Study on Green Decolorization Technology of Agar in the Extraction Process

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Abstract. To establish a green decolorization technology of agar in the extraction process, the effects of activated carbon amount, decolorization time and decolorization temperature on the decolorization of agar solution extracted from *Gracilaria rubra* were investigated through single-factor tests with the decolorization ratio as an index. On the basis of single-factor tests, the decolorization process was optimized using orthogonal experiments. The results showed that the optimum conditions for decolorization with activated carbon were as follows: amount of activated carbon 0.6 g/100mL, decolorization time 5 min and decolorization temperature 70 ºC. When the optimum conditions of decolorization with activated carbon were used on the basis of pre-treatment of diatomite, the decolorization rate of agar solution reached 81.6%, and the agar solution was nearly colourless, clear and transparent. The diatomite-pretreated decolorization of activated carbon overcame the disadvantages that chemicals are needed to acidify and bleach *Gracilaria* in the traditional agar extraction process. It not only avoided the use of chemicals, but also effectively decreased the water consumption and the amount of produced chemical-containing waste water.

1. Introduction

Agar is a hydrophilic polysaccharide with high gel property. It is mainly composed of repeat units of D-galactose and 3,6-anhydro-β-galactose. It is widely used in such industries as food, medicine, and cosmetics [1-2]. In Guangdong, Fujian, Guangxi and Hainan, *Gracilaria* is used as a raw material for agar extraction. The agar contents in different dried *Gracilaria* species range from 9.0% to 34.0% [3]. To obtain whitish or yellowish products, *Gracilaria* must be bleached in the agar extraction process. There are mainly two process steps to complete the bleaching. The first is acidification, in which HCl, H₂SO₄, or H₂C₂O₄, etc. are mainly used to acidify *Gracilaria* in production, so as to facilitate the next bleaching and agar extraction. The second is bleaching process, in which *Gracilaria* is bleached with NaClO or H₂O, so that the extracted agar with light colour is suitable for different uses [4-7]. The acidification and bleaching processes have great influences on the quality and yield of agar. If they are not strictly controlled by technicians, these process steps will lead to great fluctuation in such important indexes of product as yield, colour, and gel strength [8]. In addition, the water consumption in the acidification and bleaching process account for about 50% of that in the whole agar extraction process, and there is a large amount of waste water generated in the acidification and bleaching process, which increases the pressure of environmental protection and reduces the economic benefits of the enterprises. In this paper, a physic adsorption process of activated carbon was developed to decolorize agar so that the chemical acidification and bleaching process was eliminated. It could not only enhance the product quality control and maintain a high yield of agar, but also eliminate such chemicals as acids and bleaching agents, and greatly reduce the produced chemical waste water. It was
conducive to environment-friendly production and had good economic and social benefits in production.

2. Materials and Methods

2.1. Materials

Gracilaria rubra, produced along the coast of Wanning City, Hainan Province. Food-grade caustic soda flakes (99%), diatomite filter aid (AR, 200 mesh), activated carbon powders (AR, 200 mesh).

2.2. Preparation of Agar

2.2.1. Pretreatment. Such impurities as mud, sand, and shells, were removed from Gracilaria rubra. The solution of NaOH with a mass fraction of 20% was heated to 65 º C, Gracilaria rubra was added, and the temperature was maintained for 10 hours. The alkali solution was removed, and the Gracilaria rubra was washed and soaked until the pH was 6.5.

2.2.2. Mechanical crushing. Water with 5 times the weight of dried Gracilaria rubra was added, and crushing was conducted until there were no obviously visible solids.

2.2.3. Filtering. Water was added into the Gracilaria rubra solution until 15 times the weight of the dried Gracilaria rubra was reached, and the mixture was heated to extract agar. Coarse filtering was conducted with a centrifugal filtering machine to remove most Gracilaria rubra cellulose and insoluble particles. And then, suction filtering was carried out with medium-speed filter paper as filtering medium to obtain agar solution.

2.2.4. Decolorization. The agar solution was decolorized with diatomite and activated carbon powders according to the following experimental design, and then was suction filtered, the decolorized agar solutions were obtained.

2.2.5. Cooling and dewatering. Cooling, strip-cutting, freeze dewatering and drying were carried out to get products.

2.3. Determination of Decolorization Ratio and Loss Ratio

The decolorization ratio was determined using an ultraviolet-visible spectrophotometric method. The agar solution was scanned in the wavelength range of 380‒780 nm to obtain the maximum absorption wavelength. The absorbance values of the agar solution before and after decolorization were measured respectively at the maximum absorption wavelength. The decolorization ratio and loss ratio were calculated with the following equations respectively:

\[
\text{Decolorization ratio (\%)} = \frac{A_0 - A_1}{A_0} \times 100\% \quad (1)
\]

where, \(A_0\) and \(A_1\) were the absorbance of the agar solution before and after decolorization, respectively.

\[
\text{Loss ratio (\%)} = \frac{B_0 - B_1}{B_0} \times 100\% \quad (2)
\]

where, \(B_0\) and \(B_1\) were the content of agar before and after decolorization, respectively.

