MEDWIN PUBLISHERS
Committed to create value for Researchers

International Journal of Biochemistry & Physiology
ISSN: 2577-4360

Biofilm Effect and Growth of Waste Frying Oil (WFO) on Bacteria

Kahramana H[1]* and Canan Karaderi C[2]

[1]Department of Biology, Faculty of Art and Science, Inonu University, Turkey

[2]Department of Biology, Institute of Science, Inonu University, Turkey

***Corresponding author:** Department of Biology, Faculty of Art and Science, Inonu University, Malatya 44280, Turkey, Tel: +90 422 377 37 94; Fax: +90 422 341 00 37; Email: huseyin.kahraman@inonu.edu.tr

Research article

Volume 3 Issue 4

Received Date: July 18, 2018

Published Date: August 10, 2018

Abstract

Every year in the world, waste frying oils that are released into the environment without using a large amount or recycling can create a big environmental problem. Waste oils may be an alternative, especially for studies where biofilm formation is not desired or should be reduced. Production for biofilm from waste oil, which is used cheap source. In this study, only 10% of waste frying oil was added to the buffer environment; the effects of *Pseudomonas aeruginosa* on biofilm (also referred to as "biofilm formation index"), viable cell count and biomass formation were investigated under different aeration conditions. As a result, increases in biofilm formation up to 14-fold, increased number of viable cells and increased biomass formation were observed. This work we have done, it has been thought that waste frying oils can be used as the medium for fattening.

Keywords: Waste frying oil; Biofilm formation; *P. aeruginosa*

Abbreviations: WFO: Waste Frying Oil; cfu/ml (log): Cell numbers/ ml; LB: Luria Bertani Broth; BFI: Biofilm Index; OD600: Cell Density; PBS: Phosphate Buffered Saline.

Introduction

Vegetable oil industry's main source of low-cost fermentative wastes rich in nutrients is cooking or frying oil. However, these oils can be used for microbial growth as high-energy sources and biosurfactants, so that these wastes can be recycled [1-3].

The constant of increases in WFO (waste frying oil) from domestic wastes is becoming a growing problem all over the world.

However, WFO is generally used for animal feed. In recent years, studies on the production of biodiesel from waste oil, which is a cheap source, are more common [4].

Biofilms are found in almost all aquatic ecosystems that can support microbial growth, such as industrial or potable water system pipes. A biofilm is a collection of microbial cells that are associated with a surface and can contain non-cellular materials that are contained in a matrix of polysaccharide materials and incorporated into

biofilms from the surrounding environment from which the biofilm is formed. The environment composition, temperature, presence of antimicrobial agents, other organisms, inoculum quantity, hydrodynamic forces, and substrate properties affect the development of the biofilm system [5-8].

P. aeruginosa is a free living Gram-negative microorganism living in the environment. *P. aeruginosa* may also produce biofilm, e.g. in the lungs of patients with cystic fibrosis, on diabetic wounds and on inert surfaces such as medical devices, prostheses and catheters. Biofilms are known to develop tolerance to antibiotics and biocides. At the depth of biofilms, the concentration of nutrients and oxygen is low; because of this, bacteria found in biofilms exhibit low physiological activity and are tolerates to antibiotics targeting growing bacteria [9].

With this study, we used waste frying oil (sunflower) as an alternate low-cost carbon source for the fermentative production of growth and biofilm production by *Pseudomonas aeruginosa* in experiments.

Methods

Chemicals

KCl, KH_2PO_4 was purchased from Carlo-Erba. NaCl, Na_2HPO_4, crystal violet were purchased from Merck. Yeast and peptone were purchased from Mast Diagnostics. Acetic acid was purchased from Acros. Ethanol was purchased from Riedel de Haen. All chemicals used were of analytical grade.

Bacterial strains

Bacterial strain *Pseudomonas aeruginosa* (ATCC 27853) was used in this study. Cells were maintained on agar plates at 4 ºC with transfers at monthly intervals.

