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Biodecolourization of Textile Dye and Wastewaters by Crude Laccase from *Pleurotus florida* ITDI 6003 Cultivated in Wheat Grains

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Abstract

The continuous growth of textile industry in the Philippines resulted in an increase in the discharge of textile wastewater which includes environmental contaminants such as dyes. The current most common decolourization treatment of textile wastewater is through the use of chemicals, which in turn, also adds to environmental contamination. Thus, this study aims to employ biological treatment through the use of enzyme, particularly of crude laccase, extracted from *Pleurotus florida* ITDI 6003 grown on wheat grains. The crude laccase from *P. florida* ITDI 6003 yielded an enzyme activity of 5.36 U/mL based on the oxidation of 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS). Furthermore, the crude laccase was able to significantly decolourize the textile dye Remazol Blue RGB, and four textile wastewaters originally collected from the dyeing facility of the Philippine Textile Research Institute (DOST-PTRI). UV-Vis spectrophotometric analyses of the laccase-treated textile dye and wastewater samples also showed decrease in absorbances at their respective specific λ_{max}. Full wavescan analyses indicated hypsochromic shifts in the λ_{max} as also observed on the changes in colour on the visual analyses of the treatment setups. The results show the potential of using crude laccase from *P. florida* ITDI 6003 in the decolourization of textile dye and wastewaters discharged from the textile industry.

Key words – Biodecolourization – Laccase – *Pleurotus florida* – Textile Dye – Textile Wastewater

Introduction

Several industries including cosmetics, pharmaceutical, food, and textile industries, widely use synthetic dyes to put colour into their products (Sathishkumar et al. 2010). As the textile industry in the Philippines continues to grow, the amount of wastewater discharges from these industrial plants, which contain different dye stuff, also increase. This is because during the dyeing of fabrics, not all of the dyes used are fixed onto the fabric, thereby getting washed out and contributing to the amount of dyes on the textile effluents (Ghaly et al. 2013). Thus, the

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Department of Environment and Natural Resources – Environmental Management Bureau (DENR-EMB) implemented effluent standards which include colour as a parameter (Environmental Management Bureau 2008).

One of the commonly-used dyes in the textile industry is reactive dyes such as azo dyes. Reactive dyes are preferred over other kinds of dyes because they form covalent bonds with the fabric, thus, allowing them to permanently dye the fabrics. Moreso, they are able to withstand different temperatures. Azo dyes, which include Remazol, are the most widely-used reactive dyes in the textile industry. However, they pose great concern to the environment and health due to their structural properties which provide them resistance to degradation using conventional wastewater treatment systems (Ekambaram et al. 2016).

Chemical treatments of textile wastewater are surrounded by environmental concerns and health hazards due to the introduction of toxic chemicals. Thus, biological treatment provides a safe and more economical way to treat textile wastewater, which includes enzymatic treatments (Banat et al. 1997). Laccase is a copper-containing 1,4-benzenediol: oxygen oxidoreductase (EC 1.10.3.2) commonly produced by fungi and other microorganisms. It catalyses by the oxidation of phenolic compounds and reduction of oxygen to produce water (Shraddha et al. 2011). However, laccases sourced from different species as well as the production conditions may result in variations in their decolourization activities which may be due to the differences in their redox potentials. Thus, researches on different sources of laccase have been growing (Yesilada et al. 2018). Laccases from different sources have been previously tested for their decolourization activity such as those from *Cerrena unicolor* (Zhang et al. 2018), *Podoscypha elegans* (Pramanik & Chaudhuri 2018) and *Streptomyces* sp. (Blánquez et al. 2019) to name a few. Bacterial consortium has also been tested before for their synergistic decolourization activity (Karim et al. 2018).

However, laccases from white rot fungi such as *Pleurotus* spp. remain as one of the most in-demand due to their broad applications (Jeon & Lim 2017; Sathishkumar et al. 2010; Yesilada et al. 2018). *Pleurotus florida*, commonly called "oyster mushroom" is a basidiomycete widely cultivated as food in the Philippines (Ragasa et al. 2015). Previous studies on different strains of *P. florida* showed their potential application on dye decolourization (Balan et al. 2012, 2013; Sathishkumar et al. 2010, 2013). Hence, in this study, crude laccase extracted from *P. florida* ITDI 6003, a locally-isolated strain, was tested for its efficacy in decolourizing textile dye and wastewaters.

