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The synergetic effect of a fungal biofilm and an adsorbent media in a fluidized bed system, on the wastewater treatment

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Abstract

The present study aims to determine the ability of three promising materials; suspended fungal biomass, alginate beads and immobilized fungal biomass as a bio-sorbent film on alginate beads; in a Fluidized Bed System (FBS) to remove most of organic and inorganic pollutants from wastewater and also determine the ability of immobilized fungal biomass for recirculation. Wastewater was derived from Kitchener drain, Kafr El-Sheikh Governorate, Egypt. This drain is characterized by a high concentration of mixed heavy metal and organic matter. This experiment was conducted in three phases using nontraditional technique in wastewater treatment. In the first phase, the suspended fungal biomass remove COD, BOD, TDS, TSS, TN, TP and total heavy metals up to 69%, 54%, 36%, 67%, 66%, 72% and 75%, respectively. . In the second phase, the alginate beads remove COD, BOD, TDS, TSS, TN, TP and total heavy metals up to 81%, 58%, 38%, 71%, 69%, 79% and 83%, respectively. In the third phase, the immobilized fungal beads remove COD, BOD, TDS, TSS, TN, TP and total heavy metals up to 85%, 76%, 42%, 79%, 73%, 80% and 92%, respectively. The results showed that the immobilization of the fungal biomass by Alginate had enhanced the bio-sorption capacity of all measured contaminants more than suspended fungus alone or alginate alone. Results also showed the efficacy of recycling of immobilized fungal biomass up to three cycles to reach the sorption capacities of COD, BOD, TDS, TSS, TN, TP and total heavy metals up to 67%, 55%, 24%, 58%, 48%, 61% and 69%, respectively in third cycle. Studies of bio-sorbent films include; SEM and FT-IR examinations indicating the advantage of using FBS immobilized fungal media as promising system for wastewater treatment.

Key words: Fluidized Bed System, Wastewater, Heavy metal, Suspended biomass

INTRODUCTION

The increases in environmental concerns and water demands as well as Egyptian water scarcity and Egypt's water stable share lead to a strong direction towards wastewater treatment. The reuse of wastewater has a number of benefits that conserve the needed resources, providing sound solution to water scarcity and climate change (Abdin & Gaafar 2009). Factories are typically characterized as pollution intensive industrial complexes which generate widely varying and high-strength wastewater (Uberoi 2003). Kitchener canal extended about 108 km and collects agricultural drainage, industrial drainage including spinning factories of Kafr El-Sheikh and sewage water of Kafr El-Sheikh city and El-Gharbia governorate. In Egypt, Kitchener canal is considered as one of the most polluted water branches. This wastewater is contaminated with agricultural chemicals, heavy metals, salts and domestic wastewater pathogens (Doaa & Sabreen 2015). Some industrial drainage is discharged directly to the main canal without proper treatment which adds difficulties to the environment and aqueous system (Abdulla et al 2010).

Treatment of highly polluted water can be done by different methods such as chemical, physical, biological or combination of these methods (Durai & Rajasimman 2011). The common methods were filtration, reverse osmosis, chemical precipitation, chemical oxidation or reduction (electrochemical treatment), evaporative recovery, ion exchange, membrane technologies and adsorption on activated carbon (Durai & Rajasimman 2011; Mahmoud et al 2017). These techniques have significant disadvantages including the need for expensive equipment, incomplete metal removal and monitoring systems (Volesky & Holan 1995).

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Biomass cost is a valuable criterion that should be considered when selecting materials. A sorbent can be considered as "low cost" if it is available in nature, industrial processes byproduct or if it requires a minimal processing (Alluri et al 2005; El-Deen et al 2017). Fungal bioremediation have several advantages of degradation capabilities of organic constituents and metal trapping through mechanisms of bio-sorption; metal binding to cell walls, absorption into the cells or keeping metals in the cytosol and metal-binding proteins (Gadd et al 1999).

The attachment of biological films on a supporting material such as alginate helps in maintaining viability and treatment efficiency. Alginate polymer is manufactured from brown algae (Pawar & Edgar 2012; Mahmoud & Mohamed 2017). Fungal biofilm is immobilized in alginate polymer in beads like structure. These beads can be reused for many treatment cycles (Alluri et al 2007).

FBS using immobilized fungal biofilm attached on alginate provides a better efficacy than suspended biomass as it requires less complex separation, allows a high biomass density to be maintained, provides a great opportunity for reuse and recovery and avoids the channeling problems (Ilamathi *et al*., 2014).

All recent studies aim to find an easily available and eco-sorbent materials of natural origin as bio-sorbents (Li et al 2011), therefore, the aim of this study was to investigate the ability of suspended fungal biomass, alginate beads and immobilized fungal beads in a FBS to remove most of organic and inorganic contaminants present in wastewater and determine the ability of immobilized fungal biomass for recycling and reusability.