Waste frying oil

Waste frying oil (WFO) was obtained and collected from the food Restaurant Malatya, Turkey. Waste frying oil was filtered for removing crude impurities and then, WFO autoclaved, and then used.

Growth conditions

Pseudomonas aeruginosa was firstly cultured in Luria-Bertani (LB) broth medium (g l^{-1}); peptone (10), NaCl (10), and yeast extract (5). The final pH values of broth media was adjusted to 7.0. The same amounts of cells were grown at 37°C, 0 rpm on incubator for overnight (O/N). 100 µl of overnight cultures (OD_{600} nm ~0,2-0,3) grown tube filled with 5 ml in 10 ml tubes was inoculated, and incubated for 24 h of time. Phosphate-buffered saline (PBS buffer) (gl^{-1}; 8,0 NaCl,0,2 KCl, 1,44 Na_2HPO_4, 0,24 KH_2PO_4 and pH 7,4) and PBS+10% waste cooking oil (WFO), and cells were cultured at 37°C for 24 h; 0, 100 rpm or 200 rpm shaker.

Biofilm formation

After the incubation, the supernatant was removed. Biofilm tubes were washed four times with 1 x phosphate buffered saline (PBS) to eliminate any remaining cells. Cells attached to the tubes were then fixed with ethanol (%99) for 15 min room temperature and stained with 1% crystal violet. After staining, excess crystal violet was eliminated with water, and 33% acetic acid was used to dissolve the remaining dye. Biofilm mass was finally determined as a function of the concentration of this dye based on the absorbance at 570 nm [10-14].

Biofilm formation was standardized to growth with the biofilm index (BFI), which was calculated. The extent of biofilm formation was determined by applying this formula: "BFI = (AB - CW) / G in which BFI is the "Biofilm Formation Index was defined as the average percentage of bacteria grown as biofilms [15]", AB is the OD_{570} nm of stained attached bacteria and CW is the OD_{570} nm of stained control tubes containing only bacteria-free medium, G is the OD_{600} nm of cells growth in suspended culture" [6,7]. OD_{600} and OD_{570} were measured using a spectrophotometer directly from tubes. OD_{600} and OD_{570} versus time were plotted to obtain growth curves and biofilm formation, respectively. Each value is the average of three independent experiments.

Results

Biofilm

The addition of WFO in all experimental conditions prevented biofilm formation and caused declines. This decline was seen in 14-fold to 100 rpm air agitation condition, with a minimum reduction of 2.7 folds to 200 rpm in air agitation condition. When we look at the difference; cell counts under non-shake conditions and 100 rpm air agitation condition, a 1.4-fold increase was observed at only 200 rpm air agitation condition. At 100 rpm shaking conditions a significant decline is evident (Figure 1).

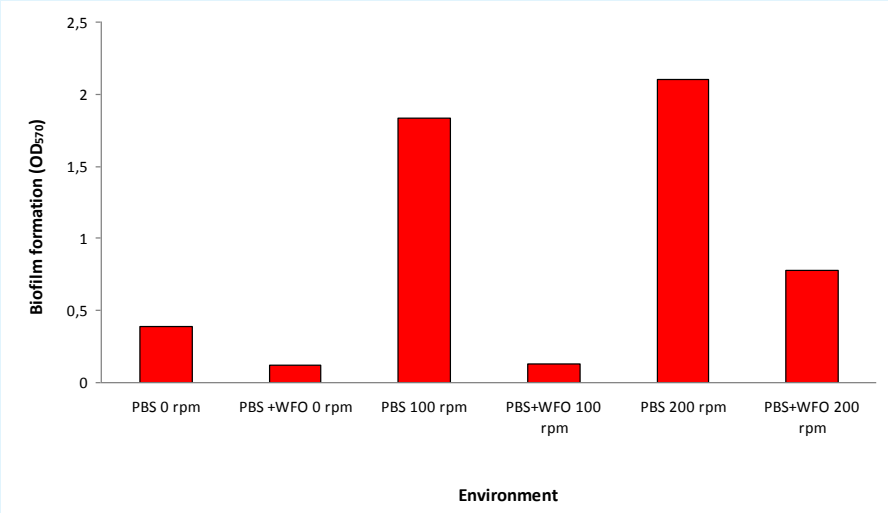

Figure 1: Biofilm formation on tubes by *P. aeruginosa* with different gentle swirling at 37°C for 24 h.

