Natural Indigo Production and Dyeing

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1. INTRODUCTION

Indigo was the only natural blue dye until when synthetic indigo completely displaced the natural one at the end of 19th century. Since then, synthetic indigo has been widely used to dye cellulosic textiles especially for denims and blue jeans. High popularity of denim makes indigo one of the most important dyes commercially. Recently, the European Union (EU) promoted some research projects aiming to reintroduce efficient indigo-delivering crops into European agriculture and to develop the process design for large-scale indigo production. With the embodiment of cultivation skill, some indigo plants may provide a source of natural indigo as an alternative to the synthetic one[1].

Natural indigo are obtained normally from cultivated species by water extraction and the following alkali precipitation. In eastern Asia, main indigo producing plant species is *Polygonum tinctorium*, a herbaceous subtropical annual plant. The precursor of natural indigo is indican (indoxyl-β-D-glucoside), a colorless glucoside representing a major leaf secondary metabolite. Some indigo plant species produce some other indigo precursors such as isatan A and B as well as indican[2]. Indican or isatan is hydrolyzed to indoxyl and glucose with the help of native β-glucosidase. With the addition of alkali to the steep water, released indoxyl is formed to indigo after a vigorous aeration[3]. In traditional indigo process, introduction of storing technique is important in terms of the level of dyeing technology and the flow of culture. Some typical examples are woad ball in Europe, sukumo in Japan, lan dian in China, or niram in Korea[2].

Considerable researches are carried out to replace chemical synthesis of indigo by an application of bio-technological approaches, such as β-glucosidase-catalyzed hydrolysis of indican[4], indigo reduction by bacteria[5-6]. Recently, the approaches such as process design[7] or process balance on large-scale production[8]. Related to indigo reduction, recent researches are focused on the use of reducing sugars[9-10], electrochemical or electro-catalytic reduction[11-12].

The objective of this study is to provide the optimization of extraction time and dye formation. Based on the traditional niram method, the process variables were chosen to get maximum indigo yield with less energy consumption. We suggest an alternative process of natural indigo production by applying biotechnology. Traditional, conventional, and more eco-friendly indigo dyeing methods are compared according to experimental dyeing condition.

2. EXPERIMENTAL

Cultivation and harvest of indigo plants and extraction in lab-scale

Indigo seeds were sowed at the end of March 2007 in Naju (Chonnam Province, southwest of Korea) and indigo plants were harvested during July and August 2007. To obtain optimal extraction condition, lab scale extraction was carried out. Only leaves (50 g) were steeped in distilled water (1 L) at 25-26℃. The extraction was made by maintaining leaves in distilled water at various time and pH. For comparison, the pH of extraction condition was adjusted as 3.5 by using acetic acid. After filtering the extract, $Ca(OH)_2$ was added with stirring for 20 min using a homogenizer, and left it overnight for sedimentation. Clear supernatant was siphoned off and then the sediment in slurry was dried in a convection oven at 50℃ and weighed. The dried sediment was finely ground and used for analytical tests and dyeing process. In lab scale extraction, all analytical grade of $Ca(OH)_2$ and NaOH was used.

Extraction of indigo in large scale

Indigo plants were harvested for large scale production of indigo dye on August 14, 2007. Into a 1000 L stainless steel tank, rain/ground water of 27℃ was poured over the plants with leaves and stems (80 Kg, 10 L of water to 1 Kg of plant materials). The steeped plants were weighted down with 6 stone-blocks (weighing 5 Kg each) to hold plants

under water to exclude air for maintaining anaerobic condition. After steeping for 2.5 days, the extract (790 L) was pumped into three settling tanks and added different amount of $Ca(OH)_2$ (1.5, 2.0, 2.5, and 3.0 g/L) in each tank respectively, and aerated for 30 min by using a compressor to help oxidation of indigo precursors and precipitation of indigo. The indigo was settled down for 12 hrs and the supernatant was removed. The sediment was collected and filtered through layers of a cotton fabric. After drying in a vacuum oven at 50℃, the amount of crude indigo dye was weighed and pulverized for analytical tests and dyeing experiments.