MATERIALS AND METHODS

1.1 Samples collection

Composite wastewater samples were collected from Kitchener drain – Kafr El-Sheikh Governorate, Egypt, which is contaminated site with industrial, agricultural and municipal wastewater in sterilized bottles of 1.5L capacity, transported on ice-box then mixed to become one sample that is analyzed within 8 hours.

Kitchener drain wastewater sample characteristics are shown in Table 1. The COD/N/P ratio averaged 120/0.72/0.35 that indicated high organics, nitrogen and phosphorus. The inhibition of biodegradation which represented by low BOD value to less than 1/3 of COD value because of high metal ions and dissolved salts concentrations. This is also confirmed by Ros & Ganter 1998 and Song et al 2000.

Table 1: Characteristic Analysis of Wastewater Sample

Parameters	Value	Parameters	Value
COD (mg/L)	1200	Total Nitrogen (mg/L)	7.2
BOD5 (mg/L)	372	Phosphorous (mg/L)	3.5
TDS (mg/L)	850	Total heavy metal (mg/L)	8.7
TSS (mg/L)	715	DO (mg/L)	0.43
Temp (°C)	29.4	PH	6.8

1.2 Isolation of fungal strain

Collected sample were examined using serial dilution and plate count technique (Benson 1985). Greenish brown colonies reaching 45mm in diameter on Czapek's-Dox Agar media were developed after seven days at 28°C. Conidia and vesicle are globosely to sub-globosely in shape and have diameter 3.4μm and 25μm, respectively. Conidiophore is 10μm in diameter. The dimensions of first and second sterigmata are 11.7×6.1μm and 9.4×5.1μm, respectively. Therefore, it's identified as *Aspergillus tamarii* as shown in Fig.1. It was then purified by streaking colonies repeatedly on PDA medium and observed under light microscopy using Soft-Imaging GmbH software (analysis Pro ver. 3.0) present at the regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

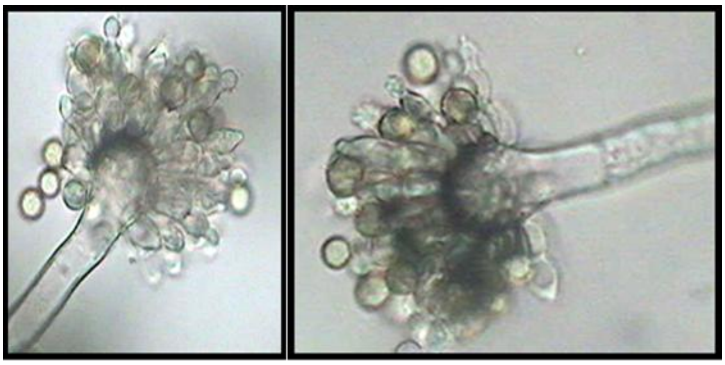

Fig. 1: Aspergillus tamarii isolate

1.3 Biofilm and absorbent media Preparation in a FBS

The method of preparing alginate gel beads was described by El-Naggar (2006). 30 g of Sodium alginate powder C6H7O6Na were mixed with 1 liter distilled water to prepare 3% solution of Sodium alginate gel shown in Fig. 2. The solution was refrigerated for 30 minutes then added as drops into 2% solution of calcium chloride $CaCl_2$ with stirring to prepare beads.

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Fig. 2: Alginate and immobilized fungal beads preparations

Spheres of alginate hydro gel were formed into equal size of diameter ranged from 3.5-4.0 mm. Distilled water was used to wash the alginate beads then dried at 60°C for two hours then used as an effective adsorbent particle in a FBS in second and third phases of treatment. After drying, shape, size and weight of the beads were changed. *A. tamari* biomasses were immobilized by its inclusion in the alginate polymer. The biomass / polymer spheres were used as a media with attached biofilm in a FBS in the third phase of treatment.

1.4 Biosorption experiments

Biosorption studies were conducted in three phases, in each phase 1gm of biosorbent were immersed in one liter of Kitchener drain wastewater sample in a FBS. In the first phase the biosorbent was A. Tamari which used as suspended biomass. In the second phase the biosorbent was the alginate beads. In the third phase the biosorbent was immobilized fungal beads to enhance the biosorption efficacy. To examine the reusability and recycling ability of the immobilized fungal beads in FBS, three consecutive cycles of the immobilized fungal beads were carried out to the third phase.

Experiments were conducted by mixing of raw wastewater with the studied biosorbent in FBS at 120 rpm for two hours of contact at room temperature. After equilibrium time, a sample from the supernatant was analyzed chemically including total heavy metals concentrations that were measured by Atomic Absorption Spectrophotometer. TDS, TSS, BOD, COD, Total Nitrogen and total Phosphorous concentrations were analyzed according to standard method for examination of water and wastewater, 23rd ed. (APHA, 2017).

1.5 Recycling or Reusability

In order to show the reusability of the biosorbent (Immobilized fungal beads), 0.05N HNO_3 was used for elution. The biosorbent was then studied for recycling and reusability. This process was carried out for three subsequent cycles. Recycling or reuses of the immobilized beads was one of the most important advantages over the suspended biomasses.

