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Bacterial Decolourization and Degradation of Azo Dye

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ABSTRACT

The compositions of dyes and pigments used in textiles and other industries generate the hazardous and toxic waste. Frequently, a dye is used to impart colour to materials which it becomes an important part in the textile industry. The dyes commonly found to contain the organic and inorganic substances, which contaminant that leading to the hazard and toxic to ecosystem and biodiversity causing impact on the environment. Nowadays, the most worried phenomenon when the amount of azo dyes concentration present in wastewater varied from lower to higher concentration that lead to colour dye effluent causing toxicity to biological ecosystem. The physical and chemical treatment does not remove the colour and reduce the dye compound concentration. Adsorption on the microbial biomass and bioremediation by the cells can takes place the decolourization of the dye. Bioremediation takes place by anaerobic and aerobic process. This experiment deals with the decolourization and degradation of Congo Red (CR) dye by bacteria, Pseudomonas euroginosa by aerobic conditions. The maximum degradation of 87.64 % was observed under aerobic condition within 5 days at pH 9 and temperature 26ºC. The degraded metabolites of CR dye were analysed by UV-Vis spectrophotometer. The effectiveness of decolourization and degradation of CR dye of bacteria is within 5 days in 25 ppm. Bacteria, Pseudomonas euroginosa decolorized several individual textile dyes, dye mixtures and textile industry effluent. Therefore, it is useful strain for the development of effluent treatment methods in the textile industries.

KEYWORDS: Bacteria Pseudomonas Euroginosa, Azo Dyes, Bacterial Decolourization, Bacterial Degradation, Wastewater Treatment

INTRODUCTION

The textile industry is one of the most problematic industries among manufacturing process. The industry uses an extremely large amount of water and also produces large volume of wastewater from its processes. Wastewater from textile industries constitutes a threat to environment in large parts of the world. It because wastewater comes from textile industries produces in large volume due to the processes such as dyeing and finishing processes. Thus, the industry uses huge amount of water and produces large volume of wastewater from its processes. Mostly wastewater generated from textile industries is contaminated mainly with synthetic dyes [1].

Azo dyes is the largest group of synthetic colorants and the most usual synthetic dyes released into the environment [2]. Azo dyes are characterized by one or more azo groups which nitrogen to nitrogen double bond (-N=N-) and it absorb light in the visible spectrum directly to their chemical structure [3]. Azo dyes are major group more than 3000 different varieties of all textile dyestuffs produces because of the ease and cost effectiveness of their synthesis, their stability and the variety of colour available compared to natural dyes [4]. They are spread widely used in the textile, paper, food, leather, cosmetics and also pharmaceutical industries [3]. Reactive azo dye used is Congo Red. The IUPAC name of Congo Red dye is sodium 3,3'-([1,1'-biphenyl]-4,4'-diyl) bis(4-aminonaphthalene-1-sulfonate). Congo Red is a synthetic azo dye used widely in textile dyeing process. The removal of dyes from effluents is very required because many azo dyes and their breakdown products from the process are toxic to aquatic life and mutagenic to humans [5]. This problem can affect the aquatic life and creates serious problem on human health.

Each industry has different types of treatment methods for wastewater. Nowadays, the general treatment methods for removal of dyes from wastewater effluent are divided into three methods which are chemical methods, physical methods and biological methods. Among all methods, the biological methods is the most effective and being environmentally-friendly compare to the other two methods which proven by researches on their advance investigation. The textile effluents comes from textile industries contain large concentration of colour. Nowadays, the release of coloured dyes into rivers affects photosynthesis which decreasing oxygen levels and pH in water bodies. This problem will cause the aquatic ecosystem harmful [6]. Thus, azo dyes may be toxic and produce carcinogenic aromatic amines after the metabolic reduction of azo bond.