Materials & Methods

Cultivation of Pleurotus florida ITDI 6003

Pleurotus florida ITDI 6003, obtained from the Culture Collection of the Industrial Technology Development Institute, Department of Science and Technology, was cultivated on wheat grains. Approximately 150 g of wheat grains were steamed for 30 min for sterilization. Thereafter, *P. florid*a ITDI 6003 mycelial plugs were inoculated, and then incubated for 14 d at 25°C until full ramification of the substrate was observed.

Extraction of Crude Laccase

The crude enzyme of *Pleurotus florida* ITDI 6003 was extracted according to Nakajima et al. (2018) with few modifications. First, the mycelia were mixed with approximately 50 mL sterile distilled water. The mixture was then filtered through a sterile cheese cloth and the filtrates were subjected to centrifugation at 10,000 rpm for 10 min. The supernatant was further filtered using sterile 0.22-µm syringe filter.

Preparation of Dyes and Collection of Textile Wastewater

Fifty milligrams of textile dye Remazol Blue RGB were dissolved in 1 L distilled water to obtain a concentration of 0.05%. On the other hand, samples of textile wastewater were collected from the Innovation Center for Yarns and Textiles, Philippine Textile Research Institute (PTRI) of

the Department of Science and Technology, Bicutan, Taguig City. Samples were collected at four points (1st wash (WW-1), 2nd wash (WW-2), 3rd wash (WW-3), and effluent) during the dyeing of textile with Remazol Blue RGB. According to a personal communication with M.E. Ablan (2018), WW-1 involves the addition of water and boiling for 5 min, while WW-2 and WW-3 involve further addition of water.

Determination of Laccase Activity

Laccase activity was determined using 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) as substrate. One hundred microlitres of crude enzyme was combined with 900 µL 0.5 mM ABTS dissolved in 100 mM *N*-acetate buffer (pH 5.0). The oxidation of ABTS substrate was monitored at 1-min intervals by an increase in absorbance at 420 nm for 10 min. One unit (1 U) of laccase activity was determined by the amount of enzyme required to oxidize 1 µM of ABTS per minute and was computed using this formula (Vantamuri & Kaliwal 2016) and the extinction coefficient of ABTS at 420 nm (Kenzom et al. 2014):

$$Enzyme\ activity\ (U/mL) = \frac{\Delta Abs420\ (4)(V\text{-}t)(df)}{\varepsilon(Vs)}$$

Where:
$\Delta Abs420$ = change in absorbance at 420 nm
4 = derived from unit definition and principle
Vt = total volume of reaction mixture
Df = dilution factor
ε = extinction coefficient of ABTS at 420 nm (36,000 M^{-1} cm^{-1})
Vs = volume of enzyme

Dye Decolourization Assays

Preparation of Reaction Mixtures and Visual Analysis of Dye Decolourization

Crude enzymes were added to the dye solution and textile wastewaters (30:70; v/v) and were incubated statically at room temperature for 24-72 h. The changes in colour were visually observed over time. Dye solution and wastewater samples without crude enzymes served as the control.

Percentage Dye Decolourization

The maximum absorbances (λ_{max}) of the dye solution and wastewater samples were first determined by doing a full wavescan (200 nm-900 nm) using UV-Vis Spectrophotometer (Implen Nanophotometer C40). Afterwards, 100 µL of the reaction mixtures were read at their specific λ_{max} at time intervals from 0 h up to 72 h to determine the decolourization activity over time.

Percentage dye decolourization was computed as:

$$Dye\ Decolourization\ (\%) = \left[\frac{(Ai - Af)}{Ai}\right] x\ 100$$

Where:
Ai = Initial Absorbance of reaction mixture at 0 min.
Af = Final Absorbance of reaction mixture after *n* min.

Determination of Shifts in λ_{max}

Shifts in the maximum absorbances (λ_{max}) of the dye solution and wastewater samples were monitored by conducting a full wavescan (200 nm-900 nm) of the solutions at 0 h and after treatment at 72 h.

Statistical Analyses

Statistical analyses were done using IBM SPSS Statistics 2.0 software. One-Way Analysis of Variance (ANOVA) was used to determine if there is a significant difference among the decolourization activities of the crude laccase and the controls on the different samples. Tukey's *post hoc* test was also performed to identify where the significant difference lies. Values are presented as means ± standard deviation (SD).

