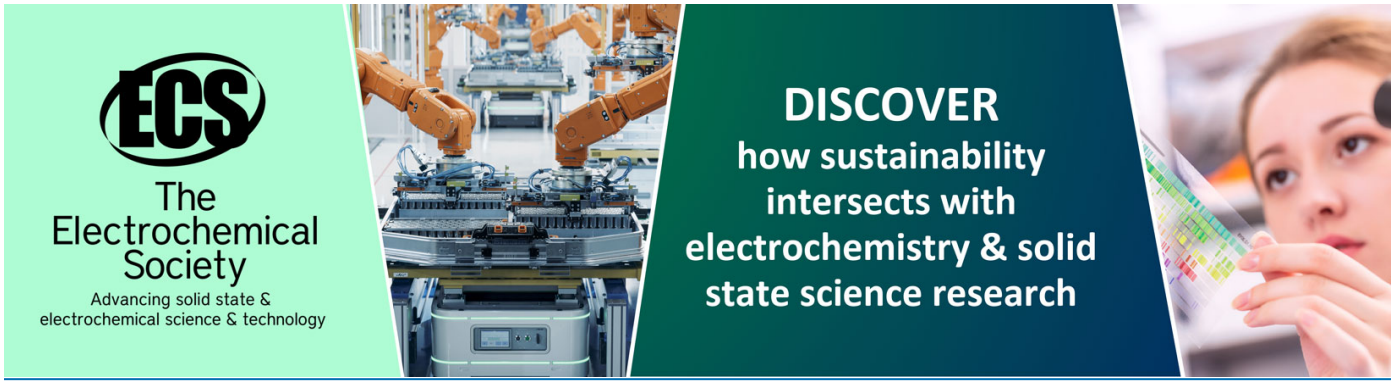
Phenol Biodegradation by Free and Immobilized *Candida tropicalis RETL-Crl* on Coconut Husk and Loofah Packed in Biofilter Column



Phenol Biodegradation by Free and Immobilized *Candida tropicalis RETL-Cr1* on Coconut Husk and Loofah Packed in Biofilter Column

D Shazryenna, R Ruzanna, M S Jessica and M T Piakong
Faculty of Science and Natural Resources, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia.

E-mail: shazryennadalang@gmail.com

Abstract. Phenols and its derivatives are environmental pollutant commonly found in many industrial effluents. It is toxic in nature and causes various health hazards. However, they are poorly removed in conventional biological processes due to their toxicity. Immobilization of microbial cells has received increasing interest in the field of waste treatment and creates opportunities in a wide range of sectors including environmental pollution control. Live cells of phenol-degrading yeast, *Candida tropicalis RETL-Cr1*, were immobilized on coconut husk and loofah by adsorption. The immobolized particle was packed into biofilter column which used for continuous treatment of a phenol with initial phenol concentration of 3mM. Both loofah and coconut husk have similar phenol biodegradation rate of 0.0188 $gL^{-1}h^{-1}$ within 15 hours to achieve a phenol removal efficiency of 100 %. However loofah have lower biomass concentration of 4.22 gL^{-1} compared to biomass concentration on coconut husk, 4.39 gL^{-1}. Coconut husk contain higher biomass concentration which makes it better support material than loofah. Fibrous matrices such as loofah and coconut husk provide adequate supporting surfaces for cell adsorption, due to their high specific surface area. Therefore, coconut husk and loofah being an agricultural waste product have the potential to be used as low-cost adsorbent and supportmatrix for microbial culture immobilization for the removal of organic pollutant from wastewater.

1. Introduction

Phenols are among the most common water pollutants. They impact taste and odour to water and are highly toxic to aquatic life, animal and human beings [1]. Consequently, many treatment processes have been applied for the removal of phenols from waste waters. Some of these processes include: adsorption [2], photo-fenten degradation [3], photocatalytic degradation [4], and biodegradation [5].Combined methods like biochemical, electrochemical, physicochemical or simultaneous adsorption and biodegradation is gaining importance. Simultaneous adsorption and biodegradation increases the life of adsorbent, as biofilm on adsorbent degrade the adsorbate [6].