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In this research, the treatment method focuses under biological methods which microorganism process using bacterial by aerobic conditions. The mechanism of microbial degradation of azo dyes involves the reductive cleavage of azo bonds (–N=N–) with the help of azoreductase enzymes under anaerobic conditions that resulted in the formation of colourless solutions containing potentially hazardous-aromatic amines [7]. The presence of the pollutant in the water system creates very serious environmental problem. Thus, the discharge of azo dye is a major concern due to the colorization of the water which will interferes the growth of plants in the water ecosystem. In this study, microorganisms including bacteria can decolorize and even completely mineralize many azo dyes under certain environmental conditions. Many reviews are available on the microbiological methods for decolourization of azo dyes [8]. It focuses on the pathways and mechanisms by which aerobic and anaerobic bacteria decolorize azo dyes and degrade the aromatic amines generated by the reaction.

The target pollutant is Congo Red (CR), a synthetic azo dye used widely in textile dyeing process. This study focused on the efficiency of removal decolourization through biological treatment by bacteria on stimulated industrial wastewater as a culture medium. This study also to investigate the degradation under aerobic conditions. The main objective of this study is to investigate the decolourization and degradation of azo dyes of bacteria by aerobic conditions. The influence of the different experimental conditions that affect the decolourization of azo dyes, which is pH and temperature were investigated.

MATERIALS AND METHODS

Materials

All materials in this experiment were prepared in the laboratory and of analytical grade. The model dye, Congo Red was purchased from manufacturing company. The Congo Red dye used because it solubility in water. The Congo Red dye was prepared in distilled water and used for all experiments. The instrument UV-Vis Spectrophometer-UV 1800 Shimadzu was used in this experiment. The model dye is Congo Red will be purchased from manufacturing company and a stock solution of the dye (10^{-3}M) was prepared in distilled water. All materials was prepared in the laboratory.

Methods

Bacteria, Pseudomonas euroginosa were used in this research. The percentage of degradation of Congo Red dyes in certain parameters was studied. 250 ml of conical flask which contain 0.4 ml of nutrient broth and 10 ml of 5 ppm of Congo Red solution was autoclaved under 121^{o}C within 15 minutes. 1 ml of bacteria was added into 250 ml of conical flask which contains the mixture of solution. The pH meter was dipped into the solution and adjusted at pH 7. The solution was incubated for 3 days by using the incubate shaker. The reading of pH and temperature was recorded. The suspension was centrifuged under 4000 rpm within 30 minutes. The supernatant liquid was filtrated using filter funnel with filter paper. 3 ml of the sample liquid was obtained and monitored by UV/Vis spectrophotometer instrument to get the absorbance reading of the degradation of the samples. All the method was repeated with present of bacteria in a concentration of 10 ppm, 15 ppm, 20 ppm and 25 ppm respectively in 5 days and 7 days.

RESULTS AND DISCUSSION

Effect of Different pH of Congo Red Dye in Different Concentration

Based on the experiment, it was proven that bacteria Pseudomonas euroginosa effectively degrades the Congo Red dye in a several days. The effectiveness of the degradation also increases with the increases of concentration. Figure 1-5 shown the graph of pH against days interval at 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm respectively.

