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Surface Methodological Approach of *Pleurotus florida* Biowaste Towards Aspirin Drug

S. PADMAVATHY[1,*] and P. PUNGAYEE ALIAS AMIRTHAM[2]

[1]PG and Research Department of Chemistry, Bishop Heber College, Tiruchirappalli-620 017, India
[2]Department of Chemistry, Cauvery College for Women, Tiruchirappalli-620 018, India

*Corresponding author: Fax: +91 431 2770293; Tel: +91 431 2770136; E-mail: padmavathymanju@yahoo.co.in

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Microbial bioremediation covers a wide range of recalcitrant degradation of pharmaceutical waste. The present study aims to inspect the dried, nonliving *Pleurotus florida* biowaste efficacy for bioremediation of aspirin in an ecofriendly manner. The equilibrium uptake of aspirin was investigated using batch experiments which were carried out as a function of contact time, initial concentration, pH and biomass dose. The optimal conditions for the highest percentage removal of aspirin was achieved at 2 h contact time, 100 mg/L of aspirin concentration, at pH 5 and 4.0 g/L biomass dose. The best fit was obtained by Langmuir isotherm model with high correlation coefficient (R^2 = 0.989). The *Pleurotus florida* biowaste was characterized using Fourier transform infrared spectroscopy, X-ray diffraction and thermogravimetric analyzer and their interaction between the aspirin was illustrated with Fourier transform infrared spectroscopy and scanning electron microscope.

Keywords: Aspirin, *Pleurotus florida*, Bioremediation, XRD, TGA, FTIR, SEM.

INTRODUCTION

Global population growth and economic development lead to rapid changes in the structure and functioning of world's ecosystems. Pharmaceuticals are the diverse class of emerging pollutants in water comprises ecological impact even at trace levels [1,2]. Pharmaceuticals have been continuously pierced into the environment either in complete or dissociated form from discarding the unused expired drugs in trash, disposal from manufacturing industries or from hospital waste water for more than two decades, but currently the researchers have focused additional attentions on the identification of their concentration level [3-6]. Some of the biologically non-degradable drug found their way in the food chain. Pharmaceuticals are only partially consumed by the human or animals and the remaining are excreted through urine or feces and are easily transmitted to water or soil and the latest screening reveals a wide range of it in surface and in ground water. The bioactive pharmaceuticals in aqueous environment have severe impact on the human, aquatic flora and fauna [7]. It includes the inhibition or stimulation of growth in aquatic plant species, increase in antibacterial resistance of microbes, fertility and developmental behaviour of aquatic living organisms like vertebrates and invertebrates [8-11]. The most important worldwide pharmaceuticals present in the aqueous environment are the non-steroidal anti-inflammatory drug [12]. Forty thousand metric tons of aspirin has been consumed worldwide as an analgesic, antipyretic, anti-inflammatory and antirheumatic drug per annum [13,14]. Trace levels of aspirin and their metabolites salicyluric acid and gentisic acid in water exhibit developmental resistance in microbes, geno-toxicity and endocrine disruption [15-17]. Among the variety of treatment technologies, research has attained more focusing on the ecofriendly bioremediation process. The main aspiration of bioremediation process is to minimize the concentration of pollutants to harmless, undetectable or acceptable limit [18]. Microbial biomass including bacteria, fungi and algae are the prominent biomaterial available in large quantities for the biodegradation of contaminants. Chemical behaviour of the drug, biological property of the microorganisms and climatic conditions are the vital factors responsible for the degradation of pharmaceutical substances by microorganisms [19]. The enhanced waste water treatment biological degradation by microorganisms of drugs leads to the reduction of parental pharmaceutical compounds and their metabolites. Fungi are unique and ubiquitous organisms playing a key role in balancing the ecosystems [20]. Fungal mycelium is well suited for the breakdown of pharmaceutical compounds [21,22]. *Pleurotus florida* fungal ravage biomass was chosen as an imperative biomaterial for the removal of aspirin under different experimental conditions due to its large availability, tough texture when dried and high biological efficiency [23]. The fungal cell walls of the biomass are mainly composed of proteins and cellulose and the functional groups such as amine, amide, sulfates, hydroxyl, carboxyl and phosphate groups offer an excellent binding property with aspirin [24,25].