Indigo and indirubin quantification

Indigo and indirubin contents were determined according Liau et al.[13]. HPLC analysis was carried out on a Agilent 1200 liquid chromatography system (Agilent technologies Inc., Waldbronn, Germany) equipped with two pumps, UV detector and Rheodyne injector (50L loop). Chromatographic conditions were used as follows: The LC column was a Zorbax Eclipse XDB-C18 (4.5 mm × 150 mm, 5m) (Palo Alto, CA, USA). Two mobile phases A and B were filtered through a 0.45 m filter and run at flow rate of 1.0 mL/min and room temperature of 25℃. Mobile phase A consisted of water with 0.1% trifluoroacetic acid (TFA) and mobile phase B was acetonitrile (ACN) containing 0.1% TFA. A linear gradient was maintained from 40% B to 85% B for 15 min. The measurement of each sample was tried and averaged in triplicate. Synthetic indigo (Vat. Blue 1, Aldrich) and indirubin (Alexis Biochemical, USA) were used of analytical grade as standard dyestuffs for calibration.

Dyeing process

Dyeing of indigo-alkali complex onto some fabrics was performed by using Automatic Dyeing machine(Ahiba Nuance, USA). 1 g of the scoured fabrics were dyed in a solution containing 0.4-20 g/L of natural indigo, 1-5 g of $Na_2S_2O_4$, 0.1 g of NaOH in a liquor ratio 1:100 at the conditions of 30-80℃ and 5-80 min. After dyeing, oxidation was followed in air for 15 min, rinsed in water, neutralized in 0.1% acetic acid solution, rinsed, and dried. The chemicals used in dyeing process were all of analytical grade.

Color strength and shade

Color strength (indigo content on the fabric) was determined according to the Kubelka-Munk equation from the reflectance at 660 nm and expressed as K/S values using a spectroscope (Coloreye 3100, Macbeth). CIELab coordinates (Illuminant D65/ 10° Observer) were measured with a Macbeth Coloreye 3100 spectrophotometer at 660 nm (λmax). H V/C values were obtained from L*a*b* data by using CIE Munsell conversion program.

3. RESULTS AND DISCUSSION

Traditional niram method

There have been two different ways of traditional indigo dyeing in Korea, *i.e.*, the banmul method and the niram method[14]. The former is the dyeing from an indigo extract by water directly in summer season and the latter is the dyeing by using an indigo-alkali complex available for all season. The latter has some advantages of getting deeper color and trading an indigo dye. With the worldwide concern for sustainability and the demand for natural products, there has been a revival of traditional technology in Korea. In recent years, some masters of traditional technology including natural indigo dyeing are registered officially by the Korean government.

In the niram method, indican is extracted by steeping the plant leaves and stems for 1-3 days. As the second step of this method, the powder of oyster shell or shellfish is added onto the extract while stirring manually for the introduction of air oxygen as much as possible until blue color developed, and then left it overnight. The sediment is collected and filtered through a cotton fabric. Thick paste obtained is called niram, which means indigo mud. The introduction of indigo paste was a revolutionary stage to indigo dyeing a storage capability and deep dyeing. However, indigo yield of the niram paste (normally 10-15%) was varied by the extraction conditions and subsequent conversion into indigo depending on the dyer's experience.

For the dyeing, the reduction of indigo was carried out by adding 1-2 kg of niram paste, 10 L of lye onto a ceramic jar and keeping it closed in warm place (25-30℃) for a certain period of time, 7-10 days depending on environmental conditions. Makgulri (Korean rice wine) is sometimes added to ensure a good fermentation. The color of sufficiently reduced indigo solution is greenish to yellowish. When the reduced indigo is prepared, the fibers are immersed in the liquid and exposed to the air for drying. This process is repeated until desired color of dyed fibers is obtained. For 1 kg of niram, 1 pil (the unit of fabric size used in Korea) of ramie fabric is dyed with a medium color.

Modified lab and large scale extraction

In this study, we modified the niram method by using calcium hydroxide instead of oyster shell powder and dried the niram of mud-like form into a crude dye of powder form. Indigo plant cultivated in Korea is *Polygonum tintorium*, which produces greater quantity of indigo precursor(s) than *Isatis* species[15]. The crude dye obtained is a mixture of indigo and calcium hydroxide. Some process variables (such as extraction time, storing time, pH,

calcium hydroxide concentration) were applied to modify the traditional one in lab-scale and then farm-mill scale. The formation of indigo is shown in Fig. 1.

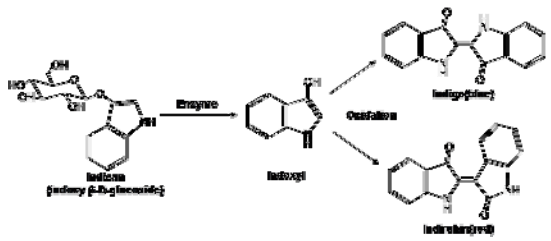

Fig. 1. The formation of indigo and indirubin from indican via oxidative coupling of indoxyl.

