Factors affecting the immobilization of fungal biomass on CNT as a biosorbent for textile dyes removal

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Abstract. Effluents from dye and textile industries are highly contaminated and toxic to the environment. High concentration of non-biodegradable compounds contributes to increased biochemical oxygen demand (BOD) and chemical oxygen demand (COD) of the wastewater bodies. Dyes found in wastewater from textile industries are carcinogenic, mutagenic or teratogenic. Biological processes involving certain bacteria, fungi and activated carbon have been employed in treating wastewater. These methods are either inefficient or ineffective. These complexities necessitates search for new approaches that will offset all the shortcomings of the present solutions to the challenges faced with textile wastewater management. This study produced a new biosorbent by the immobilization of fungal biomass on carbon nanotubes. The new biosorbent is called “carbon nanotubes immobilized biomass (CNTIB)” which was produced by immobilization technique. A potential fungal strain, Aspergillus niger was selected on the basis of biomass production. It was found out in this studies that fungal biomass were better produced in acidic medium. Aspergillus niger was immobilized on carbon nanotubes. One-factor-at-a time (OFAT) was employed to determine the effect of different factors on the immobilization of fungal biomass on carbon nanotubes and optimum levels at which the three selected parameters (pH, culture time and agitation rate) would perform. Findings from OFAT showed that the optimum conditions for immobilization are a pH of 5, agitation rate of 150rpm and a culture time of 5 days.

1. Introduction

Immiscobilization is a process whereby cells are attached or ensnared in a torpid support. Usually, the substrate is converted to a product by the immobilized cells. There is often a retention of the immobilized cells which is continuously utilized in the bioreactor. Over the years, it has been shown that in many processes, using immobilized cells bring about more efficiency when compared to suspended cells [1]. In this research, fungal biomass was immobilized on carbon nanotubes for the production of a novel biosorbent; carbon nanotubes immobilized biomass for the removal of textile dyes.
2. Experimental

Aspergillus niger and Penicillium sp were cultivated in a liquid medium using a shake flask method. The liquid medium was 98 ml of (17g/1000ml) malt extract, (0.1-0.5) g of carbon nanotubes and 2mls of inoculum. The malt extract solution was prepared by dissolving 17g of malt extract powder into 1000mls distilled water. It was placed on a magnetic stirrer and allowed to make a uniform mixture. Varying quantities of carbon nanotubes were measured and poured into the liquid media in the Erlenmeyer conical flask. The pH of each samples were recorded and then autoclaved at 121ºC for 2-3 hours. The liquid medium was allowed to cool down to room temperature after autoclaving. The inoculum, 2% (2mls) of each fungus was used to inoculate the liquid medium and transferred to the incubator shaker at 150 rpm for 3 days. Immobilized activated carbon was filtered with whatman filter paper (100mm) and dried at 105ºC in a drier. The weight of dried biomass was recorded, crushed and then stored in a dry container.

![Image](image1.jpg)  
(A) immobilized carbon nanotubes by Aspergillus niger in culture broth (B) filtered CNTIB (C) dried CNTIB (D) crushed CNTIB (E) stored CNTIB

The growth of fungus (Aspergillus niger) was observed after two days as it grew into pellicles. The pellicles increased in diameter subsequently. After the third day of growth (72 hours), the carbon nanotubes immobilized biomass was harvested by filtering through a 150µm filter. It was then washed thoroughly with distilled water to ensure that CNTs were tightly attached to the biomass. The filtrate which contained the unattached CNTs was passed through the pump, washed thoroughly with deionized water to remove the growth medium sticking on to its surface and filtered to get a clear solution. The CNTIB and unattached CNTs were transferred to the drier for 24 hours at a temperature of 100 C. The weight of dried CNTIB and CNTs were taken. The mass of biomass and mass of attached CNTs were also determined. The percentage of carbon nanotubes attached to fungal biomass was then calculated thus:

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\begin{align*}
M_{uCNT} &= M_{CNT}^0 - M_{CNT}^1 \\
M_{CNT}^1 &= (M_{CNT}^0 - M_{uCNT}) \\
M_B &= (M_{CNTIB} - M_{uCNT}) \\
E_{B-CNT} &= \frac{M_B}{M_{CNTIB}} \times 100
\end{align*}
\]

Where

\( M_B \) = Mass of biomass

\( M_{uCNT} \) = Mass of unattached Carbon nanotubes
$M^{0}_{CNT} =$ Initial mass of carbon nanotubes  \\
$M^{1}_{CNT} =$ Mass of attached carbon nanotubes  \\
$M_{CNTIB} =$ Mass of carbon nanotubes immobilized biomass  \\
$E_{B\rightarrow CNT} =$ Efficiency of biomass immobilization on CNT

3. Results and discussion

3.1. Effect of pH on biomass production

The effect of pH on *Aspergillus niger* for the immobilization on carbon nanotubes was studied. The mass of biomass produced and percentage of carbon nanotubes attached after immobilization were also determined.

