STM JOURNALS

International Journal of
Pollution Prevention & Control

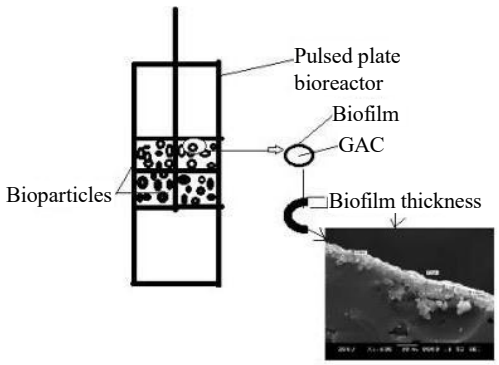
ISSN: 3048-6750
Volume 2, Issue 2, 2024
DOI (Journal):10.37591/IJPPC
July–December

https://journals.stmjournals.com/ijppc

Research IJPPC

Effect of Cell Carrier Loading on Phenol Degradation in Association with Production of Exopolymeric Substances and Biofilm Characteristics during Start up and Steady State in a Pulsed Plate Bioreactor

B.R. Veena*



Graphical abstract

Highlights

- Cell carrier load influences greatly the biodegradation of phenol
- Greater the cell carrier loading thinner the biofilm and greater the biodegradation
- Biofilms are proactive when the thickness of film is thinner
- Almost complete degradation is possible with higher cell carrier loading
- Both physical and chemical characteristics of biofilm influences the biodegradation of phenol

Abstract

Biodegradation of phenol in a continuous pulsed plate bioreactor (PPBR) using immobilized bacterial cells has been shown to be a highly efficient process.The present paper reports the studies on the effect physical characteristics of biofilm (biofilm thickness, attached dry biomass and biofilm dry density) and chemical characteristics in terms of exopolymers viz. protein, carbohydrate and humic substance produced by Pseudomonas desmolyticum (NCIM 2112) bacterial cells immobilized on granular activated carbon during phenol degradation. The start-up time reduced from 12 to 10 h and the degradation of phenol was above 99% with increase in cell carrier loading from 80 to 120g of granular activated carbon (GAC). The increase in percentage degradation could be due to higher inoculum size. Also the net phenol removal rate was increased that could be due to increase in number of immobilized cells. The production of exopolymers increased and biofilm thickness decreased with increase in cell carrier loading. Increase in exopolymers could be due to higher consumption of phenol by microbial cells leading to higher production of exopolymers and also could be due to shear caused by cell to cell collision thus reducing biofilm thickness, increasing biomass and biofilm density.

***Author for Correspondence**
B.R. Veena
E-mail: veena-cheml@dayanandasagar.edu

Associate Professor, Department of Chemical Engineering, Dayananda Sagar College of Engineering, Kumaraswamy Layout, Bangalore, Karnataka, India

Received Date: September 04, 2024
Accepted Date: September 26, 2024
Published Date: October 11, 2024

Citation: B.R. Veena. Effect of Cell Carrier Loading on Phenol Degradation in Association with Production of Exopolymeric Substances and Biofilm Characteristics during Start up and Steady State in a Pulsed Plate Bioreactor. International Journal of Pollution: Prevention and Control. 2024; 2(2): 24–30p

Keywords: Biofilm, cell carrier loading, phenol, exopolymeric substance, pulsed plate bioreactor

INTRODUCTION

One of the toxic pollutants, phenol, present in industrial effluents viz. refineries, paper and pulp, coal etc. is harmful to human and other living things [1] and according to [2] it is considered priority organic pollutant. Maximum phenol concentration

of 1 mg/l is permissible in industrial effluent and is set by central pollution control board (CPCB, India) and 1 μg/l in drinking water [3]. Biological method for the degradation of phenol seems to be advantageous over other methods which are cost effective and environmental friendly. The major factor to be considered in biological process is complete mineralization of phenol that produces simple elements using microbial cells in suspended form or immobilized form in the bioreactor [4, 5]. One of the most common techniques used wastewater treatment is using attached growth cells [6].

