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REVIEW PAPER

Solubility enhancement of indigo dye through biochemical reduction and structural modification

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Abstract–Indigo is one of the most widely used dyes for textiles and is thus produced in large quantities. Owing to its low solubility, a chemical-dependent continuous reduction-oxidation process is often used for indigo dyeing. Unfortunately, the indigo dyeing process has adopted a hazardous reducing agent such as $Na_2S_2O_4$, to reduce indigo into leucoindigo, thus causing serious water pollution in the process. To avoid this, the use of chemical reducing reagents was banned, creating a need to identify alternative reducing agents or to develop more eco-friendly dyeing processes. In this review, alternative reducing reagents such as biochemicals, plant fruits, biocatalysts, and microbes, which are less harmful to the environment than chemical reducing reagents, are summarized with their reducing reactions and performance. In addition, alternative modifications of indigo that bypass the use of reducing reagents have also been briefly introduced. The reducing chemicals and processes summarized have their respective merits and drawbacks; however, further research is required to obtain profitable dyeing performance that meets economic goals.

Keywords: Indigo, Indigo Reduction, Reductase, Eco-friendly, Indigo Dyeing

INTRODUCTION

Indigo is one of the oldest vat dyes used and remains the main compound used to dye denim [1,2]. Recent research on carbon-neutral policies and regulations has focused on the eco-friendly production of chemicals and sustainable development. Various environmental regulations have been strengthened to overcome the environmental pollution caused by the release of dye pigments (such as indigo) into aquatic environments. Chemical-based synthetic indigo dye production uses petroleum-based raw materials such as aniline as well as harmful chemical catalysts and organic solvents that require additional treatment after use [3]. Therefore, attempts have been made to replace conventional indigo processing with an eco-friendly alternative [1,2].

One example is to use indigo-producing biocatalysts that are responsible for the conversion of indole into 3-hydroxyindole (later sequentially oxidized into bio-indigo) that have been identified and engineered to obtain desirable catalytic activity and production yields [4]. These biocatalysts include flavin monooxygenase, toluene dioxygenase, naphthalene dioxygenase, and cytochrome P450 monooxygenases [1,5]. Biocatalyst-based bio-indigo production and their current manufacturing levels have been well summarized in previous studies [1,3,4,6].

Irrespective of the chemical or biological production of indigo, however, the use of strong reducing reagents in the vat dyeing process cannot be avoided [7]. Owing to the low solubility of indigo, it must be reduced to its soluble form, leucoindigo [2]. Through continuous oxidation and repeated reduction cycles, a robust vat dyeing process can then be achieved (Fig. 1).

Although vat dyeing varies depending on its dyeing process, it could employ chemicals and produce harmful byproducts and unreacted dyeing materials; there are still more challenges to overcome to achieve a completely eco-friendly process. Sodium dithionite ($Na_2S_2O_4$) has often been used as a reducing reagent for vat dyeing because of its high reducing potential, low cost, and ease of handling [2]. However, the use of $Na_2S_2O_4$ in indigo dyeing requires an exhaustive treatment process involving indigo residue, a strong reductant, and sulfur additives, all of which pose high risks to aqueous environments [7,8]. Several nations have thus banned the use of reducing reagents or even prohibited indigo dyeing outright.

In accordance with recently strengthened environmental regulations and improved awareness, additional research is being conducted into eco-friendly dyeing processes using chemical, electrical, physical, and biological methods [2,9-11]. As an alternative to chemical reductants, the so-called green reductases, such as reducing sugars and microbes, have been extensively studied [2]. In addition

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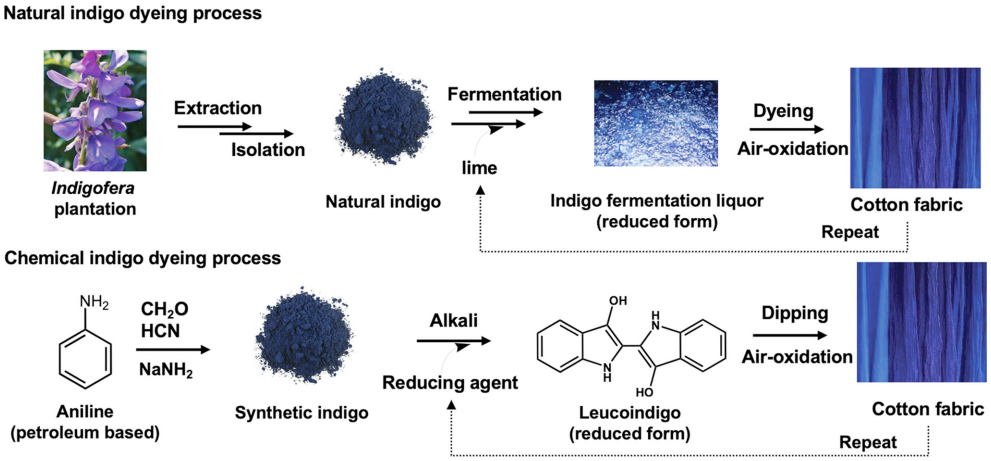

Fig. 1. Securing and dyeing scheme of natural and synthetic indigo.

