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Potentials of Biosurfactant and Biomass from Locally Isolated *Panus tigrinus* and *Klebsiella pneumonia* Strains on Decolorization of Selected Industrials Dye

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Pollution problem posed by dye contamination, resulting from errant human technological activities has for long posed a challenge for researchers. Biosorption is becoming a promising alternative to replace or supplement the present dye removal processes, which are used for removing dyes from wastewater. In this study, the removal of four dyes; Congo Red, Malachite Green, Neutral Red and Reactive *Blue* 19,by biosorption using biomass of fungus and bacteria, *panustigrinus*, *klebsiellapneumoniae* were investigated. Bacterial biosurfactant, obtained from *klebsiella pneumonia,* was also tested for dye removal. Fungal biomass proved to be the best remover amongst the three bioactive biosurfactant and biomass, which removed 96.948% of Congo Redfollowed by 89.231% removal of Reactive Blue 19.

Key words: Biosorption; *Panustigrinus*; *Klebsiella pneumonia;* Azo dyes.

Dyes are used extensively in several industries, including textile, leather tanning, plastic, cosmetics, food processing, carpet, printing, paper and pharmaceutical industries[1]. They are found in different structural varieties, such as, acidic, basic, disperse, azo, diazo, triphenylmethane, phthalocyanine, anthraquinone based and metal complex dyes. On the basis of the dying process, textile dyes are classified as reactive dyes, direct dyes, disperse dyes, acid dyes, basic dyes and vat dyes[1].

The main focus of this research is the removal of azo dyes since they are the largest chemical class of dyes frequently used for textile dyeing, paper printing, food, and cosmetics. They are characterized by the presence of one or more azo bonds (-N-N-) in association with one or more aromatic systems, which may also carry sulfonic acid groups[2]. When these dyes are present in the effluent, they are not normally removed by conventional wastewater treatment systems. Therefore, the employment of these dyes must be controlled and the effluents must be treated before being released into the aquatic and terrestrial environment[3, 4].

The dyes present in wastewater are extremely difficult to treat and are stable under exposure to light and washing, also resistant to aerobic biodegradation by bacteria[5]. This is due to their complex aromatic structure, considerable structural variety, synthetic origin and fade-resistant design of the dyes[6], especially the brightly colored, water-soluble reactive dyes, since

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they tend to pass through conventional treatment systems unaffected[1, 7].

There is a growing interest in using low cost, easily available materials for the adsorption of dyes[8]. Thus, this research focuses on the ability of biomass, and biosurfactants produced from low cost raw materials, cassava wastes and Sludge palm oil, using *panustigrinus*, and *klebsiella pneumoniae* respectively to decolorize four Azo dyes, namely Reactive *Blue* 19, Malachite Green CI 42000, Neutral Red and Congo Red.

MATERIALS AND METHOD

Microorganisms

Both strains used in this work were maintained by the Department of biotechnology, IIUM, Malaysia. The *Panustigrinus*cells were preserved by continuous plating on malt extract agar at 32ºC for one week and the *klebsiella pneumonia* cells were preserved by plating on nutrient agar at the same temperature for 24 hours.

Substrates

Cassava peel

Cassava peel was collected from a small scale kerepek (local snack) industry in Kuala Langat, Selangor Malaysia, where cassava is processed to produce a local snack called KerepekUbiKayu. The collected Cassava peel was washed properly to remove sand and tuber head and dried immediately at 60°C in an oven for 48 hrs to avoid growth of unwanted microbes. The dry peels were then grinded and sieved to 1 mm particle size.

Sludge palm oil (SPO)

Sludge palm oil (SPO) was obtained from West Palm Oil Mill, Carey Island, Selangor, Malaysia. SPO was dark orange in colour and existed in semi-solid form at room temperature. The samples were stored at 4ºC and were thawed for 24 hours before use.

Inoculum preparation

Panustigrinus

To prepare inoculum, all flasks, L-shaped glass rods, funnels and distilled water were sterilized prior to use. Four plates of fungal culture were washed with 15 mL of distilled water; which was poured on each MEA plate and the spores were gently scrapped using sterile glass rod. The suspended fungal spores were then collected into an Erlenmayer flask.

Klebsiella pneumonia

To prepare the bacterial inoculum, the nutrient broth was autoclaved first. Then 100 ml of the nutrient broth was poured into each flask, and 3 loops filled with bacteria were taken from one bacterial plate and dispersed into the media. The culture was then placed in an incubator shaker for 12 hours at 37ºC at an agitation speed of 180 rpm.