![Figure 2: Effect of pH on the biomass production at temperature 32°C, 150 rpm and 72 hours culture time by Aspergillus niger.](image)

The effect of pH was studied on two major types of fungal strains with respect to carbon nanotubes immobilization. As shown in Figure 2, the mass of biomass production was plotted against various pH ranging from 4 to 8. The mass of biomass produced was determined after 3 days of culturing. From the result, it is shown that at pH 4, there was a gradual increase in the mass of biomass produced from 0.42g and 0.38g for *Aspergillus niger* and *Penicillium sp* respectively. At about pH 5.5, the biomass of *Aspergillus niger* and *Penicillium sp* were highest, producing a biomass of 0.65g and 0.58g respectively. Further increase in pH resulted into a decline in production of biomass.

Based on results from the experiment, *Aspergillus niger* was chosen as the fungal strain to be used in the immobilization process. A pH of 5.5 was also observed to be the optimum for biomass production because most of the biomass was produced at that point. It was also observed that growth of fungal biomass was best in acidic media.
The effect of pH was studied on the germination and growth of seven xerophilic fungi - *Eurotium rubrum*, *E. repens*, *Wallemia sebi*, *Aspergillus penicillioides*, *Penicillium roqueforti*, *Chrysosporium xerophilum* and *Xeromyces bisporus*. They were grown at varying temperatures and on media with varying pH values. From the results of the experiment, it was found out that all fungal strains grew faster under acidic than neutral or alkaline pH conditions [2].

Furthermore, according to [3], in the studies of the effects of operational parameters including pH on maturity of *Aspergillus niger* and *Spirogyra sp* in aqueous solution on dye removal, the results showed that the maximum performance of fungal biomass was obtained at pH 3 (acidic). [5] also suggested an acidic pH of 3 for growth of fungal biomass. The optimum pH for the biomass growth of *P. ostreatus*, in the determination of biodegradation products from sulfonated dyes was also suggested at 5 [1].

3.2. Effect of agitation rate

The percentage of biomass attached to carbon nanotubes was examined. The agitation rates were varied from 100 i.e., immobilization increased with corresponding increase in agitation. From the graph, immobilization increased with an increase in agitation rate. Agitation of 100 – 150 rpm gave corresponding increase in immobilization. After agitation of 150 rpm, further increase to 180-200 rpm led to a fall in percentage immobilization. The immobilization was highest (85.1%) at 150 rpm for *Aspergillus niger*. In the study of the influence of agitation rate on the performance of a stirred anaerobic sequencing batch reactor containing immobilized biomass, [6] found out that the use of agitation increased the efficiency of the reactor and enabled reduction of the total cycle time. Moreover, it was found that the biomass of Aspergillus niger was obtained at 150 rpm in accordance with 5 days culturing time [4]. The study by [7] on a white rot fungus, *Pleurotus ostreatus* was also conducted at an agitation rate of 150 rpm.

The effect of agitation rate on the immobilization of fungal biomass on carbon nanotubes is shown in figure 3.

![Figure 3: Effect of Agitation rate on the immobilization of carbon nanotubes at temperature 32°C, 150 rpm and 72 hours culture time by *Aspergillus niger*](image)
3.3 Effect of pH on immobilization of CNTs

The effect of pH was studied on Aspergillus niger for the immobilization of its biomass on carbon nanotubes as shown in Figure 4.

![Figure 4: Effect of pH on the immobilization of carbon nanotubes at temperature 32°C, 150 rpm and 72 hours culture time](image)

The percentage of biomass immobilized on carbon nanotubes was determined. The pH ranges were varied from 4 to 8. After 72 hours of culturing, the percentage immobilization was determined. At pH 5, the carbon nanotubes showed the highest percentage immobilization in which the strain of Aspergillus niger showed about 96% attachment. Further increase in pH from 5 to 8 brought about a decrease in immobilization. This suggests acidic medium as the best medium for growth and immobilization of fungal biomass. Various studies have shown that the acidic media is the most favorable for fungal biomass growth. T. versicolor, P. ostreatus, P. sajor-caju and P. chrysosporium strains were studied by some researchers and they suggested the optimum pH of 4-5 for these fungal biomass growth [5]. Based on the results observed, pH 5 was selected for immobilization of fungal biomass onto carbon nanotubes for the production of the biosorbent, carbon nanotubes immobilized biomass.

3.4 Effect of time on Immobilization of Aspergillus niger

The effect of culture time on the immobilization of carbon nanotubes was studied as shown in Figure 5.
The culture time was varied for 3, 5, 7 and 9 days. From the result, it was shown that the highest immobilization was achieved on the 5th day (120 hours culture time). The 5 day culturing time was also supported by [4] in his research which involved fungal chitosan production from potato processing wastewater and its characterization.

4. Conclusion

The results obtained in this study showed that Aspergillus niger was able to immobilize its biomass on carbon by pallet formation. The effective pH was pH 5, agitation rate was 150 rpm and culture time was found to be 5 days for optimum immobilization of biomass on carbon nanotubes. The highest biomass production in this research for Aspergillus niger was found to be 89% while the highest immobilization was at 96%.

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