Studies on some selected microorganisms for biodegradation of congo red

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Abstract. The color pollution is typical in the textile wastewater and requires a lot of costs for treatment. This study aim to investigate the ability to degrade Congo Red dye in the group of popular diazo dyes by selected strains which were isolated from typical textile dyes in Vietnam. Some influence factors such as shaking conditions, adding sugar sources, changing culture media and combination of single strains to decolorization performance were also assessed using the Congo Red calibration through UV-Vis spectrometers. The results show that the isolation obtained in textile dyes wastewater 8 strains capable of Congo Red decolorization, the best strain was \textit{Aspergillus Niger} which degraded nearly 90\% of Congo Red 200mg/L after 14 days. The influence of shaking conditions of 180 rpm will better 15\% decolor performance than static conditions and the addition of glucose also increases treatment time efficiency. The changing medium of PDA and mineral medium Czapez will have certain effects on the effectiveness of the treatment process. Expecially, the combination of strains of \textit{Aspergillus Niger} and \textit{Streptomyces Thermodiastaticus} illustrated that the Congo Red degradation performance increased by nearly 10\% and the contact time was reduced to 50\%. Overall, some selected strains showed good decolorization ability after 14 days, which compares to the control sample.

1. Introduction
Textile industry is one of Vietnam's high profit industries with more achievements which have the second largest export turnover with export value contributing from 10 to 15\% to the country's GDP, accounting for more than 10\% of industrial workers nationwiden. Thus, Vietnam's textile is an important industry for the country's development [1]. Beside the greatly contribution to the economic development of the country, synthetic dyes bring many negative impacts on the ecological environment. The most common groups of dyes in the world today were recorded such as the azo
group (N=N), the ethylene group (=C=C=), the methine group (-CH=), the carbonyl group (=C=O), carbon-nitrogen (=C=NH;CH=N), carbon-sulfur (=C=S; CSSC=), nitro (-NO2; -NO-OH), nitroso (-N=O;=N-OH) or chinoid group [2].

According to some research statistics, azo dyes group are the largest and most versatile organic dyes. It's also one of the most commonly used dye groups in the world [3] which widely used in textile, printing, cosmetics, pharmaceutical, food processing industries and laboratories [4]. Today, faster industrialization leads to the increasing demand for azo dyes because of their high color characteristics and stable N-N= arene structure. [5]. At least 3,000 azo dyes are used for various purposes in the textile, paper and food industries and to a lesser extent than are used in laboratory studies. [6]. There are more than 10,000 common color index names (CI) assigned to commercial pigments; about 4,500 are being used and more than 50% of these belong to the azo dye group [7]. It is worth noting that the loss of dyestuffs during dyeing has resulted in 10% - 15% of dyestuffs not being used which is flowing directly into wastewater. [8] Residue of dyes bring the risk of affecting the environment and human health. Thereby, some data are recorded that shows that in countries with high industrialization, cancer becomes more common and correlates between the increase in cancer cases and the amount of use of azo dyes [9]. Direct exposure to polluted water sources exposed to human skin or through daily activities that is a very common occurrence of people living along the river. Thus, concerns about the greatest pollution potential of textile dyes are primarily due to the apparent toxicity and carcinogenicity of the originating ingredients such as benzidine and other aromatic compounds. [10]. The chemical structure of many azo dyes is mainly to compounds containing p-phenylenediamine and benzidine radicals [11] which are carcinogenic components [12]. Many studies have reported a high incidence of bladder cancer among workers exposed to aromatic amines 2-naphthylamine, benzidine and 4-aminobiphenyl [13] through respiratory exposure and eating. Therefore, the treatment of the Azo group such as Congo Red is particularly concerned when this group of dyes enters natural water sources, which will lead to bioaccumulation causing malignant diseases of human health [14].