Over suspended cell reactors attached growth bioreactors are advantageous with respect to various aspects viz. high cell concentration, high dilution rate and resistance to toxic loads [7, 8] and stability of the process [9]. In attached growth, microbial cells are immobilized on appropriate solid support and these cells first attach to solid support and produce exopolymeric substances (EPS) and they are embedded in these polymeric substances to protect themselves from harsh environment [10]. The microbial cells either in living or dead form present with EPS and other substances on solid support is known as biofilm [11]. The EPS thus produced consists of protein, carbohydrate and humic substance as main substances and minute compounds are lipids and nucleic acids that could be present due to cell lysis. The proteins thus produced are helpful in binding cells with adhesion and cohesion, while the humic substance and carbohydrates gives structural strength and stability to biofilm. Some of the parameters viz. biofilm thickness, biofilm dry density, excreted polymeric substances influence the performance of bioreactor and hence these parameters are considered to be important in tailoring the biofilm structure. The other important factors that influence the biofilm structure is shear force that results in detachment of biofilm from the surface of biofilm. The formation, metabolic activity and, structure of biofilm depends on the detachment forces and this could happen due to hydrodynamic conditions. Collision between particles etc. [12] hence the balancing of these attachment and detachment forces are important in the production of viable biofilm and thus they influence the performance of biofilm [13, 14].

The use of biofilm reactors for wastewater treatment is increasing in popularity. A key factor affecting the formation, structure, and stability of biofilm under hydrodynamic conditions is the detachment of the biofilm, which can occur due to hydraulic forces and/or particle collisions. [12].

Hence it is necessary to understand the biofilm formation and its composition and factors that influence the formation and detachment of biofilm or the prevention of contamination or clogging of bioreactor.

MATERIALS AND METHODS

Microorganism

In this study, the bacterium *Pseudomonas desmolyticum* (NCIM 2112) was utilized sourced from the National Collection of Industrial Microorganisms (NCIM) in Pune, India. The culture was preserved by subculturing every 15 days on agar slants and storing it at 4°C

Culture Medium

Mineral medium, Bushnell Haas broth, was used to grow the microorganisms and was procured from HIMEDIA, Mumbai, India. The maintenance and growth of bacteria was followed as described by [15]. Phenol was used as sole carbon and energy source for the microorganisms.

Inoculum

The bacterial cells were acclimatized to phenol and were immobilized on granular activated carbon (GAC) according to procedure presented by Veena *et al.* [15]. Synthetic wastewater was created by mixing tap water with the desired concentration of phenol and Bushnell Haas medium.

Chemicals

Granular activated carbon was procured from NICE Chemicals, Cochin, India. Analytical grade phenol, humic substance, copper sulphate and potassium ferricyanide were procured from LOBA

Chemie, Mumbai, India; sodium hydroxide and disodium tartrate from Merck, Mumbai, India; Ammonium chloride, sodium carbonate from NICE Chemicals, Cochin, India; 4 amino antipyrine from Spectrochem Pvt. Ltd., Mumbai, India.

Experimental Setup and Experimentation

Pulsed Plate Bioreactor consisted of 56 cm tall Perspex vertical column having inner and outer diameter of 6.8 and 7.5 cm respectively. It consisted of five pulsating plates mounted on a central shaft. 20 gm of bio particles (GAC with immobilized cells) were placed between plates and covered with wire mesh and space between each plate was occupied with 33% bio particles. The schematic representation of Pulsed Plate Bioreactor and methodology are presented by [15]. The frequency of pulsation of the reactor was set at 0.08 s^{-1}, and amplitude of pulsation at 3.3 cm. 200 ppm of synthetic water was pumped using peristaltic pump in upward direction at dilution rate of 0.33 h^{-1}. The cell carrier loading of 80 and 120g of GAC with immobilized cells were used in the present study. Sufficient air flow rate of 1.7-1.8 litres per minute was maintained in the reactor. Effluent was withdrawn from outlet port and phenol concentration was determined until steady state was reached. The duration required to achieve a steady state was defined as the start-up time. Once start-up time was determined, it was divided into equal interval of time. For each interval of time, fresh experiments were conducted and the concentration of phenol and biofilm characteristics (in terms of physical characteristics viz. biofilm thickness, attached dry biomass and biofilm dry density and chemical characteristics viz. composition of EPS in terms of protein, carbohydrate and humic substance) were analysed. All experiments were carried out with a pulsation frequency of 0.08 s^{-1}, a pulsation amplitude of 3.5 cm, a dilution rate of 0.33 h^{-1}, and an influent phenol concentration of 200 ppm.

