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An Improved Method for the Large-Scale Processing of Woad (*Isatis tinctoria*) for Possible Commercial Production of Woad Indigo

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The increasing use of alternative crops has meant the introduction of new technologies to process their products. In this work, an alternative method is presented for the extraction of natural indigo from woad (*Isatis tinctoria*) based on a technique used to extract indigo from *Indigofera* spp. This method does not rely on the old fermentation procedure used throughout Northern Europe and is cheap, clean and efficient. Evolved from laboratory-based tests, it involves steeping the leaves at low pH in warm water and extracting the indigo at a higher pH, followed by ultra-filtration of the product, which is then left to air-dry. Problems encountered during the development of the technique and how they were overcome are discussed. © 1998 Silsoe Research Institute

1. Introduction

1.1. Indigo

Indigo is a blue coloured dyestuff which can be derived from certain plants or made synthetically from by-products of the oil and coal-tar industry. The total world market for dyestuffs is 800 kt, of which, indigo represents 80 kt (Prof. D M Lewis, University of Leeds, personal communication). The European market represents approximately 10% of this. Unpublished market research suggests that approximately 5% of consumers expressed a preference for products dyed with natural dyes. Working on these numerical assumptions and the current market price of £30 kg⁻¹ for natural indigo, the estimated size of the European market for indigo would be £12 M. In the next 10 y this market is expected to grow to a possible total of £36 M.

At present small amounts of naturally derived indigo are produced in tropical and sub-tropical countries, from various indigo-producing plants. This source provides enough indigo to satisfy the demands of small-scale craft dyers, supplying exclusive markets. These supplies vary widely in quality and no standards are in use to guarantee quality.

1.2. The chemistry of indigo

Indigo is an artefact of secondary metabolism; it is not found as a native compound in the plant. In most cases, the indigo precursor, indoxyl, is found in plants as the glucoside indican (indoxyl- β -D-glucoside, *Fig. 1a*), but in woad the main precursor is the ester, isatan B (indoxyl-5-ketogluconate, *Fig. 1b*), as outlined by Epstein *et al.*¹ To form indigo from the precursors, the carbohydrate moiety is cleaved from the indoxyl group and two of the resulting indoxyl molecules combine to produce an indigo molecule. In practice, this occurs spontaneously in aerobic conditions once the molecule has been hydrolyzed; indigo then precipitates from solution. Indigo produced from any plant source is chemically identical to synthetic indigo.

In nature, indigo precursors are broken down by hydrolases when the tissue is damaged and exposed to the air, producing indigo in the leaf, a property exploited in the medieval method of producing woad balls. However, indigo is not soluble in aqueous solution so once it is formed in the leaf tissue it is almost impossible to extract. Therefore, to improve the process, the soluble precursors should be extracted, separated from the leaf tissue and converted to indigo. In order to extract the precursors without converting them to indigo, hydrolase activity must be suppressed or the extraction done under anaerobic conditions. Once the precursors have been isolated in an extract and hydrolyzed, they must be oxidized to form indigo (*Figs 1 a–d*).