In fact, textile dye wastewater is often treated by chemical and physical processes to achieve the better color reduction, but these methods require a high treatment cost and release many hazardous wastes which are arduous to decompose. To overcome these disadvantages, biological treatment has received more attention due to cost effectiveness, less sludge generation and environmental friendliness [15]. Dye decomposition by biological processes has been developed including aerobic and anaerobic microorganisms, especially white rot fungi. However, this study has two major limiting factors when used in industry such as a lignocellulosic substrate is required and many species are difficult to grow, besides that, it gives an unclear effect because of biological absorption. The effectiveness of microbial decontamination has been of great interest in recent years such as Ruijing Li et al who studied the fading and biodegradation of CR color by Acinetobacter baumannii YNWH 226 with the aim of reducing Congo Red in aerobic conditions [16]. NaWang et al has found a new fungus, Ceriporia lacerata, isolated from decaying mulberry limbs capable of degrading the Congo Red [17]. In addition, Laila Abdelfattah Sallam also studied biological treatment of textile wastewater with Chlorella Vulgaris algae also obtained initial positive results [18]. In overall, the complete biological treatment of dyes is a new trend in the world because this treatment method is affordable and ecological. However, in Vietnam there are still few and many limitations, most textile wastewater treatment currently focus on chemistry and physiochemistry methods, although them spent huge processing costs.

In this study, the main objectives are to isolate and select strains which are capable to degrade Congo Red dye in a typical Vietnam's textile dye wastewater. In addition, it’s also search of various influences impact to decolorization performance. In the future, we would like to have a biological treatment solution with a synthetic strain capable of handling multiple colors, especially in the azo dye group. Experimental results can be used as a premise for methods of treating textile wastewater by bio-process that are more environmentally friendly and economical for the dyeing industry in Vietnam.
2. Materials and methods

2.1. Research materials

The textile wastewater before treatment was taken in Hung Phat Dat Dyeing Textile Co., Ltd. at Road No. 3, Xuyen A Industrial Park, My Hanh Bac Commune, Duc Hoa District, Long An Province, Vietnam. All chemicals (with the highest purity available) and culture media were purchased from Merck (Germany). The tested microorganism was grown on Potato Dextrose Agar (PDA) medium.

2.2. Methodological approach and experimental design

E1. Isolation of microbial strains from textile wastewater

Textile waste water is collected and enriched in PDA medium, then it was implanted onto PDA agar medium with 200mg/L Congo Red added. Medium discs after transplanting were stored in a microbiological incubator at 30 degrees in 2 days. Identification signs to catch strains through color resolution rings are created by strains on Congo Red background in agar plates.

E2. Qualitative assessment of laccase enzymes of VSV strains isolated on PDA with reagent guaiacol

To detect the production of laccase, guaiacol (0.01%) was added to the PDA medium as an indicator. Laccase production is revealed by a zone of red precipitation around the fungal colony, and the size and shade of the red zone can be used to estimate the amount of laccase produced [19]. Microorganisms are cultivated on microbiological media and stored in a microbiological incubator at 30 degrees. The experiment repeated triple times for each microbial strain and the comparison sample doing the same but not added microbial strain.

E3. Quantitative testing of the ability of Congo Red dye decolorization of strains with standard Congo Red calibration lines

Measuring 100 mL of PDA medium and 100 mL Congo Red 200mg/L in erlenmeyer triangle flask 250ml. After that, adding 100µl/strains proliferation (at concentration 10^7) of isolated strains. The experiment repeated triple times. The control sample doing the same added material but not added any microbial strain. Finally, using the spectrometers (UV-VIS Thermo scientific- Evolution 60S) with wavelengths 498 nm to measure Congo Red concentration.

E4. Evaluating of shaking on decolorization

Assessing on the effect of experiment condition: shaking state and static state to the ability decolor of selected strains, experimental processes are operated on the shaker at 185 rpm and doing under laboratory temperature conditions. Assessing on the effect of adding a different kind of carbon source into the culture on the decolorization ability of different carbon sources: Glucose and Sucrose, which were added into samples with an additional amount of 5g/L. Assessing on the effect of changing the culture medium by comparing the decorization performance of selected strains on PDA culture and Czapek culture.

E5. Assessing on Congo Red degradation by strains combination
Study of Aransiola et al shows that the effectiveness of microbial strains can be improved through the combination of strains [20]. Our experiment used 200 mL of PDA medium supplemented with 100mL (concentration of 200 mg/L) dye into a 250ml sterile flask. After that, using pipette draw 50µl/strains of microorganisms into the flask, each treatment combines 2 strains follow table 1. The experiment was performed with triple replicates for each pair of strains and each type of dye. The comparison sample doing as the same process but not adding any strains of microorganisms. The duration of experiment was 7 days.

