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Congo red biosorption with live and dead biomass of thermophilic *Aspergillus fumigatus*

ABSTRACT:
Biosystems are the most promising processes for dyes sorption because they seem to be eco-friendly. Decolorization of Congo red with live and dead biomass of thermophilic *Aspergillus fumigatus* was studied. The biosorption percentage by dead biomass increases with increasing pH up to 6 at 10, 60, and 70 mg/100 ml dye initial concentration, and up to pH7 at dye initial concentrations of 20, 30, 40, 50, and 60 mg/100 ml. The mount of Congo red biosorbed onto dead biomass increases with increasing temperature from 10 to 30°C then decreases at 40°C and sharply at 50°C. Increase in dead biomass concentration above 0.8 (g/100 ml) is not effective in biosorption of Congo red. Maximum decolourization was observed at 180min of contact time. The study concluded that dead fungal biomass possesses various advantages such as absence of nutrient needs, therefore the findings offer potential for the development of a cost effective for biosorption of Congo red.

KEY WORDS:
Dead biomass, *Aspergillus fumigatus*, Congo red, biosorption

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INTRODUCTION:

Control of pollution is one of the prime concerns of society today, since in both developing and industrialized nations a growing number of contaminants enter water supplies from human activity (Shannon *et al*., 2008). There are more than 100,000 commercially available dyes with over 7 x 10^7 tons of dyestuff produced annually worldwide (Akhtar *et al*., 2005). Synthetic dyes are used extensively for textile dyeing, paper printing, leather dyeing, colour photography and as additives in petroleum products. Pollution from the effluents has become increasingly alarming with the usage of a wide variety of dyes in industries (Zollinger, 1991). The presence of very small amounts of dyes in water (less than 1 ppm for certain dyes) is highly visible and undesirable (Lian *et al*., 2009).

Many genera of fungi either in living or dead form have been employed for the dye decolourization and heavy metals treatments (Abdel-Razek *et al*., 2009a&b; Prachi and Anushree, 2009; Mahmooda *et al*., 2014; Abdel Ghany *et al*., 2014). Fungi like as *Aspergillus niger, A. flavus, A. fumigatus, Fusarium oxysporum, Penicillium chrysogenum, Trichoderma viride, T. asperellum, Cunninghamella elegans* and *Mucor* sp. were used as a good microbial source for waste treatment (Saranraj *et al.,* 2010; Abdel Ghany and Masmali, 2016; Abdel Ghany *et al*., 2018a&b). In previous study, *Mucor mucedo* decolourised 78% of the crystal violet and 65% of malachite green (Moturi and Singara-Charya, 2009).

Fungal systems appear to be most appropriate biological agent in the treatment of coloured and metallic effluents (Ezeronye *et al*., 1999; Abdel Ghany *et al*., 2016; Saraf and Vaidya, 2016). Biomaterials of microbial origin have been very effective in dye removing because of their cell wall constituents. Biosorption capacity of the dead biosorbent is due to high surface to volume ratio (Magyarosy *et al*., 2002). Being metabolic independent process, there are no

restrictions of enzymatic activities of adsorption (Makky and Abdel Ghany, 2009; Hemambika *et al*., 2011). It is harmless, cheap, eco-friendly, highly efficient (Isil and Tugba, 2008). Asma *et al*. (2006) showed that it was possible to remove textile dyes by dead biomass of *Phanerochaete chrysosporium*. An increase in the amount of dead biomass positively affected of the dye removing. Isil and Tugba (2008) stated that methyl orange decolorization with dead fungal biomass will be more economic process. Whereas, in living biomass, adding nutrients in the medium growth increase costs. In addition, products of living biomass may cause problems in adsorption. Biomass species such as *Aspergillus niger* and *A. foeditus* have various functional groups such as carboxyl, amino, phosphate and sulfonate. These groups act as excellent binding sites in the biosorption of heavy metals and various dyes (Kapoor and Viraraghavan, 1998). Binding of dye to adsorbent involves interaction between dye and adsorbent. The present work aims to study the decolourisation potential of Congo red dye by dead and live mycelia of *Aspergillus fumigates* with study the optimal pH, temperature, initial dye concentration.

MATERIAL AND METHODS:

Fungal used:

Five fungal isolates were isolated from the soil contaminated industrial site located in Jazan area, Saudi Arabia. The five isolates were cultivated at different temperature up to 50°C, the highest strain resistance to temperature was selected for dye sorption and identified as *Aspergillus fumigatus* according to Raper and Fennell (1965) and Domsch *et al*. (1980).