2.4. Single-factor Tests

The influences of such process parameters as amount of activated carbon, decolorization time, and decolorization temperature on decolorization ratio were investigated, respectively. The decolorization ratio was determined at different amounts of activated carbon ranged from 0.15, 0.30, 0.60, 0.75, 0.90, 1.05 to 1.20 g/100mL, with the decolorization temperature fixed at 60 º C and the decolorization time fixed as 15 minutes. The different decolorization time with 0, 5, 15, 25, 35, 45 and 55 minutes was designed with the decolorization temperature fixed at 60 º C and the amount of activated carbon fixed at 0.60 g/100mL. The different decolorization temperatures at 60, 65, 70, 75, 80, 85, 90 and 95 º C
were investigated with the decolorization time fixed at 15 minutes and the amount of activated carbon fixed at 0.60 g/100ml.

2.5. Orthogonal Experiments
On the basis of the results of single-factor tests, L9(3)3 orthogonal experiments (three factors at three levels) were carried out with decolorization ratio as an evaluation index.

2.6. Diatomite-pretreated Decolorization

2.6.1 Slurry mixing method. 1.5% 200-mesh diatomite was added into the agar solution, the container was placed into a water bath at 75 ºC, the agar solution was stirred for 5 minutes at the constant temperature, and then suction filtering was conducted.

2.6.2 Pre-coating method. A proper amount of agar solution was taken and heated to 75 ºC, which was maintained, and 200-mesh diatomite was pre-coated with a thickness of 2‒3 mm on the filter paper in a Buchner funnel. The diatomite filter layer was wetted with distilled water at 75 ºC, and then suction filtering was conducted.

3. Results and Discussion

3.1. Determination of the Maximum Absorption Wavelength of Agar Solution
With distilled water as blank control, the original agar solution was scanned in the wavelength range of 380-780 nm. The absorbance decreased rapidly with the increase of wavelength in the range of 380-500 nm, and decreased gently with the increase of wavelength in the range of 500-780 nm. There was a small peak at the wavelength of 663 nm. According to the colour complementarity principle, the wavelength of 663 nm was selected as the maximum absorption wavelength for detecting the absorbance of agar solution. The determined absorbance of the original agar solution was 0.3362.

3.2. Effects of Amounts of Activated Carbon on Decolorization Rate and Loss ratio
The influences of different amounts of activated carbon on the decolorization rate with the decolorization temperature 60 ºC and the decolorization time 15 min were shown in Fig. 1. As can be discerned from Fig. 1 that both decolorization ratio of agar solution and the loss ratio of agar increased with the increase of the amount of activated carbon, but the increase rate of the decolorization ratio was greater than that of the loss ratio of agar. When the amount of activated carbon reached above 0.60 g/100mL, the loss ratio of agar increases rapidly. In respect of the yield of agar product, the amount of activated carbon should not exceed 0.60 g/100mL.
Figure 1. Effects of amounts of activated carbon on decolorization rate (a) and loss ratio (b)

3.3. Effects of Decolorization Time on Decolorization Rate
The effects of decolorization time with activated carbon on decolorization rate were shown in Fig. 2. With the increase of treatment time with activated carbon, the decolorization effect exhibited an ascending trend. When the decolorization time exceeds 30 min, the change in decolorization ratio tended to be gentle and the decolorization ratio did not increase evidently. In respect of improving production efficiency and reducing production cost, the decolorization time with activated carbon should not be over 30 min.

Figure 2. Effects of decolorization time with activated carbon on decolorization rate

3.4. Effects of Decolorization Temperatures with Activated Carbon on Decolorization Rate
The effects of different decolorization temperatures with activated carbon on the decolorization rate with the decolorization time 15 min and the amount of activated carbon 0.60 g/100mL were shown in Fig.3. Decolorization temperatures had some influence on the decolorization rate with activated
carbon as shown in Fig 3. With the increase of temperature, the decolorization rate increased gradually, reached its maximum at 80 °C, and then started to decrease. That may be that there was a dynamic process of adsorption and desorption for the combination of activated carbon and pigments. With the increase in temperature, the adsorption process surpassed the desorption process. However, after the temperature exceeded 80 °C, the desorption process surpassed the adsorption process, thus to reduce the adsorption of pigments on activated carbon, leading to the decrease of decolorization rate.

![Figure 3. Effects of decolorization temperatures on decolorization rate](image)

Based on the analysis of the above data and the comprehensive consideration of the lagging of agar coagulation, energy consumption in production, production efficiency and production cost, the following levels of three factors were selected for the orthogonal experiments study on the decolorization process with activated carbon: amount of activated carbon of 0.15-0.60 g/100mL, temperature of 60-80 °C, and decolorization time of 5-30 min.