Change of crude dye and indigo contents plotted for some extraction conditions. Total mass of crude dye obtained was dependent on extraction time and the concentration of Ca(OH) , as shown in Figs. 2 and 3.

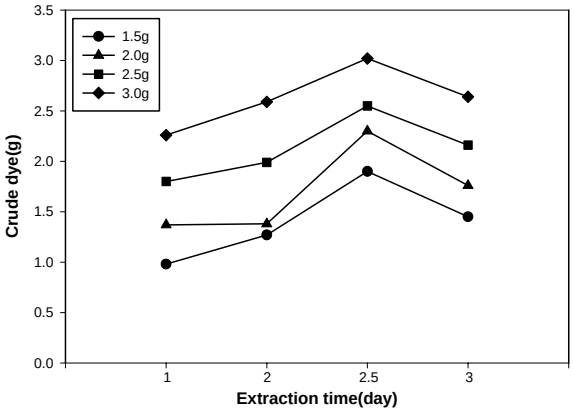

Fig. 2. Effect of extraction time on the mass of crude dye.

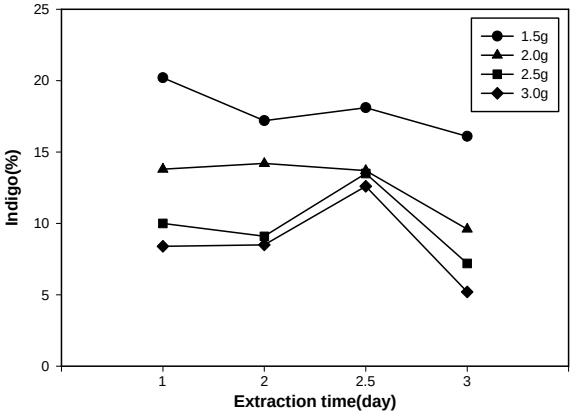

Fig. 3. Effect of extraction time on the content of indigo.

Increase of the amount of crude dye within 1-2.5 days of extraction was observed with the extraction time. Extraction time of longer than 2.5 days caused a slight decrease in the amount of crude dye. The quantity of dye extracted was increased with the concentration of $Ca(OH)_2$. But indigo content in the crude dye decreased with the increase of the concentration of $Ca(OH)_{2.}$, irrespective of extraction time. The content of indirubin was increased considerably at the extraction time of longer than 2.5 days. However, pH affects scarcely on extraction efficiency, as seen in Figs. 4 and 5.

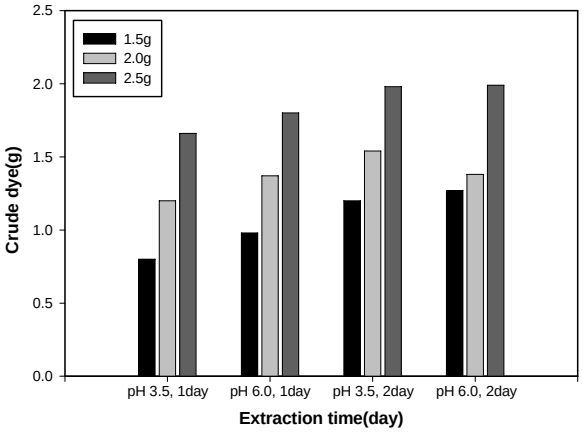

Fig. 4. Effect of extraction pH on the mass of crude dye.

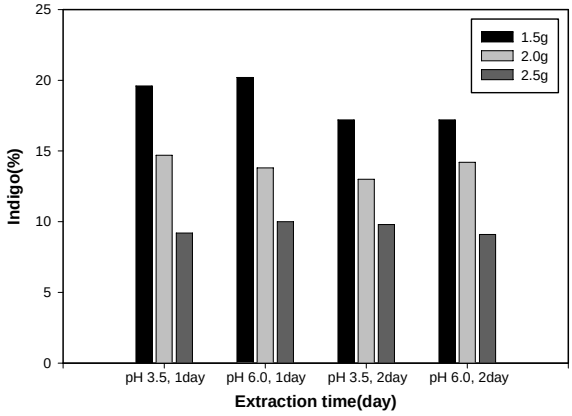

Fig. 5. Effect of extraction pH on the content of indigo.