Analytical Methods

The effluent sample from reactor after a set time was centrifuged to remove any biomass present and further was used for phenol analysis by using direct photometric method based on rapid condensation of phenol with 4-aminoantipyrine, followed by oxidation with potassium ferricyanide to form a red colour dye under alkaline condition. The resulting solution was used for measuring the absorbance at the wave length of 510 nm using Hitachi UV-VIS spectrophotometer.

Protein was analysed by using Lowry's method [16], Carbohydrate was analyzed by Dubois method [17] (1956) and Humic Substance was analysed by modified Lowry's method [18]. Extraction of EPS and the contents of protein, carbohydrate and humic substance were determined as presented by Veena *et al.* 2015[15].

RESULTS AND DISCUSSION

Effect of Cell Carrier Loading on Phenol Degradation During Start-up and at Steady State

Figure 1(a) and (b), represents variation of phenol during start-up and at steady state when the reactor was operated at frequency of pulsation (f) of 0.08s^{-1}, amplitude of pulsation (A) of 3.5 cm, initial influent phenol concentration (C_i) of 200 ppm, dilution rate (D) of 0.33 h^{-1} and with cell carrier loading of 80 and 120 of GAC with immobilized cells placed 20 and 30g between each spacing respectively.

The start-up time reduced from 12 to 10h and degradation of phenol increased from 99% to almost 100% with increase in cell carrier loading from 80 to 120g of GAC. An increase in cell carrier loading boosts the available surface area and the number of initially immobilized cells. Increase in surface increases substrate and oxygen transfer between liquid for biochemical reaction that increases phenol removal rate. Also gas holdup increases with increase in cell carrier loading which affects interfacial area and oxygen mass transfer rate in pulsed plate columns [19].

In the present work there exists marginal increase in phenol removal which could be due to sufficient surface are availability at cell carrier loading of 80g of GAC. The phenol concentration at outlet remained constant after start-up period indicating steady state condition. Higher cell carrier loading resulted in increased EPS production, potentially due to the enhanced phenol removal rate.

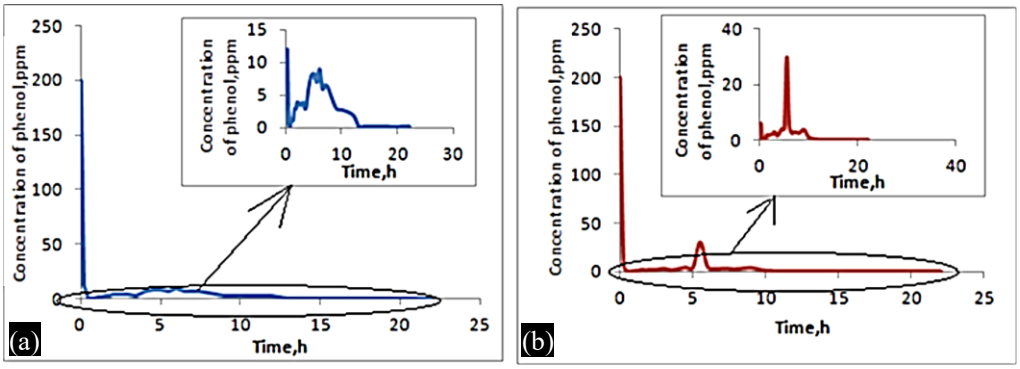


Figure 1. Variation in phenol concentration during the Start-up period for different cell carrier loading of (a) 80g. Conditions: f=0.08 s-1; A=3.5 cm; D=0.33 h-1 ; Ci=200 ppm and (b) 120 g.

Effect of Cell Carrier Loading on Chemical Characteristics of Biofilm

The effect of cell carrier loading on composition of EPS is presented in Figures 2 and 3. The production of protein, humic substance and carbohydrate increases with increase in time and reached a steady value for both cell carrier loading values. Increased cell carrier loading led to higher EPS production, likely due to the elevated overall phenol removal rate. Also owing to large surface area available for adhesion, cohesion and for structural strength of biofilm, production of EPS also is higher [20].