EXPERIMENTAL

All the chemicals used in the study were of analytical grade and all the solutions were prepared with double distilled water. Aspirin (USV Ltd, India), sodium hydroxide (Merck), hydrochloric acid (Merck) and ferric chloride (Qualigens) were procured and used as received.

Biomass preparation: The ravage biomass *Pleurotus florida* used in this study was collected from the mushroom cultivation center located at Tiruchirappalli, Tamil Nadu, India. The biomass was washed thoroughly with distilled water to remove the dirt and impurities present in it and oven dried at 80 °C for about 5 h. The dry mass obtained was then grounded, soaked in distilled water for about 12 h, filtered, dried and stored in an air tight container for further use.

Aspirin solution: The stock solution of aspirin 1000 mg/L used in this study was prepared by dissolving it in distilled water. Desired concentrations were prepared by diluting the stock solution with distilled water.

Aspirin quantification: Aspirin concentrations were determined by iron(III) chloride using spectrophotometer at 530 nm. Aspirin produces violet blue complex of tetraaquosalycylatroiron(III) complex which displays maximum absorption at about 530 nm. The initial and final concentration of aspirin used in batch studies were calculated by estimating the concentration of aspirin spectrophotometrically and the difference in concentration reveals the removal efficiency of *Pleurotus florida* biomass.

Characterization of biomass: The phyto-chemical analysis of the methanolic *Pleurotus florida* extracts were carried out using standard methods [26] and the result is given in Table-1. The FTIR spectra of *Pleurotus florida* biomass before and after loading aspirin solution were recorded on Perkin Elmer instrument in the range of 4000-400 cm^{-1} in order to provide a qualitative and preliminary characterization of main functional groups present on the fungal biomass. Scanning electron microscopic studies was employed to investigate the surface morphology such as pore structure and texture of *Pleurotus florida* biomass before and after aspirin treatment. The size and the thermal stability of the biomass was determined using XRD pattern and thermo gravimetric analyzer.

TABLE-1
PHYTO-CHEMICAL SCREENING OF METHANOLIC EXTRACT OF *Pleurotus florida* BIOMASS

Bioactive component	Concentration
Carbohydrate	Medium
Flavonoids	Medium
Oil	Low
Phenols	High
Protein	High
Alkaloids	Absence