Table 1. Code of combining strain in experiment

| NUM | CODE | NUM | CODE |
|-----|------|-----|------|
| 1   | R7-2 | 6   | R2-4 |
| 2   | R7-5 | 7   | R2-3 |
| 3   | R7-4 | 8   | R5-4 |
| 4   | R7-3 | 9   | R3-5 |
| 5   | R2-5 | 10  | R4-3 |

3. Results and discussion

3.1. Isolation result of microbial strains from textile wastewater

The isolation result from textile wastewater has obtained 8 capable microorganism strains of resolving CongoRed color, the average resolution diameter ranges from 17 mm to 47 mm. Details of strains are described in the following table:

Table 2. Description and qualitative color resolution ring diameter of capable microorganism strains of resolving Congo Red color from textile wastewater

| Code | Decolor ring diameter (mm) | Photos of strains on Congo Red agar plate | Description of the strain |
|------|---------------------------|------------------------------------------|--------------------------|
| R1   | 26                        | ![Image](image1.png)                    | Fungus, grow in surface, edges rounded edges stretch, the yellow circle |

R2   | 17                        | ![Image](image2.png)                    | Fungus, grow in surface, edges rounded edges stretch, the red circle |
Table 2. Continued

| Code | Decolor ring diameter (mm) | Photos of strains on Congo Red agar plate | Description of the strain |
|------|----------------------------|------------------------------------------|---------------------------|
| R3   | 32                        | ![R3](image)                             | Fungus, grow in surface, edges rounded, stretch, the red circle |
| R4   | 37                        | ![R4](image)                             | Fungus, grow close to the surface, with the edges rounded and red colour |
| R5   | 33                        | ![R5](image)                             | Fungus, grow close to the surface, fringe edge distortion, spread, red round |
| R6   | 30                        | ![R6](image)                             | Fungus, grow in surface, edges rounded, stretch, the red circle |
Table 2. Continued

| Num | Code | Enzyme generation ability |
|-----|------|--------------------------|
| 1   | R1   | ++                       |
| 2   | R2   | ++                       |
| 3   | R3   | +++                      |
| 4   | R4   | +++                      |
| 5   | R5   | +++                      |
| 6   | R6   | +++                      |
| 7   | R7   | +++                      |
| 8   | R8   | +++                      |

*Note: Hierarchical levels of laccase enzyme secretion as follows: "++": brown ring diameter around the colonies in 10-30 mm; +++": 30-50 mm; ++++: 50-60 mm.*

From the results of color resolution analysis on PDA culture medium, all isolated microorganism strains have the ability to resolve Congo Red color, the average resolution diameter ranges from 17 mm to 47 mm in which R2 and R6 strain has the lowest resolution with a resolution diameter of 17 mm and the R7 has the best resolution with a resolution diameter of 47 mm.

3.2. Qualitative test on laccase enzyme of isolated microorganism strains on PDA culture medium with guaiacol reagent

Qualitative test results in the secretion ability of laccase enzyme of 8 isolated microorganism strains in PDA culture medium supplemented with 4 mM of guaiacol reagent after 6 days are presented in table 3:

Table 3: The ability identification of laccase enzyme secretion of 8 isolated microorganism strains on PDA culture medium after 6 days of culture.

| Num | Code | Enzyme generation ability |
|-----|------|--------------------------|
| 1   | R1   | ++                       |
| 2   | R2   | ++                       |
| 3   | R3   | +++                      |
| 4   | R4   | +++                      |
| 5   | R5   | +++                      |
| 6   | R6   | +++                      |
| 7   | R7   | +++                      |
| 8   | R8   | +++                      |

*Note: Hierarchical levels of laccase enzyme secretion as follows: "++": brown ring diameter around the colonies in 10-30 mm; +++": 30-50 mm; ++++: 50-60 mm.*
The results showed that 8 isolated microorganism strains were able to secrete the laccase enzyme to oxidize the guaiacol and resulted in a brown halo ring around and below the microorganism strain. In the 8 microbial strains showing the positive ability to secrete the laccase enzyme, R7 and R3 strains created high activity of laccase enzyme. The strains of R4, R5, R6 and R8 have the ability to create enzyme laccase in medium level and the strains of R1 and R2 in low level.