With the optimal condition in lab scale extraction, the process was scaled up in a farm-mill near indigo plant cultivation field. We used the plants including leaves and stems to save labor of removing stems. Based on the lab scale extraction, large scale extraction was performed for 2.5 days and 2.0 g/L of $Ca(OH)_2$. Even though stems were included during the extraction, indigo content was comparable to that

of lab scale production using leaves only. The climate condition, post-harvest treatment may affect the contents of indican or indigo as well as indigo plant genotypes[16-19]. Extraction temperature is regarded another variable in extraction process. At higher temperature, the extraction time becomes shorter. Because control of temperature in large scale is not so easy in large scale process, we disregarded it in this research.

Contents of indigo and indirubin in the crude dye

The total quantity of indigo and the composition of the natural indigo obtained depend on the glycosides and enzymes present and the conditions of pH, temperature, oxygen, in which the indigo plants are processed. Indigotin, the main coloring substance, is formed by the combination of two indoxyl molecules by oxidation. Isatin is favored by special circumstances such as oxidation of the indoxyl in the presence of alkali or acid. Indirubin is purplish colorant isomeric with indigotin produced by the condensation of isatin and indoxyl. We quantified the contents of indigo and indirubin according to HPLC analysis. Fig. 6 shows the HPLC profile of natural indigo dye prepared in this study. Indigo component was eluted at 9.18 min, showing peaks at 280, 610, and 550nm. Whereas indirubin was eluted at 10.02 min, showing peaks at 280 and 550nm. They were well separated and all the peaks had good shape. For the quantification of indigo and indirubin contents of the prepared indigo dye, the peak at 280nm was used because the peak signal was strong and also it was a common peak for two components. The peak areas at 280nm were determined and calculated the concentrations of indigo and indirubin, respectively, by using the standard calibration curves.

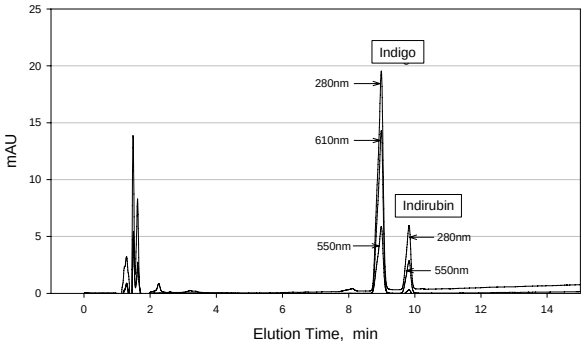

Fig. 6. HPLC profile of indigo and indirubin.

An alternative process of indigo production: Application of biotechnology

As explained above the traditional technology is long, multi-step process, thus high cost, and not easy to control quantity and quality. Considering the fact that indican is converted to indoxyl by the activity of β-glucosidase in the indican-containing plant itself, we tried to find β-glucosidase with high activity for applying indigo production. We successfully mined a glucosidase family enzyme with high activity toward indican. Indigo blue was produced with the growth of *E. coli* harboring the genes of *S. meliloti*, *T. caldophilus*, and *T. thermophilus* HB8, except *F. johnsoniae*. The screened enzymes were applied to pure indican and indigo plant extracts from *Polygonum tinctorium*. The β-glucosidase deduced from *Shinorhizobium meliloti* had the highest activity. The purified enzyme had the best activity at pH 7 and 30℃(Fig. 7). On the basis of results, an alternative process for the indigo production from natural resources can be provided. This single enzyme process has the advantages of cost, reproducibility, and uniformity in the indigo production.