Results and Discussion

Laccase Activity

An increase in the absorbance values per minute of the ABTS solution was observed over 10 min at 420 nm (Fig. 1). The laccase activity of *P. florida* ITDI 6003 was calculated to be 5.36 U/mL which is similar to the results obtained from *P. florida* NCIM 1243 which was able to yield the highest enzyme activity of 5.4 U/g when cultivated in banana peel. They credited the observed differences on the carbohydrate content of the substrates (Sathishkumar et al. 2010). Interestingly, the enzyme activity obtained from *P. florida* ITDI 6003 is greater than that of *P. florida* grown in artificial medium (Sathiya Moorthi et al. 2013). Previous studies showed that the use of different substrates affects laccase activity (Pramanik & Chaudhuri 2018; Sathishkumar et al. 2010). A previous study has shown that the binding affinity of the enzyme to particular substrates affects the enzyme activity of laccase (Mehra et al. 2018). Thus, the use of other agricultural substrates may further enhance the laccase activity of *P. florida* ITDI 6003.

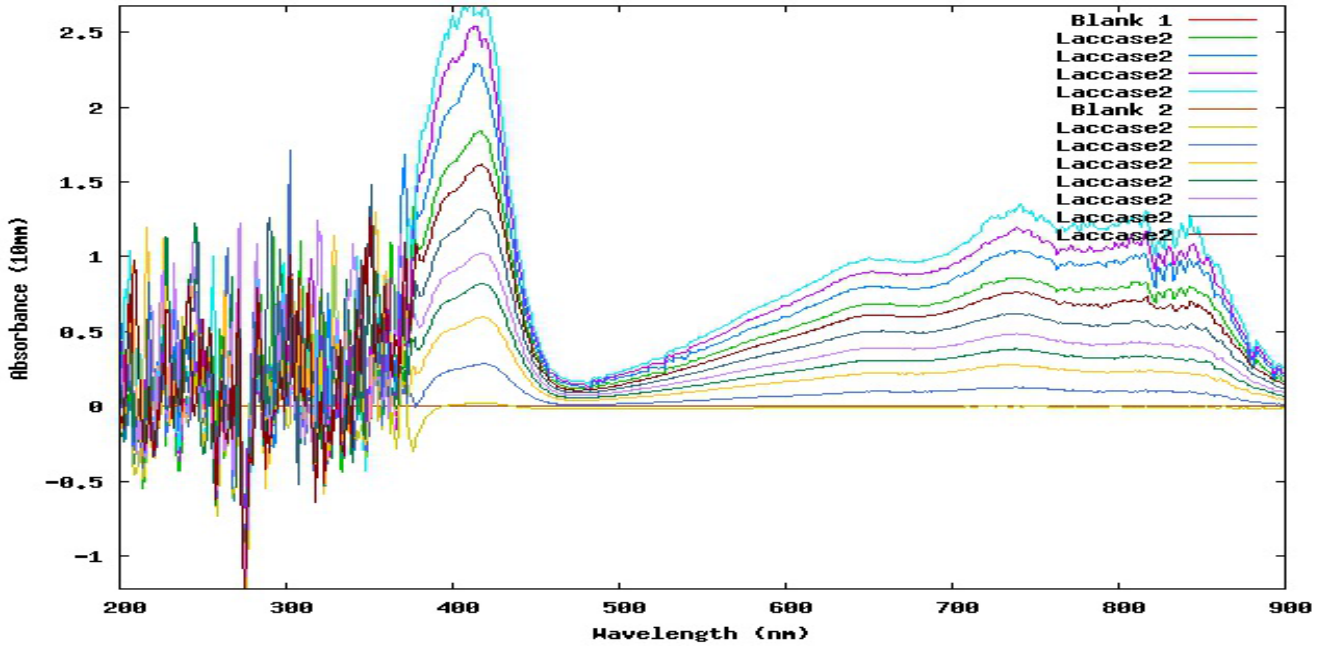

Fig. 1 – Oxidation of ABTS by Laccase

Dye Decolourization

The crude laccase extracted from *P. florida* ITDI 6003 was homogenized with textile dye 0.05% Remazol Blue RGB and textile wastewaters collected from PTRI (30:70; v/v). Drastic changes in the colour of the mixtures were observed after treatment for 72 h (Fig. 2). However, the crude laccase yielded greater decolourization activity against the wastewater samples than the prepared dye solution. This observed difference may be due to the treatment applied to the wastewaters prior to disposal, such as the subsequent addition of water which resulted in the dilution of the wastewaters. It can also be deduced that the difference in the results of WW-1 and of the prepared dye solution was due to the boiling involved in the 1st washing. Boiling hydrolyses