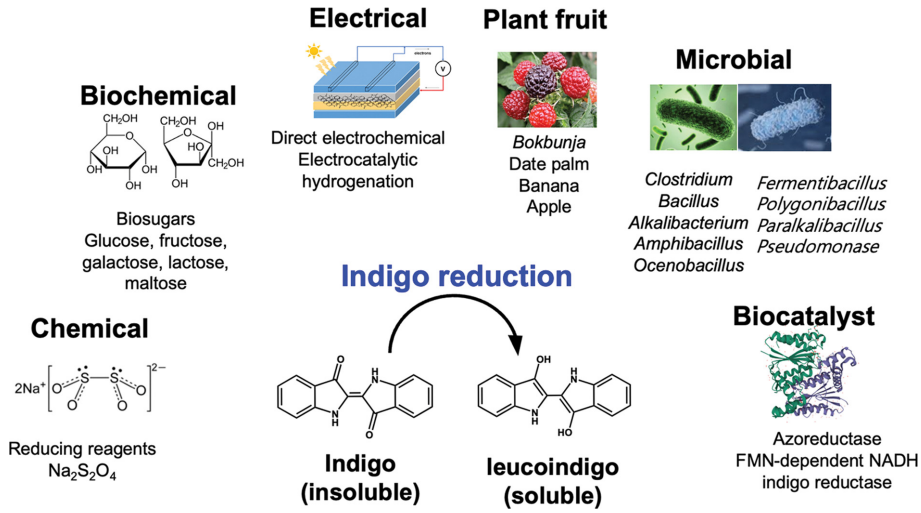

Fig. 2. Reducing reagents that include chemicals, biochemicals, plant fruits, microbes, electrochemicals, and biocatalysts used to solubilize indigo for its conversion into leucoindigo.

to biological dyeing processes, electrochemical and electroreduction have attracted considerable attention for vat dyeing because of their rapid reducing action and cost-effectiveness [7]. Modification of the chemical structure of indigo, by blocking or unblocking the indole C3 carbon using glucose or biocatalyst-dependent functional group insertion into the C5 to C7 carbon atoms, has been attempted to achieve a consolidated, eco-friendly vat dyeing process [12-14].

However, though these processes have several merits, they have drawbacks as well. In this review, several eco-friendly indigo reduction processes, with emphasis on modification of indigo, are discussed to assess their limitations as well as potential for future research (Fig. 2). This review will likely provide useful insights for the eco-friendly production and dyeing of indigo.

CHEMICAL AND BIOCHEMICAL SUPPLY OF REDUCING POTENTIAL FOR INDIGO REDUCTION AND DYEING

The chemistry of indigo solubilization involves its reduction to the leucoindigo form, as achieved by reducing agents (Fig. 3). This reaction generally requires alkaline conditions [15], as the solubility of leucoindigo is highly dependent on the pH at ionization. The optimal pH for high absorption into fibers is approximately 11, wherein the mono-ionic state of leucoindigo is dominant. A potential less than 700 mV (vs. Ag/AgCl, 3 M KCl) is required for indigo reduction [2]. To obtain desirable reduction performance, several studies have attempted to identify sustainable reducing reagents with adequate potential and ecofriendly dyeing processes at optimized environments.

1. Chemical Reduction of Indigo

The most commonly used reducing agent is sodium dithionite ($Na_2S_2O_4$), since it is an affordable, easy-to-handle chemical with effective reduction performance [15,16]. Considering the traditional time-consuming indigo reduction process during fermentation, a cutback of several hours is a great advantage to the dyeing process. However, the continuous oxidation of $Na_2S_2O_4$ produces unwanted oxidative byproducts, i.e., sulfite (SO_3^{2-}) and sulfate ($S_2O_3^{2-}$) ions, which are toxic and corrosive to aquatic ecosystems [17,18].