Adsorbate and their preparation:

Congo red is the sodium salt of benzidinediazo-bis-1-naphthylamine-4-sulfonic acid (Merck Germany) (formula: $C_{32}H_{22}N_6Na_2O_6S_2$; molecular weight: 696.66 g/mol) (Fig. 1), it was used as adsorbate at different concentration including10, 20, 30, 40, 50, 60, and 70 mg/100 ml.

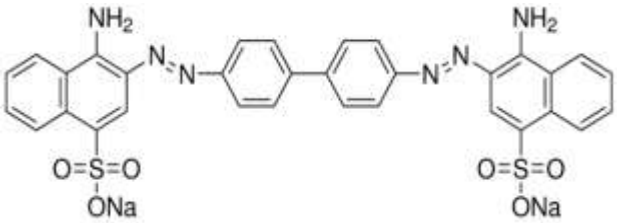

Fig. 1. Chemical structure of Congo red

Biosorption with live biomass:

Czapek-Dox medium supplemented with various concentrations of working dye solutions was autoclaved, then inoculated with fungal mycelia/spores and incubated for 8 days or at different incubation periods at 30°C in order to determine the decolorization % of Congo red.

Biosorption with dead biomass:

Disc (0.6 mm) of actively margin of *A. fumigatus* colony inoculated into 100 ml of sterile Czapek-Dox medium in 250 ml Erlenmeyer flaks and incubated at 30°C for 7 days. Mycelium developed was separated by filtration through Whatman No.1 filter paper and washed by de-ionized water for several times until free from the media components. The washed mycelial pellets were autoclaved and dried as dead biomass to Congo red biosorption.

Batch mode studies:

Batch mode experiments were carried out to investigate different factors such as contact time (20 up to 200 min), initial concentration of dyes (10 up to 70 mg/100 ml), dosage of the biosorbent (0.2 up to 1.0 mg/100 ml), temperature (10 up to 50°C) and pH (3 up to 9) influencing the rate and extent of uptake of Congo red by dead fungal biosorbent.

Estimation of dye biosorption o by dead biomass:

After contact of dead fungal biomass with medium containing Congo red with different concentration, centrifuged and tested for their ability to decolourise Congo red by spectrophotometer (JENWAY, Model 6300, EU) analysis at wavelength 530 nm. Biosorption efficiency of biosorbent was calculated by using the following expression:

$$\%R = \left[\frac{Co-Ct}{Co}\right] \times 100$$

Where C_o and Ct represent the initial and final (after adsorption) concentrations of dye in mg/L, respectively.

RESULTS AND DISCUSSION:

In the present study, *Aspergillus fumigates* (Fig. 2) was cultivated at different temperature up to 50°C and their growth appeared at 50°C, therefore it considered thermophilic/thermotolerance and selected for Congo red sorption. According to Córdova (2003) *A. fumigates* was characterized as thermophilic fungus.

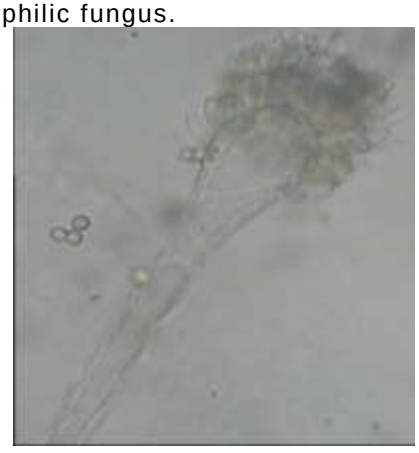

Fig. 2. Microscopic structure of *A. fumigates* (40x)

It has been found that the increase in biomass concentration decreases the biosorption efficiency (Gupta and Suhas, 2009). This occurs due to decrease in surface area on biosorbent for binding of adsorbent (Gupta and Suhas, 2009). Our findings are almost comparable with the previous findings. Comparison between growing/live and dead fungal mycelia was evaluated for Congo red sorption at different concentrations (Fig. 3). It is clear from figure 3 that Congo red sorption with dead mycelia was greater than those of growing/live biomass. Previously, Mahmooda *et al.* (2014) reported that *Aspergillus flavus* dead biomass shows maximum biosorption (53.62%) of methyl orange. Nakajima-Kambe *et al*. (1999) stated that decolorization with microorganisms is carried out either with adsorption of the pigment on to mycelia or its enzymatic degradation. In the current study, *A. fumigatus* showed decolourisation activities of Congo red indicating the role of mycelial biomass responsible for the decolourisation of the dye.