This adsorption-biodegradation process using suitable adsorbent has shown high efficiency for phenol removal. The adsorption-biodegradation process involves immobilization of microbial culture on solid porous support matrix [7]. Adsorbents used as solid support matrix for immobilization should be stable both physically and chemically and have a high mechanical strength or resistance [8]. Activated carbon is most widely used as adsorbent and solid support matrix for microbial cell in the removal of heavy metals, phenols and other hazardous chemicals [9] which may be found in waste waters, but its high cost and difficulty in regeneration limits its commercial application in large-scale waste water treatment [10].

In recent years, extensive research has been undertaken to develop alternative and economic adsorbents from microbial biomass [11] and agricultural by- products which is a ubiquitous green waste in the environment and may cause some serious environmental pollution when filling at a fixed site. Some of the agricultural waste products that has been developed as adsorbents include, orange and banana peels [12] spent tea leaves [9], tamarind fruit shell [10], soya bean hull [13], cotton seed

hull and corn cobs [14], rubber fruit pericarp [15] and pineaple peel [16]. More and more interests are focused on developing these agricultural wastes as adsorbent for wastewater treatment due to their relative high sorption affinity, ubiquitous presence in the environment, and the ease of being modified to materials with higher efficiency [17].

However, the use of raw agricultural waste adsorbent for the immobilization of cells has rarely been reported for the biodegradation of phenol. The objective of this study is to investigate the potential of loofah and coconut husk, an abundantly available agricultural by-products as a non-conventional adsorbent used as support matrix for *Candida tropicalis* RETL-Cr1 immobilization to degrade phenol.

2. Materials and methods

2.1 Miccroorganism

A pure strain of *Candida tropicalis* RETL-Cr1 was obtained from the Environmental Microbiology Laboratory of university Malaysia Sabah. The culture was maintained on nutrient agar (NA) slants at 4ºC.

2.2 Medium and culture conditions

Yeast was grown on nutrient agar. For adaptation experiments of the cell to phenol, which was also used as a sole carbon source, a Ramsay media was used as described by Ramsay (1983) containing (g/L): 2.0g NH_4NO_3 , 0.5g KH_2PO_4 , 1.0g K_2HPO_4 , 0.5g $MgSO_4 .7H_2O$, 0.01g CaCl .2H20, 0.1g KCl and 0.06g yeast extract. Loop full activated culture on NA was inoculated into an Erlenmeyer flask containing 100 ml of Ramsay broth. The inoculum was incubated at 30 ºC in rotary shaker (200 rpm) for 9 to 10 hour prior to analysis

2.3 Adsorbent (support material)

There are two adsorbents had been prepared prior to this phenol biodegradation study; coconut husk and loofah. Both materials were cut into desired sizes of 1.0 x 1.0 x 0.5 cm and 1.5 x 1.5 x 0.5 cm for coconut husk and loofah respectively. All materials were soaked in distilled water and sterilized by autoclaved at 121 ºC for 15 minutes.

2.4 Phenol biodegradation

The study of phenol biodegradation by *C.tropicalis* RET-Cr1 was done by using the column packed with loofah and coconut husk. The schematic drawing of biofilter set-up is shown in figure 1.

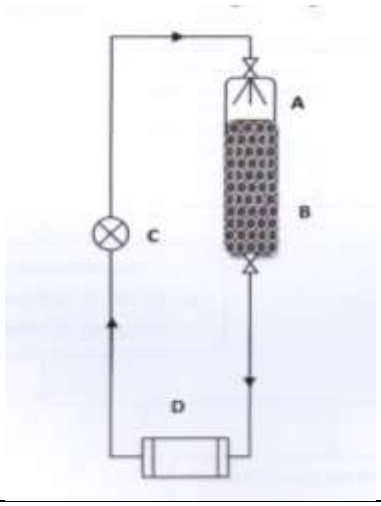


Figure 1. Schematic drawing of biofilter set-up for the degradation of phenol by C.tropicalis RETL-Cr1. (A) biofilter column (length = 1m, working volume = 1 L); (B) coconut husk (size ≈ 1.0 cm x 1.0 cm x 0.5cm, weight ≈ 334.07g); (C) tap; (D) peristaltic pump (250mL min^{-1})