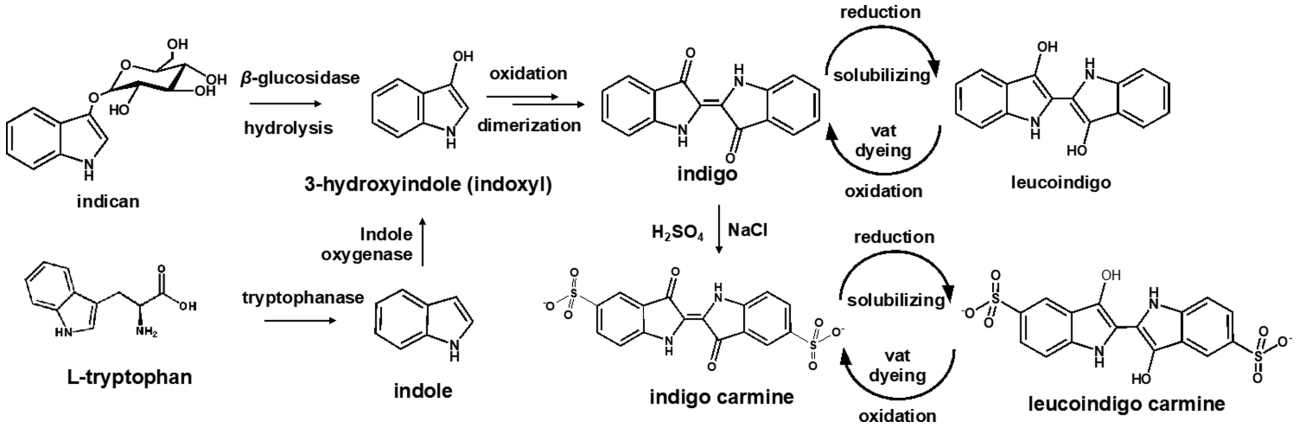

Fig. 3. Conversion of insoluble indigo into the soluble form, leucoindigo, by reduction and the biotransformation of glycosylated indican into indigo via hydrolysis.

Several attempts have therefore been made to find alternative reducing agents [2,15].

These alternatives have targeted several dyes, which require reducing potential. There are, however, requirements that must be met for their use as reducing agents. First, appropriate alternatives should have sufficient redox potential to transfer two electrons consecutively to the oxidized state of the indigo dye, and second, they must be sufficiently stable under highly alkaline conditions. More specific requirements such as biocompatibility, sustainability, and avirulence, should also be considered. Several chemicals and biochemicals that meet these requirements have been investigated. Among them, alkanediones such as 2,3-butanedione and cyclohexanedione, have been reported to reduce dyes owing to their high redox potential [19]. Sulfur-based chemicals such as thiourea were tested as alternatives to $Na_2S_2O_4$ because they have enough reduction potential and relatively low sulfur content [20,21]. However, these chemical-based reducing agents have the intrinsic disadvantage of being petroleum-based, which is contradictory to an eco-friendly dyeing process. Moreover, their low biocompatibility caused by chemical toxicity has led to the discovery of more suitable reducing agents.

2. Biosugars as Reducing Agents

One interesting alternative to reducing biochemicals is the use of biosugars, such as those used for reduction during sulfur dying [9]. The study included the redox potential of hexose and pentose monosaccharides, reducing disaccharides, and dyeing performance. Their redox potential against the color strength of the dye was approximately −650 mV for maximum color strength. The feasibility of using biosugars as reducing agents is based on their structural and potential electrochemical properties. For example, a monosaccharide glucose, wherein the aldehyde functional group is oxidized to carboxylic acid while reducing indigo in alkaline solution, was also successfully employed as a reducing sugar. The reduction potential (vs. Ag/AgCl, 3 M KCl) by the biosugars was less than −700 mV, which makes them capable of indigo reduction [2]. Further, a reduction in glucose (−703.9 mV), fructose (−716.3 mV), galactose (−702.5 mV), lactose (−704.8 mV), and maltose (−704.0 mV) was reported for indigo vat dyeing by Saikhao et al. [2]. In contrast, color fastness to washing and rubbing depended on the reducing agents used (NaOH or $Ca(OH)_2$). According to their dyeing experiments, dyeing fastness obtained by $Ca(OH)_2$ as an alkali was inferior to that obtained with NaOH, so it seems highly feasible to use biosugars as alternative reducing agents, although the rubbing fastness by $Na_2S_2O_4$ is inferior to that by reducing sugars [2].