Adsorbent

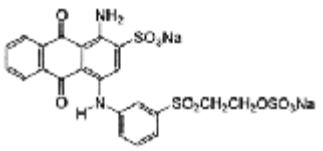
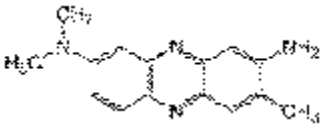
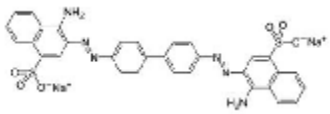
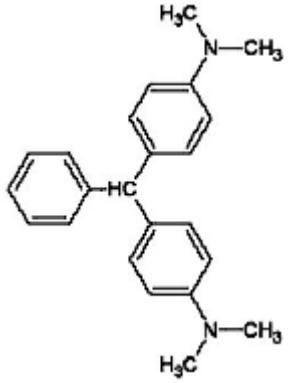
Biosorbent from klebsiella pneumonia

Biosurfactants and biomass were produced by growing *klebsiella pneumonia* in a 2 liter bioreactor using liquid state fermentation; with palm oil sludge as the substrate[9]. The products were obtained after 36 hours of fermentation, and were extracted and purified[10]. The production process began with the addition of 915 ml of the mineral solution (Sucrose 2 g/L; $MgSO_4$ 0.2 g/L; $FeSO_4$ 0.3 g/L; $NaNO_3$ 2 g/L; K_2HPO_4 7 g/L) to 85 ml of SPO. The vessel, inoculum flask, measuring cylinder, anti-foam probe, acid and base were autoclaved for 15 minutes at 121ºC. After autoclaving, the pH probe, temperature probe, air flow, anti-foam probe, agitator and vessel jacket were all connected and adjusted. The Bacterial inoculum was then transferred into the vessel through the coupling connected to the inoculum flask and the fermentation process conducted for 36 hours. Later the pallet (Bacterial biomass) was separated from the supernatant (which contained the biosurfactants), by centrifuging at 8500 rpm using 50 ml falcon tubes. The biomass was preserved. To obtain the biosurfactants found in the supernatant, a pH of 2 had to be maintained by addition of HCl, and samples were kept overnight at 4ºC. The extracellular products (biosurfactants) were obtained by extraction with chloroform: methanol (2:1) ratio[11].

Biosorbent frompanustigrinus

To obtain the biosorbent the cells were grown in 50 ml shake flasks using solid state fermentation. Cassava peel was used as the main substrate (5.14 g) together with wheat flour as the co-substrate (0.68 g) and 11.8 ml of water was added followed by 1 ml of the mineral solution ($(NH_4)_2SO_4$,1.5; $MgSO_4$,0.45; $MnSO_4$,0.5; KH_2PO_4,0.8 g/l). The samples were then auto-claved at 121ºC for 15 minutes. Inoculum (1.2 ml) was added in 150 ml flasks and incubated at 32ºC for 6 days[12]. Samples were placed in 50 ml centrifuge tubes, with

Table 1. Chemical formulae for the selected dyes

Name	Chemical Formula
Reactive Blue	
Neutral Red	
Congo Red	
Malachite Green	

an equal volume of hexane and centrifuged at 8500 rpm to remove the unwanted oily residue. A pH of 2 was maintained and the samples were kept overnight at 4ºC. Biosurfactants were obtained by extraction with chloroform: methanol and thereafter preserved and the biomass was dried at 60ºC for 1 day[11].

Adsorbate

Stock solution was prepared by dissolving 0.05 g of the four dyes in 1 L distilled water[13]. It was soaked slowly to make sure that all the dye powder has been dissolved in the distilled water. The pH of the stock solution was adjusted to 10.5 and stored for further use. This pH was used because normally it is the pH of dye effluent water[14, 15]. Table 1 provides the chemical formulae for the selected dyes.

Preparation of concentration curves

Stock solutions for each of the four dyes where prepared at different concentration ranging from 0.005 to 0.05 mg/l. The absorbance was then measured for each dye at its suitable wavelength (Table 2).

Equilibrium studies

Adsorption was carried out by suspending 0.375 g of adsorbent into 25 ml of dye solution[16, 17, 18].All flasks were placed in an orbital shaker and agitated at 200 rpm for 60 min[19, 20] at a pH of 10.5. The final concentration of the dye solutions were analyzed using UV-

Table 2. Wave length for each dye

Name	C.A.S.*	F.W.**	UV absorption
Neutral Red	9167309	288.78	λmax=428nm
Reactive Blue 19	2580-78-1	626.54	λmax=596nm
Congo Red	573-58-0	696.7	λmax=500nm
Malachite Green	2437-29-8	382.93	λmax=425nm

Note: *C.A.S: Chemical Abstracts Service;
**F.W.: Formula or molecular mass

spectrophotometer prior to filtration of the bacterial products. The amount of equilibrium uptake of dyes was calculated by using:

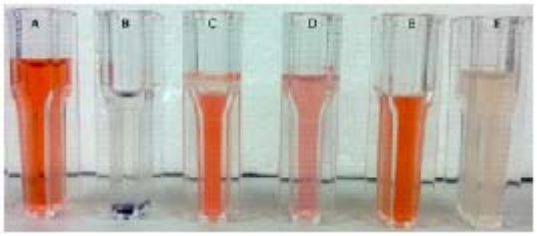
$$q_e = \frac{(C_i - C_f)}{W} V \quad ...(1)$$