1.6 Experiment setup

This study was conducted to understand the optimum parameters of non-conventional treatment technique that considered environmental friendly, available and inexpensive technology to remove contaminants from wastewater. Fig. 3 illustrates the schematic diagram of the treatment system and Fig. 4 shows a picture of the FBS reactor.

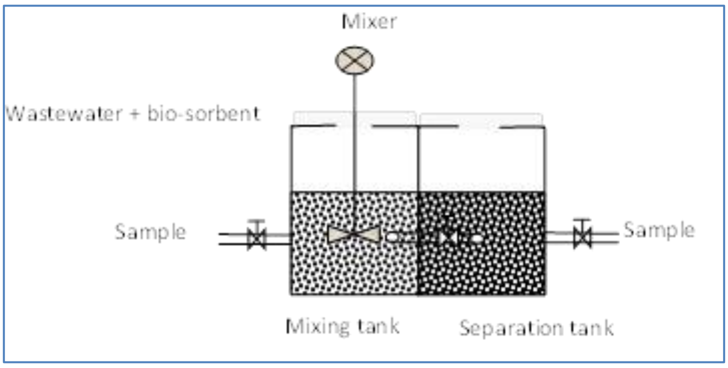

Fig. 3: Schematic diagram of the Fluidized Bed System

Fig. 4: Experimental Fluidized Bed System

The incoming wastewater, called influent, passes through 2 liter reactor tank, where wastewater and suspended fungal biomass (in phase 1), alginate beads (in phase 2) or immobilized fungal beads (in phase 3) are mixed together by a jar test mixer. The treated wastewater then flows to the 2 liter secondary tank which acts as a separation tank.

1.7 Biosorption / desorption cycles

The reuses of the immobilized fungal beads in the third phase were done for three consecutive cycles. The accumulated pollutants were detached from the biosorbent beads by using 0.05N nitric acid for approximately one hour at 150 rpm orbital shaker and washed continuously by distilled water till the pH of the washing solution reaches 6 – 6.5 then the biosorbent beads were ready for reuse in subsequent cycle. The removal efficiency was recorded after each cycle and the weight of pollutant removed in mg / g of dry biosorbent (mg/g dry biosorbent) can be calculated as follow;

$$Y = V \ \frac{X_0 \ - \ X_e}{\text{W}}$$

Where, (Y) is the mg of contaminants removed per dry biosorbent in grams; (V) is the sample volume (Liter), (X_0) and (X_e) are the initial and residual contaminants concentration (mg/Liter), respectively, and (W) is the weight of dry biosorbent (g) (Gadd et al 1999).

1.8 Statistical analyses

All analyses were done in triplicate. 2007 Excel worksheets were used for drawing the experimental figures.

RESULTS AND DISCUSSION

Kitchener drain wastewater is one of drains that cause severe environmental problems in Kafr El-Sheikh state, Egypt as it characterized by high contaminants Include; COD, BOD, total nitrogen, total phosphorous, heavy metals, suspended and dissolved solids.

2.1 Treatment by suspended fungal biomass (A. tamarii)

In the first phase of FBS, the removal percentages after treatment by suspended fungal biomass (*A. tamarii*) remove COD, BOD, TDS, TSS, TN, TP and total heavy metals up to 69%, 54%, 36%, 67%, 66%, 72% and 75%, respectively as shown in Fig. 5a. The biosorption capacities of COD, BOD, TDS, TSS, TN, TP and total heavy metals were 828, 200.9, 306, 479.05, 4.752, 2.52, 6.525 mg/g dry suspended fungal biomass (*A. tamarii*), respectively as shown in Fig. 5b. Results were in line with those reported by Durai & Rajasimman, 2011.

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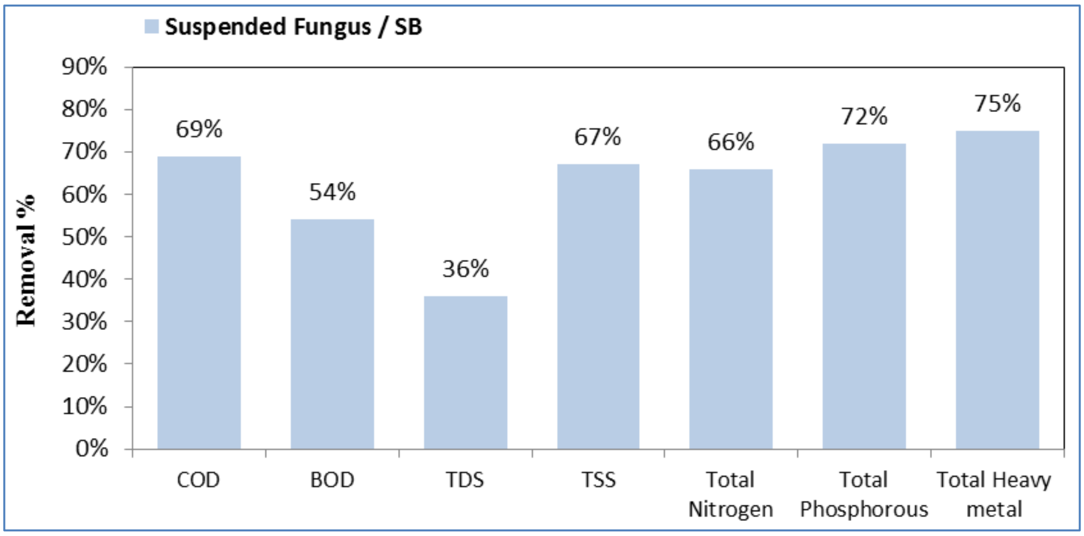