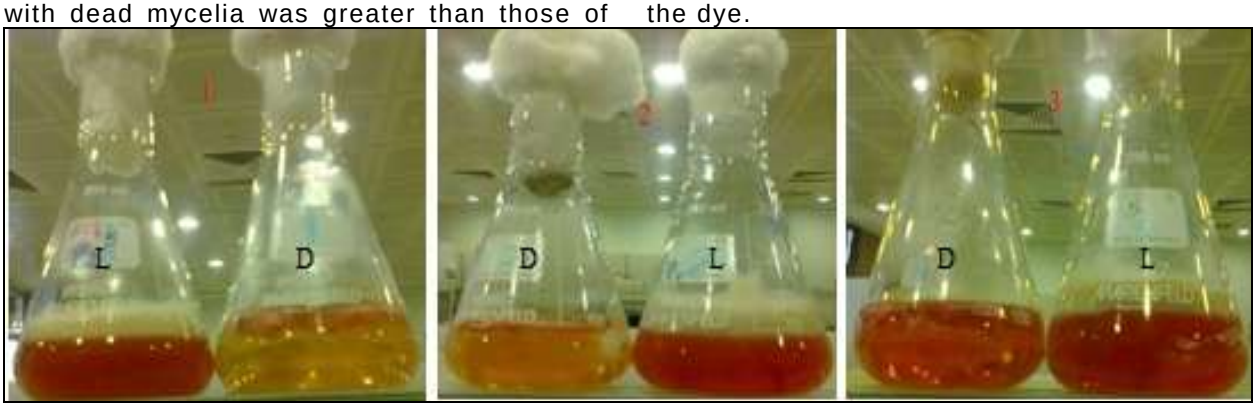

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Fig. 3. Congo red biosorption by growing (L) and dead biomass (D) of A. fumigatus at 10 (1), 30 (2), and 50 mg (3)/100 ml incubated for 7 days

The effect of the initial pH of dye solution on biosorption was studied at different pH among 3 - 9 for the different initial dye concentration among 10-70 mg/100ml at 25°C and a contact time of 180 min. As shown in table (1), the biosorption percent increases with increasing pH up to 6 at dye initial concentration 10, 60, and 70 mg/100 ml, and up to pH 7 at dye initial concentration 20, 30, 40, 50, and 60 mg/100 ml. Alkaline pH has a higher effect than acidic on biosorption. Kalaiarasi *et al*. (2012) reported that the dried non-viable fungal biomass exhibited maximum dye removal at pH 7.0 with temperature of 30ºC.

Table 1. Effect of pH on the removing of Congo red using dead fungus biomass (contact time: 180 min, temperature: 25°C)

pH	% Decolorization at different dye concentration (mg/100 ml)						
	10	20	30	40	50	60	70
3	45.43	40.41	35.80	33.22	20.32	18.70	13.65
4	49.67	42.30	41.98	36.51	33.45	30.00	16.78
5	70.50	55.35	53.12	50.50	40.67	35.05	20.90
6	77.51	59.31	55.43	52.66	46.80	39.46	23.00
7	69.07	62.08	60.70	55.54	48.50	38.50	20.12
8	65.00	5542	53.55	44.32	39.00	29.56	16.13
9	61.45	42.10	35.50	33.11	26.08	24.65	14.00

As shown in table 2, the results indicate that the amount of Congo red biosorbed onto dead biomass increased with increasing temperature from 10 to 30°C, then decreased at 40°C and sharply at 50°C. The fact that the biosorption decreases with an increase in temperature indicates that lower temperature is in favour of biosorption. Similar trend was observed by Khalaf (2008) and Kalaiarasi *et al*. (2012) for biosorption of reactive dye from textile dye effluent by non-viable biomass of fungi. If the temperature increase above 30°C it may alter the surface activity of biomass which results in a decrease in removal value, indicating that this process is exothermic in nature. The exothermic nature of dye biosorption has also been reported for the biosorption of Acid Red 274 dyes by *Enteromorpha prolifera* (Ozer *et al*., 2005).

Table 2. Effect of Temperature (°C) on the removing of Congo red using dead fungus biomass (contact time: 180 min, pH 6).