the reactive group of the dye thereby allowing it to fix on the fibres (Lewis & Vo 2007). The alteration in the structure of the dye due to boiling may have also rendered it susceptible to treatment. On the other hand, the effluent is non-discriminatory and therefore contains different wastewaters. Furthermore, the visible colour left after treatment was due to the colour of the crude laccase used.

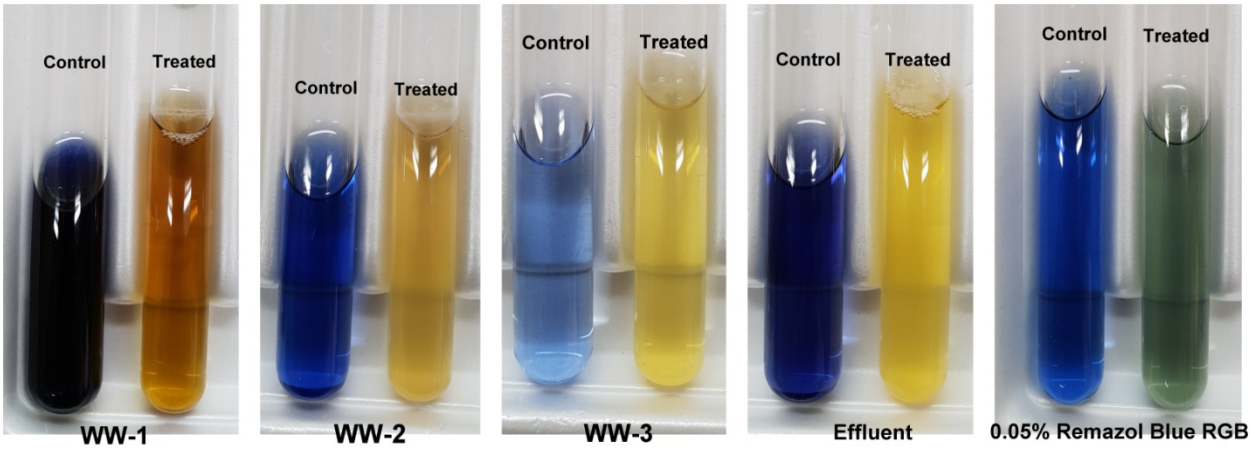

Fig. 2 – Decolourized Textile Dye and Wastewaters

To observe the decolourization of the dye solution and wastewaters over time, λ_{max} of each was first determined using full wavescan (Table 1). The absorbance values obtained at the specific λ_{max} of the samples were then plotted against incubation time (Fig. 3) and the percentage decolouurization for each was computed. The results show that the crude laccase was able to significantly decolorize WW-1 (94.66%) and WW-2 (78.19%) after 72 h of treatment. Further extending the incubation time did not enhance the decolourization (data not shown). Meanwhile, WW-3 was decolourized only for up to 27.72% after 1 h and no further decrease in the absorbance values was observed until 72 h. Both the effluent and the 0.05% Remazol Blue RGB were decolourized by 84.98% and 59.35%, respectively, after 22 h of treatment. Extending the treatment duration did not result in increase in decolourization. Interestingly, Remazol Brilliant Blue R was previously decolourized by laccase from a different white-rot fungus, *Marasmius scorodonius*, only in the presence of a mediator (Jeon & Lim 2017). In this study, no mediators were used during the decolourization process. The results, therefore, agree with previous studies stating that laccases from different sources may have differences in their activities (Yesilada et al. 2018). This might be due to the differences in their binding affinities (Mehra et al. 2018). Nonetheless, based on the results, the decolourization of crude laccase from the strain of *P. florida* grown in wheat grains used in this study may be proposed to be applied to wastewaters from the 1st washing for at least 72 h to achieve the maximum efficiency.