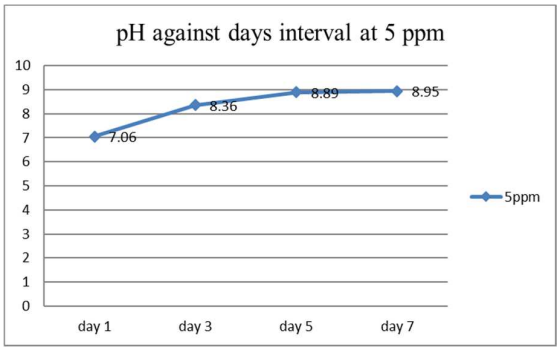


Figure 1: The graph of pH against days interval at 5 ppm

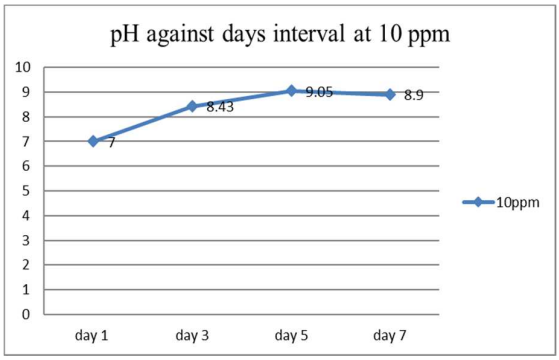


Figure 2: The graph of pH against days interval at 10 ppm

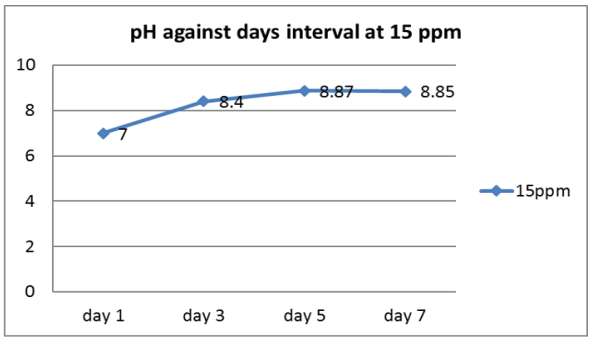


Figure 3: The graph of pH against days interval at 15 ppm

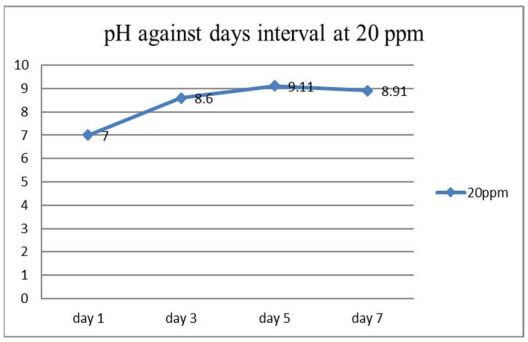


Figure 4: The graph of pH against days interval at 20 ppm

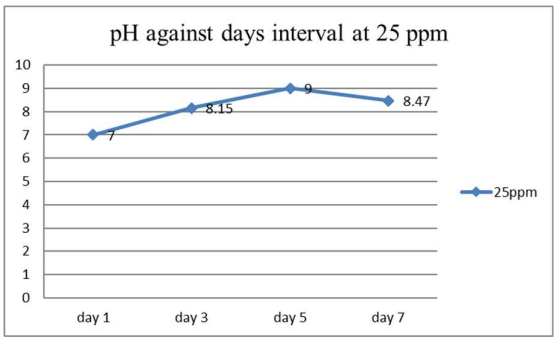


Figure 5: The graph of pH against days interval at 25 ppm

The important factor in decolourization of azo dye is pH medium. It has a major effect on the efficiency of dye decolourization. Generally, the optimal pH for colour removal is between 6.0 and 10.0 [9-11]. The rate of colour removal is increase at the optimum pH. It may tend to decrease rapidly at strong acid or strong base pH. This may be related to the transport of dye molecules across the cell membrane, which considered as the rate limiting step for the decolourization [2, 12]. Biological reduction of the azo bond can also increase in pH due to the formation of aromatic amine metabolites which are more alkaline than the raw azo compound.

Effect of Different Temperature of Congo Red Dye in Different Concentration

Based on the experiment, it was proven that bacteria, Pseudomonas euroginosa effectively degrades the Congo Red dye in a several days. The effectiveness of the degradation also increases with the increases of concentration. Figure 6-10 shown the graph of temperature against days interval at 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm respectively.