3.3. The quantitative evaluation results of the ability to Congo Red color treatment of strains

In the control sample at the initial time, the control sample had a concentration of 0.193 g/L. After 7 days of culture, the control sample decreased to 0.155 g/L (19.69% reduction compared to the original control sample) and the following days the control sample continued to decrease slightly. By 14 days, the concentration remaining had 0.133g/L (31.09% reduction compared to the original control sample). In the remaining samples at the beginning, 8 samples containing microorganism had an initial concentration of 0.193 g/L. After 7 days, Congo Red dye concentration significantly decreased, ranging from 0.052 to 0.110 g/L in which the R7 strain was able to resolve Red Congo color best with the remaining concentration of 0.052 g/L (73.06% reduction compared to the original control sample), whereas the R4 strain was able to resolve Red Congo color weakest with the remaining dye concentration of 0.110 g/L (43.01% reduction compared to the original sample). In the following days, Congo Red dye concentrations continued to decrease. By 14 days, the concentration of Congo red dye in the samples ranged from 0.021 to 0.088 g/L, in which the R7 strain reduced best with the remaining concentration of 0.021 mg/L (89.12% reduction compared to the original control sample), the R4 strain is the weakest strain with the remaining concentration of 0.088 g/L (54.40% reduction compared to the original control sample).

In addition, this research also evaluated on the Pt-Co scale, the original Congo Red concentration was 0.193 g/L corresponding to 9100 units on the Pt-Co calibration curve. As a result, the evaluation results on the Congo Red and Pt-Co scales are almost proportional and same attenuation. Therefore, the following experiments will focus on evaluating and comparing based on the concentration calibration curve of Congo red. Thus, all of the 8 tested microorganism strains showed that the Congo red resolution is quite good, especially the R7 strain with the highest resolution of Congo red dyes (89.12% reduction compared to the original control sample).
3.4. Evaluate the effect of environmental factors on the ability of Congo red decolorization

3.4.1. Effect of the shaking conditions on decolorization productivity

After 14 days, the results showed that the resolution of R7 strain in the shaking state is better in non-shaking state (1); Additional carbon source of Glucose is better than Sucrose (2); the resolution of czapek culture is better than liquid PDA culture for microorganism (3).

Experimental results affecting by shaking and non-shaking conditions (Figure 7A) showed that the non-shaking Control sample is almost unchanged with an initial concentration of 0.197 g/l. With shaking samples, the Control model dropped slightly after 7 days with 0.169 g/l (14.21% reduction compared to the original sample), after the 14th day, the concentration dropped to 0.15 g/l (23.86% reduction compared to the original sample). For strain of R7, it is shown that shaking conditions has the result in better of color removal than non-shaking conditions. After 7 days of culture, the shaking state sample was significantly decolored with a concentration of 0.089 g/L (corresponding to 54.82% compared to the original), the non-shaking state sample also decreased significantly with the remaining concentration of 0.101 g/L (48.73% reduction compared to the original). However, on the 14th day, the dye concentration of R7 strain was only 0.045 g/L (77.16% reduction compared to the original) in shaking state and 0.074 g/L (62.44% reduction compared to the original) in a non-shaking state. Statistical results show that the difference of shaking method was significant compared to
Control state with Sig error = 0.03 <0.05. There was no significant difference between the non-shaking state and Control sample due to Sig error of 0.142 > 0.05. From that, it can be seen that PDA shaking state has a significant difference compared to PDA non-shaking state and this difference is positive.

Therefore, the decolorization results in shaking condition are better than in non-shaking condition, which can partly be explained by the shaking condition that increases the amount of transferred oxygen from the culture medium to the cell, thus generating the microbial biomass and enzyme production [21]. The shaking state on the shaker showed the best decolorization ability which is consistent with the research results of Faison and Kirk [22] and Ge et al [23]. The authors argue that fungy is aerobic organisms that show the best decolorization under dynamic conditions with regular stirring. The results published by Knapp et al [24], show that the decolorization ability reached only 45% when the experiment is arranged in the non-shaking condition, while 97.5% of the dye is resolved when the experiment is performed under shaking condition.

3.4.2. Effect of adding Glucose and Sucrose sugar on decolorization performance.

The results showed that the amount of dye remaining in the 14 culture days of the state with supplement Glucozo sugar was much lower than the rest of the state followed by the supplement with Sucrose sugar. In detail:

At the beginning of the Control sample, the initial concentration was 0.199 g/L. The Control sample decreased slightly after culture days. On day 7, the concentration in Control sample decreased by 0.18 g/L (9.55% reduction compared to the original sample). By day 14, the control sample was only 0.165 g/L (17.06% reduction compared to the original sample).