Changes in the peaks at the λ_{max} of each sample were also observed. Fig. 4 shows the disappearance of the peaks at the λ_{max} of all samples after 72 h of treatment. This coincides with the results of the visual analyses that the visible blue colour of the solution which is attributed to the dye disappeared after 72 h. Furthermore, the stationary activities observed over the course of time may be explained by the flat lines shown on the spectra of the dye solution and wastewaters after treatment which indicate that the reactions have already ceased since the peaks at the λ_{max} of each samples were already absent, and no further reactions occurred. Moreover, no new peaks appeared in the spectra of all the samples after a full wavescan. Interestingly, hypsochromic shifts were observed for all the samples. Table 1 shows the λ_{max} of each sample pre- and post-treatment for 72 h. All samples whose λ_{max} were ~600 nm shifted to a λ_{max} of ~250 nm after treatment for 72 h. Similar results were obtained in the crude and purified laccase of *P. nebrodensis* ACCC 50867 which was able to elicit hypsochromic shifts in the λ_{max} of the tested dye solutions since the peaks

shifted to a shorter wavelength (Yuan et al. 2016). These results agree with the visual analyses which showed the changes in colour of the textile dye Remazol Blue RGB, and the textile dye wastewaters after subjecting to treatment by crude laccase for 72 h.

Table 1 Shifts in λ_{max} of each Textile Dye and Wastewaters

Sample	λ_{max}	
	0 h	**72 h**
WW-1	620	283
WW-2	606	264
WW-3	604	255
Effluent	605	265
0.05% Remazol Blue RGB	603	268

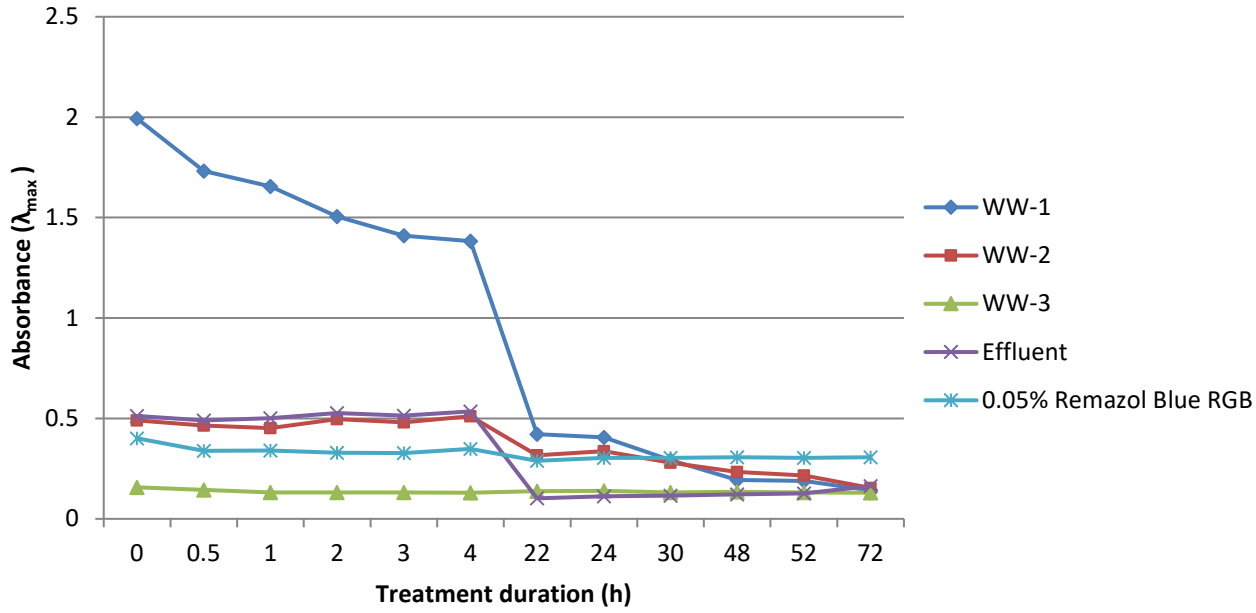

Fig. 3 – Decolourization of Textile Dye and Wastewaters over Time

Conclusions

The growth of the Philippine textile industry calls for the need to cope with the environmental impacts that come along with it, and the utilization of environment-friendly and non-hazardous materials may help. Crude laccase from locally-isolated *Pleurotus florida* ITDI 6003 grown in wheat grains was able to yield a laccase activity of 5.36 U/mL. Furthermore, it was able to decolourize Remazol Blue RGB textile dye, and the wastewaters produced by textile dyeing process. Further purification and characterization of the enzyme, as well as optimization of its production, may enhance its decolourizing efficiency.