2.5 Analytical method

2.5.1 Determination of phenol concentration

Phenol will be determined by isocratic elution high performance liquid chromatography (HPLC) (W600 2487) using a Waters Hypersil C18 5µm (4.6mmx 250 mm) column with UV detector at 280 nm

2.5.2 Determination of biomass concentration

Biomass concentrations will be determined by monitoring the optical density (OD). The OD will be monitored spectrophotometrically by measuring the absorbance at wavelength 600nm using the UV–Visible spectrophotometer [Model E 1011, 1000 series].

2.5.3 Determination of phenol biodegradation rate

According to [18], the phenol biodegradation rate can be calculated by dividing the total amount of phenol consumed with the time required for the consumption.

Phenol degradation rate = Amount of phenol consumed / Time required for consumption

2.5.4 Determination of specific growth rate

The exponential increase in biomass after inoculation is measured as a function of time and analyzed to obtained specific growth rate for that substrate concentration [19]. The specific growth rate was measured from the slope of the biomass curved by delineating points between the log growth phase represented by the equation (1).

$$\mu = (\ln X_t - \ln X_0) / t \qquad (1)$$

where, X_t and X_0 is biomass concentration at time t and 0, and t is operational time.

3. Result and discussion

For the purpose of result accuracy, the control treatment for the phenol degradation (suspension condition) was run twice and simultaneously with the phenol degradation by loofah and coconut husk. As the purpose of simplification, the control experiments in the suspension condition, with absence of loofah would be referred as LC, whereas that in the absence of coconut husk is referred as CC. Similar for experiments run with the presence of adsorbents, it would be referred as L1 for experiment with the presence of loofah, whereas that in the presence of coconut husk is C1.

3.1 Growth pattern of C.tropicalis RETL-Cr1

The growth pattern of the yeast *C.Tropicalis* RETL-Cr1 was conducted inside a biofilter column with the absence and presence of both support material. The presence of support material affects the growth pattern of the yeast. Generally, at the first 3 hours, all four experiments LC, L1, CC and C1 experienced slow cell growth as the cells are adapting to the new environment. Apparent increase in cell growth can be seen at the following hours until 12^{th} to 14^{th} hour, where the cells are consuming the available nutrients. The cell growth declines after the 15^{th} hour because of the nutrient depletion. Figure 2 shows the absence of loofah causes higher cell density at the 12^{th} hour of 0.477 and eventually declined. It is higher than L1, where the presence of loofah reached highest cell density of 0.422 on the 13^{th} hour. The different amount of cell density achieved is because of the physical properties of loofah. Loofah has many network lines which provide large surface area to the cells to bud more. According to [20], the biomass grows thicker on the surface on which it attached and consequently becomes difficult for the innermost cells to receive enough nutrients. Eventually, this leads to the loss of biomass contained on the surface area