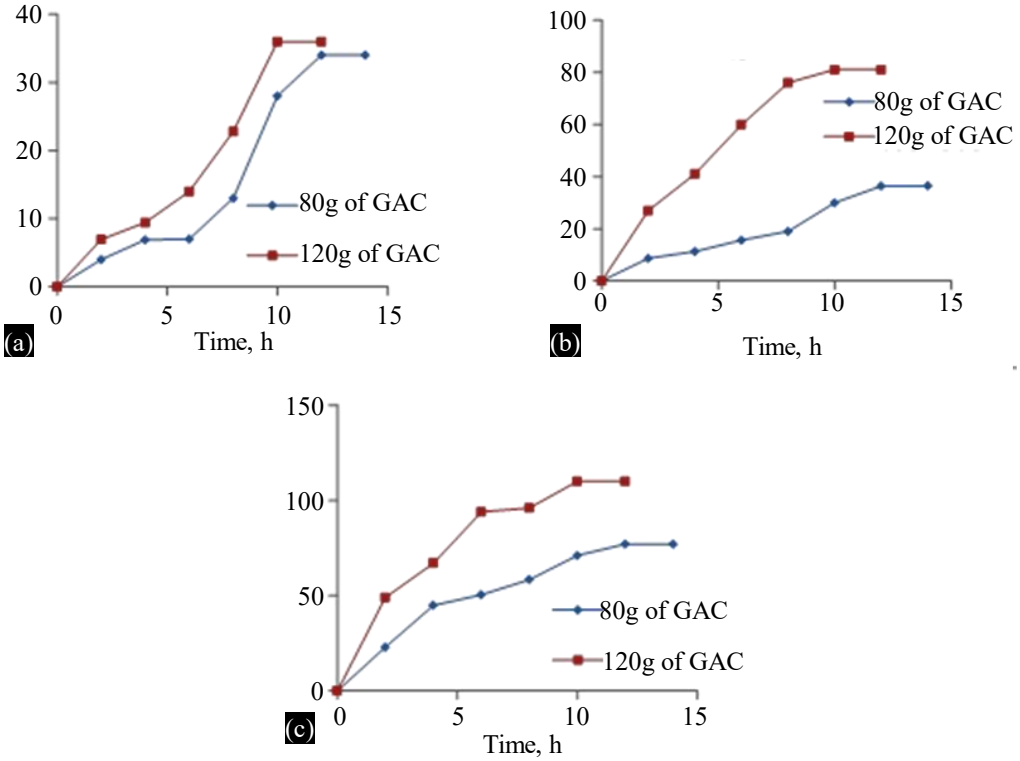


Figure 2. Effect of cell carrier loading on production EPS components during start up (a) protein (b) carbohydrate and (c) humic substance content of EPS for influent phenol concentration of 200 ppm. Conditions: f= 0.08 s-1; D=0.33 h-1; A=3.5 cm.

Effect of Cell Carrier Loading on Physical Characteristics of Biofilm

The effect of cell carrier loading on physical characteristics is presented in Figure 4. It may be observed from Figure 4 that the biofilm thickness decreased with increase in cell carrier loading whereas biofilm dry weight and biofilm density increased with increase in cell carrier loading. Increased cell

growth with increase in availability of cells due to increase in cell carrier loading, leads to higher biomass dry weight and high cell carrier loading increases cell detachment leading to faster growth and compacting biofilm that leads to high density biofilm.