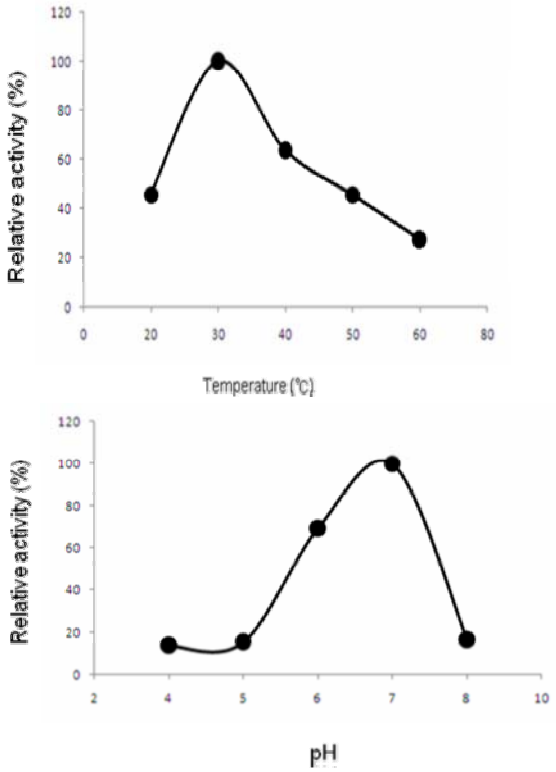

Fig. 7. Effects of temperature and pH on β-glucosidase activity.

Reduction to leuco-indigo and dyeing

In vat dyeing, indigo molecules are reduced to a colorless, water-soluble leuco form to absorb and diffuse into the fiber and then oxidize back to blue, water-insoluble forms shown in Fig. 8. The control of reduction in natural dyeing (fermentation) depends on the time and temperature required for conversion. In the niram method, normally 1-4 weeks are required depending on seasonal variation to get enough conversion to leuco-indigo form.

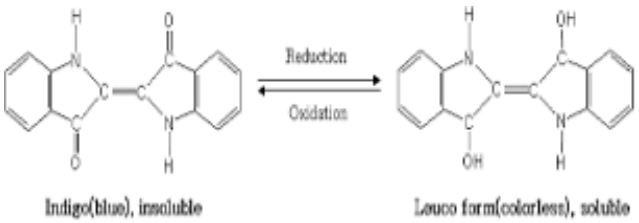

Fig. 8. Reduction/oxidation reaction of indigo.

Nowadays, chemical reduction by using reducing agent is used predominantly than the bacterial reduction by fermentation. Sodium dithionite is mainly used as a reducing agent in the denim dyeing. However, it causes a significant environmental problem. Thus, sodium dithionite should be replaced with more environmentally friendly reducing system; these would have to be economically feasible and secure the same quality dyeing to be a viable alternative. Recently, carbohydrates such as reducing sugars[9-10] or borohydride[20] are developed. It is known that glucose in conjunction with caustic soda above 90℃ has sufficient reducing potential sulfur dyes[9].

For comparison, we chose two reducing agents of sodium dithionite and glucose on behalf of bacteria reduction in the traditional niram method. For dyeing using sodium dithionite, optimum dyeing temperature was different with various fibers; 40℃ for cotton, 50℃ for rayon, and 60℃ for silk, wool, ramie, and Tencel. At above 70℃ of dyeing temperature, unstable reduction leads to the decrease of dye uptake. By controlling dye concentration without repeated dyeing, strong PB color was obtained except cotton, but mercerized cotton was capable to get strong color strength. For dyeing using sodium dithionite, optimum dyeing temperature was different from fiber to fiber; 40℃ for cotton, 50℃ for rayon, and 60℃ for silk, wool, ramie, and Tencel. At similar dyeing condition, dye uptake was high in order of wool > Tencel > mercerized cotton > rayon > ramie > silk > cotton. Fig. 9 shows the effect of sodium dithionite concentration on the dye uptake of ramie. The dyed fabrics showed PB color irrespective of fiber type.

For reduction with glucose, the highest color strength (K/S) was obtained at 70℃ for ramie, as shown in Fig. 10. As the reduction/penetration time increased, color strength increased up to maximum value at 50 min and rather decreased at longer reaction time. However, the shade of dyed fabrics became duller with increasing temperature.

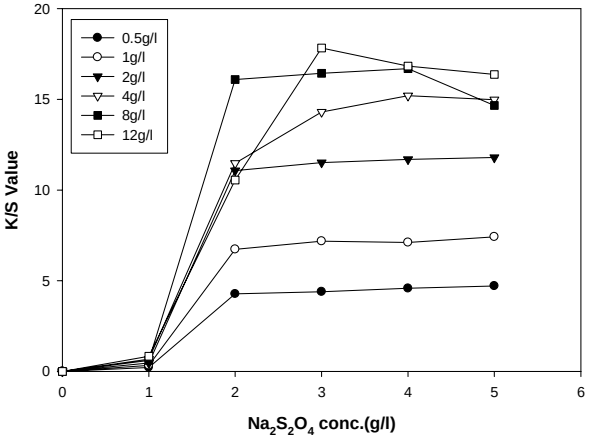

Fig. 9. Effect of sodium hydrosulfite concentration on the dye uptake of ramie(60℃, 30min).