However, there is scope for engineering desirable dyeing performance and reproducibility. Eco-friendly processes require another necessary condition to be truly useful. Harsh pH and temperature conditions during reduction using biosugars must be overcome to make them a sustainable alternative. Considering the optimum dyeing pH of 11.5, the high alkaline conditions required to achieve a sufficient rate of reduction could lead to low sugar-based dyeing performance. Moreover, the high temperature requirement during production of >70 °C is challenging to maintain and is directly related to the economic feasibility of biosugars; hence, a compensation for the use of reducing sugars instead of $Na_2S_2O_4$ should be considered. The use of biosugars has non-toxic and biocompatible advantages, thus making it more eco-friendly than the previously used reducing agents. However, the supply of glucose nutrients during the biochemical processes remains an issue, and recovery or recycling in the overall process needs to be thoroughly considered as well.

3. Plant Fruit Extracts as Reducing Agents

Plant extracts and fruits are also good sources of indigo reduction. As biosugars can be successfully employed as reducing agents, several fruit extracts consist of a mixture of up to 50 biosugars (Table 1). Moreover, fruit extracts can provide a redox potential of approximately −600 mV [22]. Fruits can thus be an excellent alternative to $Na_2S_2O_4$ and have thus been utilized for indigo reduction. Bokbunja (*Rubus coreanus*) sludge has been used as a reductant, reaching a maximum color yield at 80 °C over 1-2 days [22]. Similarly, Hossain et al. utilized plant extracts of date palm, banana, and apples as reducing agents [23]. The dyeing performance varied depending on the type and quantity of fruit used as well as $Ca(OH)_2$ concentration during dyeing. Among the tested fruit extracts, date palms showed the highest K/S value with 13.9 K/S color yield, 104.67% strength difference, and 52.56% exhaustion [23]. These results demonstrate that satisfactory color fastness and shed depth can be obtained using natural fruit extracts as reducing agents.

The use of plant-derived fruit extracts as reducing agents is highly

Table 1. Plant sources for indigo reduction

Plant fruit extract	Sources	Sugar content	Reduction potential	Dyeing conditions	Reduction activity (extract concentration)	Reference
Bokbunja	*Rubus coreanus* Miq.	50.87% (water)	−550~−600 mV	0.5 g indigo, 5 g $Ca(OH)_2$, 80 °C (reduction), 60 °C for 20 min (dyeing)	K/S[a] 13.3 (3% extract)	[22]
Date palm	Local fruit	Not defined	Not defined	10 g/L indigo, 15 g/L $Ca(OH)_2$, pH 10.8, 30 °C, 30 min, yarn count (Tex)	K/S[a] 13.9, strength difference 104.67, F[c] 52.56%, (200 g/L)	[23]
Banana	Local fruit	Not defined	Not defined	10 g/L indigo, 20 g/L $Ca(OH)_2$, pH 10.8, 30 °C, 30 min, yarn count (Tex)	K/S[a] 7.0, strength difference 52.75, F[c] 41.41%, (250 g/L)	[23]
Apple	Local fruit	Not defined	Not defined	10 g/L indigo, 20 g/L $Ca(OH)_2$, pH 10.8, 30 °C, 30 min, yarn count (Tex)	K/S[a] 12.1, strength difference 91.19, F[c] 48.23%, (150 g/L)	[23]

[a]K/S: depth of color, absorption coefficient (K) and scattering coefficient (S), [b]$K/S=(1-R)^2/2R$; R=decimal fraction of the reflectance of the dyed cloth, [c]F%=(I−P−Q)/I, F; exhaustion, I; initial concentration of dye liquor, P; concentration of post dye liquor, Q; dye concentration in the post-wash liquor.

eco-friendly because of their biocompatibility and biodegradable properties. Recycling fruit waste as a valuable factor in the dyeing process is particularly noteworthy. However, apart from dyeing performance and efficiency, the direct incorporation of fruit components such as fruit pigments, plant cell metabolites, and unidentified ingredients is inevitable during the reduction and dyeing process [24,25]. This may lead to unexpected side effects on dyed fabrics, and thus must be strictly controlled through permissions and regulations of the final fabric products with respect to toxicity. In addition, these unknown compositions could result in inconsistencies in the dyeing process itself.