Fig. 5a: Percentage of contaminants removal after treatment by suspended fungal biomass

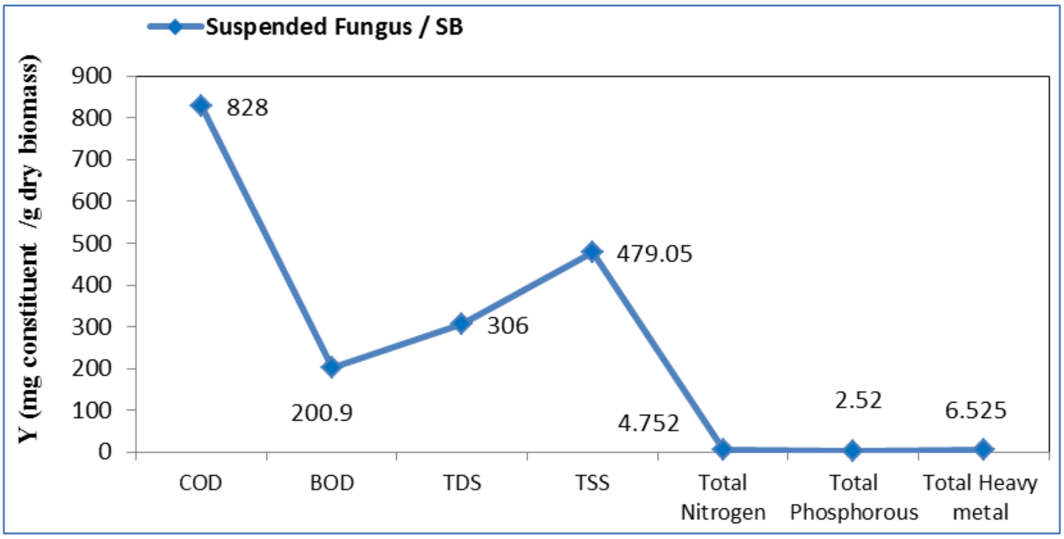

Fig. 5b: Adsorption capacity of suspended fungal biomass

2.2 Treatment by Alginate beads

In the second phase of FBS, the removal percentages after treatment by alginate beads remove COD, BOD, TDS, TSS, TN, TP and total heavy metals up to 81%, 58%, 38%, 71%, 69%, 79% and 83%, respectively as shown in Fig. 6a. The biosorption capacities of COD, BOD, TDS, TSS, TN, TP and total heavy metals were 977.5, 215.5, 326.5, 506.65, 5.001, 2.76, 7.208 mg/g dry alginate beads, respectively as shown in Fig. 6b.

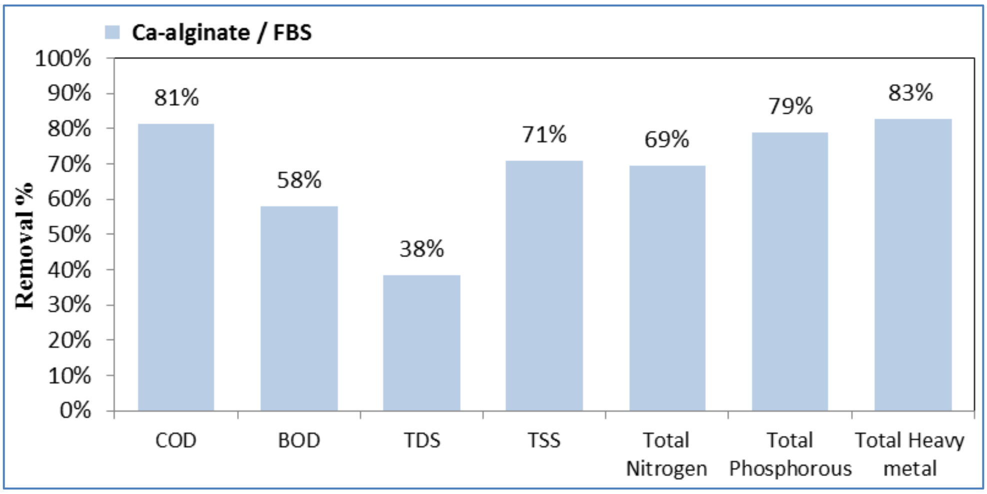

Fig. 6a: Percentage of contaminants removal after treatment by dry Alginate beads as a FBS media.