Where, C_i and C_f (mg/L) are the liquid-phase concentrations of dye at initial level and at equilibrium, respectively. V (L) is the volume of the solution and W (g) is the amount of biomass used. The percentage removal dye is defined as the ratio of difference in dye concentration before and after adsorption (C_i"C_f) to the initial concentration of dye in the aqueous solution (C_i) and was calculated using the following equation 2:

$$\text{Removal Percentage} = \frac{(C_i - C_f)}{C_i} \times 100 \quad ...(2)$$

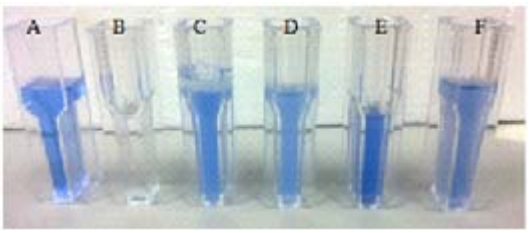
RESULTS AND DISCUSSION

Figure 1 and 2 shows the physical effect of Congo Red and Reactive Blue decolourization when using fungal biomass, bacterial biomass and bacterial biosurfactant. Moreover, Figure 3 provides the percentage of dye removed during the screening process (1 hour, 200 rpm, 15 g/l of adsorbent, and 50 mg/l of adsorbate). The best result was observed for Congo red removal by fungal biomass, which was 96.95% followed by Reactive Blue (87.38%), Malachite Green (64.57%) and Neutral Red (54.79%). The result obtained for fungal biomass was in agreement with the study reported by Khalaf[8], Pazarlioglu& Urek[21], where *A. Niger, Spirogyra sp. Phanerochaetechry sosporium* were immobilized on $ZrOCl_2$-activated pumice and exhibited a maximum removal of 88%, 85% and 98% respectively when used for treating reactive dyes present in textile wastewater and for Congo Red removal.



A) Control. B) Treatment with fungal biomass. C) Treatment with Bacterial biosurfactants. D) Treatment with Bacterial biosurfactants (Filtered). E) Treatment with Bacterial biomass. F) Treatment with Bacterial biomass (Filtered)

Fig. 1. Congo red (50mg/l, pH10.5)



A) Control. B) Treatment with fungal biomass. C) Treatment with Bacterial biosurfactants. D) Treatment with Bacterial biosurfactants (Filtered). E) Treatment with Bacterial biomass. F) Treatment with Bacterial biomass (Filtered).

Fig. 2. Reactive blue (50mg/l, pH10.5)

On the other hand, when using bacterial biosurfactants and biomass, only Congo Red and Malachite Green obtained a certain level of removal as shown in (Fig. 3). Reactive Blue and Neutral Red where not affected by the biosorption process. These results were very low when compared to fungal biomass decolourisation; the reason behind this was that bacterial biosurfactants and biomass were responsible for turbidity of the dye solution, even after filtration of the solution. Turbidity caused the reduction in UV- absorbance and thus a lesser percentage of dye was removed. In contrast, the fungal biomass directly settled at the bottom of the flask after the shaking process was over. Hence, the mixture was clear and no turbidity was visible. In another study, higher removal efficiency (94%) for Reactive Red 198 was obtained[10, 22]. The adsorption capacity shown in (Fig. 4) for Reactive Blue was (2.912 mg/g) when using fungal biomass, this was somewhat close to

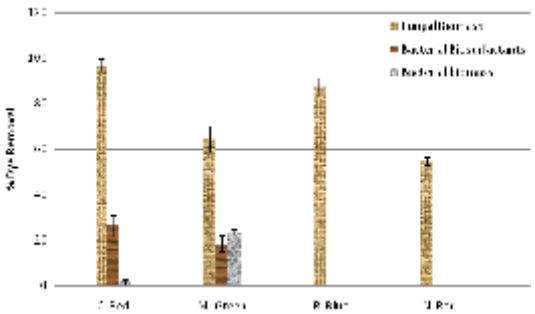


Fig. 3. Percentage of dye removal

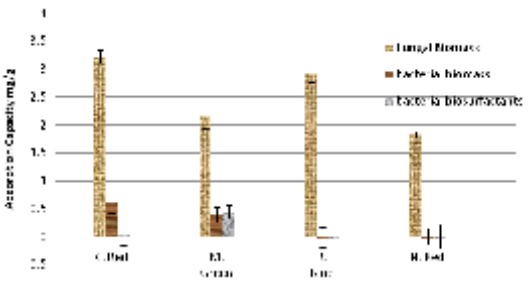


Fig. 4. Adsorption capacity (mg/g) vs dyes removed

what Polman[23] obtained (5 mg/g) when removing the same dye using *Pichiacarsonii*. Moreover, Fu & Viraraghavan also obtained an adsorption capacity that was not far off (1.4 mg/g) when removing Acid Blue 19[13].

CONCLUSION

In this study, the effect of three adsorbents (produced by using the two strains *panustigrinus* and *klebsiellapneumoniae*) on four dyes was investigated at constant conditions. The most effective adsorbent was the fungal biomass, as it showed colour removal of 96.94%, 89.23%, 64.58% and 54.79% for Congo Red, Reactive *Blue* 19, Malachite Green and Neutral Red respectively.

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