BIOTECHNOLOGICAL SUPPLY OF REDUCING POTENTIAL IN INDIGO REDUCTION

1. Understanding Microbial Indigo Reduction Mechanisms

The microbial reduction of indigo is highly ineffective without generating other mediators, considering that the insoluble indigo particle size is generally 50 times the diameter of the microbes themselves. A good understanding of the mechanism was proposed

Table 2. Microorganisms used for indigo reduction

Microorganism	Source	Feature	Reduction condition	Reduction activity (%)	Reference
Clostridium isatidis	IFL	AN	pH 9, 47 °C	Not defined	[26]
Bacillus fermenti sp. nov.	IFL	OA	pH 10, 33-40 °C (optimum)	Not defined	[55]
Alkalibacterium psychrotolerans sp.	IFL	AN, OA	pH 9-12, 34 °C (optimum)	Not defined	[29]
Alkalibacterium iburiense sp.	IFL	AN, OA	pH 9-12, 30-37 °C (optimum)	Not defined	[27]
Alkalibacterium indicireducens sp.	IFL	AN, OA	pH 9-12.3, 20-30 °C (optimum)	Not defined	[28]
Amphibacillus iburiensis sp.	IFL	FAN	pH 8.9-9.1, 36 °C (optimum)	Not defined	[33]
Amphibacillus indicireducens sp.	IFL	FAN	pH 9-12, 35 °C (optimum)	Not defined	[30]
Oceanobacillus indicireducens	IFL	FAN	pH 7-12, 39 °C (optimum)	Not defined	[31]
Fermentibacillus polygoni	IFL	FAN	pH 7.5-12, 30-33 °C (optimum)	Not defined	[34]
Polygonibacillus indicireducens	IFL	FAN	pH 10, 35-37 °C (optimum)	Not defined	[36]
Paralkalibacillus indicireducens	IFL	FAN	pH 9-10, 33 °C (optimum)	Not defined	[35]
Fundicoccus fermenti sp. nov.	IFL	FAN	pH 9-10.5, 28-32 °C (optimum)	Not defined	[32]
Alkalibacterium sp.	IFL (6 years)	AN	pH 10, 50 °C, 0.1 mM indigo, 10 mM glucose, 30 d, anaerobic	0.032 mM leucoindigo (32%)	[37]
Pseudomonas sp.	IFL (6 years)	AN	pH 10, 50 °C, 0.1 mM indigo, 10 mM glucose, 30 d, anaerobic	0.015 mM leucoindigo (15%)	[37]

[a]IFL, indigo fermentation liquor; [b]AN, anaerobic; [c]AE, aerobic; FAN, facultative anaerobic; OA, obligate alkaliphile; AT, aerotolerant

using a *Clostridium isatidis* strain that was capable of reducing indigo dye [26]. According to Nicholson and John [10], the mechanism of indigo reduction by *C. isatidis* is that it can decrease the particle size of indigo dye and generate sufficient reduction potential [10]. The redox potential of *C. isatidis* was measured to be −600 mV (SCE) in the presence of indigo. Hence, mediators of anthraquinone-2,6-disulfonic acid and humic acid can stimulate indigo reduction.

2. Indigo Fermentation by Alkaliphilic Microorganisms

Microbial indigo reduction has been widely used as a traditional indigo dyeing process (Table 2). Isolated indicans from indigo plants are generally fermented using a mixture of microorganisms under alkaline conditions. This reduction leads to the conversion of insoluble indigo to its enol forms, which are still insoluble; deprotonation by a strong base can ionize and solubilize this enol form. In this research context, Yumoto's group pioneered the screening, identification, and characterization of indigo reducing microbes from indigo fermentation broth. They have reported interesting studies on various alkaliphilic microbes that have been isolated and characterized from fermentation sludge. Up to now, several *Alkalibacterium* strains were isolated from sukumo fermentation such as *Alkalibacterium indicireducens* sp., *A. iburiense* sp., and *A. psychrotolerans* sp., all of which are obligate alkaliphiles and capable of reducing indigo dye [27-29]. The isolated *Alkalibacterium* species were most closely related by phylogenetic analysis, suggesting that a specific group of microbes was responsible for indigo fermentation and reduction [28]. Similarly, alkaliphilic *Amphibacillus indicireducens* sp., *A. iburiensis* sp., and *Fundicoccus fermenti* sp. nov. have been isolated with similar fermentation and reduction outcomes [30-32].

In addition to *Alkalibacterium* and *Amphibacillus*, the facultative anaerobic bacteria *Oceanobacillus indicireducens*, *Fermentibacillus polygoni*, *Polygonibacillus indicireducens*, and *Paralkalibacillus indicireducens* were isolated and verified to have the capacity to reduce indigo dye [33-36]. *Oceanobacillus indicireducens* displayed isoprenoid quinones-deficient aerobic metabolism, which is very rare in *Oceanobacillus*.