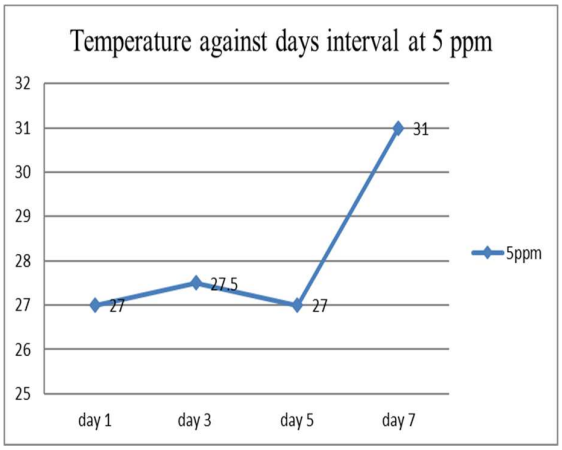


Figure 6: The graph of temperature against days interval at 5 ppm

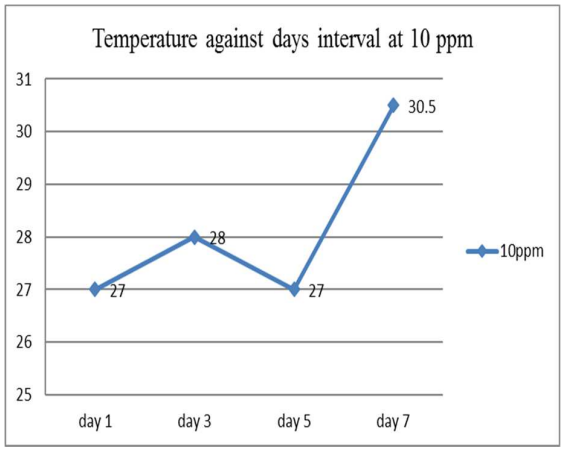


Figure 7: The graph of temperature against days interval at 10 ppm

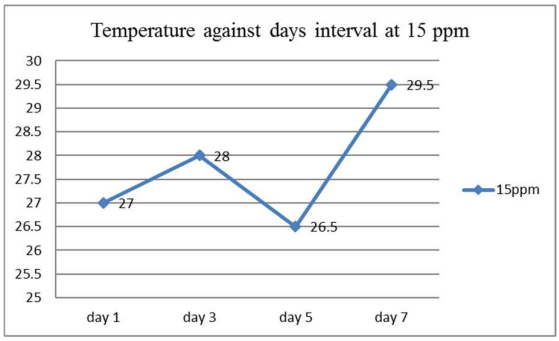


Figure 8: The graph of temperature against days interval at 15 ppm

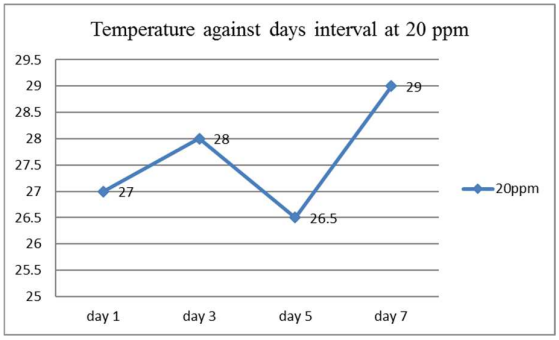


Figure 9: The graph of temperature against days interval at 20 ppm

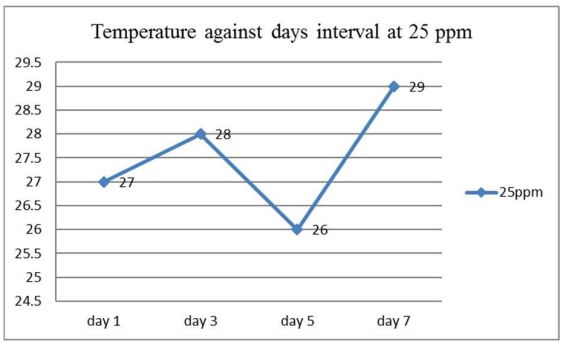


Figure 10: The graph of temperature against days interval at 25 ppm.

Temperature is also an important factor for all processes associated with microbial vitality. There are some studies of microbial decolourization of azo dyes dealing with the activation energy, which give narrow temperature ranges for decolourization of azo dyes by extremely complex consortia of microorganisms inhabiting active sludge [7, 13]. In addition, microbial physiology temperature changes lead to a sudden alteration of the activation energy [14]. The decline at higher temperature can be attributed to the loss of cell viability or the denaturation of an azo reductase enzyme [2, 1]. However, certain whole bacterial cell preparations the azoreductase enzyme is relatively thermostable and can maintain active up to 60^{o}C over short periods of time [15].