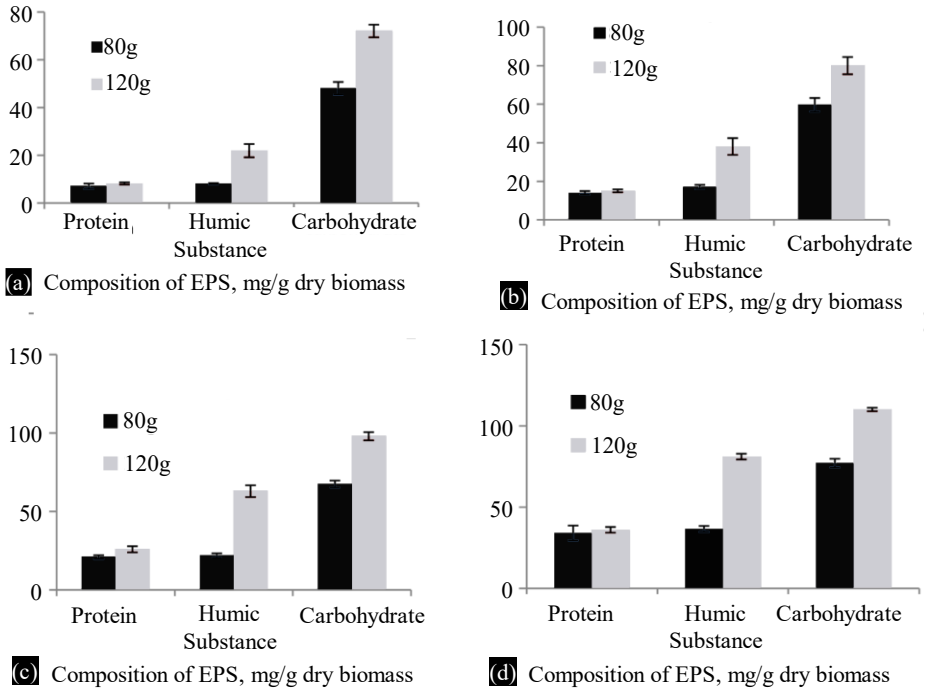


Figure 3. Effect of cell carrier loading on quantity of EPS components at steady state with influent phenol concentration of (a) 200 ppm (b) 400 ppm (c) 600ppm and d) 800ppm. Conditions: f= 0.08 s-1; A= 3.5 cm; D= 0.33h-1.

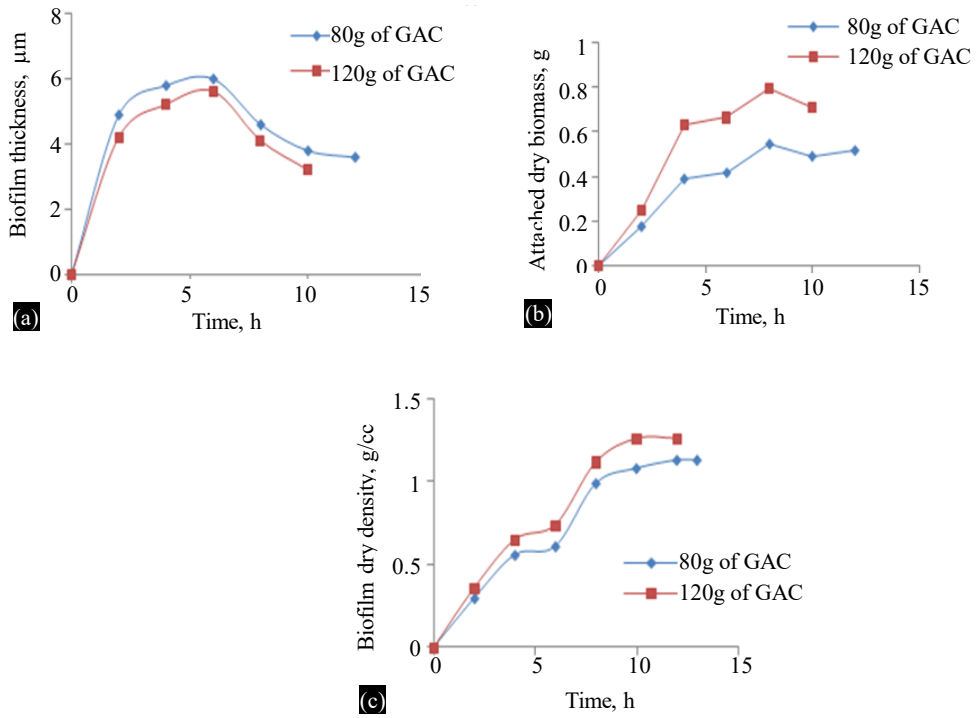


Figure 4. Effect of cell carrier loading on physical characteristics of biofilm during the start-up (a) biofilm thickness (b) attached biomass and (c) biofilm dry density for the influent phenol concentration of 200ppm. Conditions: f=0.08 s-1; A=3.5cm; D=0.33 h-1.