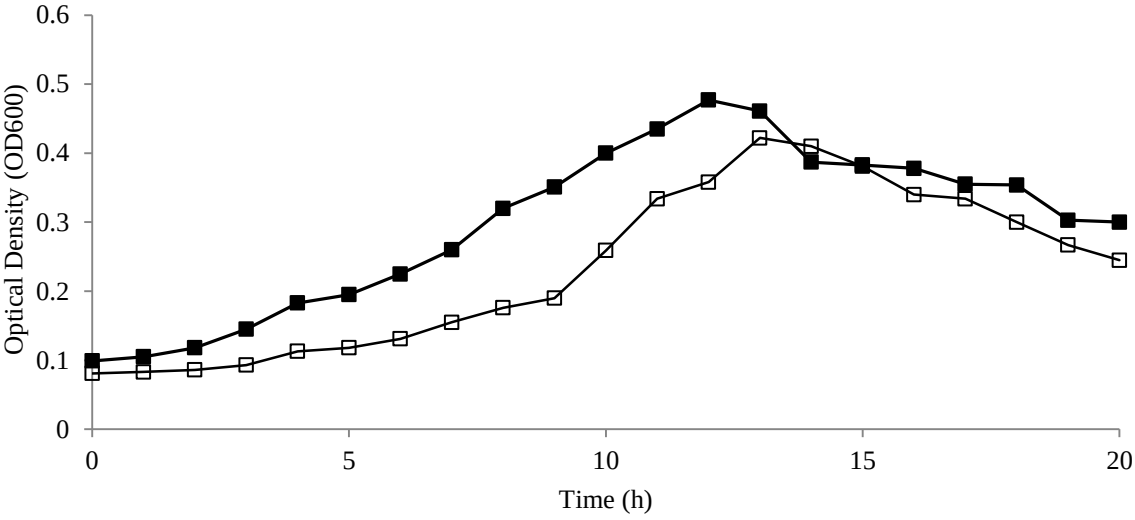


Figure 2. Growth pattern of yeast *C.tropicalis* RETL-Cr1 on Ramsay media broth with phenol (3mM) in biofilter column with the absence of loofah, LC (■) and the presence of loofah, L1 (□) as support material.

Figure 3 shows the growth curve of the yeast with the presence of coconut husk is slightly lower than with the absence of coconut husk. The highest cell density for CC was at 13th hour by 5.03, where as the highest cell density achieved by C1 was 4.39 at 15th hour. Coconut husk are known to have many voids which provide large surface area for cell budding and sustain growth and for yeast activity to occur [21]. Biomass grows on the exterior surface of the coconut husk which prevents nutrient and oxygen from going into the interior of the coconut husk [22] which makes it more difficult for the innermost cell to receive sufficient nutrient as the competition is increased. Cells are attached to the support material resulting on low cell density. Whereas, in suspension LC and CC, cell density is higher.

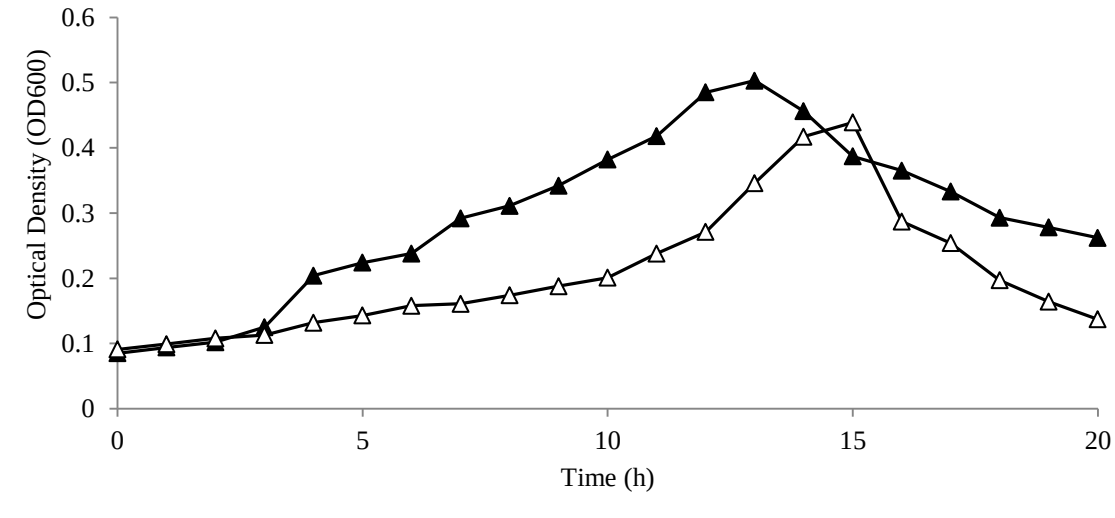


Figure 3. Growth pattern of yeast *C.tropicalis* RETL-Cr1 on Ramsay media broth with phenol (3mM) in biofilter column with the absence of coconut husk, CC (▲) and the presence of coconut husk, C1 (Δ) as support material

3.2 Phenol biodegradation

Phenol biodegradation was studied in batch experiments with initial concentration of 3mM by immobilized *C. tropicalis* RETL-Cr1. The phenol concentration in the medium decreased clearly when the adsorbed microorganism started to grow (Figure 4 and 5). Phenol biodegradation were 100% completed for all four treatments, LC, L1, CC and C1.