BFI

An increase is observed at 200 rpm air agitation condition while a decrease is observed at 100 rpm air agitation condition at 0 rpm non-shake conditions. The smallest value is 0.84, while the highest value is 0.98. The difference was only 1.2 times. This corresponds to only 16% difference (Figure 2).

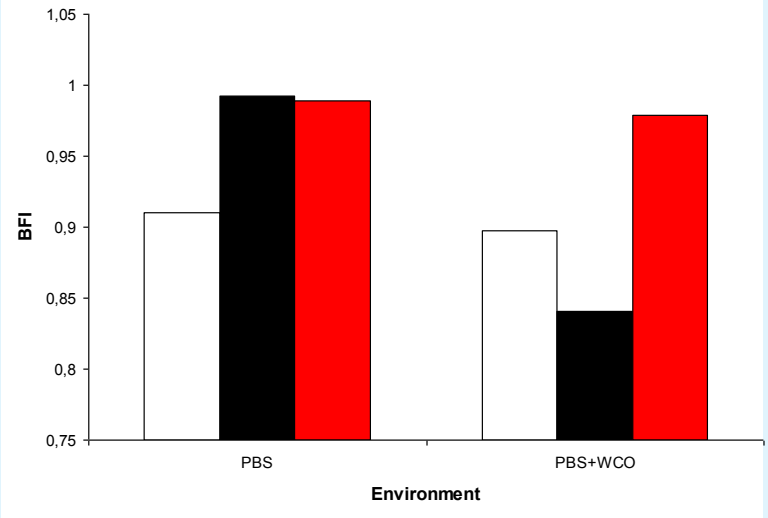

Figure 2: Biofilm index. Adhesion to glass surfaces of *P. aeruginosa* with different gentle swirling at 37°C for 24 h.

cfu

When the PBS medium was not taken into account, the increase in cell count in the WFO-supplemented medium occurred at a maximum of 200 rpm in air agitation condition (4.90E + 07) while the lowest increase (1.29E + 07) in the non-shake environment occurred at 0 rpm. As

can be seen, as the ventilation conditions increase, the number of cells increases. When the 24th and the final time were compared, the highest difference was observed in 19 times and 100 rpm shaking conditions, while the lowest difference was observed in the non-shake conditions of 2.7 times and 0 rpm (Figure 3).

When we look at the difference; a 6-fold reduction in cell count was achieved in non-shake conditions, a 3-fold increase at 100 rpm shaking conditions, and a 7-fold increase at 200 rpm shaking conditions. The biggest headlights were at 5735% at 100 rpm. This was followed by 754% and 200 rpm, respectively (Figure 4).