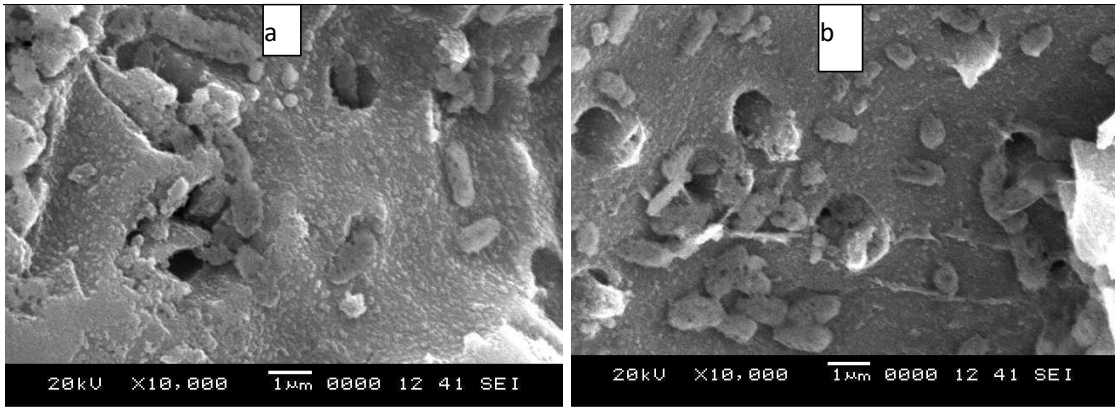


Figure 5. Effect of cell carrier loading on morphological characteristics of biofilm at steady state.(a) 80 g of GAC loading and (b) 120 g of GAC loading. Conditions:f=0.08 s-1; A=3.5cm; D=0.33 h-1; Ci =200 ppm.

Effect of Cell Carrier Loading on Morphological Characteristics of Biofilm

Images of morphological characteristics of biofilm using scanning electron microscope are shown in Figure 5, for cell carrier loadings of 80g and 120g of GAC. The biofilm formed for 80g of GAC was rougher than that of 120g of GAC and are uneven with more pores indicating loose structure. The biofilm formed with a cell carrier loading of 120 g exhibited a smooth, compact surface with uniformly sized small micropores. These morphological features align with the findings on biofilm thickness and density. At 120 g cell carrier loading, a higher number of single, unattached cells were observed on the biofilm, suggesting they were in the early stages of colonization. In contrast, fewer unattached cells were seen on the biofilm at 80 g cell carrier loading, indicating that reduced particle movement at lower loadings may cause loose cells to detach more easily

CONCLUSIONS

A slight improvement in percentage degradation was observed with an increase in cell carrier loading. The production of EPS and the biofilm's physical properties are significantly affected by the amount of cell carrier used. Higher cell carrier loading promoted EPS production, resulting in a denser, thinner biofilm. The impact of improved mass transfer due to particle movement at lower carrier loading appears minimal compared to the reduced surface area available for phenol biodegradation and biomass growth. Therefore, the bioreactor's performance is primarily determined by the greater surface area available for microbial growth

REFERENCES

1. Tepe, Ozlem, and Arzu Y Dursun (2008) Combined effects of external mass transfer and biodegradation rates on removal of phenol by immobilized Ralstonia eutropha in a packed bed reactor. *Journal of hazardous materials* 151.1: 9-16.
2. EPA “Priority Pollutant List” Epa US. Priority pollutant list. Federal Register. 1979;44:69514-7. http://www.epa.gov/sites/production/files/2015- 09/documents/priority-pollutant-list-epa.pdf (date of access, Feb 01 2016).
3. Edition F. Guidelines for drinking-water quality. WHO chronicle. 2011;38(4):104-8.http://apps.who.int/iris/bitstream/10665/44584/1/9789241548151_eng.pdf
4. Tziotzios, G Teliou, M, Kaltsouni, V, Lyberatos, G and Vayenas, D V (2005) Biological phenol removal using suspended growth and packed bed reactors. *Biochemical engineering journal*, *26*(1), 65-71.
5. Loh, Kai-Chee, Tai-Shung Chung, and Wei-Fern Ang (2000) "Immobilized-cell membrane bioreactor for high-strength phenol wastewater." *Journal of Environmental engineering* 126.1: 75–79.
6. Seker S, Beneyal, H, Salih B and Tanyolac A (1995) Multi-Substrate growth kinetics of *Pseudomonas putida* for phenol removal. *Applied microbiology and biotechnology* 47(5): 610-614.