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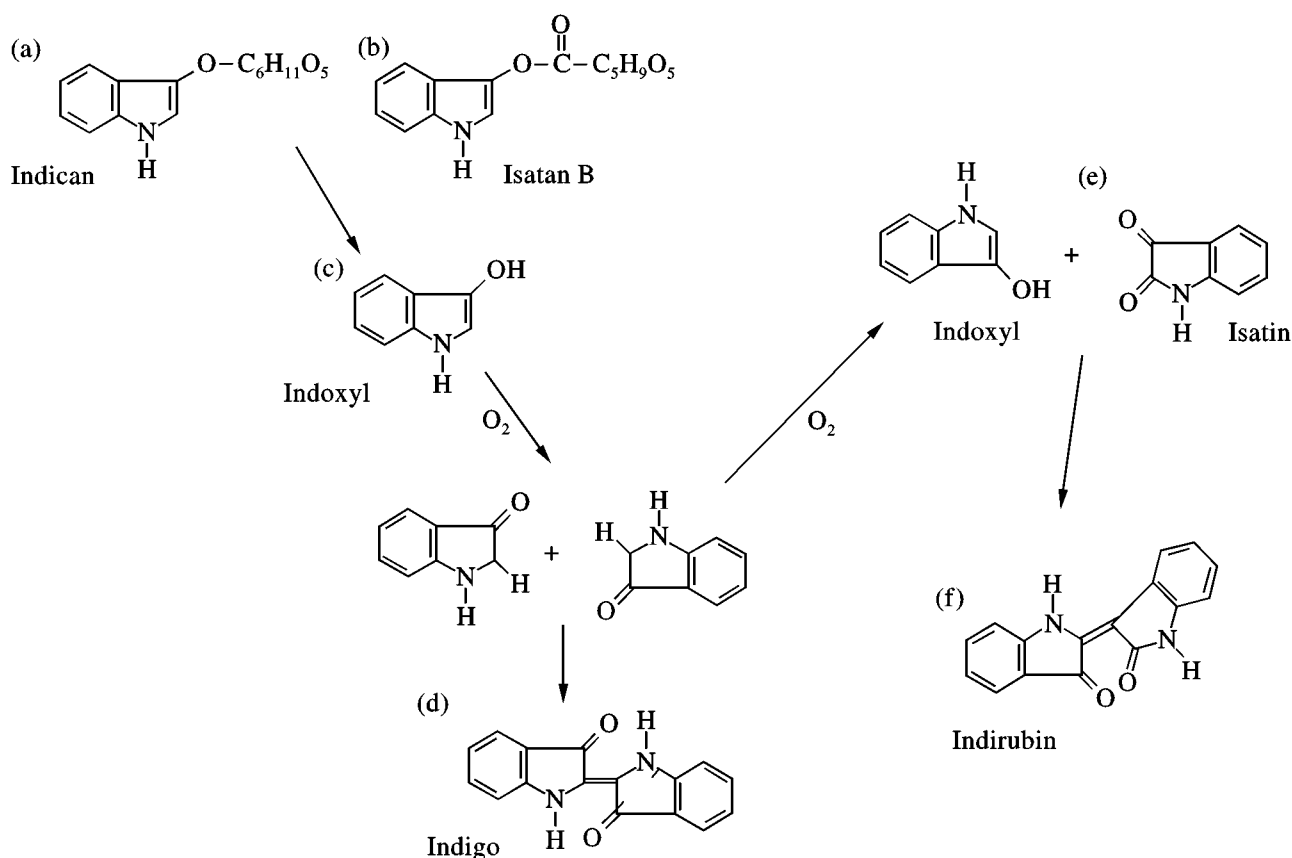


Fig. 1. Mechanism of conversion of indican and isatan B into indigo via indoxyl at pH 9.5 and with aeration. Indirubin is formed if isatan B or indican is oxidized before indigo is formed

1.3. Existing techniques and their limitations

Woad (*Isatis tinctoria*) was grown and used, as the sole source of indigo in Europe, from medieval times and continued to be grown until the first part of the twentieth century. The traditional method for the extraction of indigo was by making "woad balls", as described by Hurry.² This was done by crushing the freshly harvested leaves, forming them into balls (about 10–12 cm diameter) and allowing them to dry over a period of 4–6 weeks, by which time the indigo had formed by oxidation. The balls were then crushed, heaped into piles, water added and allowed to ferment aerobically over a period of some weeks. The indigo was then ready to be used by the dyer. However, this method is now unacceptable to modern dyers, as it relies on "dirty" processing, is inefficient and produces a putrid odour. Modern craft-dyers rely on the more recent method of Plowright.³

Apart from woad, indigo can also be obtained from many other plants across a wide range of species, families and genera,⁴ including the tropical and sub-tropical spe-

cies, *Indigofera* and *Polygonum*, respectively. Although, indigo can be made from these species by the method of making "indigo balls" in the same way as "woad balls", traditional methods have included steeping the leaves of the plant in water and adjusting the pH during this process to form indigo.⁵ A similar process was also reported as being used in Shanghai in 1846 as a way of extracting indigo from tein-ching or Chinese woad (*Isatis indigotica*) by steeping the leaves in water in round tanks for "a certain period of time" and then treating them with lime.⁶

Previously, the conditions for extraction of indigo precursors from woad and their subsequent conversion into indigo have been imprecise. The methodology used to date is relatively successful in providing some indigo, but the quantity and quality of the dye is variable. To produce indigo on a large-scale, it is necessary to standardize the extraction and conversion into indigo of the precursors. Indigo can be extracted from woad by boiling the leaves in water for a few minutes, cooling the extract to room temperature and adding ammonia solution

followed by aeration.⁷ By filtering the extract, this method produces a "clean" indigo product but it cannot be used for large-scale extraction because it is energetically expensive and, thus, uneconomical for commercial indigo production.