Although several indigo-reducing microbes have been identified and classified, their reducing capacity has not been clearly demonstrated. Considering that it takes a relatively longer period in anaerobic fermentation than other reducing methods as well as other factors such as electron mediating agents, it does not seem clear to define their reducing capability. Park et al. [37] explored the capability of using isolated microbes of *Alkalibacterium* sp. and *Pseudomonas* sp. for indigo fermentation liquid [37]. During 30 days after anaerobic fermentation of each strain at pH 10 and 50 °C, the active cell broth reduced 0.1 mM of indigo into 0.032 mM and 0.015 mM of leucoindigo, with the supplementation of 10 mM glucose, respectively [37].

The potential uses of these microbes in indigo reduction process are long and complicated, as not a single microbe but a crowding of several genus and species, acting similar to an activated sludge, are involved in the fermentation process. According to Tu et al. [38], several microbial consortia are involved in *sukumo* fermentation in stepwise aerobic-anaerobic phases [38]. One possible approach to engineering the capability and performance of indigo reduction would be the control of electron mediators that can transfer released electrons to indigo or other stepwise mediators. However, this could affect the dyeing performance directly related to dye fixation and fastness.

If the production of bio-indigo using microorganisms and microbial reduction-staining process is employed continuously, a consolidated bioprocess can be achieved. Recently, studies on various enzymes related to indole oxidation have been reported, and since bio-indigo production is possible at a several g/L level, the indigo dyeing process using the reducing power of microorganisms is also highly likely to be scaled up to industrial levels [4]. Using microorganisms is advantageous as a long-term reaction is required for the desired level of dyeing performance because of its low reducing power. In addition, it is essential to consider the biological safety of various toxins produced by recombinant strains or microorganisms, since they come into direct contact with the skin through dyed fibers [39,40].

3. Biocatalyst for Indigo Reduction

Since the identification of indigo-reducing strains, an attempt to verify the key enzymes responsible for indigo reduction and their application has been reported, such as azoreductases, which have been utilized for the reductive degradation of a variety of azo-type dyes (Table 3) [11]. Indigo-reducing biocatalysts reported to date are listed on Table 2. In 2018, Suzuki et al. [41] identified a new flavin-dependent NADH-azoreductase (EC. 1.7.1.6), encoded by the *azoA* gene in *Bacillus* sp. AO1 for indigo carmine reduction [41]. Azoreductase was the first isolated indigo reductase that showed

Table 3. Biocatalysts for indigo reduction

Source	Microorganism	Encoding gene	Reduction condition	Reduction activity	Reference
Flavin-dependent azoreductase	*Alkaliphilic Bacillus* sp. AO1	*azoA*	pH 10.5, 262 mg/L indigo, indigo carmine, 0.2 mM NADH, 7 mg/L azoA, 30 °C	$(148\pm2)\times10^3\ s^{-1}M^{-1}$ k_{cat}/K_m (indigo carmine), ~0.8 mM NADH decrease/12 h (indigo)	[41]
FMN-dependent NADH-indigo reductase and Y151F	*Bacillus smithii*	WP_003354211.1	pH 7.0, 50 μM indigo carmine, 80 μM NADH, 50 °C	30.7 $\mu mol\cdot min^{-1}\cdot mg^{-1}$, 6.9 μM of K_m (indigo carmine), >95% complete activity retained after 10 min at 100 °C	[42]
FMN-dependent NADH-indigo reductase and W60A	*Bacillus wakoensis*	*azoA*	Not defined	Not defined	[44]

$148\pm2\times10^3\ s^{-1}M^{-1}$ k_{cat}/K_m specific activity against indigo carmine at alkaline and room temperature conditions [41]. These results demonstrate the feasibility of facilitating biocatalyst-based indigo reduction.

Recently, an FMN-dependent NADH-indigo reductase from *Bacillus smithii* was isolated with specific activity against indigo carmine as 30.7 $\mu mol \cdot min^{-1} \cdot mg^{-1}$ [42]. The produced enzyme was remarkably thermostable, as it retained its activity even after 10 min of incubation at 100 °C. In addition, it showed high stability over broad pH ranges and long storage periods. The crystal structure was obtained and a homology model of another azoreductase enzyme of *B. cohnii* was constructed, which was less stable than that of *B. smithii*. By comparing the crystal and model structures of these enzymes, the key intersubunit aromatic interactions (F105-F172′ and F172-F105′) responsible for the extreme characteristics of *B. smithii* indigo reductase were identified. Later, the same group reported the stereospecificity of H^- ion transfer by *B. smithii* indigo reductase enzyme [43].