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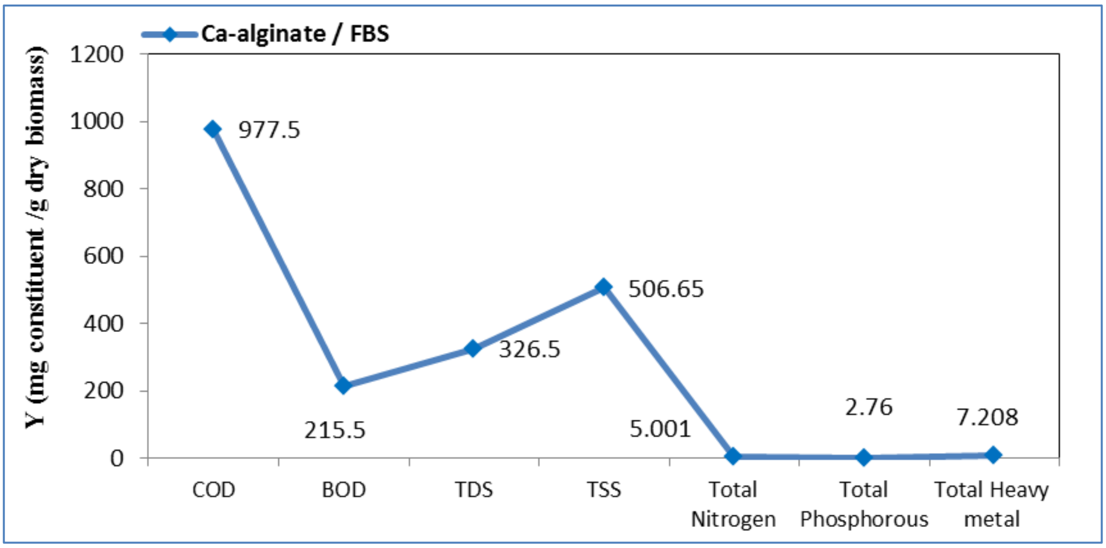

Fig. 6b: Adsorption capacity of dry Alginate beads.

2.3 Treatment by immobilized fungal beads (fungal biomass as a bio-sorbent film attached on alginate beads)

In the third phase of FBS, the removal percentages after treatment by immobilized fungal beads achieve removal of COD, BOD, TDS, TSS, TN, TP and total heavy metals up to 85%, 76%, 42%, 79%, 73%, 80% and 92%, respectively as shown in Fig. 7a. The biosorption capacities of COD, BOD, TDS, TSS, TN, TP and total heavy metals were 1020, 282.7, 357, 564.85, 5.256, 2.8, 8.004 mg/g dry immobilized fungal beads, respectively as shown in Fig. 7b.

The removal efficiencies and biosorption capacities for the three phases were in the following order; Suspended fungal biomass < alginate beads < immobilized fungal beads as shown in Fig. 8. This may explained by the availability of active sites needed for biosorption and effective remediation. These results and explanations were in agreement with Muhammad et al 2009; Chatterjee et al 2010; Mahmoud &Mohamed 2017.

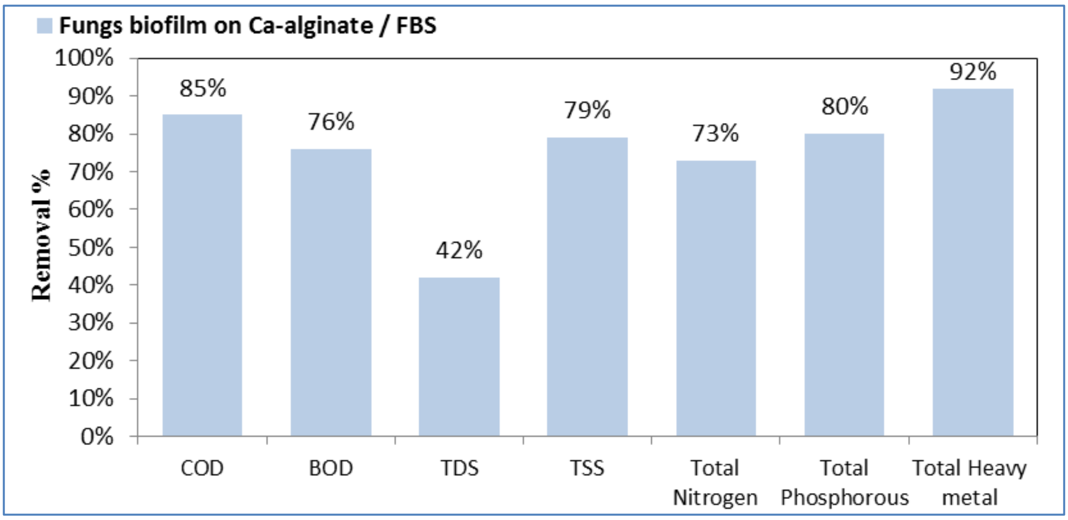

Fig. 7a: Percentage of contaminants removal after treatment by immobilized fungal beads.

