STUDYING EFFECTIVE METHODS FOR OBTAINING INDIGO DYE FROM THE INDIGOFERA L PLANT TRADITIONALLY

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ABSTRACT

In this article, the use of natural dyes and the creation of methods for their effective extraction, as well as the extraction of indigo dye from the Indigofera tinctoria L plant traditionally grown in the Khorezm region during our research, and the indigo dye particles in solution after product extraction options are provided. Extraction of indoxyl-β-D-glucoside from the leaves of Indigofera tinctoria L plant was carried out in aqueous medium, and hydrolysis of indoxyl-β-D-glucoside released into the solution was carried out with the presence of β-glucosidase enzyme. Lactobacillus acidophilus bacteria were used to obtain β-glucosidase enzyme. According to the obtained results, the formation of dye was observed in the remaining solution even after the indigo dye was extracted. In order to solve these problems, we conducted research.

Keywords: *Indigofera tinctoria L, indigo, solution, indican, β-glucosidase, extraction, Lactobacillus acidophilus, indoxyl.*

ИЗУЧЕНИЕ ЭФФЕКТИВНЫХ МЕТОДОВ ПОЛУЧЕНИЯ КРАСИТЕЛЯ ИНДИГО ИЗ РАСТЕНИЯ ИНДИГОФЕРА ТРАДИЦИОННЫМ СРЕДСТВОМ

АННОТАЦИЯ

В данной статье рассмотрено использование природных красителей и создание методов их эффективного извлечения, а также извлечение красителя индиго из растения Indigofera tinctoria L, традиционно выращиваемого в

Хорезмской области во время наших исследований, и частиц красителя индиго. В растворе после предоставления вариантов экстракции продукта. Экстракцию индоксил-β-D-глюкозида из листьев растения Indigofera tinctoria L проводили в водной среде, а гидролиз выделившегося в раствор индоксил-β-D-глюкозида осуществляли в присутствии фермента β-глюкозидазы. Для получения фермента β-глюкозидазы использовали бактерии Lactobacillus acidophilus. Согласно полученным результатам, образование красителя наблюдалось в оставшемся растворе даже после экстракции красителя индиго. Для решения этих проблем мы провели исследование.

***Ключевые слова**: Indigofera tinctoria L, индиго, раствор, индикан, β-глюкозидаза, экстракция, Lactobacillus acidophilus, индоксил.*

INTRODUCTION

Today, the production and use of synthetic dyes cause many environmental and everyday problems. Due to the pollution caused by the production and use of many synthetic dyes, there is a renewed interest in obtaining and using natural dyes that do not harm the environment. The use of indigo dye obtained by synthetic and natural methods is still a large part. The use of synthetic dyes can cause harmful effects on the environment and the human body. It encourages research on the use of natural dyes and energy-saving methods to avoid harming the natural environment.

Natural indigo dye can be obtained from tropical and subtropical plants belonging to the genus Indigofera [1-8]. In our experiments, it was found that the biomass of the *Indigofera tinctoria L* plant is used as a raw material in indigo dye, and the concentration of dye compounds in the leaves is high. In the Indigofera plant, Indigo dye is not found in the plant as a free form, but in the form of an indican (indoxyl-β-D-glucoside) compound. Extraction of indigo from plant biomass involves several steps; indican located in cell vacuole is released from plant biomass by water and fermentation, and β-glucosidase enzyme hydrolyzes indican into indoxyl and glucose[2].

The formation of indigo is observed when two molecules of indoxyl combine in the presence of oxygen in an alkaline environment [3]. Under the catalytic of the β-glucosidase enzyme, inhibitions were carried out at different temperatures and pH values of the medium to hydrolyze the indican contained in the Indigofera plant. In these studies, the process's duration is very long and is 10–36 hours [4-6].

Taking into account the research data presented above, experiments were conducted to obtain indigo from the plant *Indigofera tinctoria L* traditionally.

MATERIALS AND EQUIPMENT

Dried indigofera leaves, distilled water, 0.1 N NaOH solution, *Lactobacillus acidophilus* colony, dimethylformamide, Bante 210 digital stationary pH meter, 1 L conical flask, 1 L beaker, digital magnetic stirrer hot plate (MS7-H550-S, China), Schott funnel, 6 50 ml volumetric flasks, 200 ml volumetric flask, UV-1800 Shimadzu spectrophotometer.