Another study on indigo-reducing azoreductase identified azoA from *Bacillus wakoensis* A01 [44]. The azoA enzyme displayed considerable solvent-tolerant and thermostable characteristics, very similar to *B. smithii* reductase; this enzyme could also reduce several azo-type dyes. Of the several indigo reductases reported to date, most are dependent on the NAD(P)H cofactor supply for redox potential, which limits their applicability at the industrial level. In addition, direct reduction by the enzyme itself has not been achieved. This method thus needs to be engineered to supply cost-effective reducing potential in the enzymatic reduction process to obtain desirable process outcomes.

SOLUBILIZATION OF INDIGO DYE THROUGH ENZYMATIC MODIFICATION

1. Indigo Solubilization with Hydrophilic Functional Groups

Various attempts have been made to increase the solubility of indigo by introducing hydrophilic functional groups such as hydroxyl and carboxylic groups. Recently, Namgung et al. reported the one-pot production of 7,7′-dichloroindigo by co-expressing indole 3-hydroxylase of CYP102G4 (cytochrome P450 monooxygenase) with tryptophan 7-halogenase of PrnA in *Escherichia coli* [13]. However, the introduction of a chloro moiety into indigo did not substantially affect its solubility. Instead, 7,7′-dichloroindigo showed a different coloration and varying biological activity such as antioxidant and antibacterial activity. Later, 5,5′-dihydroxyindigo and 5,5′-dicarboxyindigo were synthesized through the biotransformation of 5-hydroxyindole and 5-carboxyindole using a CYP102G4-expressing *E. coli* host [1,13]. Similarly, Mikas et al. demonstrated enzymatic modification of indigo into water-soluble one through the

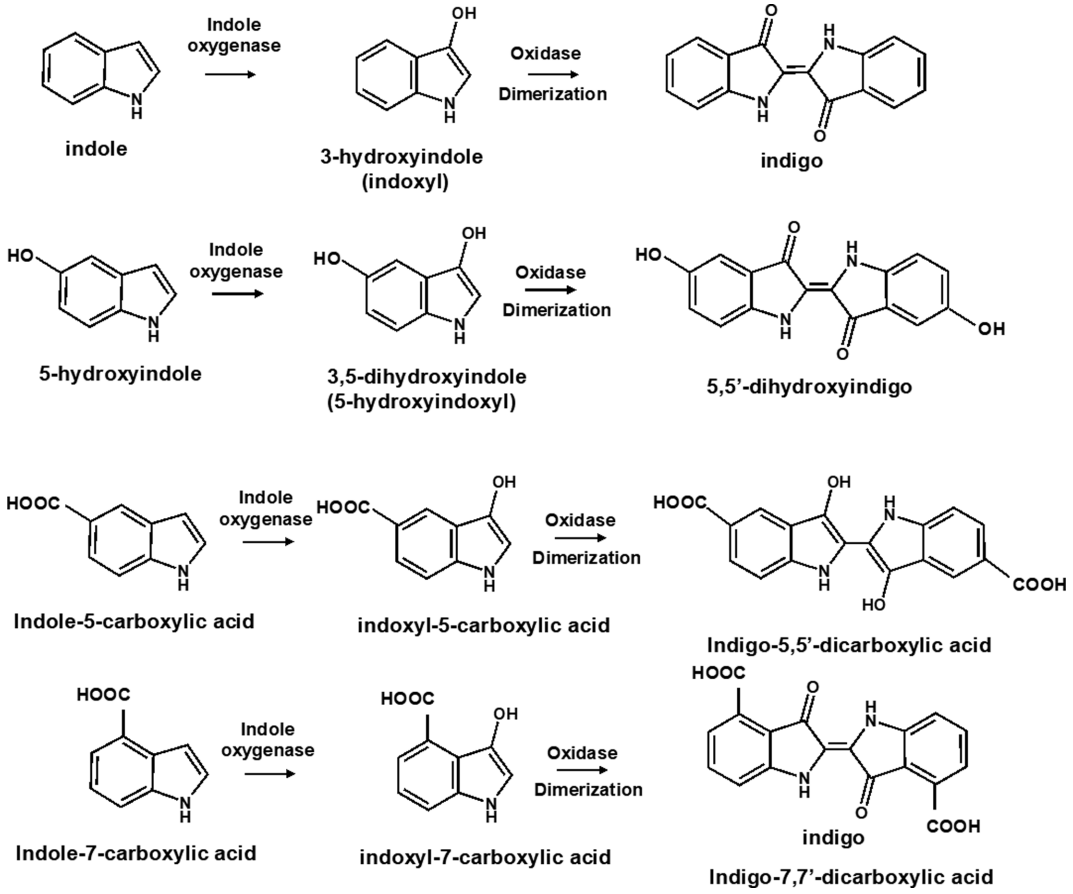

Fig. 4. Structural modification of indigo via biotransformation of 5-hydroxyindole into 3,5-dihydroxyindole (5-hydroxyindoxyl) followed by oxidation, which can result in the production of soluble 5, 5′-dihydroxyindigo.