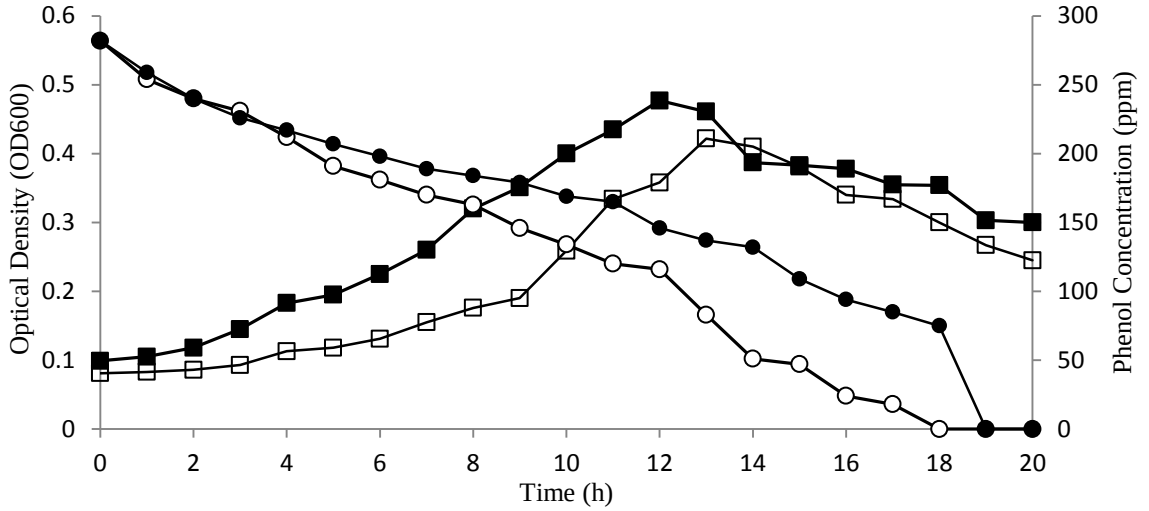


Figure 4. Profile of phenol biodegradation by C.tropicalis RETL-Cr1 with the absence of loofah (●) and with the presence of loofah (○) against time and growth pattern with the absence of loofah (■) and with the presence of loofah (□).