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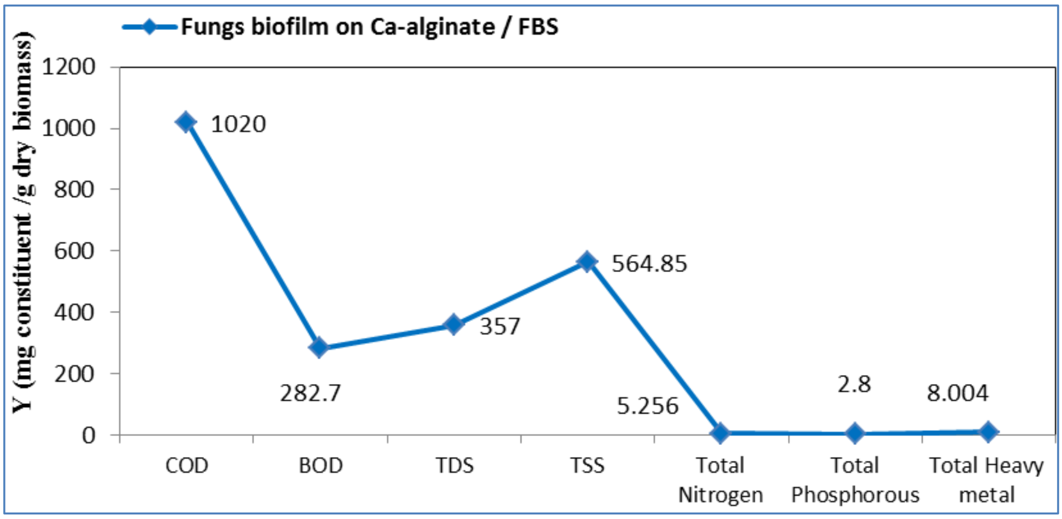

Fig. 7b: Adsorption capacity of immobilized fungal beads.

The removal efficiencies and biosorption capacities for the three phases were in the following order; Suspended fungal biomass < alginate beads < immobilized fungal beads as shown in Fig. 8. This may be explained by the availability of active sites needed for biosorption and effective remediation. These results and explanations were in agreement with Muhammad et al 2009; Chatterjee et al 2010; Mahmoud &Mohamed 2017.

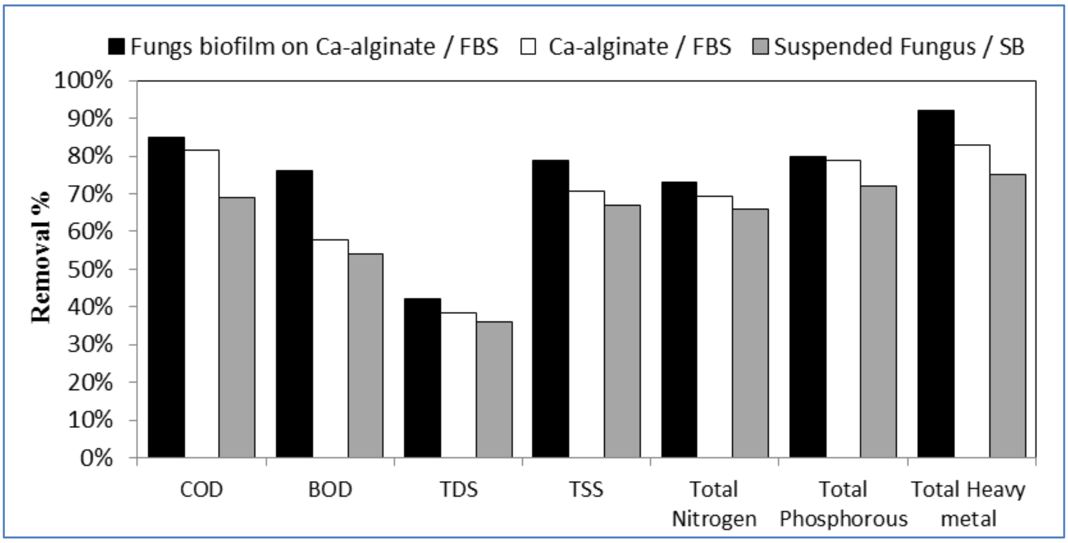

Fig. 8: Contaminants removal percentage after treatment by suspended fungal biomass, alginate beads and immobilized fungal beads.

2.4 Recycling of immobilized fungal beads

A considerable advantage of the FBS is the ability to reuse the fluidized bed media. To investigate the reusability of the immobilized fungal beads, three recycles were applied and tested. Our results showed that the immobilized fungal beads can be reused and recycled but with a decrease to some extent in the absorption capacity. This could be explained by the decrease of free active adsorption sites available for bioremediation (Sag et al 2000). Results showed that the efficacy of recycling of immobilized fungal biomass up to three cycles to reach the sorption percentage of COD, BOD, TDS, TSS, TN, TP and total heavy metals up to 67%, 55%, 24%, 58%, 48%, 61% and 69%, respectively in third cycle as shown in Fig. 9a. Also, the biosorption capacities of COD, BOD, TDS, TSS, TN, TP and total heavy metals reaches 804, 204.6, 204, 414.7, 3.456, 2.135, 6.003 mg/g dry immobilized fungal beads, respectively in third cycle as shown in Fig. (9b).