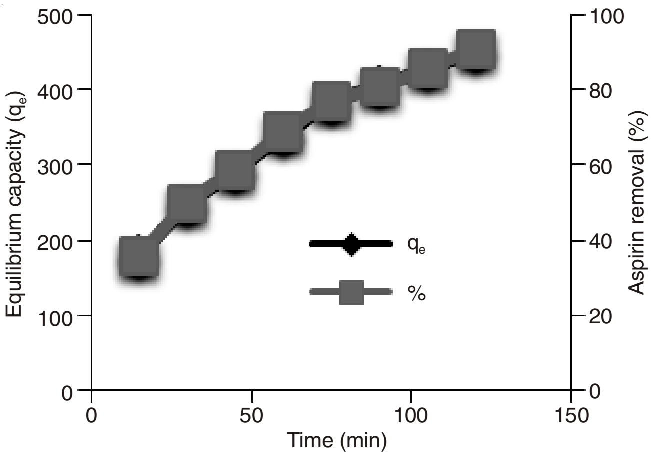

Fig. 1. Effect of time of contact

RESULTS AND DISCUSSION

Batch mode experiments: The contact time was investigated first to determine the equilibrium time required for the binding of aspirin onto the *Pleurotus florida* biomass at 30 ± 2 °C. The maximum percentage removal 91 % was achieved at 2 h (Fig. 1). The rapid removal rate at the initial stages of contact time could be attributed to the availability of a large number of active sites on the surface of *Pleurotus florida*. Later there was a gradual occupancy of these sites by aspirin and the removal efficiency became less efficient [27]. The effect of aspirin concentration by *Pleurotus florida* biomass waste was evaluated under the reaction conditions such as pH 5, dose 4 g/L, 30 ± 2 °C at optimum contact time and illustrated in Fig. 2. The data reveal that the maximum per cent removal at lower concentration was due to the strong and quick interaction of aspirin with the binding sites of biomass. The decrease in percentage uptake of aspirin with the increase in concentration was accredited with the lack of availability of free active binding sites [28]. Initial pH is an important factor which plays a vital role in evaluating the competence of *Pleurotus florida* biomass. Maintaining other parameters constant the effect of pH on aspirin was carried out in the range from 1.0 to 10 and the results are shown in Fig. 3. The sharp increase in the aspirin removal reveals high concentration of H^+ ions compete for the vacant active sites of microbial waste biomass. The percentage removal of aspirin at pH 4 was found to be 37.02 % and pH 5 resulted in the increase of removal efficiency to 81.98 %. Beyond the pH 5 there was no sufficient increase indicating that the process had reached the saturation point [29,30]. Biomass dose determines its capacity for a given initial concentration of aspirin at the operating conditions. The per cent uptake of aspirin (100 mg/L) with varying dosages of *Pleurotus florida* is shown in Fig. 4 and the maximum per cent removal 90.99 % was observed for 400 mg of biomass. The increase in the per cent removal could be attributed to the increase of more adsorptive surface area and the availability of more binding sites with the increase in biomass dose. After the equilibrium time, there is no change in per cent removal due to the saturation of available biomass sites [31].

Isothermal studies: The sorption process involves the interaction between the solid phase of biomass and the liquid phase of aspirin solution. The ionic distribution between the biomass and the solution determines the affinity of biomass for aspirin. The isothermal curve is well fit into the Langmuir

