light produces anthocyanins.

Production of Pigment from Cranberry Cell Suspension

Although callus provides more accessible uniform cells than the intact plant, the callus tissue is not uniform since only the base of the callus is exposed to the medium and the callus mass may contain cells at various stages of development. The alternative approach is to use cell suspensions. Cell suspensions show a faster growth rate, and all cells are exposed uniformly to the medium, and environment such as light. Cell suspensions are preferred for large scale and commercial production of secondary metabolic products.

We initiated cranberry cell suspension culture by transferring callus to liquid media of the same composition as the callus medium and gently agitating the suspension on a horizontal shaker at 150 rpm and 20°C (Figures 2 and 3). We have found out that the growth of biomass of cranberry cell suspension culture in WP and MS liquid media were greater than B5 liquid medium (Figure 4). Interestingly, anthocyanin content of cranberry cell suspension culture in MS liquid medium was higher than in WP liquid medium (Figure 5). However, the flavonol content of cranberry cell suspension culture in WP liquid medium was higher than in MS liquid medium.

Figure 2. Initiation of Suspension Cell Culture
Suspension cell cultures were initiated by transferring fresh mass callus to 15 ml of WP liquid medium and incubated on a rotary shaker at 150 rpm, 20 °C.

Figure 3. Examination of Different Media (WP, B5, and MS)

Suspension cell cultures were under a continuous photosynthetic photon flux of 25μM $m^{-2}s^{-1}$ provided by cool white fluorescent lamps (F40CW-RS, General Electric Company, USA) plus continuous red light at a photon fluence rate of 12 μM $m^{-2}s^{-1}$, was obtained from six 40-w bulbs (F48T12/R-660/HO, Red, General Electric Company, USA) filtered through a red plastic sheet (Roscolux color filter # 27, ROSCO Laboratories, Port Chester, NY)

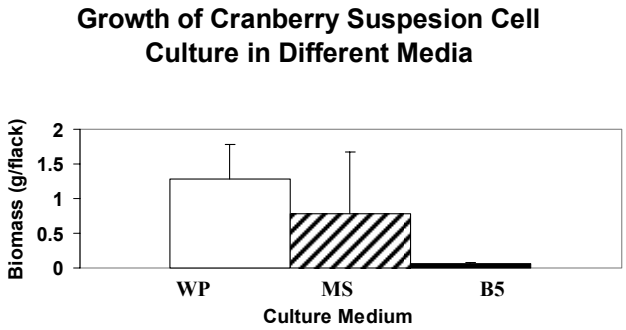

Figure 4. Growth of Cranberry Suspension Cells in Different Media (WP, B5, and MS)

Growth was evaluated quantitatively in term of biomass, Fr.Wt. (g/flask) under different media. Cells were separated from cell suspension medium by vacuum filtration through 3 mm Whatman filter paper, just until the point when free liquid was no longer expressed, and Fr.Wt. was immediately recorded.

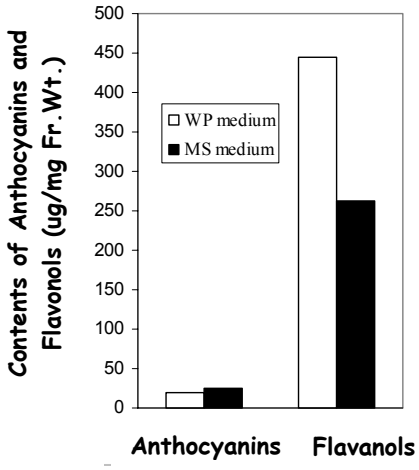

Figure 5. Anthocyanin and Flavonol Contents in Cranberry Suspension Cell Culture
Comparison of Different Liquid Media

Experimental Approach

We used the following approach to assess the potential value of using cranberry cell suspensions as a production source of environmentally friendly red dyes:

- Preparation of cranberry extract from the red cranberry fruits containing the anthocyanin and flavonol pigments;
- Extract prepared by crushing the cranberry in frozen form by the use of liquid nitrogen and formation of the solution by common solvents (HCl, CH_3OH and water);
- Suitable and industrially accepted pre-treatments of the fabrics to be used for dyeing from the cranberry extract;
- Dyeing of the most widely used polyamide fabrics (Nylon-66 and wool) by the exhaustion method of acid dyeing;
- Determination of the exhaustion curves of the dyeing process;
- Comparison of the dyeing exhaustion curves with the standard acid dyes having the wavelength of maximum absorption in the range of the wavelength of maximum absorption of the cranberry extract;
- Establishment of the equations between the exhaustion of dye and the concentration of the dye solution;

- Matching of the shade of the dyed fabrics by the use of spectrophotometry and computer color matching.

Preparation of Anthocyanin and Flavonols:

Cranberry anthocyanins and flavonols were extracted in a mini-blender with 20 ml of extraction solvent (85:15, ethanol: 1.5 M HCl) according to Boulanger and Singh [11]. The resulting homogenate was allowed to incubate at 4°C overnight. Homogenates were filtered, and residue on the filter paper was washed with the extraction solvent to remove all pigments. The filtrate was diluted to 250 ml in a volumetric flask, and the solution spectrally analyzed. Flavonoid contents were estimated using published methods of cranberry anthocyanin and flavonol analysis [4-7].