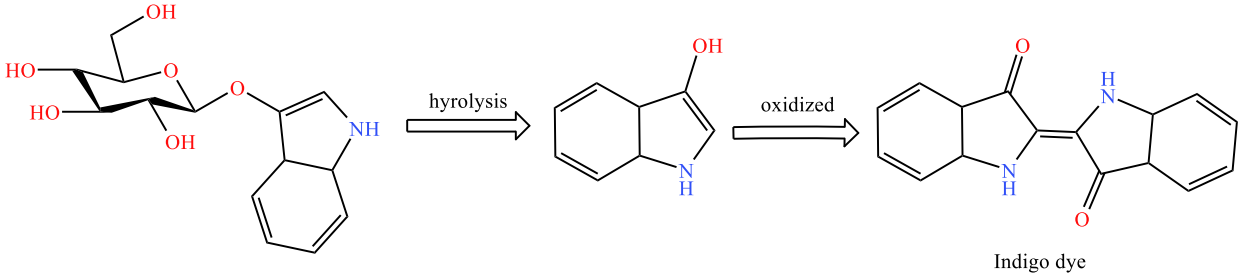
SEPARATION OF INDIGO DYE

10 g of dried Indigofera leaves were cut and placed in a 1 L flask, 150 ml of distilled water was poured over it, and extraction was carried out for 90 minutes at a temperature of 50°C. After the plant mass in the flask was mixed with water, 1 ml of the *Lactobacillus acidophilus* colony was added to the mixture in the flask and placed in a thermostat at a temperature of 37°C for 24 hours. At the end of the specified time, the solution in the flask passes through a Schott funnel and separates. 0.1 N NaOH solution was added until the pH of the solution reached 8-8.5. Air was passed for 1-1.5 hours until blue precipitates were formed through the solution, the pH indicator of which was brought to the specified value. The blue precipitates were separated by filtration. The mass of the obtained sample was measured on an analytical balance.

RESULTS AND DISCUSSION

It is released as a result of the extraction of indoxyl-β-D-glucoside contained in the Indigofera plant into an aqueous solution at a temperature of 50°C. The reason for keeping the mixture in a thermostat for 24 hours at a temperature of 37°C is that it is most convenient for *Lactobacillus acidophilus* bacteria to multiply at this temperature [7]. Indoxyl and glucose are formed due to the hydrolysis of indoxyl-β-D-glucoside released from the plant mass into the aqueous environment with the participation of the β-glucosidase enzyme produced by *Lactobacillus acidophilus* bacteria.

As a result of the oxidation of indoxyl formed during hydrolysis under the influence of air oxygen, indigo dye material is formed. The resulting blue indigo dye substance precipitates in the solution.



Indigo dye

Indigo dye is slightly soluble in water and relatively well soluble in chloroform, concentrated sulfuric acid, nitrobenzene, and dimethylformamide. Therefore, indigo precipitates in water.

The indigo precipitate was quickly separated by filtration, and the mass of the precipitate was measured on an analytical balance. Based on the above experiments, it was found that the amount of indigo dye extracted from the *Indigofera tinctoria L* plant grown in the Khorezm region is on average 0.2-0.3 g per 10 grams of leaf in laboratory conditions. It has been shown that 30-60 kg of indigo can be obtained on average from 1 ton of leaves in different phases of the plant, depending on the ontogeny periods.

When studying the processes of obtaining indigo dye traditionally obtained from the Indigofera plant, parts of the indigo dye were observed in the plant residues and the filtrate.

It is possible to increase the productivity of the product by extracting the indigo dye that is formed over time. Therefore, it is possible to obtain more dye than the 0.2-0.3 g mass of indigo dye obtained by the traditional method. Researching obtaining indigo dye from the Indigofera plant faster, in less time and a productive amount is one of the urgent issues. Therefore, research is being conducted to increase the activity of *Lactobacillus acidophilus* bacteria and the β-glucosidase enzyme produced in it to obtain indigo dye productively.

CONCLUSION

When the indigo dye obtained by the traditional method was obtained, it was observed that the dye sample was formed again over time in the plant residues and in the solution. This situation shows that it is possible to obtain more mass of paint than the mass of paint obtained by the traditional method. A low-energy and efficient method is required to perform this task. We are conducting new research to address these issues.

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