Samples containing R7 strain supplemented with Glucose and Sucrose sugar had a concentration of 0.199 g/L. In the following days, the samples all changed. By day 7, the R7 sample supplemented with Glucose sugar significantly decreased with the remaining concentration of 0.077 g/L (61.31% reduction compared to the original), the R7 sample supplemented with Sucrose sugar also decreased with the remaining concentration of 0.151 g/L (24.12% reduction compared to the original).

In 14th day of experiment, R7 strain with addition of Glucose was only 0.065 g/L (67.37% reduction compared to the original), R7 strain with addition of Sucrose was only 0.104 g/L (47.74% reduction compared to the original). ANOVA statistics showed that the difference of supplemented with Glucose sugar state was significant compared to Control state with the error of Sig = 0.003 <0.05. There were no significant differences in supplemented with Sucrose sugar state compared to Control.

![Figure 4. Evaluation results of the impact by shaking and static conditions](image-url)
sample due to Sig error = 0.051 > 0.05. Since then, the state of Glucose addition has significant differences compared to Sucrose addition and this difference is positive. Thus, the state of Glucose addition had better result than the sucrose addition, which was also consistent with the study of YangGe [25].

Figure 5. The evaluation results of the effect in adding sugar source to the decolorization performance on Congo Red

The more effective of Glucose is explained by this is a single sugar, a form that can be easily used for most strains of microorganisms growth [26]. Different microorganism strains use the different Carbon sources for the best decolorization ability. The source of Carbon is considered as the main nutrient in the decolorization ability of the microbial dye and the most readily carbon source to be used for most strains of the bacterium is Glucose [27-29].

3.4.3. Comparison of decolorization performance of microorganisms developed on PDA and Czapek culture

Figure 6. The evaluation results of the effect of different culture media on the decolorization performance on Congo Red
The results after 14 days showed the graph comparing two PDA and Czapek culture media of the R7 strain. Based on the graph, it can be seen that after 14 days of culture test, the R7 strain in PDA culture decreased to a concentration of 0.045 g/L (77.16% reduction compared to the original) whereas R7 strain in the Czapek culture dropped to a concentration of 0.05 g/L (74.62% reduction from the original).

The result of Control sample represented the average similarity between Control PDA and Control Czapek. Statistics showed that the difference of PDA shaking method is significant compared to Control shaking method with Sig error = 0.036 <0.05, while Czapek shaking method also has a significant difference compared to Control sample with wrong Sig number = 0.023 <0.05. Thus, the result showed that the decolorization ability on Congo Red in PDA and Czapek cultur e media is nearly the same, both cultures are good culture media for the R7 strain to grow. This is a little different from Torres' research that showed that in PDA culture, the decolorization ability on Crystal Violet is more effective than the Czapek culture [30]. It is also possible for each different culture to be suitable for different strains of microorganisms. Therefore, this factor needs to be tested more times and various colors.

3.5. The quantitative Congo Red discoloration test on strain combination
Through 7 days of testing, 10 experimental samples for decolourization performance are as follows:

| Num | Samples | Performance of first strain | Performance of second strain | Performance of combination strain | Note       |
|-----|---------|-----------------------------|------------------------------|----------------------------------|------------|
| 1   | R7-2    | 98%                         | 83.4%                        | 59.1%                            | Antagonism |
| 2   | R7-5    | 98%                         | 75.7%                        | 74.4%                            | Antagonism |
| 3   | R7-4    | 98%                         | 86.9%                        | 98%                              | Symbiosis  |
| 4   | R7-3    | 98%                         | 84.8%                        | 97.3%                            | Symbiosis  |
| 5   | R2-5    | 83.4%                       | 75.7%                        | 78.5%                            | Antagonism |
| 6   | R2-4    | 83.4%                       | 84.8%                        | 84.8%                            | Symbiosis  |
| 7   | R2-3    | 83.4%                       | 86.9%                        | 83.4%                            | Not effective |
| 8   | R5-4    | 75.7%                       | 84.8%                        | 88.0%                            | Symbiosis  |
| 9   | R5-3    | 75.7%                       | 86.9%                        | 92.4%                            | Symbiosis  |
| 10  | R4-3    | 84.8%                       | 86.9%                        | 69.5%                            | Antagonism |

After evaluation, 5/10 strain pairs of Congo Red dye colorants were selected including: R7-4; R7-3; R2-4; R5-4; R5-3

At the initial time, the Control sample had a concentration of 0.196 g/L. After 3 days of culture, the Control sample decreased to 0.191 g/L (2.8% reduction compared to the original Control) and the following days the Control sample continued to decrease slightly. After 7 days of culture, the residual concentration was 0.184 g/L (a slight decrease of 6.3% compared to the original).