Binding of Cranberry Pigments to Cotton and Nylon Fabrics:

Binding of dyes to fabrics is referred to as substantivity, and can be determined by measuring the reflectance of dyed cotton or nylon using spectrophotometer. Higher substantivity of the dye to cotton results in a lower reflectance (R) of the dyeing. The reflectance is measured at the wavelength of maximum absorbance of the dye concerned. Based on the f(R) value of the dyed cotton where **[f(R)]=(1-R)^2/2R**, also called Kubelka-Munk relation, the substantivity of a new dye is determined by doing a parallel dyeing against a known standard dye. The substantivity is diretly proportional to f(R). The higher the substantivity of a dye, the higher the f(R). f(R)is also expressed as K/S, where K is the coefficient of absorption and S is the coefficient of scatter.

After a series of dyeing using different dye concentrations with the same dye on same substrate (cotton) under same conditions, a relation of the function of R [f(R)] vs. the dye concentration is obtained, usually being a straight line. The slope of the line is used to estimate dye binding.

Results

- The extract was found feasible to be used as a dyestuff at higher concentrations for Nylon-66 and Wool.
- Dye-affinity of the fabric increases with the increase in the concentration of the extract used.
- The extract gave very bright and acceptable shades for fabrics of coarser yarns, comparable to synthetic dyes, such as Nylamine Red A2B (Figure 6).

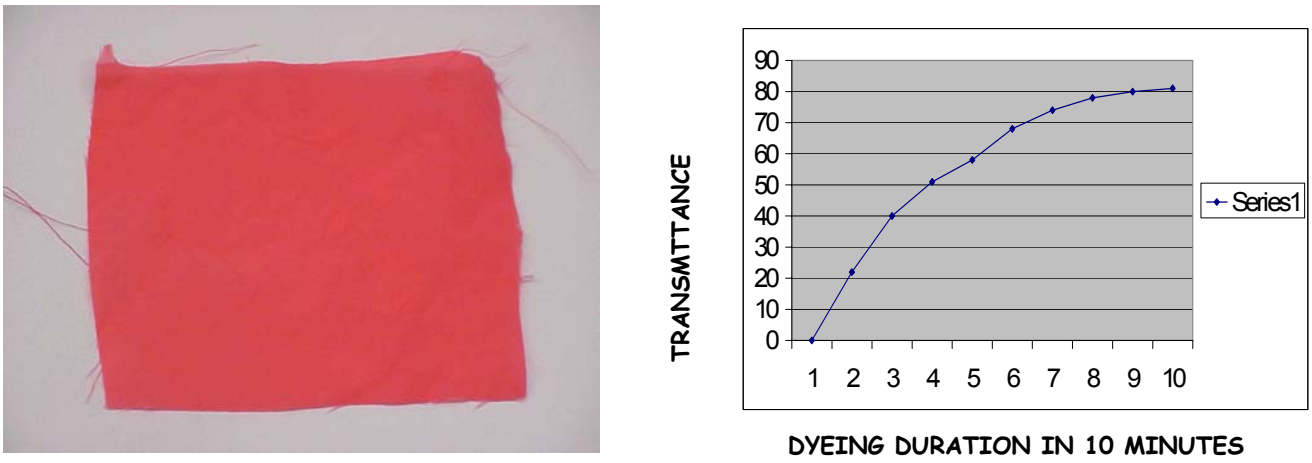

Figure 6: Cranberry extract dye on coarse yarn fabric

Technology Status

Dyes currently used for dyeing textile material are classified as soluble, disperse, and pigments [10]. These are all synthetic compounds, which are environmentally unfriendly compounds, as their degradation by organisms is not carried out naturally. The industry has to design expensive ways to remove these harmful compounds from the environment. Availability of natural dyes is a desired technology for dyeing fabrics with naturally produced compounds.

Future Research Plans

1. Because low temperature and light can induce anthocyanin biosynthesis, we plan to use a incubator with rotary shaker and temperature control under a continuous photosynthetic photon flux of 100 μmol $m^{-2}s$-1 provided by cool white fluorescent lamps in order to obtain cranberry callus, which can produce anthocyanins. The same logic will be extended to extracts from the fungi *Curvularia lunata* to produce natural, anthroquinone dyes.
2. We have obtained some informations of physiological, phytochemical and morphogenic responses of cranberry callus to environmental stresses from our preliminary experimental results. We will use the cranberry callus, which can produce anthocyanins to investigate environmental regulation (light, temperature, chemicals, etc.) of anthocyanin biosynthesis at cellular level. We will also investigate environmental regulation (light, temperature, pH, alkalinity, redox potential, etc.) to maximize anthroquinone dye production from the fungus species *Curvularia lunata*
3. Based on the results mentioned above we will design optimum conditions to produce higher quantity of anthocyanins with cranberry callus and anthroquinone dye products from the fungus *Curvularia pallescens* and *Curvularia lunata.*
4. We will also study how replacement/modification of the glucose group on the anthocyanin affects its color-fastness vis-a-vis dyeing on cotton.

5. We will also study the effect of metals, temperature, and nutrient conditions on the yield rate of cynodontin by *Curvularia pallescens* and *Curvularia lunata*.

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