2. Theoretical considerations

The methods described are intended to be used for the commercial production of natural indigo by farmers cultivating woad. Therefore, the technology has been developed to be appropriate for use on a farm, making it accessible to all growers and requiring minimal outlay of initial equipment. Although the primary techniques are aimed at woad growers, the technology would be transferable, with some modification to the conditions of extraction, *e.g.* pH, to other indigo-producing crops.

In practice, indigo precursors are readily released from the leaf tissue into an aqueous medium on heating. However, certain difficulties are encountered if the water is maintained at too high a temperature, as some of the released indoxyl is oxidised to isatin, (*Fig. 1e*), which can react with another indoxyl molecule to produce indirubin (*Fig. 1f*). This isomer of indigo is a red colour, which is of limited use as a dyestuff, since it has poor light-fastness. The reaction which produces indirubin is poorly understood, variable and irreversible. If the starting temperature is insufficiently high (< 60°C), then the enzymes which cleave the sugar or acid moiety from the indoxyl glucoside to form indoxyl, remain active and indoxyl is formed prematurely. Therefore, it is necessary to optimize the conditions of extraction to maximize indigo production and minimize indirubin formation. Furthermore, the process of extraction should be cheap and consume as little energy as possible. The indigo precursors must not be stored for long periods in the aqueous phase, because they are highly reactive and sensitive to light. Hence, the indigo-forming capacity of the aqueous extract is reduced over time. Isatan B is more stable in acid conditions and possibly extracted optimally at pH 3.5.¹ Therefore, extraction at this pH was tested on a laboratory-scale and subsequently used in the pilot-scale operation. The indigo precursors were converted to indigo in a separate "settling" tank at alkaline pH and the system aerated; this aided the formation of indigo. To provide a solid product, indigo must be removed from the suspension. Indigo is formed as a fine particulate solid in suspension, the particle size was of the order of 0.5–1 µm as measured by scanning electron microscopy. This meant that the time for the indigo to settle out of suspension was of the order of days, allowing contamination of the aqueous phase by microorganisms. Therefore, separation by filtration would be preferable.

The principles of large-scale indigo production, outlined above, were first tried on a laboratory scale, in order to increase the understanding of extraction processes and to assess the possible efficiency of large-scale operation.

3. Methods

3.1. Laboratory-scale extraction

Woad leaves were sampled from plants grown in the field or from stocks kept under glass. Fresh leaves were cut into strips about 1 cm wide and put into Quick-fit test-tubes. Indigo precursors were extracted by taking 2 g of cut fresh leaves and treating them with boiling water to break down the surface wax. The water was cooled so that the resulting solution was maintained at a final temperature of either 30, 60 or 90°C and the leaves were in a volume of 20 ml. Ten percent HCl (5 µl) was added to maintain the stability of isatan B in solution; stoppers were then put in the tubes and the leaves steeped at the defined temperatures, overnight, in the dark. After about 17 h, the extracts were rapidly cooled on ice to 25°C, the leaves removed, ammonia solution added to adjust the pH to between 9 and 10 and the extract aerated using a small compressor until development of the blue colour was complete.

3.2. Pilot-scale extraction

Woad leaves (50–100 kg) were harvested from the field using a Haldrup harvester and immediately transferred into mesh bags 70 cm × 100 cm, with 10 kg leaves per bag. These were put into a 500 l tank fitted with a pump-driven circulation system (*Fig. 2*). Hot water (60°C) was poured over the leaves (2 l of water to every 1 kg of leaves) to damage the wax surface sufficiently to facilitate precursor extraction. Cold water was then added to reduce the temperature to 38°C and the pH adjusted to 3.5 with sulphuric acid. The steeping leaves were then covered with a piece of wire mesh and weighted down with four stone blocks, each weighing about 3 kg, to hold the plant material under water. This was done to exclude air, thus maintaining anaerobic conditions, so preventing hydrolytic enzyme activity. Water was circulated around the tank for about 2 h (*Fig. 2*); after 24 h, following overnight steeping, the leaves were rinsed thoroughly with more cold water and removed.