3.5. Orthogonal Experiments for Decolorization with Activated Carbon

On the basis of single-factor test results, L₉(3)³ orthogonal experiments were carried out at different levels of amounts of activated carbon, decolorization times and temperatures. The levels of experimental factors were shown in Table 1, and the orthogonal experiment results were shown in Table 2.

### Table 1. Factors and levels for orthogonal design of decolorization with activated carbon

| Levels | Factors                  | Amount of activated carbon (g/100mL) | Decolorization time (min) | Decolorization temperature (°C) |
|--------|--------------------------|--------------------------------------|---------------------------|---------------------------------|
| 1      | A                        | 0.15                                 | 5                         | 60                              |
| 2      | B                        | 0.35                                 | 15                        | 70                              |
| 3      | C                        | 0.60                                 | 30                        | 80                              |
Table 2. Orthogonal experimental results and analysis of activated carbon decolorization

| Experiment No. | A | B | C | Decolorization ratio (%) |
|----------------|---|---|---|--------------------------|
|                | Amount of activated carbon (g/100mL) | Decolorization time (min) | Decolorization temperature (ºC) |
| 1              | 1 | 1 | 1 | 48.36 |
| 2              | 1 | 2 | 2 | 60.71 |
| 3              | 1 | 3 | 3 | 37.83 |
| 4              | 1 | 1 | 2 | 75.55 |
| 5              | 2 | 2 | 3 | 65.73 |
| 6              | 2 | 3 | 1 | 67.82 |
| 7              | 3 | 3 | 2 | 69.54 |
| 8              | 3 | 3 | 3 | 63.8 |
| 9              | 3 | 3 | 2 | 76.65 |
| k1             | 48.97 | 64.48 | 59.99 |
| k2             | 69.70 | 63.41 | 70.97 |
| k3             | 69.99 | 60.77 | 57.70 |
| R              | 21.02 | 3.71 | 13.27 |

Note: ki is the experiment value of i value of this factor. R is the maximum difference between the three ki values.

As can be seen from the results in Table 2, the optimum process scheme is A3B1C2, i.e., amount of activated carbon 0.6 g/100mL, decolorization time 5 min, and decolorization temperature 70 ºC. In addition, according to the range analysis, the sequence of the factors affecting the decolorization ratio of agar was A > C > B, that was, amount of activated carbon > decolorization temperature > decolorization time.

Table 3. Analysis of variance of orthogonal experiment results

| Factor                        | Sum of squares of deviations | Degree of freedom | F ratio  | F critical value | Significance |
|-------------------------------|------------------------------|-------------------|----------|-----------------|--------------|
| Amount of activated carbon (g/100mL) | 872.220                      | 2                 | 11.647   | 9.000           | *            |
| Decolorization time (min)     | 21.963                       | 2                 | 0.293    | 9.000           |              |
| Decolorization temperature (ºC) | 301.839                      | 2                 | 4.031    | 9.000           |              |
| Error                         | 74.89                        | 2                 |          |                 |              |

According to the analysis of variance, amount of activated carbon had significant influences on the decolorization ratio, while the other two factors had no significant influence. The optimum conditions did not fall in the scope of the above experiment combinations, so validation was carried out three times under these conditions to investigate the stability of the experiment. The obtained decolorization ratio of the agar solution was 76.85% and the colour of product was yellowish. It can be seen that the orthogonal experiment method was accurate and reliable in optimizing the conditions for decolorization with activated carbon.

3.6. Diatomite-pretreated Decolorization of Activated Carbon

Diatomite not only plays a role of filtration aiding, but also can effectively adsorb coloured substances, helping the decolorization of agar solution to lighten its colour. The decolorization ratios using the slurry mixing method and the pre-coating method were 75.5% and 74.7%, respectively. When
Diatomite was used as adsorption medium, the decolorization effect using the slurry mixing method was very close to that using the filter layer pre-coating method. Therefore, which one to use can be determined according to the experimental environment.

The agar solution was filtered using the diatomite slurry mixing method firstly, and then decolorized under the optimum conditions of activated carbon mentioned above. The decolorization rate of the obtained agar solution was 81.6%, and the agar solution was nearly colourless, clear and transparent. Therefore, it could be seen that a satisfactory decolorization result can be achieved when the optimum decolorization process with activated carbon was used on the basis of pre-treatment of diatomite.

4. Conclusions
The optimum conditions for decolorization with activated carbon were the amount of activated carbon 0.6 g/100mL, decolorization time 5 min, and decolorization temperature 70 ºC. On the basis of pre-treatment by using the diatomite slurry mixing method, the optimum decolorization conditions with activated carbon was used to decolorize agar solution, the best decolorization results were obtained and the agar solution was nearly colourless. The green process technology was simple, easy to operate and environmentally friendly.

5. References
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