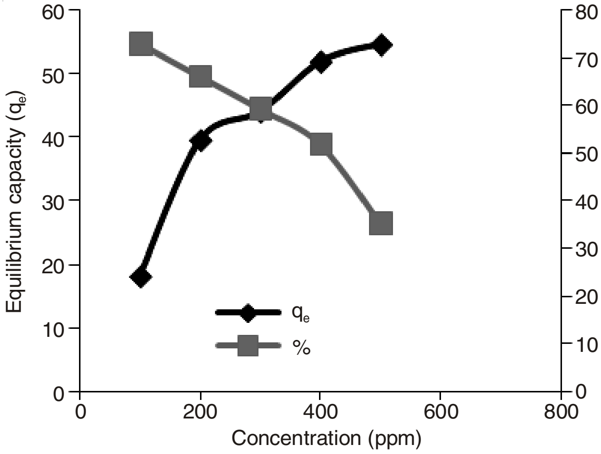

Fig. 2. Effect of initial concentration of aspirin

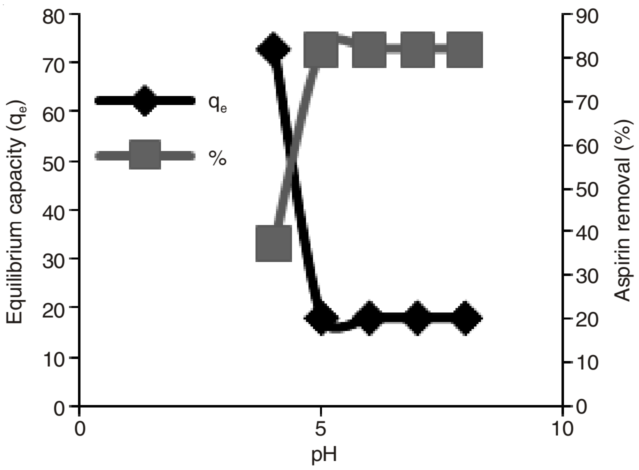

Fig. 3. Effect of pH

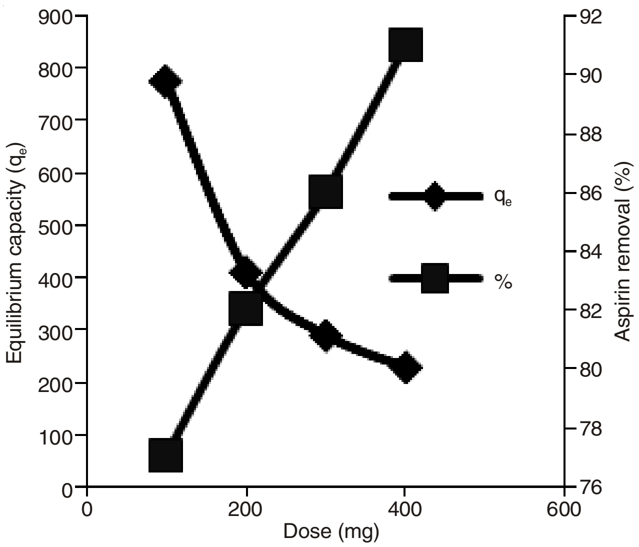

Fig. 4. Effect of biomass dose

pattern [32] where the active sites of fungal biomass hold the aspirin ion and it usually serves to estimate the maximum aspirin uptake value (q_{max}). The experimental results are in good agreement with Langmuir equation ($R^2 = 0.989$) and the linear transformation of the data were expressed in Fig. 5. The result suggests that bioremediation of aspirin on *Pleurotus florida* biomass is homogeneous and multilayer in nature.

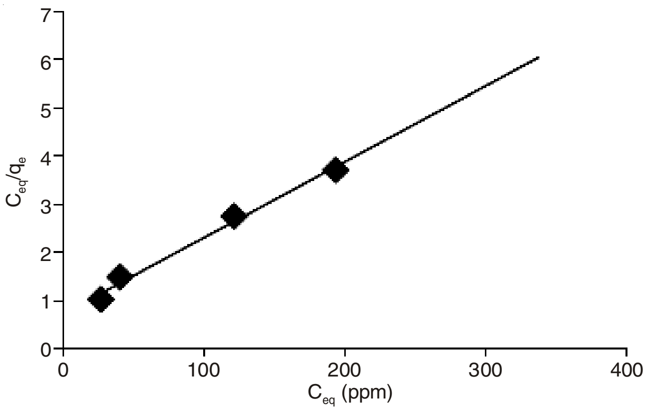

Fig. 5. Langmuir isothermal plot

Characterization of biomass: The preliminary phytochemical screening results revealed the presence of varying quantity of bioactive components such as phenols, proteins, carbohydrates, flavanoids, tannins and oil with the absence of alkaloids in the methanolic extracts of *Pleurotus florida* biomass. The XRD pattern of aspirin loaded *Pleurotus florida* biomass (Fig. 6), exhibits the sharp reflection at 2θ = 19.49° and this corresponds to the characteristic features of cellulose present in the *Pleurotus florida* biomass [33]. The descending TGA curve accounts for the weight loss of biomass with variation in temperature. The initial degradation is associated with the loss of water molecules which occur around 100 °C. The latter degradation from 200-600 °C in the Fig. 7, describes the decomposition of hemicelluloses, cellulose, proteins and lignin compounds in the *Pleurotus florida* biomass. Both the TGA and DTA curve of *Pleurotus florida* confirm the high thermal stability of biomass up to 600 °C [34,35].

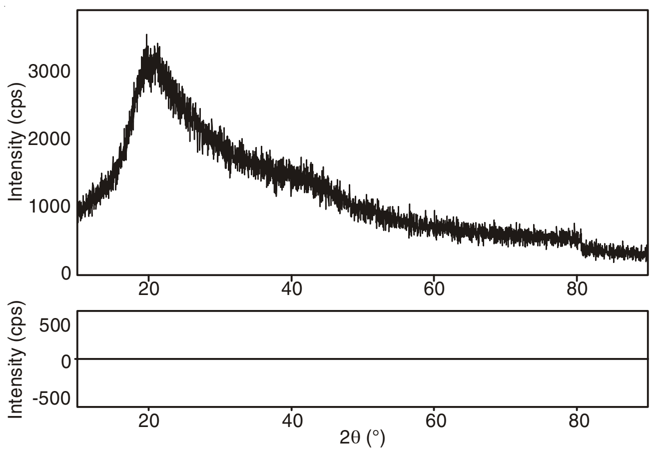

Fig. 6. XRD pattern for *Pleurotus florida* biomass

FTIR spectroscopy: The FTIR analysis was carried out to determine the contribution of functional groups for the binding with aspirin. Fig. 8 shows the FTIR spectrum of unloaded and loaded aspirin- *Pleurotus florida* samples. Table-2 lists the band assignments of different functional groups present in the unloaded biomass and aspirin loaded biomass. The band at 3406.02 cm^{-1} in unloaded biomass was shifted to 3398.28 cm^{-1} after binding with aspirin characterizes the interaction of hydroxyl (phenolic) group. The –CN stretching vibrations might be expected in the region 2400-2100 cm^{-1}. The peak at