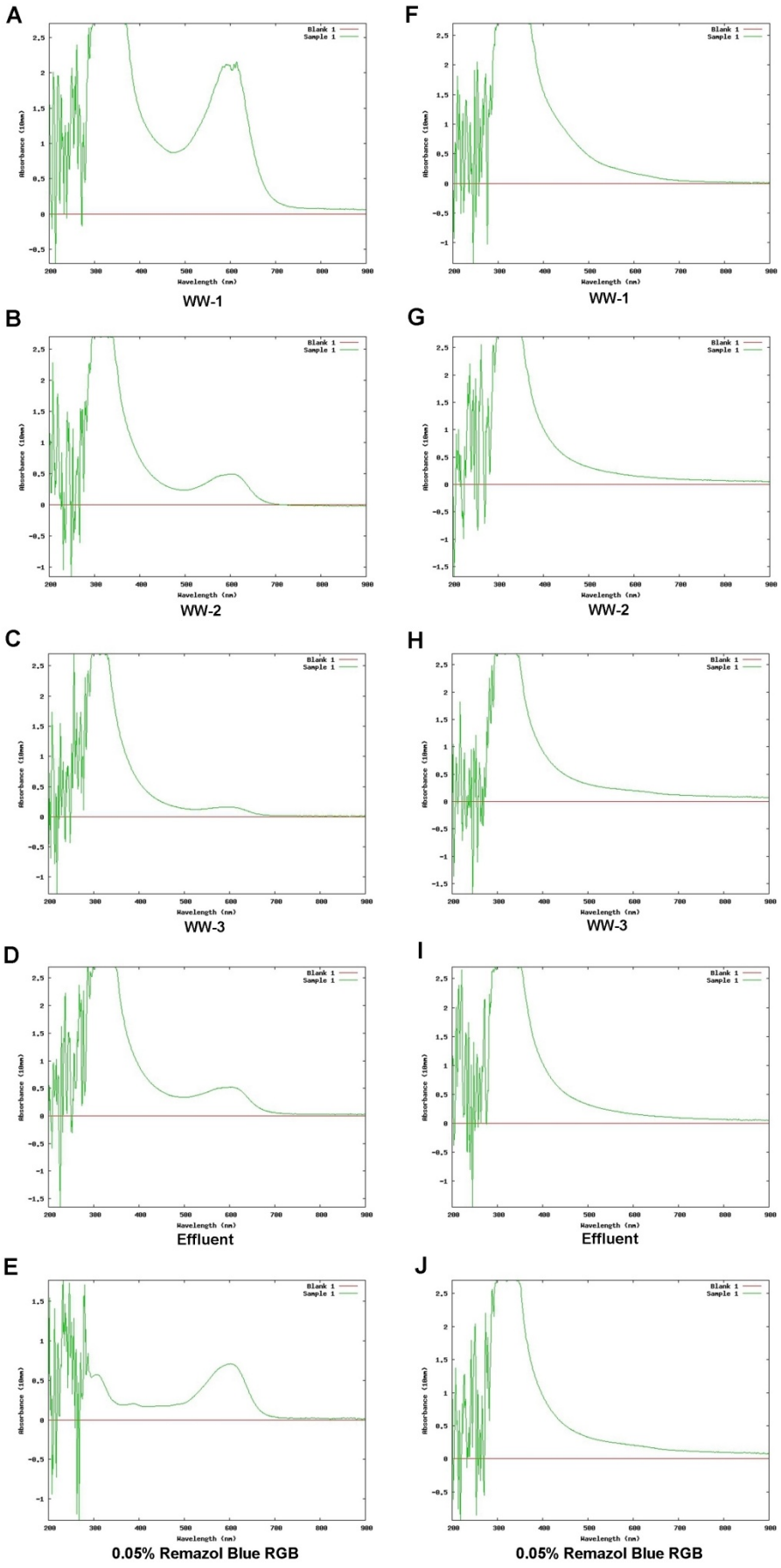

Fig. 4 – Absorbance Spectra (λ_{max}) of Textile Dye and Wastewaters at 0 h (A-E) and after 72 h (F-J)

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