The fabric showed more vivid PB color at 60℃. Considering color strength and shade of the dyed fabrics, reduction/penetration temperature and time were balanced at 60℃ and 50 min. Vuorema *et al.* obtained the maximum rate of leuco-indigo formation at 65℃[9]. $Ca(OH)_2$ also affected reduction efficiency, eventually dye uptake, and the shade of dyed fabrics. As $Ca(OH)_2$ concentration increased, color strength increased up to 8 g/L and further increase lowered dye uptake and the shade of fabrics got duller.

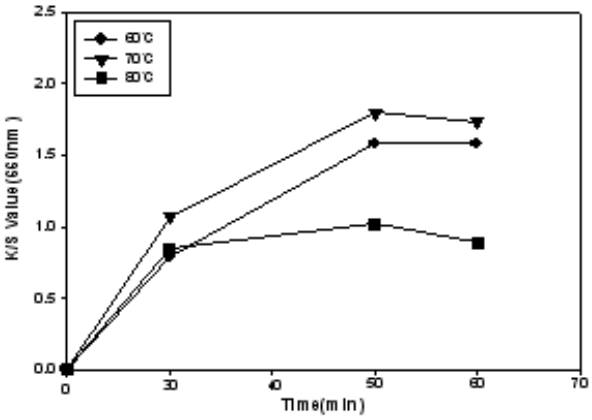

Fig. 10. Effect of reduction condition on the dye uptake of ramie.

Fig. 11 shows the effect of glucose to indigo concentrations on dye uptake of ramie at 60ºC and 50 min. At the same glucose concentration, higher dye uptake was obtained at higher indigo concentration. This result indicates that leuco-indigo formation is proportional to the amount of indigo present, or the surface area of indigo particles in suspension. At less than 6 g/L of indigo concentration, maximum color

strength was obtained at 8 g/L of glucose concentration, while at more than 8 g/L of indigo concentration, it was obtained at 10 g/L of glucose concentration. The dyed ramie fabrics showed PB colors and the shade got darker and duller as indigo concentration increased. On the other hand, the shade of dyed silk fabrics were varied with dyeing temperature; B color at 60℃, BG color at 70℃, and G color at 80℃. From the results obtained, reduction process using glucose as a reducing agent in indigo dyeing may be limited to medium strength of color. Compared with the dyeing results of sodium dithionite system, maximum dye uptake was much lower in reduction for glucose system.

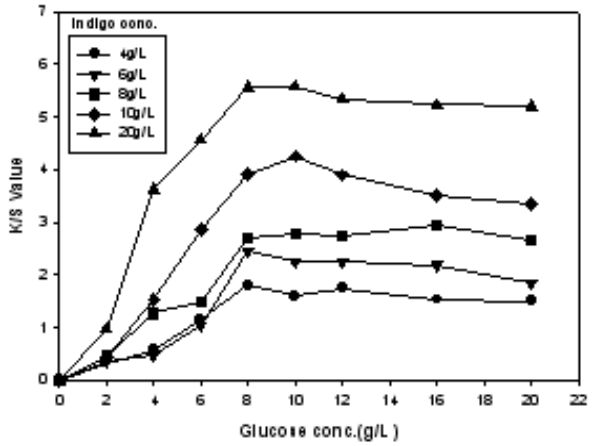

Fig. 11. Effect of indigo and glucose concentration on the dye uptake of ramie.

4. SUMMARY

Natural indigo produced by traditional technology had 10-21% of indigo content and 0.06-0.2% of indirubin. We successfully mined a glucosidase family enzyme with high activity toward indican deduced from *Polygonum tinctorium*. The β-glucosidase deduced from *S. meliloti* was the most effective, showing the highest activity at pH 7 and 30ºC. Based on the results, an alternative process for the indigo production from natural resources can be provided. For reduction by using sodium dithionite, optimum dyeing temperature was different from fiber to fiber; 40℃ for cotton, 50℃ for rayon, and 60℃ for silk, wool, ramie, and Tencel. The dyed fabrics showed PB color irrespective of fiber type. For reduction by using glucose, maximum dye uptake was much lower and limited to get medium strength of color. The dyed ramie fabrics showed PB colors, while the shade of dyed silk fabrics were varied with dyeing temperature, i.e., B color at 60℃, BG color at 70℃, and G color at 80℃.

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5. REFERENCES

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