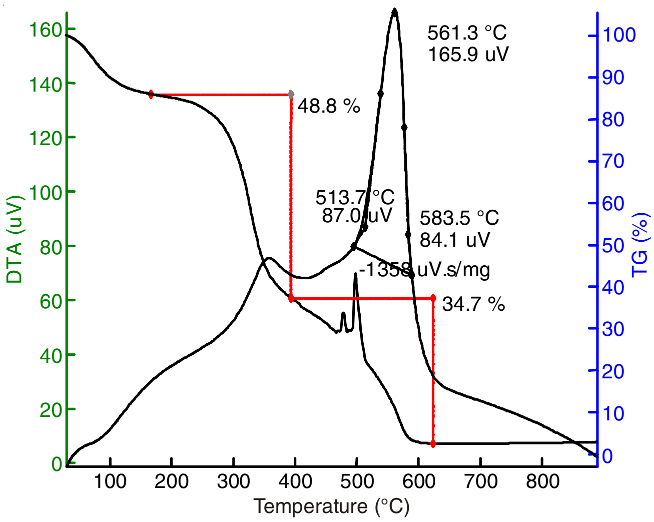

Fig. 7. TGA and DTA pattern of *Pleurotus florida* biomass

TABLE-2
FREQUENCY REGION AND ASSIGNMENTS OF FTIR BANDS OF *Pleurotus florida* AND ASPIRIN LOADED BIOMASS

Frequency region (cm^{-1})		
Pleurotus florida biomass	Aspirin loaded biomass	Assignment
3406.02	3398.28	O-H stretching
2365.01	2358.72	Triple bond stretching in -CN
1567.51	1577.45	-NO stretching
1125.74	1124.76	-PO (phosphine oxide)
829.79	831.28	Saturated acid (–COO-) group
696.47	697.61	Nitro compounds and disulfide groups

1577.45-1567.51 cm^{-1} attributed to an interaction between aspirin and N-containing bio-ligands. On the other hand, band observed at 800-600 cm^{-1} correspond to the S-O linking of the C-SO_3 group. The results showed that the important activation site enhancing the binding of biomass with aspirin were N-H of amines, -C=O of amides, carboxyl, hydroxyl, sulphonate and phosphate groups [36].

Scanning electron microscope analysis: Scanning electron microscope is an analytical technique widely used to study the surface morphology and the characteristics of the biomass. Fig. 9 represents the SEM micrographs illustrating the morphology of *Pleurotus florida* biomass before and after loading with aspirin. It was observed that small particle size provides larger surface area for the contaminant and cell-surface morphology has been considerably distorted due to the aspirin sorption on to the *Pleurotus florida* texture.

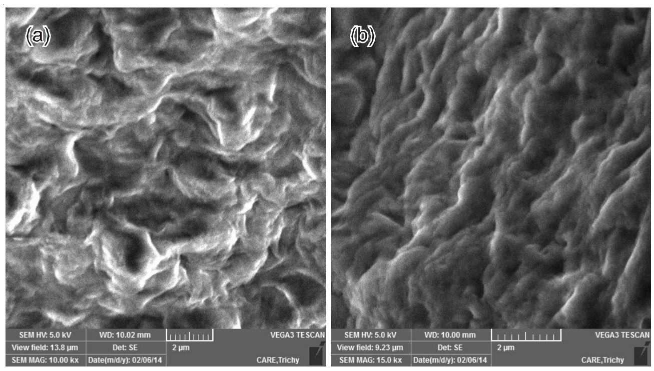

Fig. 9. SEM micrograph (A) *Pleurotus florida* biomass (B) Aspirin loaded *Pleurotus florida* biomass