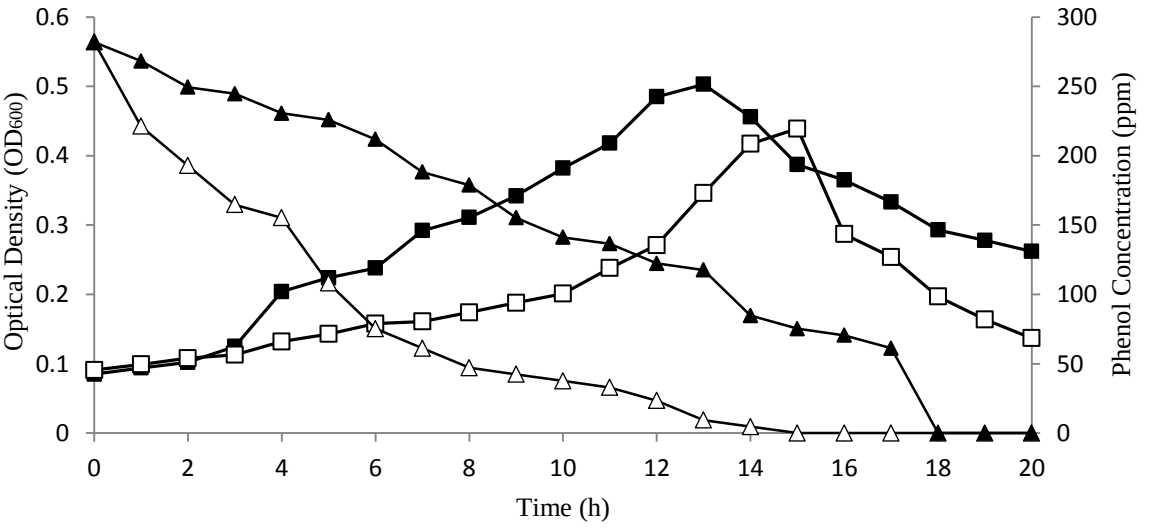


Figure 5. Profile of phenol biodegradation by C.tropicalis RETL-Cr1 with the absence of coconut husk (▲) and with the presence of coconut husk (Δ) against time and growth pattern with the absence of loofah (■) and with the presence of loofah (□).

Based on Table 1, L1 and C1 degraded phenol earlier by 1 and 2 hours respectively. The presence of support materials increase the contact between phenol and the yeast *C.tropicalis* RETL-Cr1, and hence degrade phenol faster than with the absence of support material. Phenol biodegradation rate was higher in the present of support material. L1 degrades phenol faster by the rate of 0.0188 $gL^{-1}h^{-1}$ compared to LC with the rate of 0.0176$gL^{-1}h^{-1}$. Whereas C1 degrades phenol at a rate of 0.0188 $gL^{-1}h^{-1}$ which also faster than CC as it rate is 0.0166 $gL^{-1}h^{-1}$.

Table 1. Performance of phenol biodegradation rate by *C.tropicalis* RETL-Cr1 in biofilter column with the absence and presence of support materials.

Performance	LC (Absence of loofah)	L1 (Presence of loofah)	CC (Absence of coconut husk)	C1 (Presence of coconut husk)
X_{max} (gL^{-1})	4.77	4.22	5.03	4.39
Specific growth rate, μ (h^{-1})	0.3928	0.3000	0.4906	0.3405
Operational time (h)	19	18	18	15
Lag time (h)	3	3	1	0
Biodegradation time (h)	16	15	17	15
Phenol biodegradation rate ($gL^{-1}h^{-1}$)	0.0176	0.0188	0.0166	0.0188
Phenol removal efficiency (%)	100	100	100	100

4. Conclusion

The biodegradation of phenol by free and immobilized *Candida tropicalis RET-Cr1* on loofah and coconut husk packed in biofilter column has been investigated. Biodegradation for all experiments of LC, L1, CC and C1 were 100% completed, with shorter biodegradation time for L1 and C1. Both loofah and coconut husk have similar phenol biodegradation rate of 0.0188 $gL^{-1}h^{-1}$ within 15 hours. However, It can be conclude that coconut husk is a better support material than the loofah as it enhanced the yeast *C.tropicalis* RETL-Cr1 growth better than loofah. Loofah have lower biomass concentration of 4.22 gL^{-1} compared to biomass concentration on coconut husk, 4.39 gL^{-1}. Overall, coconut husk and loofah is agricultural by-product which shows a potential as an alternative and economic adsorbent.

5. References

[1] Annadurai A, Ling L Y and Lee J F 2007 Biodegradation of phenol by Pseudomonas pictorum on immobilized with chitin *Afr. J. Biotechnol.* **6(3)** 296-303.
[2] Zhao G H, Li Y F, Liu X X and Liu X L 2010 Preparation of capsules containing 1- nonanol for rapidly removing high concentration phenol from aqueous solution *J. Hazard Mater.* **175** 715 – 725.
[3] Babuponnusami A and Muthukumar K 2011 Degradation of phenol in aqueous solution by fenton, sono-fenton and sono-photo fenton methods *Clean-Soil, Air, Water,* **39** 142- 147.
[4] Devipriya S P and Yesodharan S 2010 Photo catalytic degradation of phenol in water using TiO2 and ZnO *J. Environ. Biol.* **31** 247-249.
[5] Agarry S E, Audu T O K and Solomon BO 2009 Substrate inhibition kinetics of phenol degradation by Pseudomonas fluorescence from steady state and washout data *Int. J. Environ. Sci. Tech.* **6(3)** 443 – 450
[6] Dabhade M A, Saidutta M B and Murthy D V R 2009 Adsorption of phenol on granular activated carbon from nutrient medium: equilibrium and kinetic study *Int. J. Environ. Res.* **3(4)** 557–568.