Temperature (°C)	% Decolorization at different dye concentration (mg/100 ml)						
	10	20	30	40	50	60	70
10	45.43	41.31	23.04	23.22	17.00	15.31	9.61
20	69.67	48.46	45.99	43.50	34.36	30.01	17.85
30	77.50	59.36	53.42	54.60	45.57	38.00	22.63
40	46.51	55.38	44.13	50.61	41.29	34.07	21.13
50	20.07	32.00	40.15	45.51	40.50	30.41	20.81

In the current study further increase in biomass concentration above 0.8 g/100 ml did not led to in biosorption of Congo red (Table 3). This was due to the fact that almost all the ions were bound to the biomass at the establishment of equilibrium between the dye molecules bound to the biomass and those remaining un-adsorbed in the solution (Kumar *et al*., 2006). Maximum decolourization was observed at 180min of contact time (Table 4). Although Kalaiarasi *et al*. (2012) reported that the dead *A. fumigates* biomass exhibited maximum dye removal after 24 h contact time.

Table 3. Effect of adsorbent dosage on the removal of Congo red using dead fungus biomass (contact time: 180 min, pH: 6.0, temperature: 25°C)

Adsorbent dosage (g/100 ml)	% Decolorization at different dye concentration (mg/100 ml)						
	10	20	30	40	50	60	70
0	00.00	00.00	00.00	00.00	00.00	00.00	00.00
0.2	54.56	42.50	38.05	36.80	30.56	19.65	12.65
0.4	59.00	49.60	47.00	39.78	36.76	26.54	15.12
0.6	76.80	60.50	55.12	53.00	40.43	31.45	21.10
0.8	78.50	63.55	58.34	55.05	45.23	38.80	26.14
1.0	82.00	63.31	60.11	54.04	47.23	40.00	27.14

Table 4. Effect of agitation time and initial Congo red concentration removal of dye using dead fungus biomass (pH: 6.0, temperature, 25°C, adsorbent dosage 0.8g/100 ml)

Time (min)	% Decolorization at different initial dye concentration (mg/100 ml)						
	10	20	30	40	50	60	70
0.0	00.00	00.00	00.00	00.00	00.00	00.00	00.00
20	17.34	15.11	14.90	13.12	13.02	14.40	13.00
40	25.10	23.43	22.12	18.23	15.34	14.54	11.05
60	37.00	35.12	29.13	25.16	21.12	17.67	16.45
80	39.01	38.50	37.00	33.08	28.34	25.21	22.00
100	55.30	53.89	53.15	45.34	36.10	27.11	23.11
120	68.12	66.09	58.23	51.03	45.00	29.10	24.23
140	77.37	66.03	59.10	52.05	45.02	31.03	26.03
160	80.13	60.01	62.34	54.00	47.34	38.21	26.09
180	82.23	63.20	60.09	55.03	47.45	40.50	27.03
200	80.02	63.00	58.89	54.90	45.00	40.02	25.98

CONCLUSIONS:

A. fumigatus showed a high ability to decolorize Congo red, where using dead biomass is advantageous. Compared with live fungal cells, dead fungal biomass possesses various advantages such as absence of nutrient needs and ease of regeneration, therefore this finding offers a potential trend for the development of a cost effective for biosorption of Congo red.

Conflict of Interest:

The authors declare that they have no conflict of interest.

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الامتصاص الحيوي لصبغة أحمر الكنجو باستخدام الكتلة الحيوية والميتة لفطر اسبرجيللس فيوميجاتس المحب للحرارة

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تم دراسة إزالة لون صبغة الكنجو الأحمر باستخدام الكتلة الحيوية النامية والميتة لفطر اسبرجيللس فيوميجاتس المحب للحرارة، وجد أن نسبة الامتصاص الحيوي للصبغة تزداد بزيادة درجة الأس الهيدروجيني حتى 7 عند تركيزات 10 و 60 و 70 ملج/100 مل وتزداد حتى أس هيدروجيني 7 عند تركيزات 20، 30، 40، 50، و 60 ملج/100 مل. ووجد بزيادة درجة الحرارة تزداد الكمية الممتصة من الصبغة حتى 30 درجة مئوية ثم تقل بعد تلك الدرجة، وبزيادة كمية الكتلة الميتة عن 0.8 جم لكل 100 مل كانت غير مؤثرة في نسبة امتصاص الصبغة. ووجد أن أعلى نسبة امتصاص للصبغة كانت عند 180 دقيقة. ودعمت تلك الدراسة قدرة الكتلة الميتة للفطر على امتصاص الصبغة التي تتميز بعدم احتياجها للمواد الغذائية مقارنة باستخدام الفطر النامي.