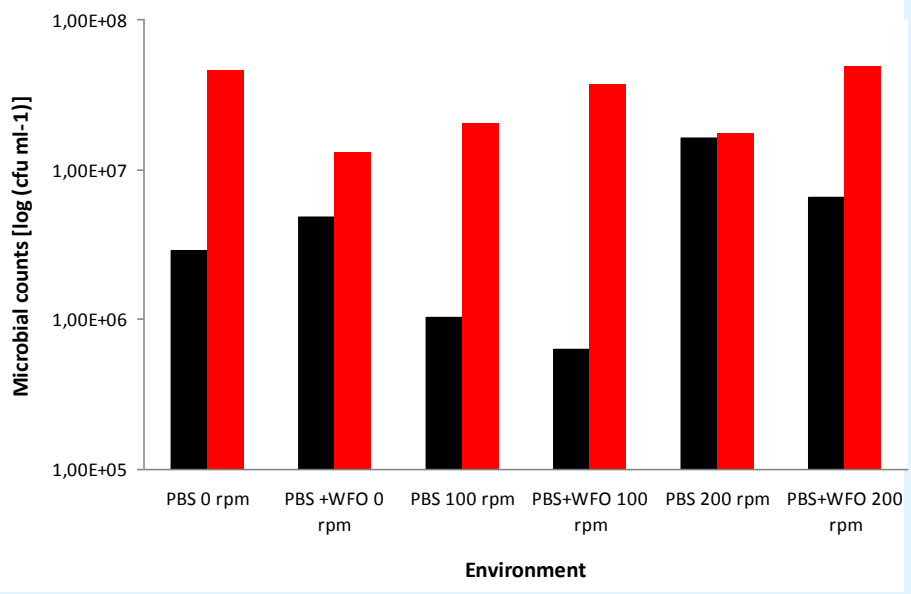

Figure 3: Microbial counts of *P. aeruginosa* with different gentle swirling at 37°C for 24 h.

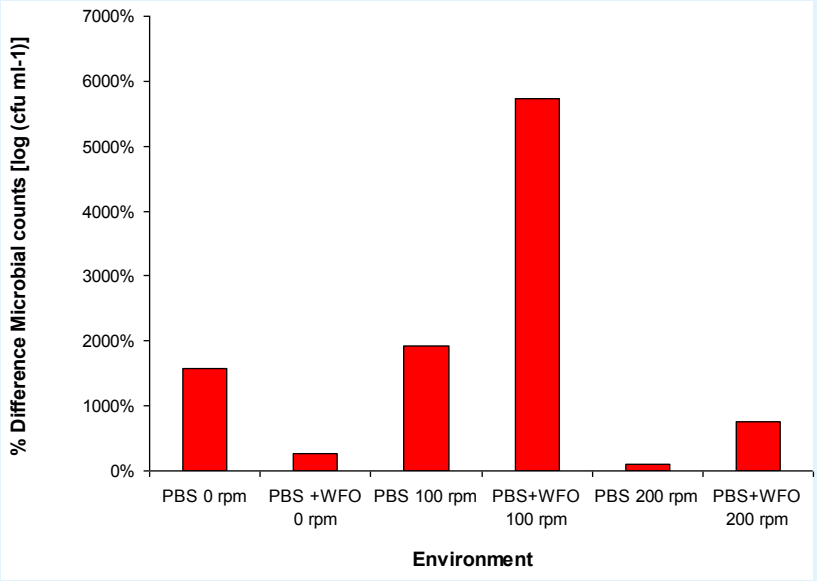

Figure 4: OD_{600} values of *P. aeruginosa* with different gentle swirling at 37°C for 24 h.

OD_{600}

However, when we look at the OD_{600} values we call biomass; again the greatest increase occurred at 100 rpm air agitation (0.025) while the lowest rate and even decreased at 100 rpm air agitation condition. As a different point of view; the highest rate was observed in the shake conditions at 1.4 times and 200 rpm, while the lowest rate was observed in the non-shake conditions at 0.7 times and 100 rpm (Figure 5). The reason for this is not fully understood.

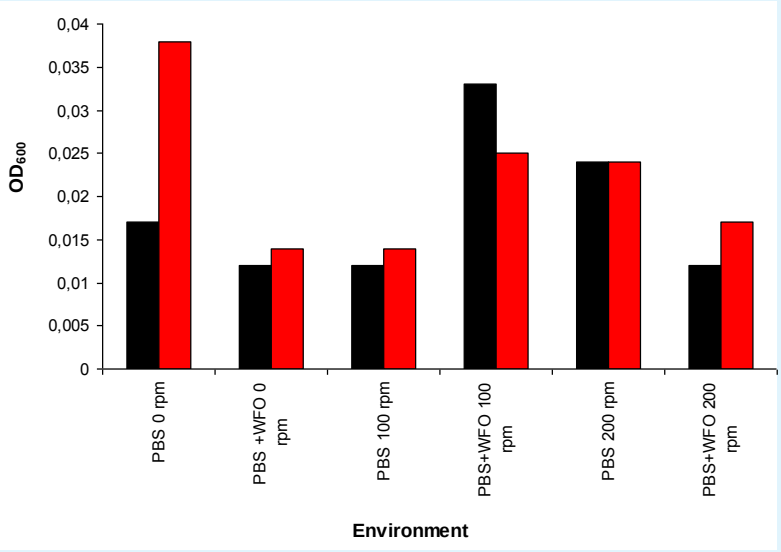

Figure 5: Percentage of the difference between cell numbers at initial and after 24 hours.