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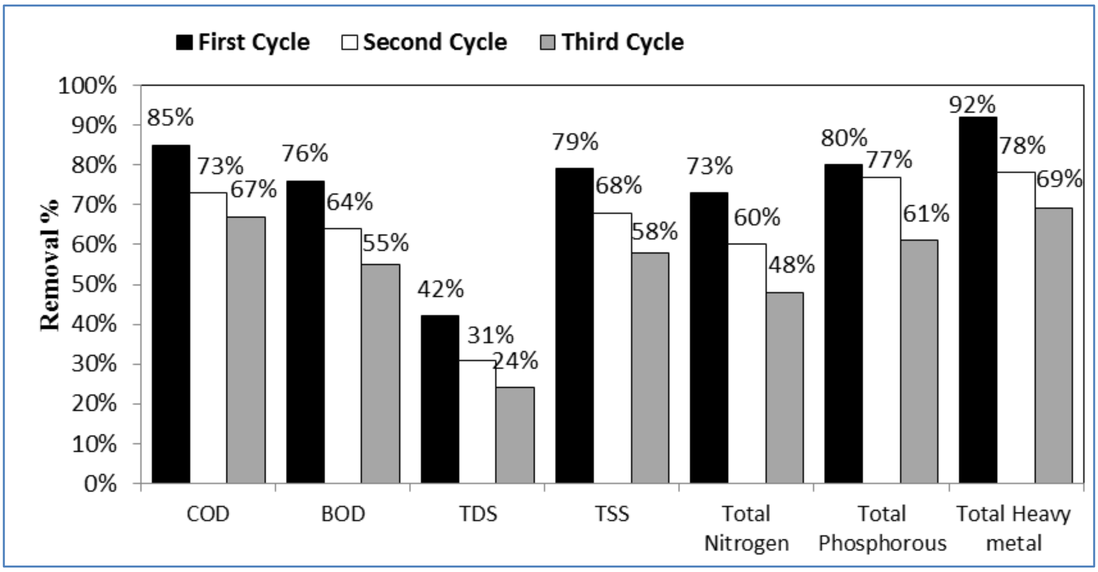

Fig. 9a: Contaminants removal percentage after recycling of immobilized fungal beads for three subsequent cycles.

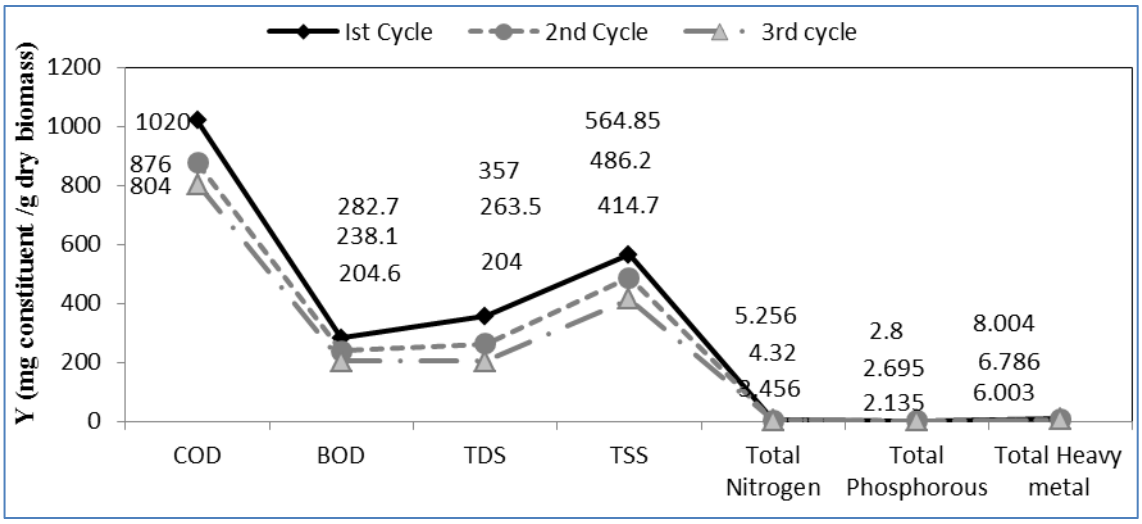

Fig. 9b: Adsorption capacity of immobilized fungal beads for three subsequent cycles.

2.5 SEM Examination

SEM micrographs were done for suspended fungal biomass (*A. tamarii*), alginate beads and Immobilized fungal beads before treatment of wastewater as shown in Fig. 10.