Changes of Colour of Congo Red Dye in Different Concentration

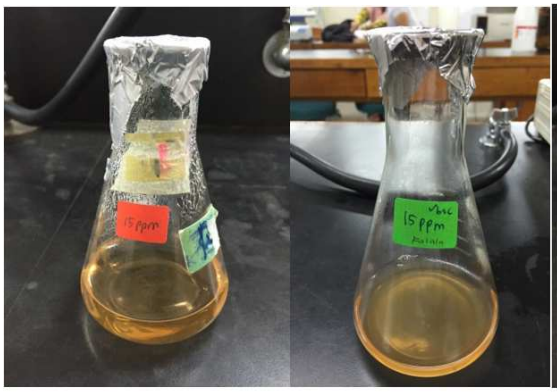
Based on the experiment, it was proven that bacteria Pseudomonas euroginosa effectively degrades the Congo Red dye in a several days. The effectiveness of the degradation also increases with the increases of concentration. Figure 11-15 shown the changes of colour of Congo Red dye in the absence of bacteria and Congo Red dye in the presence of bacteria in 7 days at 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm respectively.

A B

Figure 11: (A) Congo Red dye in the absence of bacteria in 5 ppm, (B) Congo Red dye in the presence of bacteria in 5 ppm

A B

Figure 12: (A) Congo Red dye in the absence of bacteria in 10 ppm, (B) Congo Red dye in the presence of bacteria in 10 ppm



A B

Figure 13: (A) Congo Red dye in the absence of bacteria in 15 ppm, (B) Congo Red dye in the presence of bacteria in 15 ppm

A B

Figure 14: (A) Congo Red dye in the absence of bacteria in 20 ppm, (B) Congo Red dye in the presence of bacteria in 20 ppm

A B

Figure 15: (A) Congo Red dye in the absence of bacteria in 25 ppm, (B) Congo Red dye in the presence of bacteria in 25 ppm

Degradation of Congo Red Dye

A control concentration was done using Congo Red dye, which was reacted with the presence of bacteria and the absence of bacteria. The absorbance and the wavelength of the dye at 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm for 3 days, 5 days and 7 days were analysed using HACH DR/4000 UV-Vis spectrophotometer.

Table 1: The blank absorbance of Congo Red dye in absence in bacteria

Concentration (ppm)	Absorbance
5	0.224
10	0.451
15	0.662
20	0.897
25	1.116

From Table 1, the absorbance read for 5ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm was 0.224, 0.451, 0.662, 0.897 and 1.116. The absorbance then gradually increased from 0.224 to 1.116 by the increased of the Congo Red dye. All this value was used throughout the experiment as the blank absorbance of the dye which was Congo Red absence in bacteria before the reaction process begin. The UV-Vis spectrum of the dye was recorded from 350 nm to 500 nm using a UV-Vis spectrophotometer with a spectrometric quartz cuvette (1 cm path length). The maximum absorbance wavelength (λ_{max}) of Congo Red is 497 nm. Therefore, the concentration of the dye in the solution and its degradation rate as different in days were determined by measuring the absorption intensity at (λ_{max}) = 497 nm.

The results of the efficiency in the dye degradation rate were expressed in percentage (%) according to the following relation:

$$\% \text{ Degradation} = 1 - \frac{C}{C_O} \times 100\% \quad (1)$$

where C_o the initial Congo Red dye absorbance concentration and C is the absorbance concentration of Congo Red dye at several days. From Table 2, the absorbance of Congo Red dye in presence of bacteria for 5 ppm, 10 ppm, 15 ppm, 20 ppm and 25 ppm were not constantly increased. This may be due to the overall reaction of the degradation has happened between Congo Red dye and bacteria.

Table 2: The absorbance of Congo Red dye in presence of bacteria in 3 days, 5 days and 7 days

Concentration (ppm)	Absorbance		
	3 days	5 days	7 days
5	0.187	0.162	0.166
10	0.148	0.108	0.110
15	0.181	0.121	0.140
20	0.232	0.153	0.170
25	0.160	0.138	0.158

From the tabulated data, the decreases in the absorbance of the dye in two conditions; the absence of bacteria and the presence of bacteria can be compared. The absorbance of the dye using the presence of bacteria much lower than the absence of bacteria for all the concentration in 3 days, 5 days and 7 days. From the absorbance reading, the percentage of degradation can be calculated by using the percent degradation formula. The percent degradation efficiency of Congo Red dye at both conditions is shown in Figure 16.