When we look at the differences biomass (OD_{600}); with is the biggest rate at 142% at 200 rpm and this was followed by non-shake conditions at 0 rpm with 117%, respectively (Figure 6).

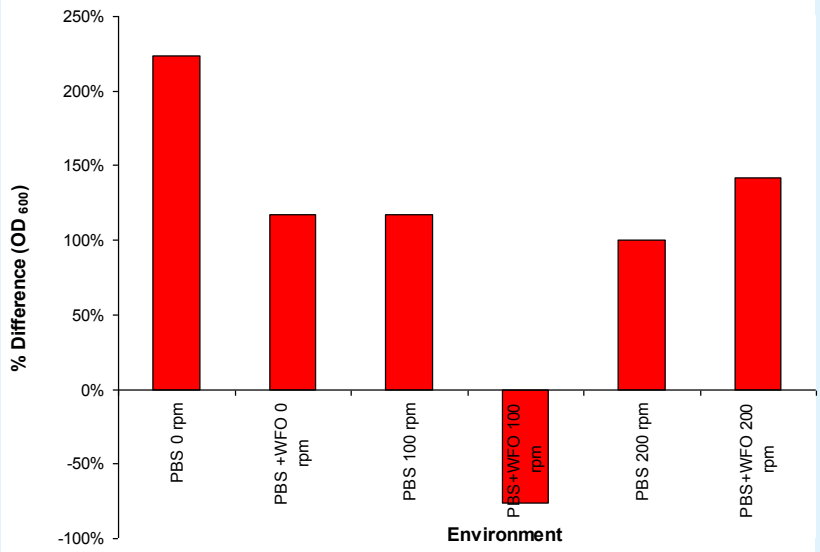

Figure 6: Percentage of the difference between cell density values at initial and after 24 hours.

The reason for the decrease in the OD_{600} value is not fully understood. The addition of WFO in all experimental conditions has prevented the formation of biofilm and blocking 2- fold more. Conversely, when WFO causes cell numbers to increase, no significant change in biomass has been observed. Under no circumstances OD_{600} = 0.05 was achieved.

The greatest increase in cell numbers (cfu/ml) occurred at 5735% at 100 rpm, while the greatest decrease (-76%) occurred in the same medium (OD_{600}) in the same medium.

Discussion

At the same time, similar studies with microorganisms will reduce the use of chemicals and open up waste oils in such works. In both cases the environment will be preserved.

In many study, in addition to waste oil, various minerals and trace elements have not been added to our work [3,16]. At the moment, these waste oils are not processed in any heat or chemical process. Waste oil is generally used in research for biodiesel production. However, we sought to find both a growth environment and possibilities for biofilm production. Waste frying oil (WFO) is, especially, a more sustainable alternative feedstock for biofilm production and growth.

Bacterial biofilm production is affected by many factors, including the surface properties of the material, the characteristics of the bacteria, and the environmental factors [7].

Our knowledge so far no study has compared the effect of WFO on biofilm formation under both static and agitated conditions. *Pseudomonas aeruginosa* is capable of using many biological and chemical wastes as carbon, energy and growth source [3]. This is at the exit of our work.

P. aeruginosa is usually a strong biofilm producer, but in a study with *P. aeruginosa*, *E. coli* and *S. agona*, it was stated that these three bacteria are capable of forming a weak biofilm. *P. aeruginosa* has been shown to have the lowest BFI value [5].