7. Veena B R, Vidya S K and Saidutta M B(2016) Effect of dilution rate on dynamic and steady-state biofilm characteristics during phenol biodegradation by immobilized *Pseudomonas desmolyticum* cells in a pulsed plate bioreactor. *Frontiers of Environmental Science & Engineering* 10(4)-16.
8. Akira Hirata, Takeshi Takemoto, Kentaro Ogawa, Joseph Auresenia, Satoshi Tsuneda (2000) Evaluation of kinetic parameters of biochemical reaction in three-phase fluidized bed biofilm reactor for wastewater treatment. *Biochemical Engineering Journal* 5: *165-171*.
9. Cheng, Kuan-Chen, Ali Demirci and Jeffrey M Catchmark(2010) Advances in biofilm reactors for production of value-added products. *Applied microbiology and biotechnology* 87(2): 445-456.
10. Yan Liu and Herbert Fang (2003) Influences of extracellular polymeric substances (EPS) on flocuulation settling, and dewatering on activated sludge. *Critical reviews in Environmental Science and Technology* 33(3) : 237-273.
11. Kumar M A, Anandapandian K T K and Parthiban K (2011) Production and Characterization of Exopolysaccharides (EPS) from Biofilm forming Marine Bacterium. *Brazilian Archives of Biology and Technology 54*(2): 259-265.
12. Yu Liu and Joo-Hwa Tay (2001) Detachment forces and their influence on the structure and metabolic behavior of biofilms. *World Journal of Microbiology and Biotechnology* 17: 111-117.
13. Characklis W G, McFeters G A and Marshall K C (1990) Physiological ecology in biofilm systems *Biofilm 37*: 67-72.
14. Van Loosdrecht M C M, Eikelboom D, Gjaltema A, Mulder A, Tijhuis L and Heijnen J J(1995) Biofilm Structures. *Water Science and Technology 32*(8): 35-43.
15. Veena B R, Vidya S K, and Saidutta M B (2015) Shear Stress Effects on Production of Exopolymeric Substances and Biofilm Characteristics During Phenol Biodegradation by Immobilized Pseudomonas desmolyticum (NCIM2112) Cells in a Pulsed Plate Bioreactor. *Preparative Biochemistry and Biotechnology* 46(5):421-428.
16. Lowry OH, Rosebrough NJ, Farr A L and Randall RJ (1951) Protein Measurement with the Folin Phenol Reagent. *Journal Biological Chemistry 193*(1): 265-275.
17. [17] Dubois M, Gilles K A, Hamilton JK, Rebers PT and Smith F (1956) Colorimetric Method for Determination of Sugars and Related Substances. *Analytical Chemistry 28*(3): 350-356.
18. Frolund B. Griebe T and Nielsen P H (1995) Enzymatic activity in the activated-sludge floc matrix. *Applied Microbiology and Biotechnology 43*(4): 755-761.
19. Stamenković I S, Stamenković O S, Banković-Ilić I B, Lazić M L, Veljković V B and Skala D U (2005) The gas holdup in a multiphase reciprocating plate column filled with carboxymethylcellulose solutions *Journal of the Serbian Chemical Society 70*(12):1533-1544.
20. Kwok W K, Picioreanu C, Ong S L, Van Loosdrecht M C. M, Ng W J and Heijnen J J (1998) Influence of biomass production and detachment forces on biofilm structures in a biofilm airlift suspension reactor. *Biotechnology and bioengineering 58*(4): 400-407