[7] Baoshan X, William B M and Martin J D 1994 Sorption of phenol by selected biopolymers isothanus, energetics and polarity *Environ. Sci. Technol.* **28** 466-473
[8] Annadurai A, Babu S R, Mahesh K P O and Murugesan T 2000 Adsorption and biodegradation of phenol by chitosan-immobilized Pseudomonas putida (NICM 2174) *Bioprocess Engineering.* **2** 493-501
[9] Hameed B H 2009 Spent tea leaves: A new non-conventional and low-cost adsorbent for removal of basic dye from aqueous solution *J. Hazard. Mater.* **161** 253-259
[10] Popuri S R, Jammala A, Reddy K V N and Abburi K 2007 Biosorption of hexavalent chromium using tamarind (Tamarinddus indica) fruit shell - a comparative study *Electronic J. Biotechnol.* **3** 358-367
[11] Texier A-C, Andres Y and Lecloirec P 1999 Selective biosorption of lanthanide (La, Eu, Yb) ions by Pseudomonas aeruginosa *Environ. Sci. Technol.* **33** 489-495
[12] Annadurai G R S, Juang and Lee D J 2002 Biodegradation and adsorption of phenol using activated carbon immobilized with pseudomonas putida *J. Environ. Sci. Health Part A, Toxic/Hazardous Substances Environ. Eng.* **37** 1133-1146
[13] Marshall W E, Wartelle L H, Boler D M and Toles C A 2000 Metal ion adsorption by soybean hulls modified with citric: a comparative study *Environmental Technology.* **21(6)** 601-607
[14] Reddad Z, Gerente C, Andres Y and Lecloirec P 2002 Adsorption of several metal ions onto a low cost biosorbent: Kinetic and equilibrium studies *Environ. Sci. Technol.* **36** 2067-2073
[15] Agarry S E and Owabor C N 2005 Utilization of rubber fruit pericarp – activated carbon for abattoir waste water treatment and absorption of iron (III) ions form aqueous solution *J. Sci. Technol. Res.* **4(2)** 49 -54
[16] Agarry S E and Aremu M O 2012 Batch Equilibrium and Kinetic Studies of Simultaneous Adsorption and Biodegradation of Phenol by Pineapple Peels Immobilized *Pseudomonas aeruginosa* NCIB 950 *British Biotechnology Journal.* **2(1):** 26-48
[17] Chen B, Yuana M and Liu H 2011 Removal of polycyclic aromatic hydrocarbons from aqueous solution using plant residue materials as a biosorbent *J. Hazard Mater.* **188** 436 – 442
[18] Kar S, Swaminathan T and Baadarajan A 1996 Studies on biodegradation of a mixture of toxic and non-toxic pollutant using arthrobacter species *Bioproc. Eng.* **15 (4):** 195 – 199
[19] Yoong E T, Staib C I and Lant P A 2004 Kinetic coefficient of high strengthphenolic wastewater biodegradaion. Environmental biotechnology: Advancement in water and wastewater application in the topics *Wat. Env. Manag. Ser.* 35 – 42.
[20] Levin M A and Gealt MA 1993 *Biotreatment of Industrial and Hazardous Waste* (eds). McGraw-Hill New York.
[21] Ortiz I, Revah S, and Auria R 2003. Effects of packing material on the biofiltration of benzene, toluene and xylene vapours. *Environmental Technology.* **24(3):** 265 – 275.
[22] Levstek M and Plazi I 2009 Influence of carrier type on nitrification in the moving-bed biofilm process *Water science and technology.* **59 (5)** 875 – 882