Conclusion

We have demonstrated that *Pseudomonas aeruginosa* was able to grow well on the PBS with WCO as the sole carbon source. This waste oil had significant influences on bacterial growth and biofilm synthesis. In this work, *Pseudomonas aeruginosa* also possessed good adaptability in WFO, and it was able to produce biofilm and growth when degraded the oils. Waste oils may be an alternative, especially for studies where biofilm formation is not desired or should be reduced. As a result of the work we have done, it has been thought that waste frying oils can be used as the medium for fattening.

References

1. George S, Jayachandran K (2012) Production and characterization of rhamnolipid biosurfactant from waste frying coconut oil using a novel *Pseudomonas aeruginosa* D. J of Appl Microbiol 114(2): 373-383.

2. Amin TK, Amin AS, Mazaheri H (2013) A review on novel processes of biodiesel production from waste cooking oil. Appl Energy 104: 683-710.

3. Wadekar SD, Kale SB, Lali AM, Bhowmick DN, Pratap AP (2012) Microbial synthesis of rhamnolipids by *Pseudomonas aeruginosa* (ATCC 10145) on waste frying oil as low cost carbon source. Prep Biochem Biotechnol 42(3): 249-266.

4. Lan G, Fan Q, Liu Y, Chen C, Li G, et al. (2015) Rhamnolipid production from waste cooking oil using *Pseudomonas* SWP-4. Biochem Eng 101: 44-54.

5. Teh KH, Flint S, French N (2010) Biofilm formation by Campylobacter jejuni in controlled mixed-microbial populations. Inter J Food Microbiol 143(3):118-24.

6. Naves P, del Prado G, Huelves L, Gracia M, Ruiz V, et al. (2008) Measurement of biofilm formation by clinical isolates of *Escherichia coli* is method-dependent. J Appl Microbiol 105(2): 585-590.

7. Berlanga M, Gomez-Perez L, Guerrero R (2017) Biofilm formation and antibiotic susceptibility in dispersed cells versus planktonic cells from clinical, industry and environmental origins. Antonie van Leeuwenhoek 110(12): 1691-1704.

8. Sayin Z, Ucan US, Sakmanoglu A (2016) Antibacterial and anti-biofilm effects of boron on different bacteria. Biol Trace Elem Res 173(1): 241-246.

9. Orlandi VT, Rybtke M, Caruso E, Banfi S, Tolker-Nielsen T, et al. (2014) Antimicrobial and anti-biofilm effect of a novel bodipy photosensitizer against

Pseudomonas aeruginosa PAO1. Biofouling 30(8): 883-891.

10. Kim H, Park HD (2013) Ginger extract inhibits biofilm formation by *Pseudomonas aeruginosa* PA14. Plos One 8(9): 1-16.

11. Wu C, Cheng Y, Yin H, Song X, et al. (2013) Oxygen promotes biofilm formation of *Shewanella putrefaciens* CN32 through a diguanylate cyclase and an adhesin. Nature Sci Rep 3(1945): 1-7.

12. Adamus-Bialek W, Kubiak A, Czerwonka G (2015) Analysis of uropathogenic *Escherichia coli* biofilm formation under different growth conditions. Acta Biochim Pol 62(4): 765-771.

13. Crespo A, Pedraz L, Astola J, Torrents E (2016) *Pseudomonas aeruginosa* exhibits deficient biofilm formation in the absence of class II and III ribonucleotide reductases due to hindered anaerobic growth. Frontiers Microbiol 7: 688.

14. Goncalves Mdos S, Delattre C, Balestrino D, Charbonnel N, Elboutachfaiti R, et al. (2014)Anti-biofilm activity: a function of Klebsiella pneumoniae capsular polysaccharide. Plos One 9(6): e99995.

15. Castro J, Alves P, Sousa C, Cereija T, França A, et al. (2015) Using an *in-vitro* biofilm model to assess the virulence potential of bacterial vaginosis or non-bacterial vaginosis *Gardnerella vaginalis* isolates. Sci Rep 5: 11640.

16. Aghajani M, Rahimpour A, Amani H, Taherzadeh MJ (2018) Rhamnolipid as new bio-agent for cleaning of ultrafiltration membrane fouled by whey. Eng Life Sci 18: 272-280.