In other states at initial time, 5 samples containing the pair of microorganism were at an initial concentration of 0.196 g/L. After 3 days of culture, Congo Red dye concentration decreased significantly, in which the R7-3 strain pair decoloured best with the residual concentration of 0.008 g/L (94.5% reduction from the original sample), in this pair of strains, there are strong absorption by fungi biomass, whereas in 5 pairs of symbiotic strains for high decolorization effect, 2 pairs of R7-4 and R2-4 have the lowest decolorization efficiency with the concentration of 0.048 g/L (65.4% reduction from the original sample). In the following days, Congo Red dye concentrations continued to decrease. On day 7, Congo Red dye concentrations in the samples ranged from 0.003 to 0.022 g/L
in which the R7-4 pair had the best result with the remaining concentration of 0.003 g/L (98% reduction compared to the original sample), the pair of R2-4 strain is still the lowest decolorization pair with concentration of 0.022 g/L (84.8% reduction compared to the original sample).

Figure 7. The decolorization diagram of Congo red dye of 5 pairs of symbiotic microorganism strains calculated according to the Congo Red standard curve after 7 days of culture.

Thus, all 5 pairs of symbiotic microorganism strains were able to decolour of Congo Red dye better than single strains, the decolorization effect was shortened more time, especially the pair of R7-4 with the high ability of Congo Red dyes (98% reduction compared to the original sample).

Therefore, the low decolorization strains combined with other strains with the effect of symbiotic stimulation and support leading to increase the ability of decolorization. This is consistent with the hypothesis of K.M. Oliver [31] the author argues that symbiosis originates from the proper interaction in the suitable strains or species will bring different benefits depending on the mechanism of the resonant pairs. Experimental results of Md. Ekramul Karim [32] also showed that the isolated microorganisms strains from the culture containing Novacron Super Black-G colors have the decolorization effect from 40 to 42% but when combined together, the decolorization effect increases up to 90%. Based on the quantitative results from the tests, the decolorization efficiency mainly is as follows:

Table 5. Comparison of decolorization performance by single strains and combination strains.

| Experiment                     | Code | Performance of decolorization | Time decoloration |
|--------------------------------|------|-------------------------------|------------------|
| Evaluation of single strains   | R7   | 77.16%                        | 14 days          |
| Evaluation of combination of strains | R7-R4 | 98%                          | 07 days          |

The results showed that in the multi-color dye resolution, the R4 strain is the one that gives the most satisfactory multi-color resolution among the selected strains from the qualitative results in Table 5. In addition, R4 belongs to R7-4 pair also gave the highest result of Congo Red monochrome resolution in combination strains, so the strains of R4 and R7 were selected to evaluate morphology and identification of strains.
3.6. Identifying the microorganism strains with the highest application potential by molecular biology techniques

Through PCR molecular biology method, comparing 16S rRNA and 28S rRNA sequence of microorganisms with genetic data banks using BLAST programs, based on the results of gene decoding and comparison with database through BLAST search shows that the R7 strain has a 99% similarity to the *Aspergillus niger* strain. Based on the results of genetic decoding and comparison with the database through BLAST search, the R4 strain has 99% similarity with *Streptomyces thermodiastaticus* strain. These fungal strains were also interested in some studies and appreciated the ability of decolorization.

4. Conclusion

In conclusion, the study isolated 8 strains from textile wastewater. Evaluating on showed that the shaking state at with a speed of 185 rpm have color degradation performance higher non-shaking state 1.24 times. The strain of *Aspergillus Niger* was the most outstanding single strain which has solved 77.16% of the initial concentration of Congo Red at 200mg/L. Adding different sugars also affect the ability to reduce color with Glucose sugar present better result than Sucrose sugar nearly 20%. Growth medium change also showed that the Czapek medium (mineral medium) gave the ability to treat 74.62% of the Congo Red that was not different than the PDA medium at the end point, decreased by 77.16%, however, PDA culture showed the result of decolorization occur faster at most time points. The strains combination results also showed that the intergation of *Aspergillus niger* and *Streptomyces thermodiastaticus* improved decolorization efficiency from approximately 70% to over 90% and shortened 50% of the contact time. Although the research needs to go through many stages, its environmental and economic benefits and the potential for application of color biodegradation treatment for textile wastewater are very promising.

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