The extraction liquor was pumped into a 500 l settling tank, the pH adjusted to between 9 and 10 by the addition of concentrated ammonia solution (*ca* 0.67 ml/l). It was then aerated for 2–4 h, using a compressor, to enable

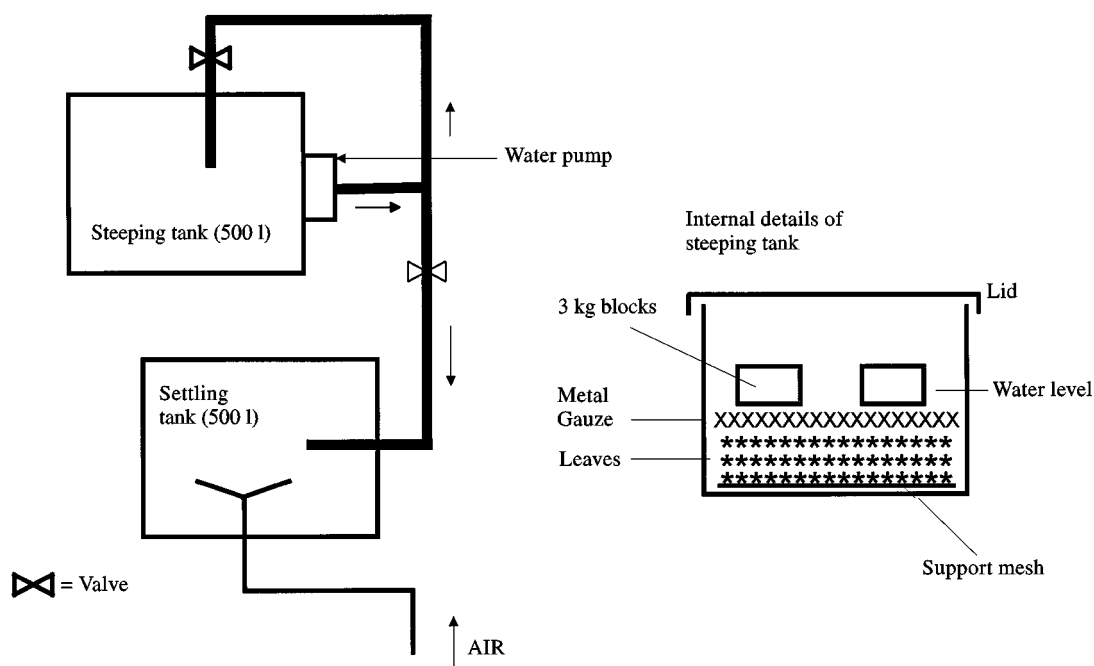


Fig. 2. Cross-section of tanks and pumping system used for the pilot-scale extraction of woad leaves. Leaf material is put into a steeping tank and allowed to steep overnight in acidified water. The extraction liquor is then pumped into a settling tank, where it is made alkaline and aerated for 2-4 h before settling overnight. The supernatant is then drained off and the indigo slurry is filtered using a Microza ultra-filtration module (not shown) and the resulting thickened slurry is allowed to air-dry

complete oxidation of the precursors and assist indigo formation. The indigo was allowed to settle for 24 h before the supernatant was siphoned off, leaving a slurry of indigo and water. This was filtered by cross-flow filtration, using a Microza ultra-filtration module, ACP 1010 (Pall Ultrafine Filtration Company) which concentrated the slurry, allowing rapid drying of the final product in trays maintained at room temperature. The efficiency of the extraction procedure was assessed by weighing the final product and determining its indigo content.

3.3. Analysis of indigo

In order to reduce the time and effort to dry the indigo, analysis was done using the indigo suspension directly from the extraction process. Thus, an appropriate amount of indigo suspension was taken and diluted to 10 ml with water and extracted with 10 ml ethyl acetate. Indigo content was determined spectrophotometrically at 600 nm, using a polynomial distribution curve derived from the absorbance of serial dilutions of a standard solution of indigo.

4. Results and discussion

4.1. Laboratory-scale extraction

The results of extracting indigo showed that the yield at 30°C was substantially greater ($380 \pm 120 \mu\text{g/g}$ fresh weight) than that at other temperatures; as temperature increased, yield decreased (47 ± 12 and $18 \pm 1.69 \mu\text{g/g}$ fresh weight at 60 and 90°C, respectively). Steeping for 24 h at high temperature may have incurred a loss of indigo, since steeping the leaves for 5 min at 100°C gave a yield of 2.1 mg/g fresh weight (± 0.14) whereas leaves steeped at 30°C overnight gave a yield of 2.08 mg/g fresh weight (± 0.28) which was not significantly different.