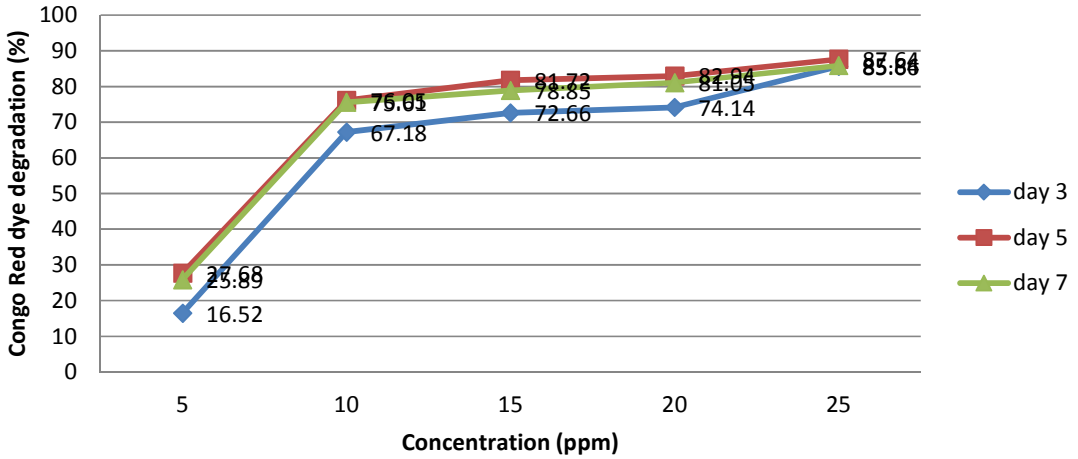


Figure 16: The percentage of efficiency of degradation at two different conditions

The percentage of efficiency of degradation at two different conditions for 5 ppm within 3 days, 5 days and 7 days are 16.52 %, 27.68 % and 25.89%. For 10 ppm, the percentage of efficiency of degradation at two different conditions within 3 days, 5 days and 7 days are 67.18%, 76.05% and 75.61%. Besides, for concentration 15 ppm, the percentage of efficiency of degradation at two different conditions within 3 days, 5 days and 7 days are 72.66%, 81.72% and 78.8%. Moreover, 74.14%, 82.94% and 81.05% are the percentage of efficiency of degradation at two different conditions within 3 days, 5 days and 7 days for concentration 25 ppm. For the last concentration 25 ppm, the percentage of efficiency of degradation at two different conditions also within 3 days, 5 days and 7 days are 85.66%, 87.64% and 85.84%.

The lowest percentage of efficiency of degradation at two different conditions is 16.52% at 5 ppm within 3 days at pH 8.36 and 27.5°C. Meanwhile, the highest percentage of efficiency of degradation at two different conditions is 87.64% at 25 ppm within 5 days at pH 9 and 26°C. The degradation of the dye is mainly due to the effect of parameters in decolourization. It is because azo dye binds to cotton fibers by addition or substitution mechanism under alkaline conditions [16]. Besides, there are nonspecific enzymes catalysing azo bond reduction have been isolated from aerobically grown cultures of Pseudomonas euroginosa [17]. If characterized, these enzymes have been shown to be flavoproteins.

According to [18], it has reported that intracellular sulfonated azo dye reduction not only required the presence of azo reductases but also required a specific transport system which allows the uptake of the dye into thee cells. Therefore, there appears to be no available information about the transport systems for these dyes. Although, there are few reports on systems which are involved in the transport into bacterial cells of other sulfonated substrates such as p-toluene sulfonate, taurine and alkane sulfonates [19].

CONCLUSION

The experiment proved that bacteria, Pseudomonas euroginosa was efficient in the degradation of Congo Red dye solution. The lowest percentage of efficiency of degradation at two different conditions is 16.52 % at 5 ppm within 3 days at pH 8.36 and 27.5ºC, whereas the highest percentage of efficiency of degradation at two different conditions is 87.64 % at 25 ppm within 5 days at pH 9 and 26ºC. The experiment also proved that the decolorization of Congo Red dye was recorded in the presence of bacteria, Pseudomonas euroginosa. The effectiveness of degradation of Congo Red dye of bacteria by aerobic condition is within 5 days in 25 ppm. The bacteria, Pseudomonas euroginosa could be potential to degrade for the treatment of textile dyestuffs and textile industry effluent as compared to other biological sources.

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