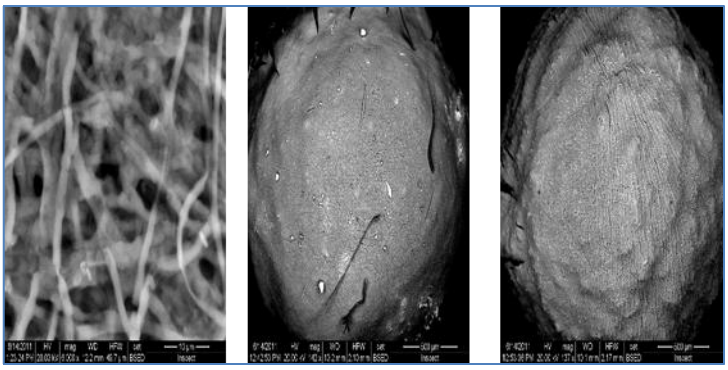

Fig. 10: *A. tamarii* suspended biomass, alginate bead and immobilized fungal beads.

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2.6 Fourier Transform Infrared Spectrometry (FT-IR)

FT-IR is another investigation related to the biosorption phenomenon. It is used for determination of functional groups in frequency range from 500 to 4000 Cm^{-1}. FT-IR is carried out to the immobilized fungal beads before and after treatment. The FBS media were dried to almost nil moisture and send for FT-IR analysis. Spectral region consists of a set of peaks. This analysis had eventually confirmed the difference between functional groups in relation to biosorption occurred and to change in chemical composition. A change of absorption bands can be seen when comparing the FT-IR spectra. After treatment, there is a deletion in the band at (721.3 and 1041.5 Cm^{-1}) which represents strong C-O stretch (Carboxylic acids or Esters). There is also an interesting phenomenon which is the shifting in the band intensity at (1342.4 to 1326.9 Cm^{-1}), shifting in the band intensity at (1407.9 to 1404.1 Cm^{-1}), shifting in the band intensity at (1531.4 to 1554.5 Cm^{-1}). There is a shifting and sharp decrease in the band intensity at (1651.0 to 1639.4 Cm^{-1}), shifting and sharp decrease in the band intensity at (3552.6 to 3375.8 Cm^{-1}) which represents Strong C=O and Strong broad dimer OH (Carboxylic acids). This decrease in the bands intensity was considered as adsorption bands after binding with contaminants as shown in Figs.11, 12.

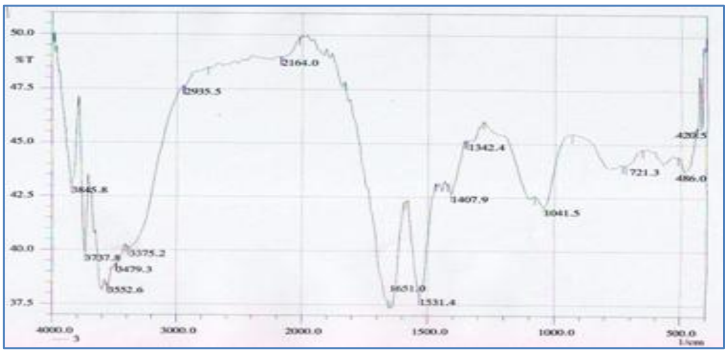

Fig. 11: FT-IR spectrum of immobilized fungal beads before wastewater treatment.

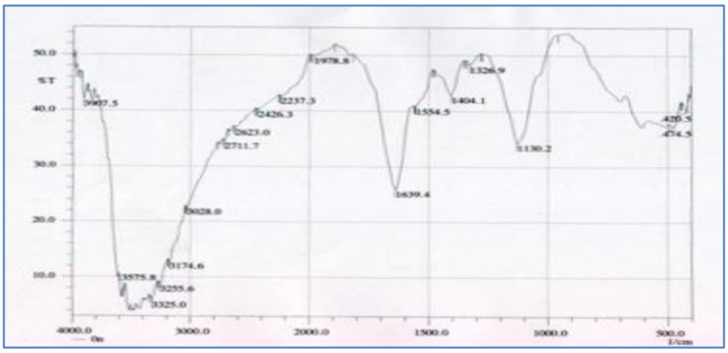

Fig. 12: FT-IR spectrum of immobilized fungal beads after wastewater treatment.

CONCLUSION

Concerning interests of environmental issues and improve the water usages as the needs for water, this research comes to evaluate the important role of microorganisms' especially fungal strains *A. tamarii* in taking up organic loads & heavy metals from aqueous solutions and wastewater. Our findings revealed that the isolated fungal strains *A. tamarii* had a potential to degrade and remove heavy metal at a laboratory scale. To overcome the suspended biomass separation problems and the mass loss after biosorption, the biomass was immobilized on the alginate matrices gel polymer.

Biosorption studies of the immobilized fungal beads have been found to be effective in removing organic and inorganic constituents from wastewater. Also, recycling of the immobilized fungal beads was studied for three subsequent cycles. So, it can be concluded that the immobilization of fungal biomass by alginate could enhance the biosorption of color, organic contents and metal-polluted industrial wastewater. The obtained data are useful in the treatment units designs concerned with the treatment of wastewater. More studies on how system characteristics enhance efficiencies, and eventually, the overall treatment cost, should be performed to be cost-competitive one.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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