biotransformation of indole-7-carboxylic acid [14]. The hydrophilic functional group slightly affected the solubility of indigo; however, the coloration of substituted indigoids changed and lost the primary purpose of indigo vat dyeing, i.e., its unique coloration (Fig. 4).

Therefore, there are numerous challenges yet to overcome. One-pot production of such hydrophilic indigoids requires additional introduction of enzymes, which might decrease the final indigoids production titer (also observed by [13]). In addition, the extraction and separation of solubilized indigo has a high separation cost to ensure that increased solubility of a target compound is obtained.

2. Glucose- and *β*-Glucosidase-dependent Protection-deprotection of Indigo Dyeing

Other efforts to find alternative chemical reducing reagents have been made to bypass the reduction step, and thus utilize soluble indicants for the dyeing process through glycosylation. Hsu et al. [12] utilized glucose as a protecting group by introducing UDP-glycosyltransferase, blocking the conversion of indoxyl into indigo through spontaneous oxidation (Fig. 3) [12]. During the dyeing process, *β*-glucosidase was introduced to deprotect the glycosylated moiety and lead indigo formation, followed by hydrolysis and natural dyeing. The dyeing process, using indigo derived from plants, includes biotransformation of a highly soluble glycoside indican (indolyl-*β*-D-glucopyranoside) by *β*-glucosidase and air oxidation [12] (Fig. 3). However, the use of biocatalysts in the dyeing process inevitably increases the costs and requires regeneration process, thus limiting its potential use in mass dyeing.

CONCLUSIONS AND FUTURE PERSPECTIVES

In this review, various efforts to provide alternative reducing reagents via chemical and biological methods were assessed and highlighted. Indigo, an important dye throughout human history, is still widely used to dye denim. However, its reducing reagent-dependent dyeing process is a threat to natural environments. Studies show that the reduction performance, in terms of rate and scale-up, is excellent when using a chemical-based reducing reagent. Despite this good performance, it is required to overcome the drawbacks of such eco-friendly dyeing processes. Alternative biochemicals, such as reducing sugars, have been utilized instead of $Na_2S_2O_4$. Nonetheless, using these reducing reagents has disadvantages, such as poor price competitiveness and lower efficiency as compared to that of chemical reducing agents. Reducing sugars are thus unlikely to be used as a dye-reducing agent because it is a major carbon source in microbial culture; they can be utilized as biomass that can be converted into other high-value-added biochemicals.

Although not covered in this paper, indigo reduction methods using direct electrochemical methods are also possible. The electrochemical reduction of indigo is simple and directive; however, it requires additional mediators capable of reaching the indigo molecule and transfer electrons unless indigo is not immobilized directly into the electrode [7,45-47]. Using these electrochemical methods for indigo reduction seems very efficient and ecofriendly. However, scale-up production and cost considerations seem to be considered as well. Another emerging technology in the indigo dyeing process includes plasma treatment, i.e., surface modification of the indigo dye. Plasma can induce physicochemical changes in polymer fibers [48-50]. Although plasma technology is actively used in various industries, it has not yet been commercialized in the dyeing process. Alternatively, it can be used by adding a plasma process to the decolorization process to remove the indigo color [51,52].

In addition to indigo, various dyes are being updated with eco-friendly production technologies such as bioconversion and biotransformation, and various bioprocesses are being tested to reduce carbon emissions [53]. Dye production and dyeing processes that have long been used have now been replaced by chemical processes and are further being replaced by eco-friendly processes to produce natural dyes biologically. The challenges that need to be gradually solved include ensuring high production yield, dyeing process efficiency, price competitiveness, and scale-up to industrial levels, securing a carbon source, recycling, and using eco-friendly treatment techniques. Besides, several reduction techniques summarized here could be also applied to various dyes such as azo dye [54]. It is expected that research in the field of bio-indigo will continue until the process can appropriately evolve through life cycle evaluation of carbon emissions in response to climate change.

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