4.2. Pilot-scale extractions

Using the information obtained from laboratory-scale extractions, a pilot plant was designed. However, in scaling-up the quantities of leaves some difficulties were encountered.

It was found that unless the waxy coating of the woad leaves was in some way compromised, before overnight

steeping, the yield of indigo from the extraction was poor. This point was particularly important because the leaves used in this process were not chopped but intact, meaning that only their surfaces were in contact with the extraction medium. Therefore, it was necessary to partially melt the leaf surface wax. This was achieved by using water heated to about 60°C, which was sufficient to allow extraction from the leaves. If the leaves are damaged at harvest, however, their surfaces release the indigo precursors and indigo forms spontaneously in the leaves. Once formed, indigo is virtually impossible to remove. Therefore, harvesting procedures must be designed to maintain the leaves intact, if they are to be stored before extraction.

It was not possible to maintain this temperature overnight, as this would have proved both energetically and financially expensive. Therefore, following the addition of hot water to damage the leaf surface wax, the extract was cooled by the addition of cold water to achieve the final volume required and a temperature of 38–40°C; overnight the latter dropped to about 25°C. This compromise did not appear to affect the yield of indigo. The indigo product was found to be 20–40% pure in pilot-scale samples, as assessed by spectrophotometric analysis, when the weight of the indigo product was compared with the spectrophotometrically calculated value. Laboratory-scale extractions do not produce a solid product, as the technique for analysis of indigo relies on the preferential partitioning of indigo into ethyl acetate from an aqueous suspension. Therefore, purity of the product made in the pilot plant cannot be directly compared with the purity of the laboratory-scale product.

In preliminary work, the pH was not adjusted with acid during steeping, resulting in a loss of yield due to the premature formation of indigo, which is characterized by a blue scum on the surface of the extract. Addition of acid stabilized the extract, preventing indigo formation. Also, reducing the pH during steeping meant that there was a large pH shift over the course of the extraction process, from pH 3.5 to 9.5. This shift appeared to have an additional beneficial effect, because the supernatant could be stored for much longer periods (up to 3 months) without the occurrence of visible microbial contamination.

Another difficulty encountered was the slow settling of indigo from the suspension. The use of a flocculent was found to be unsatisfactory, for two reasons. Firstly, although the bulk of the indigo settled more rapidly, it changed colour due to an unknown reaction with the polymer. Secondly, there was difficulty in separating the flocculent from the waste water and product, so the indigo could not be sold as a completely natural product. By increasing the pH to 9.5 or above, the settling time was considerably decreased (from 1 week to < 24 h).

This, along with the microbial stabilization of the supernatant and the introduction of the cross-flow filtration system, has meant that indigo is produced relatively quickly, which should reduce the long-term costs of production.

4.3. Waste products

The advantage of this process is that the waste products can all be recycled. The plant material can be converted to compost and used as organic matter to improve the soil used for woad growing (or other agricultural land). The waste liquid from the extraction process contains nitrogen in the form of ammonium. This can be neutralized, using sulphuric acid, to form ammonium sulphate, which can be applied to the woad crop as part of the fertilizer requirement prior to the subsequent harvest.

4.4. Success of the process in the production of indigo

This paper outlines the development of a method which opens the way for the large-scale production of woad indigo on a commercial basis. As described in the introduction, earlier methods were either inefficient (woad balls), or did not prove profitable. It uses simple equipment which would not require a large initial capital outlay by the producer. The results described here suggest that reasonable yields of indigo could be achieved (20 kg/t of fresh leaves). Preliminary data suggest that 1 ha produces about 1 t of fresh leaf material. Although this pilot system only allows the processing of up to 100 kg of leaves, the technique is suitable for use on a much larger scale being capable of processing leaves from a hectare or more of woad.

5. Conclusions

The process described above has been used successfully to produce woad indigo for use by the textile industry. Problems involved in the extraction of indigo from plant material, such as, handling of large amounts of plant material harvested from the field, extraction of precursors from plant material and removal of the product from solution, have been overcome, thus providing a method which can be scaled-up for commercial production of natural indigo from woad. Woad indigo has not been used in the commercial dyeing of textiles since the Middle Ages and the use of natural indigo from tropical sources has been limited to small-scale designer markets.

The production of natural indigo, even on a relatively small scale, will open the market and make naturally dyed products available to a much wider market. The method outlined here will enable commercial producers to fulfil the growing desire amongst consumers for natural rather than man-made products.

6. Acknowledgements

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