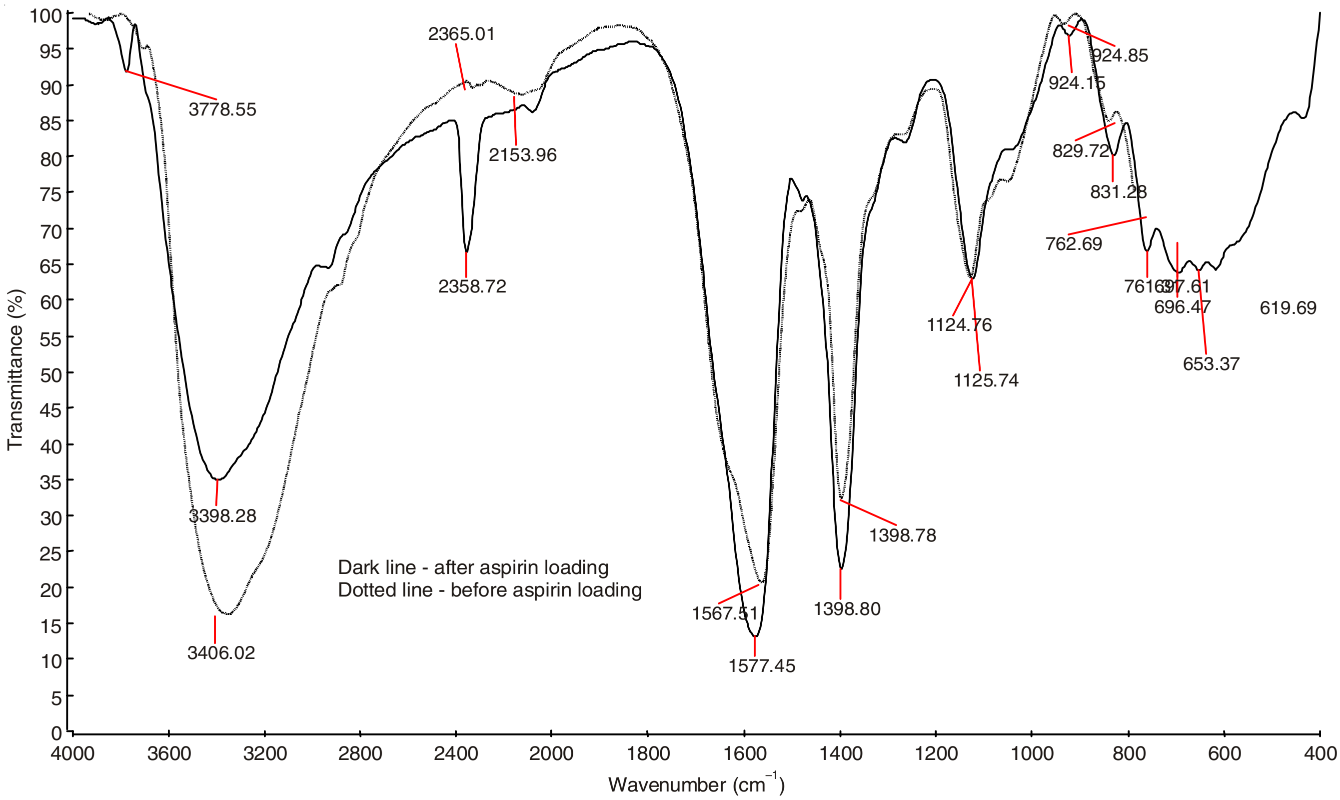

Fig. 8. FTIR spectra of *Pleurotus florida* before and after loading aspirin

Conclusion

The current study highlighted the potential of *Pleurotus florida* biomass for remediating aspirin from aqueous solution. The efficacy of biomass was studied under various parameters and the result implies that *Pleurotus florida* can be acted as an ideal alternate, cost effective and easily available potent biomass for the removal of aspirin from the waste water. In future this competent ecofriendly approach would focus on the mechanism and uptake of other pharmaceutical compounds polluting the environment.

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